# Fish consumption benefits and PFAS risks: Epidemiology and public health recommendations

Ali Hamade

*Oregon Health Authority, Portland, OR, USA*

ABSTRACT

Finfish and shellfish intake (collectively referred to as fish) has been associated with health benefits, although fish often have chemical contaminants that are separately associated with health risks. The presence of chemical contaminants, however, does not inherently pose a health risk and optimizing the benefits is desirable for individual and population health. Reference doses (RfDs) and other comparison values that estimate contaminant or pollutant safety thresholds typically do not account for the benefits of the foods that carry them (e.g., fish, eggs, fruit, vegetables). Rather, these numbers are typically applied uniformly for various media such as food, soil, and water. This paper summarizes principal epidemiology studies on per- and polyfluoroalkyl substances (PFAS)-associated noncancer health indicators used by the United States Environmental Protection Agency (EPA) to develop RfDs for PFAS and compares these with the same health outcomes associated with seafood intake. Moreover, it frames these findings in relation to varying human PFAS exposures, fish intake amount, and fish type when the information is available. Further, it presents brief overviews of both general population temporal PFAS exposure trends and PFAS fish contaminant data in the United States. Finally, it discusses approaches that risk assessors and policy makers can consider in developing their fish consumption recommendations in relation to PFAS. In brief, epidemiology studies show that the benefits of fish intake generally counter the risks of PFAS exposure based on four noncancer health endpoints that EPA identified as having the greatest strength of evidence for PFAS health effects.

## 1. Background

The United States Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) have not issued fish consumption recommendations with respect to PFAS and state and Tribal health agencies in the United States are left to address this critical need. Health agencies and other advisory groups have a responsibility to frame risk appropriately and inform the public of the benefit/risk balance. There is a wealth of knowledge on the health benefits of eating and catching fish, in addition to the food security and spiritual aspects that traditional and subsistence communities enjoy and rely on as a way of life [1–5].

In 2016, EPA released RfDs for perfluorooctane sulfonic acid (PFOS) (20 ng/kg/d) and perfluorooctanoic acid (PFOA) (20 ng/kg/d) based on developmental health outcomes observed in animal toxicology studies [6,7]. In April 2024, EPA issued the most recent RfDs for several PFAS including PFOS (0.1 ng/kg/d) and PFOA (0.03 ng/kg/d) that are several orders of magnitude lower [8,9]. EPA RfDs are estimates of daily oral exposures lasting up to a lifetime which are likely without appreciable adverse noncancer health risks among a human population, including sensitive groups [10]. In developing the RfDs for these PFAS, EPA found most relevant the epidemiology studies that reported associations with

PFAS exposure for lower birth weight (BW), lower vaccine antibody concentrations in children, and higher liver function enzyme (alanine transaminase, ALT) and total cholesterol (TC) concentrations in blood (Table 1). EPA has high confidence in the developmental studies and medium confidence in the studies for other endpoints and found the selected studies to have the greatest strength of evidence and lowest risk of bias among other studies.

Several agencies have developed comparison values for PFAS, collectively spanning several orders of magnitude [11–13]. None of these comparison values account for the benefits of eating fish.

Prior to the release of EPA's RfDs for PFOS and PFOA in 2024, several states had developed their own RfDs which they deemed more precautious or protective than the EPA RfDs from 2016 (ECOS, 2023). To develop fish intake recommendations, two main components are needed: 1) the concentration of contaminant in the fish or fish tissue to be consumed and 2) the RfD or similar value below which no adverse health effects are expected to occur with daily exposure (see equation in Table 2). State and other health agencies will develop fish intake recommendations when these components are available and might be prompted by agency or public concern or routine assessments of contaminants in fish. An illustration of how some RfDs developed by states

for PFAS may be applied to fish intake recommendations is presented by Petali et al. [14]. It is not clear if any states plan to adopt EPA's RfDs instead of their current values for this purpose, as doing so could severely limit the amount of fish recommended for intake, particularly from freshwater sources. Table 2 uses an equation and assumptions to compare fish intake recommendations based on EPA's RfD for PFOS with those based on RfDs from several states. The results show states would potentially recommend consumption of 18–50 times as much fish as relying on EPA RfDs would when PFOS is the risk driver. This is particularly concerning since the use of EPA's RfD for PFOS/PFOA would suggest recommending very little to no consumption of many freshwater fish species even when they do not come from highly contaminated waters. Recommendations for many store-bought fish could also be affected albeit to a much lower extent because these fish appear to have lower PFOS concentrations than freshwater fish [15,16]. This paper aims to provide a general scope of the fish intake benefits and PFAS risks epidemiology, with EPA RfDs in perspective, that the reader can use to inform fish consumption recommendations.

## 2. Methods

In the following sections is an evaluation of studies that EPA identified as the most important for developing RfDs, additional relevant studies on PFAS-associated outcomes, mainly meta-analyses of the RfD endpoint of concern or associated disease, and a narrative review of studies that examined the effects of fish intake on health endpoints and biomarkers. Occasionally, follow-up studies are added for context or other studies are added when there are few meta-analyses (e.g., PFAS and liver disease). This paper focuses on PFOS and PFOA, the PFAS with the most available data on human health effects, as proof of concept.

This is not meant to be a comprehensive review or a weight of evidence evaluation of the PFAS or fish intake health effects epidemiology literature. One aim of this paper is to explain the studies that EPA used to develop candidate RfDs. A second aim is to provide a broader view of the literature since EPA might have selected studies that do not necessarily reflect the range of outcomes in the literature. Therefore, presenting meta-analyses in this paper provides a broader representation of the magnitude of PFAS-health effects associations. For fish consumption health effects, the paper starts with meta-analyses and extends into studies that include outcomes based on amount and type of fish, when available.

Moreover, there are studies of other health effects of interest for both PFAS exposure and fish intake (e.g., renal, thyroid), but the focus of this paper is on the health endpoints that EPA found to have the most strength of evidence associated with PFAS exposure.

Lastly, because this paper is not a systematic review, the basic conclusions from EPA and ATSDR on the health evidence for PFOS and PFOA are summarized in Table 3. The reader can refer to the respective reports for an overall assessment of the weight of evidence in both epidemiology and animal toxicology literature. In depth analyses of fish intake epidemiology outcomes are available elsewhere [5].

**Table 2**
Comparison of recommended fish meals using EPA [8,9] and state [12] RfDs for PFOS.

| | PFOS RfD (ng/kg/d) | PFOS concentration (ng/g wet weight)* | Body weight (kg) | 4-ounce meals/ week |
|---|---|---|---|---|
| EPA 2024 | 0.1 | 3.07 | 70 | 0.14 |
| Massachusetts | 5.0 | 3.07 | 70 | 7.0 |
| Minnesota | 3.1 | 3.07 | 70 | 4.4 |
| New Jersey | 1.8 | 3.07 | 70 | 2.5 |
| Oregon | 4.1 | 3.07 | 70 | 5.8 |

$$\frac{Meals}{week} = \left(\frac{consumer\ body\ weight\ in\ kg\ x7\frac{days}{week}}{113\ \frac{g\ fish}{meal}}\right) x \frac{RfD\left(\frac{ng}{kg\ body\ weight - day}\right)}{PFOS\ concentration\ in\ fish\left(\frac{ng}{g}\right)}$$

* Median of all fish collected during 2018–2019 and tested by Stahl et al. [17] as part of the National Rivers and Streams Assessment.

## 3. Results

### 3.1. Developmental effects

The candidate RfDs for this endpoint are based on BW decrements associated with maternal PFAS exposure. Therefore, the following sub-section discusses PFAS studies used by EPA that assessed BW and other birth outcomes when presented by these studies. Subsequently, the same is done for studies of maternal fish intake and birth outcomes. Birth outcomes examined in relation to PFAS exposure (discussed in depth by EPA [8,9] and ATSDR [11]) and fish consumption include differences in BW, low birth weight (LBW), small for gestational age (SGA), preterm birth (PTB), and others. Low birth weight is usually defined as BW < 2500 g and PTB is typically a length of gestation < 37 weeks.

#### 3.1.1. PFAS studies

EPA relied on the studies by Darrow et al. [18], Sagiv et al. [19], and Wikström et al. [20] for associations between maternal plasma or serum PFAS and birth weight. As part of the C8 Health Project, Darrow et al. [18] found a natural logarithmic unit increase of maternal serum PFOS associated with lower BW, mainly among women whose serum sample was collected after their first pregnancy (-49 g; 95 % CI: −90, −8), but not among all births. The corresponding BW decrease per serum PFOS interquartile range (IQR) (10 ng/mL) was −29 g (95 % CI: −58, 0). Maternal PFOA was not associated with BW and neither PFOS nor PFOA were associated with LBW or PTB, although higher PFOS concentrations were associated with increased odds of pregnancy-induced hypertension. Sagiv et al. [19] reported the following associations between 1 ng/mL increments in maternal plasma PFAS and BW: PFOS: −1.1 g (95 % CI:-2.6, 0.3); PFOA: −4.9 g (95 % CI: −11.9, 2.2). In addition, the authors found elevated odds of PTB for PFOS (Odds Ratio, OR = 1.1; 95 % CI: 1.0, 1.3) and PFNA (OR = 1.2; 95 % CI: 1.0, 1.4) per IQR in plasma, but not for PFOA or PFHxS, during 1999–2002 in Massachusetts. Maternal median PFOS and PFOA plasma concentrations were 25.7 ng/mL and 5.8 ng/mL, respectively. In the Swedish SELMA cohort

**Table 1**
Final studies that EPA considered in proposing PFOS and PFOA RfDs [8,9].

| PFAS | Endpoint considered and study-derived candidate and final RfDs (ng/kg/d) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lower birth weight | | | Lower vaccine antibody concentrations | | | Higher total cholesterol | | Elevated alanine transaminase (ALT) | | |
| | Sagiv et al. 2018 | Wikström et al. 2020 | Darrow et al. 2013 | Budtz-Jorgensen and Grandjean 2018 | Timmerman et al. 2022 | Zhang et al. 2023 | Dong et al. 2019 | Steenland et al. 2009 | Gallo et al. 2012 | Nian et al. 2019 | Darrow et al. 2016 |
| PFOS | 0.6 | **0.1** | 0.3 | 0.2–0.3 | 0.1–0.2 | 0.4 | **0.1** | 0.1 | 0.7 | 0.2 | NA |
| PFOA | 0.1 | **0.03** | | **0.03** | 0.02–0.03 | | **0.03** | 0.05 | 0.2 | 0.05 | 0.8 |

*bold denotes final RfDs

**Table 3**

Summary of EPA and ATSDR conclusions on the epidemiological evidence for PFOS and PFOA health effects.

| Health endpoint | EPA [8,9] | ATSDR [11] |
|---|---|---|
| Developmental | Consistent adverse effects on fetal growth restriction and post-natal growth. Consistent deficits in birth weight, although potential bias due to hemodynamic differences in studies using samples from later pregnancy. Inverse effects "observed" on gestational age along with increased risk of preterm birth. However, EPA also noted overall mixed evidence of exposure to PFOA and these two outcomes. | Mixed results for birth outcomes, particularly birth weight (meta-analyses found increases in maternal PFOA or PFOS associated with 11–19 g or 1–5 g decreases in birth weight, respectively). Accounting for maternal glomerular filtration rates attenuated results by ≈ 50 %. In general, no associations between exposure and risk of adverse pregnancy or birth outcomes (miscarriage, low birth weight, preterm birth, or small for gestational age, birth length, ponderal index, sex ratio, or birth defects) or neurodevelopmental outcomes (IQ or scholastic achievement, motor skills, and risk of ADHD). |
| Cardiovascular | "Evidence for cardiovascular effects is based on […] studies reporting positive associations with serum lipids (LDL and TC) in adults from the general population." Cardiovascular disease studies of general population adults reported mixed results. | Studies suggest associations between total cholesterol and LDL cholesterol, and serum PFOS and PFOA. "In general, occupational exposure studies have not found increases in the risks of deaths from heart disease or in the risks of ischemic heart disease, cerebrovascular disease, or coronary disease." ATSDR noted inconsistent results in small number of studies of the general population and of residential areas with high PFOA in drinking water. |
| Immune | "Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus." "An increased risk of upper and lower respiratory tract infections was observed among children…" | The strongest evidence for PFOS and PFOA immunotoxicity comes from studies evaluating the antibody response to vaccines. "In general, decreases in infectious disease resistance have not been found for PFOA, PFOS, PFHxS, or PFNA." |
| Hepatic | "Evidence for hepatic effects is based on increases in ALT in adults…" Liver disease or injury only observed in low confidence studies and there was lack of coherence across measures of liver inflammation. Limited availability of high quality studies. | Inconsistent associations between serum PFOA levels (not PFOS) and increases in serum ALT, AST, and GGT and decreases in serum bilirubin. Serum enzyme levels typically in normal range. Considerable variability across studies of liver enzymes and not all studies adjusted for potential confounders. Exposures to PFOS and PFOA not consistently suggestive of an association with increased risks of liver disease in workers or highly exposed community members. |

during 2007–2010, Wikström et al. [20] found that a 1 ng/mL increase in maternal serum PFAS associated with decreased BW and increased SGA odds for girls and not boys with Quartile 4 (Q4) vs. Quartile 1 (Q1) of exposure [PFOS (BW: −142 g; 95 % CI: −231, −54; SGA OR = 2.05; 95 % CI: 1.00, 4.21) and PFOA (BW: −136 g; 95 % CI: −231, −40; SGA OR = 2.33; 95 % CI: 1.00, 5.43)]. There were no significant associations in Q2/Q3 vs. Q1. Maternal median PFOS and PFOA serum concentrations were 5.38 ng/mL and 1.61 ng/mL, respectively. Table 4a presents

studies on PFAS exposure and birth outcomes.

Several meta-analyses found lower BW associated with higher maternal PFAS exposure ranging from −1 g to −5 g BW per 1 ng/mL PFOS increment in prenatal serum or plasma and −3 g to −18 g for 1 ng/mL PFOA [21–25]. Steenland et al. [24] further assessed BW changes by exposure assessment timing and found a smaller decrease in BW (−3.3 g; 95 % CI: −9.6, 3.0) when blood was drawn early in pregnancy or shortly before conception vs. when done late in the pregnancy (−17.8 g; 95 % CI: −25.0, −10.6). Negri et al. [23] and Dzierlenga et al. [21] reported similar observations. These effect differences in timing might be related to changing gestational plasma volume and glomerular filtration rate, as stated in several of these meta-analyses.

EPA and ATSDR both found evidence for associations between higher maternal PFAS exposures and small reductions in birthweight. However, both noted the influence of hemodynamic differences in pregnancy on this association. And, while ATSDR found no association between maternal PFOS/PFOA exposure on other birth outcomes or neurodevelopmental outcomes, EPA noted potentially adverse effects on gestational age and preterm birth outcomes, albeit with mixed findings (Table 3).

*3.1.2. Fish consumption studies*

Meta-analyses investigating the effect of maternal fish intake on birth outcomes generally found small increases in BW and reduced risk/odds of adverse birth outcomes with higher intake. For example, Leventakou et al. [26] found that consuming fish ≥ 3 times/week vs < 1 time/week was associated with lower risk of PTB (RR = 0.89; 95 % CI: 0.84, 0.96) and a baby with higher BW (15.2 g; 95 % CI: 8.9, 21.5), with no association by fish type (lean, fatty, other). The BW increment was larger in mothers who smoked during pregnancy. Similarly, Zhao et al. [27] found that a 45 g/day increment in maternal seafood intake of all types was associated with reduced odds of LBW (OR = 0.65, 95 % CI: 0.47, 0.90), PTB (OR = 0.84, 95 % CI: 0.70, 1.01) and SGA (OR: 0.84, 95 % CI: 0.71, 0.98) with sustained reduced odds of PTB and SGA up to the maximum intakes of 80 g/d and 150 g/d, respectively, for these endpoints in this analysis. Also, there was a modest J-shaped association between fatty fish and PTB with the lowest odds at intake of 30 g/day and possible increased odds (> 1) starting at ≥ 60 g/day for the central estimate. Moreover, an increment of 45 g/d of lean fish intake was associated with higher odds of LBW (OR: 3.51, 95 % CI: 1.16, 10.66), which the authors noted was based on two studies and might be associated with frying that is more common with lean fish. Table 4b presents studies on fish intake and birth outcomes.

Individual studies can highlight specifics and variation in associations between fish intake and birth outcomes. With increasing fish intake, most studies showed increased BW [28–35], lower risk/odds for SGA [28,29,33,35–39], and lower risk/odds of PTB [32,40–42] . Other studies showed no associations with birth outcomes [43–45], although Rogers et al. [45] found that not eating fish was associated with increased odds for intrauterine growth restriction. However, other studies showed lower BW or increased risk/odds of LBW, SGA, or PTB [33,46–50].

Associations in individual studies varied with amount of fish intake. For example, some showed increasing benefit on PTB up to the highest reported fish intake (≥ 420 g/week for Brantsæter et al. [40]; > 350 g/week for Wang et al. [42]) while Halldorsson et al. [46] showed higher odds of SGA only in those consuming > 420 g/week of fish. Heppe et al. [43] found no consistent association between maternal intake (lean fish, fatty fish, and shellfish combined averaging > 210 g/week) and PTB, LBW, or SGA, but reported that weekly consumption of shellfish > 14 g/week was associated with lower BW (-41.7 g). Nykjaer et al. [44] found no association with intake averaging > 200 g/week of fatty fish on birth outcomes and Benjamin et al. [51] found higher odds of an SGA infant only in women having ≥ 7 meals/week of all fish (< 1 % of the cohort population) but no effect in those consuming up to 6 meals/week (ORs ≤ 1).

**Table 4a**
Birth outcomes and PFAS.

| Study and population | Maternal PFAS serum (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| ***RfD candidate studies*** | | | | | |
| Darrow et al. 2013<br>1330 women. C8 Health Project, Ohio and West Virginia. USA | Geometric mean PFOS: 13.2 PFOA: 16.2 | 2005–2010 | BW, LBW, PTB | Serum natural log (ln) unit increase vs. BW:<br>Among all births (n = 1470)<br>• PFOS: −29 g (95 % CI: −66, 7)<br>• PFOA: −8 g (95 % CI: −28, 12)<br>Among those in their first pregnancy conceived after serum measurement (n = 710)<br>• PFOS: −49 g (95 % CI: −90, −8)<br>• PFOA: 5 g (95 % CI: −22, 33)<br>No association between PFAS and LBW or PTB. | Increased odds of pregnancy-induced hypertension with higher PFOS and PFOA maternal serum. |
| Sagiv et al. 2018<br>1645 women in the Project Viva birth cohort, Massachusetts, USA | Plasma PFOS Median: 25.7 IQR: 16.0 Plasma PFOA Median: 5.8 IQR: 3.8 | 1999–2002 | BW, PTB | Maternal IQR serum PFAS increase vs. BW:<br>• PFOS: β = −17.9 (95 % CI: −40.9, 5.1)<br>• PFOA: β = −18.5 (95 % CI: −45.4, 8.3)<br>PTB risk per IQR increase:<br>• PFOS: OR = 1.1 (95 % CI: 1.0, 1.3)<br>• PFOA: OR = 1.0 (95 % CI: 0.9, 1.3) | BW change for 1 ng/mL plasma PFAS increase:<br>PFOS<br>−1.1 g (95 % CI: −2.6, 0.3)<br>PFOA<br>−4.9 g (95 % CI: −11.9, 2.2) |
| Wikström et al. 2020<br>1533 infants. SELMA study, Sweden | PFOS Median: 5.38 IQR: 3.97, 7.60 95th %ile: 10.34 PFOA Median: 1.61 IQR: 1.11, 2.30 95th %ile: 3.18 | 2007–2010 | BW, SGA | Maternal serum Q4 vs. Q1 (only in girls):<br>PFOS<br>• BW: −142 g (95 % CI: −231, −54)<br>• SGA OR = 2.05 (95 % CI: 1.00, 4.21)<br>PFOA<br>• BW: −136 g (95 % CI: −231, −40)<br>• SGA OR = 2.33 (95 % CI: 1.00, 5.43) | No association for Q2/Q3 vs. Q1 in girls. No significant association for boys in any quartile. |
| ***Meta-analyses and relevant studies*** | | | | | |
| Dzierlenga et al. 2020<br>Meta-analysis of 29 studies | | 2007–2019 | BW | 1 ng/mL increment in maternal serum PFOS vs. BW<br>• −3.22 g (95 % CI: −5.11, −1.33)– (all studies)<br>• −7.17 g (95 % CI: −10.93, −3.41) (blood collected late in pregnancy)<br>• −1.35 g (95 % CI: −2.33, −0.37) (blood collected early in pregnancy) | |
| Johnson et al. 2014<br>Meta-analysis of 9 studies | | 1991–2009 | BW | 1 ng/mL increment in maternal plasma PFOA vs. BW<br>• −18.9 g (95 % CI: −29.8, −7.9) | Includes the seven studies in Verner et al. (2015). |
| Lauritzen et al. 2017<br>424 mother–child pairs. Norway and Sweden | Median Swedish PFOA: 2.33 PFOS: 16.4 Norwegian PFOA: 1.62 PFOS: 9.74 | 1986–1988 | SGA | Swedish women<br>• Prenatal PFOA, PCB 153, HCB associated with higher odds for SGA birth.<br>Norwegian women<br>• No associations before or after adjusting for fish intake | Fish intake data not available for the Swedish contingent. |
| Negri et al. 2017<br>Meta-analysis of 16 studies | | 1991–2013 | BW | Maternal blood PFAS increase vs. BW<br>PFOS<br>• 1 ng/mL: −0.92 g (95 % CI: −3.4, 1.6)<br>• 1 $\log_e$ ng/mL: −46.1 g (95 % CI: −80.3, −11.9)<br>PFOA<br>• 1 ng/mL: −12.8 g (95 % CI: −23.2, 2.4)<br>• 1 $\log_e$ ng/mL: −27.1 g (95 % CI: −50.6, −3.6) | Increase of 1 $\log_e$ [PFAS] ≈ 2.7 times in untransformed [PFAS].<br>No consistent pattern of BW association with study location or blood sampling time. |

**Table 4a** (*continued*)

| Study and population | Maternal PFAS serum (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| Steenland et al. 2018 Meta-analysis of 24 studies | | 1990–2013 | BW | 1 ng/mL increment in maternal/cord blood PFOA vs. BW<br>• All times: −10.5 g (95 % CI: −16.7, −4.4)<br><br>• Late in pregnancy: −17.8 g (95 % CI: −25.0, −10.6)<br>• Early in pregnancy or before: −3.3 g (95 % CI: −9.6, 3.0) | Authors found reduced effect estimate in the newer studies. |
| Verner et al. 2015 Meta-analysis of 7 studies | | 1991–2006 | BW | 1 ng/mL increment in prenatal plasma PFAS vs. BW:<br>• PFOS: −5.00 g (95 % CI: −21.66, −7.78)<br>• PFOA: −14.72 g (95 % CI: −8.92, −1.09) | |

BW, birth weight; IQR, interquartile range (Q1-Q3); LBW, low birthweight; PTB, preterm birth; SGA, small for gestational age; ng/mL, nanogram(s) per milliliter.

Other study outcomes depended on type of fish (e.g., total seafood, fatty fish, lean fish, freshwater fish, or shellfish). For example, Brantsæter et al. [28,40] showed sustained reduced PTB risk with all seafood, lean fish, and shellfish intake, but fatty fish intake was neutral. Halldorsson et al. [46] found increased SGA odds and lower BW (-25.2 g) in the highest fish consumers that was related to fatty fish intake. A subsequent study by Halldorsson et al. [47] showed reduced BW in children born to women with higher PCB exposures in plasma (-155 g per IQR). Both Guldner et al. [36] and Wei et al. [35] found reduced SGA risk associated with higher combined fish and shellfish intake. While this was mainly driven by freshwater fish and shellfish in Wei et al., Guldner et al. [36] found increased SGA odds when shellfish were analyzed separately and it was driven by large crustaceans that the authors indicated had higher concentrations of dioxins, PCBs, arsenic, and cadmium than fish. Alternatively, Amezcua-Prieto et al. [52] found reduced odds of SGA with shellfish intake. Ramón et al. [33] found that eating ≥ 2 portions/week of larger oily fish (swordfish, fresh tuna, bonito) was associated with higher odds of being SGA but decreased odds with lean fish (hake, sole, gilthead) and not remarkable for smaller oily fish (mackerel, anchovy, salmon, and sardine). The authors' additional findings of higher odds of a child being SGA (OR = 5.3) and weighing less (BW, −143.7 g) for Q4 vs. Q1 cord blood mercury exposure might partially explain the association with intake of larger fish. Likewise, Mohanty et al. [49,50] found higher risk of PTB with increased lean fish intake, but not for fatty fish or shellfish and suggested that the lean fish intake might be associated with increased exposure to trans fatty acids from frying that have been associated with LBW [53], although mercury exposure was not assessed.

Other studies also found variation in birth outcomes depending on maternal BMI or time of fish intake data collection [29,54]. These are important factors to consider, although a detailed discussion is outside the scope of this paper.

Several studies showed a beneficial effect of fish intake that countered the effects of risk factors and toxic exposures. For example, a subsequent analysis of the SELMA cohort [55] confirmed the inverse relationship between BW and maternal PFOS exposure by Wikström et al. [20]; however, freshwater fish intake > 0.35 times/week decreased the slope of logPFOS related to BW from −120.5 to −48.9 indicating a benefit of maternal fish consumption. The authors noted that 95 % of pregnant women who filled the food frequency questionnaire ate fish < 3 times/week and 16 % consumed no fish. Likewise, Jedrychowski et al. [56] suggested that higher fish intake during pregnancy nullified the BW lowering effects of fine particulate matter exposure and Taylor et al. [57] found an effect of maternal blood mercury concentration on BW that was modified by fish intake (all women, −3.1 g; non-fish eaters, −58.4 g; fish eaters; −1.5 g). Lastly, Martínez-Galiano et al. [58] found that diet can counteract some risk factors

for SGA. For example, fruit intake countered the effect of smoking and fish intake countered the effect of maternal BMI < 20 kg/m$^2$ on BW.

Consideration for fish intake and health outcomes include genetic variation that is infrequently investigated in environmental epidemiology studies. More attention is needed in that area, particularly when studies have shown interactions between specific alleles and fish intake or fish contaminants on birth outcomes [54,59].

### 3.1.3. Summary of developmental effects

Overall, there is evidence of adverse associations between maternal PFAS exposure and decreased BW that depended on PFAS exposure assessment timing, but mixed evidence on risk of other birth outcomes. In contrast, higher maternal fish intake was generally associated with higher BW and lower odds of SGA, PTB, and LBW. There were few exceptions with consumption of lean fish, large oily fish, and shellfish, which in some studies were related to high seafood PCB or mercury content. Some authors also suggested that frying lean fish might have contributed to the adverse outcomes. One fish intake study that incorporated PFAS measurements showed contrasting effects of fish intake and PFAS exposure and another showed no effect of PFAS with or without fish intake. Lastly, despite contrasting effects of maternal PFAS exposure and fish intake on BW, studies on their interaction on weight gain later in life is needed.

### 3.2. Cardiovascular effects

The candidate RfDs for this endpoint are based on increments in TC associated with PFAS exposure. Because hypercholesterolemia can result in cholesterol deposits on arterial walls potentially causing coronary artery disease, a heart attack, stroke, or other cardiovascular disease (CVD), the following sections explore changes in blood cholesterol and CVD outcomes in association with each of PFAS exposure and fish intake.

### 3.2.1. PFAS studies

EPA relied on the studies by Dong et al. [60] and Steenland et al. [61] for associations between PFAS and TC in human serum. Dong et al. [60] explored associations within National Health and Nutrition Examination Survey (NHANES) data between PFAS in serum collected during 2003–2014 and TC. The authors found 1.5 mg/dL (95 % CI: 0.2, 2.8) and 0.4 mg/dL (95 % CI: 0.1, 0.6) TC increase for 1 ng/mL PFOA and PFOS increments, respectively. Steenland et al. [61] explored the association between serum PFAS (PFOA and PFOS) and lipids among adults from the C8 Health Project (2005–2006) who drank water with high PFOA contamination from chemical plant releases in West Virginia. The study population had median serum PFOA of 27 ng/mL and PFOS of 20 ng/mL. The authors reported an increase in TC from lowest to highest

**Table 4b**

Birth outcomes and fish consumption.

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| ***Meta-analyses*** | | | | |
| Leventakou et al. 2014<br>Meta-analysis of 19 birth cohorts. 151,880 women. Europe. | 1996–2011 | BW, HBW, LBW, PTB, SGA | Mothers eating fish $\geq$ 3 times/wk vs. < 1 time/wk:<br>• BW: 15.2 g (95 % CI: 8.86, 21.54)<br>• PTB RR = 0.89 (95 % CI: 0.84, 0.96)<br>1 time/wk increment in fish intake effect on BW:<br>• All fish: 1.46 g (95 % CI: 0.45, 2.46)<br>• Fatty fish: 2.38 g (95 % CI: 0.51, 4.25)<br>• Lean fish: 0.76 g (95 % CI: −2.45, 3.98)<br>No association between fish intake and SGA, LBW, HBW | BW increase larger in mothers who smoked during pregnancy.<br>Approximately two thirds of the population was from Norway and Denmark. |
| Zhao et al. 2021<br>Meta-analysis<br>21 studies with 571,641 women from India, New Zealand, Türkiye, Europe, USA. | 1973–2015 | LBW, PTB, SGA | 45 g/day increment in total maternal seafood consumption:<br>• LBW (7 studies) OR = 0.65 (95 % CI: 0.47, 0.90)<br>• PTB (7 studies) OR = 0.84 (95 % CI: 0.70, 1.01)<br>• SGA (7 studies) OR = 0.84 (95 % CI: 0.71, 0.98)<br>45 g/day increment in maternal lean fish consumption<br>• LBW (2 studies) OR = 3.51 (95 % CI: 1.16, 10.66)<br>• PTB (3 studies) OR = 0.98 (95 % CI: 0.46, 2.08)<br>• SGA (2 studies) OR = 0.64 (95 % CI: 0.22, 1.86) | Total seafood intake: Reduced PTB odds up to max. intake of 80 g/day.<br>J-shaped relation between fatty fish intake and PTB risk. Least risk at 30 g/day.<br>No other associations for fatty fish or shellfish intake with PTB, LBW, SGA. |
| ***Individual studies*** | | | | |
| Amezcua-Prieto et al. 2018<br>518 pairs of pregnant women, Spain | 2012–2015 | SGA | SGA risk:<br>• Shellfish (> once/wk): OR = 0.25 (95 % CI: 0.08, 0.76)<br>• Fish (> 29 vs. $\leq$ 8 g/d): OR = 0.63 (95 % CI: 0.41, 0.98)<br>SGA ORs < 1 for higher consumption of lean fish/fatty fish, but group sizes often small and not significant at p <0.05. | Fish intake assessed within 2 days after delivery.<br>Authors suggest pregnant women consume 3–4 servings of seafood/wk (minus highly contaminated species). |
| Benjamin et al. 2019<br>10,919 mother–infant pairs. National Birth Defects Prevention Study. USA | 1997–2011 | PTB, SGA | Eating fish $\geq$ 7 times/wk vs. <once/mo:<br>• PTB OR = 0.7 (95 % CI: 0.3, 1.6).<br>• SGA OR = 2.1 (95 % CI: 1.2, 3.4)<br>No association with intake $\leq$ 6 times/wk (OR $\leq$ 1) for SGA or PTB | Fish intake data collected in year prior to pregnancy. Fish serving = 85–140 g.<br>SGA estimates based on 21 SGA infants. Number of mothers in this group < 10 % other categories. |
| Brantsæter et al. 2012<br>62,099 women. Norwegian Mother and Child Cohort. | 2002–2008 | BW, SGA | Higher BW (up to 32 g) for > 60 vs. $\leq$ 5 g/d total seafood intake<br>o Association with lean fish and shellfish<br>o No association with fatty fish<br>• Lower risk of SGA with total seafood intake | Fish intake data collected during mid pregnancy |
| Brantsæter et al. 2017<br>67,007 women. Norwegian Mother and Child Cohort. | 2002–2008 | PTB | Total seafood intake and PTB risk vs. never/rare consumption:<br>• > 1 serving/wk: HR = 0.80 (95 % CI: 0.69, 0.93)<br>• $\geq$ 3 serving/wk: HR = 0.72 (95 % CI: 0.61, 0.85)<br>Associations true for total seafood (p < 0.001) and lean fish (p=0.005), but not fatty fish (p=0.411). | Fish intake data collected in mid to late pregnancy (weeks 12 and 30 of gestation). 1 serving $\approx$ 140 g.<br>Lean fish was 56 %, fatty fish 34 % and shellfish 10 % of seafood intake. |
| Drouillet et al. 2009<br>1805 women in the EDEN mother-child cohort study, France | 2002–2005 | BW, LGA, SGA | Seafood consumption and fetal growth:<br>• No effect for whole cohort or those with BMI $\leq$ 25.<br>• For women BMI $\geq$ 25<br>o Seafood before pregnancy: 167 g higher BW, lower odds of SGA, higher odds of LGA.<br>o Seafood at end of pregnancy: No association | Seafood intake data collected in<br>• Year before pregnancy: 8.5x/mo (60 % white fish)<br>• 3rd trimester: 8.2x/mo (less shellfish intake than pre-pregnancy) |
| Gennings et al. 2020<br>1312 women. SELMA cohort | 2007–2010 | BW | Fish intake > 0.35 times/week vs. less decreased the slope of the relationship between serum maternal logPFOS and BW from −120.5 to −48.9.<br>Fish intake questions were about amounts of "smoked fish, herring/mackerel, salmon, lake fish and other fish". | Fish intake data collected in mid pregnancy for freshwater fish.<br>Same cohort as Wikstrom et al. (2020) |
| Guldner et al. 2007<br>2398 pregnant women, (PELAGIE prospective cohort, Brittany, France) | 2002–2005 | BW, LBW, PTB, SGA | SGA risk<br>Fish and shellfish<br>$\geq$ 2x/wk vs. < 1x/mo: OR = 0.57 (95 % CI: 0.31, 1.05)<br>Shellfish alone<br>1–4x/mo vs. < 1x/mo: OR = 1.33 (95 % CI: 0.83, 2.11)<br>$\geq$ 2x/wk vs. < 1x/mo: OR = 2.14 (95 % CI: 1.13, 4.07)<br>No relation between fish/shellfish intake and BW/LBW/PTB | Fish intake data collected first trimester about fish intake just before pregnancy.<br>Authors indicated shellfish association mostly explained by consumption of large crustaceans that contained more dioxins, PCBs, arsenic, and cadmium than fish. |
| Halldorsson et al. 2007<br>44,824 women. Danish National Birth Cohort | 1996–2002 | BW, SGA | Eating > 60 g/d of fish vs. $\leq$ 5 g/d.<br>• SGA for BW: OR = 1.24 (95 % CI: 1.03, 1.49)<br>• BW: −25.2 g (95 % CI: −47.4, −3.0)<br>No association with SGA or BW for eating $\leq$ 60 g/d. | Fish intake data collected mid/late pregnancy.<br>Associations likely for fatty fish. None for lean fish including shellfish. |
| Halldorsson et al. 2008<br>100 nulliparous women. Danish National Birth Cohort | 1996–2002 | BW | Maternal plasma 75th vs. 25th PCB percentiles<br>BW: −155 g (95 % CI: −291, −19)<br>Adjustment for fish consumption and DDE did not significantly change association. | Fish intake data collected for mid to late pregnancy.<br>Plasma PCB association with daily fatty fish intake (Spearman's r=0.54) |
| Heppe et al. 2011<br>3380 pregnant women in the R | 2002–2006 | BW, LBW, PTB, SGA | No consistent association between intake of lean fish, fatty fish, or shellfish up to > 210 g/wk and PTB/LBW/ | Fish intake data collected during first trimester. |

*(continued on next page)*

**Table 4b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| Generation Study. The Netherlands. | | | SGA.<br>Intake > 14 g/wk shellfish associated with lower BW: −41.7 g (95 % CI, −81.2, −2.2) but not other fetal growth indicators. | |
| Jedrychowski et al. 2010<br> 481 pregnant women in Krakow, Poland | 2001–2004 | BW | BW decrease during pregnancy among mothers exposed to fine particulate matter exposure > 46.3 µg/m$^3$:<br>• Fish intake < 91 g/wk: −133 g (95 % CI: −267, 1)<br>• Fish intake 91– 205 g/wk: −93 g (95 % CI: −252, 65)<br>• Fish intake > 205 g/wk: −24 g (95 % CI: −220, 173) | Fish intake data collected in third trimester. PM2.5 determined by 48-hour personal air samples in second trimester. Median = 35.3 µg/m$^3$ (range: 10.4–249.9 µg/m$^3$). |
| Kamenju et al. 2022<br> 7564 women in Dar es Salaam, Tanzania | 2001–2004 | PTB | Fish intake (highest tertile vs. lowest tertile):<br>• Very PTB RR = 0.76 (95 % CI: 0.58, 0.99). | Fish intake data collected monthly in all trimesters. Fish consumed were mostly small dried lean fish. |
| Larsen et al. 2016<br> 2118 women. Danish National Birth Cohort<br> 4841 women from the ALSPAC study. UK. | 1996–2002<br>1991–1992 | Gestational weight gain (GWG) | Increment of 150 g/wk fish intake<br>Danish cohort: fatty fish GWG = 580 g (95 % CI: 0.16, 0.99) GWG among all women.<br>ALSPAC: Lean fish GWG = −1050 g (95 % CI: −1980, −130) among obese women only. | Fish intake data collected in mid/late pregnancy. Study compared obese and non-obese women. No other significant associations than ones reported here. |
| Mendez et al. 2010<br> 592 women from the Sabadell cohort, Catalonia, Spain | 2004–2006 | SGA | Maternal intake of > 1 meal/wk – SGA risk<br>• Crustaceans: OR = 3.05 (95 % CI: 1.34, 6.99)<br>• Canned fish OR = 2.49 (95 % CI: 1.04, 5.97)<br>• Fatty fish, lean fish, other shellfish: No association. | Fish intake data collected during first and third trimesters and at delivery.<br>Adjusting for PCBs/HCB/DDT/DDE/Hg/ beta-HCH did not alter associations. |
| Mitchell et al. 2004<br> 542 women living in New Zealand | 1995–1996 | SGA | Not consuming fish at conception vs. > once/week:<br>• SGA OR = 1.69 (95 % CI: 1.07, 2.69)<br>Eating fish ≤ once/week not associated with SGA risk. | Fish intake data collected retrospectively for the time of conception and the last month of pregnancy. |
| Mohanty et al. 2015, 2016<br> 3141 pregnant women, Omega study. Washington, USA | 1996–2008 | BW, LBW, PTB, pre-eclampsia | Fish intake and birth outcomes vs. < 0.2 serving/mo<br>• LBW<br>  o Lean fish (> 1 serving/wk): RR = 2.23 (95 % CI: 1.21, 4.09), but not at lower intakes.<br>  o Shellfish (0.5–1 serving/wk): RR = 0.54 (95 % CI: 0.31, 0.93), but no association at lower and higher intakes.<br>  o Total seafood (0.2/mo-< 0.5/wk): RR = 3.52 (95 % CI: 1.29, 9.57), but no association at higher intakes.<br>• PTB<br>  o Lean fish (> 1 serving/wk): RR = 1.55 (95 % CI: 1.04, 2.30), but no association at lower intakes.<br>Total seafood, fatty fish, shellfish intake not associated with PTB/BW/pre-eclampsia. Lean fish intake not associated with BW. | Fish intake data collected 3 mo before pregnancy and 3 mo after conception.<br>Median shellfish, lean fish and fatty fish intake (0.3, 0.5, and 0.5 serving/wk).<br>Authors suggested lean fish might be associated with trans fatty acids from frying.<br>No dose-response for lean fish effect on LBW and PTB.<br>Fish serving categories: (< 0.2/mo), (0.2/mo-< 0.5/wk), (0.5–1/wk), (> 1/wk) |
| Muthayya et al. 2009<br> 676 women from Bangalore, India | 2002–2006 | BW, LBW | • BW increased with maternal fish intake.<br>• Not eating fish during third trimester associated with higher risk of LBW (OR = 2.49; 95 % CI: 1.16, 5.36).<br>• No relation between fish intake and gestational duration. | Fish consumption data collected in each trimester. Median fish intake ≤ 10 g/d |
| Nykjaer et al. 2019<br> 1208 women. CARE study. UK. | 2003–2006 | BW, LBW, PTB, SGA | No association with BW, SGA, LBW, or PTB.<br>Average fatty fish portion = 101 g. | Pre-conception and trimester-specific fatty fish consumption. Multiple 24-h recalls during pregnancy. |
| Olsen et al. 1993<br> 767 women. Faroe Islands. | 1986–1987 | BW | BW of Faroese newborns increased with frequency of seafood dinner meals consumed in pregnancy, peaking or plateauing at 3 meals/wk. | Fish intake collected post-partum and calculated as sum of fish and pilot whale meals. |
| Olsen and Secher 2002<br> 8729 women in Aarhus, Denmark | 1992–1996 | IUGR, LBW, PTB | No fish intake vs. ≥ 1 meal/wk<br>• PTB OR = 3.60 (95 % CI: 1.15, 11.20)<br>• LBW OR = 3.57 (95 % CI: 1.14, 11.14)<br>• IUGR OR = 1.01 (95 % CI: 0.45, 2.26) | Fish intake data collected in second trimester |
| Ramón et al. 2009<br> 550 mother-infant pairs in the Infancia y Medio Ambiente project (INMA), Spain | 2004–2006 | BW, SGA | Eating ≥ 2 portions/mo vs. < 1 portion/mo:<br>• BW<br>  o Canned tuna: 116.4 g (95 % CI: 2.8, 230.0)<br>  o Lean fish/oily fish: nonsignificant<br>• SGA for weight<br>  o Canned tuna: OR = 0.3 (95 % CI: 0.1, 0.8)<br>  o Lean fish: OR = 0.3 (95 % CI: 0.1, 1.0)<br>  o Oily fish: OR = 4.6 (95 % CI: 1.4, 15.4) | Q4 vs. Q1 cord blood total mercury:<br>• BW = −143.7 g (95 % CI: −251.8, −35.6)<br>• OR SGA = 5.3 (95 % CI: 1.2, 23.9)<br>Lean fish: hake, sole, gilthead<br>Oily fish: swordfish, fresh tuna, bonito |
| Ricci et al. 2010<br> 555 women in Italy | 1989–1999 | SGA | Reduced SGA risk with high fish consumption 0.8 (95 % CI: 0.6, 1.0) | Fish intake data collected for period immediately before pregnancy and in last month of pregnancy. |
| Rogers et al. 2004<br> 10,040 women. ALSPAC study. UK. | 1991–1992 | IUGR, LBW, PTB | No association with PTB or LBW.<br>Frequency of intrauterine growth restriction for no fish intake vs. highest intake: OR = 1.37 (95 % CI: 1.02, 1.84) | Fish intake data collected during third trimester |
| Thorsdottir et al. 2004<br> 491 women in Reykjavik, Iceland | 1998 | BW, birth size | Women in lowest quartile of fish consumption (0–20 g/day) had children who weighed less, were shorter, and had smaller head circumference at birth than higher intakes. | Fish intake data collected after childbirth. Mean fish consumption = 47 g/d. |

**Table 4b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| Wang et al. 2022<br>10,179 women from Gansu Province, China | 2010–2012 | PTB | Fish and shellfish intake<br>• PTB OR = 0.65 (95 % CI: 0.56, 0.77) up to > 176 g/wk.<br>Reduced risk applied to freshwater fish, shellfish, and mixed fish. No association with saltwater fish. | Fish intake data collected after delivery about pregnancy period.<br>Authors recommend pregnant women consume ≥ 350 g/wk of seafood. |
| Wei et al. 2023<br>10,179 women in Gansu Province, China | 2010–2012 | BW, LBW, SGA | Total seafood<br>• BW: β = 0.027 (95 % CI:0.03, 0.11)<br>• LBW: β = 0.58 (95 % CI: 0.48, 0.69).<br>• SGA: β = 0.59 (95 % CI:0.50, 0.70)<br>LBW reduced risk for total fish, freshwater fish, shellfish, mixed fish, but not saltwater fish. | • Fish intake data collected after delivery about past intake.<br>• Fish intake range: None to > 176 g/wk. |
| Wheeler et al. 2011<br>500 pregnant adolescents. UK. | 2004–2007 | BW, SGA | Oily fish intake not associated with shorter gestation, BW, or SGA risk. | Fish intake data collected during early pregnancy and third trimester for preceding three months. |
| Zhao et al. 2022<br>1701 singleton pregnancies. Tongji Birth Cohort. China. | 2018–2021 | SGA | Maternal freshwater fish consumption (17.9–30.0 g/d vs. ≤ 3.2 g/d).<br>• OR SGA = 0.50 (95 % CI: 0.25, 0.96)<br>SGA odds ratio < 1 for intake 5–45 g/d freshwater fish. | |

BW, birth weight; HBW, high birth weight; IUGR, intrauterine growth restriction; LBW, low birth weight; LGA, large for gestational age, PTB, preterm birth; SGA, small for gestational age.

decile for either PFOS or PFOA of 11–12 mg/dL. The highest vs. lowest decile serum PFOA concentrations were approximately 340 ng/mL vs. 10 ng/mL and for PFOS, approximately 48 ng/mL vs. 7 ng/mL. The odds ratios for having TC ≥ 240 mg/dL increased by 40 % and 51 % from lowest to highest quartiles of PFOA and PFOS, respectively. The authors noted that for TC, the most important predictors were age, gender, and body mass index, not serum concentrations of PFOA or PFOS. Table 5a presents studies on PFAS exposure and cardiovascular outcomes.

In studies of PFAS-CVD associations among this C8 cohort, Winquist and Steenland [62] confirmed higher TC with increased serum PFOA for both occupational and residential exposures, but found insufficient evidence for an association between PFOA and either hypertension or coronary artery disease, although lower exposures sometimes had higher risk magnitudes than higher exposures as compared to the reference group. Similarly, Sakr et al. [63] found no association between PFOA exposure and increased mortality risk of ischemic heart disease (IHD) among occupationally-exposed individuals from this population.

Meta-analyses and reviews explored the association between serum PFAS, blood lipids, and CVD. For example, Liu et al. [64] found that an IQR increase of serum PFOA was associated with a 2.1 mg/dL increase in TC, a 1.3-mg/dL increase in triglycerides, and a 1.4 mg/dL increase in low density lipoprotein cholesterol (LDL-C). An IQR increase in serum PFOS was associated with a 2.6 mg/dL increase in TC and 1.9 mg/dL increase in LDL-C. In addition, a systematic review by Ho et al. [65] found that PFOA and PFOS exposures were more likely than not to be associated with higher LDL-C, high density lipoprotein cholesterol (HDL-C), and TC.

Other meta-analyses explored the association between PFAS exposures and hypertension, CVD, or stroke risk. For example, Pan et al. [66] and Xiao et al. [67] both found elevated odds for hypertension with higher PFOS and PFOA exposures with ORs between 1.12 and 1.31 (p < 0.05). However, Chang et al. [68] found no association between PFOS or PFOA exposure and risk of stroke in a meta-analysis of four studies. Abdullah Soheimi et al. [69] and Dunder et al. [70] also conducted meta-analyses to explore PFAS-CVD associations. Abdullah Soheimi et al. [69] found overall PFAS exposures associated with "moderate" CVD risk and PFOS (but not PFOA) associated with "large" combined CVD/CVD risk conditions (risks such as hypertension, diabetes, atherosclerosis), both with considerable heterogeneity among studies. Dunder et al. [70] found reduced CVD risk associated with high vs. low PFOA exposure (RR = 0.80; 95 % CI: 0.66, 0.94) in a meta-analysis of five studies. In additional analyses of two Swedish population-based cohorts, Dunder et al. [70] found no PFAS-CVD risk associations, except for reduced risk from PFOS in men. The meta-analyses are not exhaustive or conclusive of a PFAS-CVD association. However, both EPA

and ATSDR have concluded that the literature indicates increased TC and LDL-C with increased PFOS/PFOA in adults and mixed associations between PFOS/PFOA exposure and CVD (Table 3).

Gestational hypertension and preeclampsia risk associations with PFAS are discussed under reproductive effects by EPA and under cardiovascular effects by ATSDR. As such, these will not be discussed here. However, EPA found slight evidence for an association with PFOS/PFOA and ATSDR found suggestive evidence for the respective association.

*3.2.2. Fish consumption studies*

Several meta-analyses and reviews examined the association between fish intake and cardiovascular health. For example, meta-analyses by Larsson and Orsini [71] and Chen et al. [72] found 6–19 % reduction in risk of stroke with higher fish intake and Qin et al. [73] reported similar reductions in stroke risk by fish type - fatty fish (RR = 0.88; 95 % CI: 0.74, 1.04); lean fish (RR = 0.81; 95 % CI: 0.67, 0.99). Moreover, Zhang et al. [74] found that each 20 g fish/d intake increment was associated with a 4 % reduced risk of coronary heart disease (CHD) incidence with progressively decreasing risk up to the maximum 1260 g/week intake and Bechthold et al. [75] saw sustained lower CHD risk with intake up to > 2100 g/week and lower stroke risk up to > 700 g/week. Similarly, Zheng et al. [76] found that any fish intake was associated with reduced risk of CHD mortality in the order of 16–21 % and that a 15 g/d increment of fish intake decreased the risk by 6 % (RR = 0.94; 95 % CI 0.90, 0.98). Table 5b presents studies on fish intake and cardiovascular outcomes.

Further, Mohan et al. [77] conducted a pooled analysis of four multinational cohort studies and, separately, an analysis of 43,413 patients with vascular disease or diabetes in three multinational clinical trials. In the meta-analysis, intake of ≥ 350 g/week vs. almost no intake was not associated with risk of CVD, but intake 175 to < 350 g/week was associated with reduced risk of CVD and stroke. Similarly, in the clinical trials, the risk of major CVD showed the most benefit at total fish intake of ≥ 175 g/week vs. almost no intake with benefit from fatty fish and no association with other fish or shellfish. Risk was more reduced among patients with vascular disease. Similarly, Giosuè et al. [78] found reduced CHD risk with fatty fish intake, but no effect of lean fish. The findings of benefit from fatty fish find support from a meta-analysis of intervention trials [79] that found eating oily fish was associated with reduced plasma triglycerides (-0.11 mmol/L or 9.7 mg/dL) and increased HDL-C (0.06 mmol/L or 2.3 mg/dL).

Most individual studies reported either decreased risk of CVD outcomes with increased fish intake or no association, with some exceptions. For example, Zhang et al. [80] and Virtanen et al. [81] both found reduced CVD risk associated with higher fish intake among participants from the UK Biobank cohort and Health Professionals Follow-Up Study,

**Table 5a**

Cardiovascular outcomes and PFAS.

| Study and population | PFAS serum concentration (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| *RfD candidate studies* | | | | | |
| Dong et al. 2019 11,895 adults. NHANES. USA | Median PFOA: 2.9 PFOS: 9.4 | 2003–2014 | Total cholesterol (TC) | 1 ng/mL PFOA increase → 1.48 mg/dL (95 % CI: 0.2, 2.8) TC 1 ng/mL PFOS increase → 0.4 mg/dL (95 % CI: 0.1, 0.6) TC | Change in cholesterol per 1 ng/mL increment is < 1 % of study participants' mean |
| Steenland et al. 2009 46,294 adults. C8 Project. Ohio / West Virginia, USA | Median PFOA: 27 PFOS: 20 | 2005–2006 | TC | Lowest to highest decile exposure of PFOS/PFOA: • TC increased by 11–12 mg/dL (approximately 5 % of the study mean). Odds for having cholesterol ≥ 240 mg/dL increased 40 % and 51 % from Q1 to Q4 of PFOA and PFOS, respectively. | Highest vs. lowest decile in serum (ng/mL) • PFOA: 340 vs. 10 • PFOS: 48 vs. 7 |
| *Meta-analyses, reviews, and relevant studies* | | | | | |
| Abdullah Soheimi et al. 2021 Meta-analysis of 29 studies. | | Studies published 2009–2021 | CVD and CVD risks | Odds Ratios from graphs generally close to 1.0, but not reported numerically. Z-values reported as such: • PFAS vs. CVDs: z = 2.2, p = 0.02 • PFAS vs. CVDs + CVD risks: z = 4.03, p < 0.0001 • PFOS vs. CVDs + CVD risks: z = 3.87, p < 0.0001 • PFOA vs. CVDs + CVD risks: z = 1.56, p = 0.12 Authors reported considerable heterogeneity among studies. | CVDs: stroke, coronary artery calcium, CAD, CVD. CVD risks: atherosclerosis, diabetes mellitus I/II, gestational diabetes, hypertension |
| Chang et al. 2023 Meta-analysis. Four studies. 89,892 people. | | Studies published 2013–2022 | Stroke | Stroke risk per 1-log unit increment in serum PFAS. • PFOS: OR = 0.99 (95 % CI: 0.97, 1.02) • PFOA: OR = 1.00 (95 % CI: 0.98, 1.03) | |
| Dunder et al. 2023 Two population-based cohorts in Sweden (PIVUS and EpiHealth) + Meta-analysis | EpiHealth (relative conc) PFOS: 8.2 PFOA: 2.2 PIVUS (mean conc) PFOS: 9.4 PFOA: 2.8 | 2011–2018 2001–2004 | CVD | *Cohort analysis:* Q4 vs. Q1 serum PFAS and CVD risk EpiHealth • No associations except for PFOS-CVD o Men: HR = 0.68 (95 % CI: 0.52, 0.89) o Women: HR = 1.13 (95 % CI: 0.82, 1.55) PIVUS • No associations, overall, between PFAS and CVD *Meta-analysis* of PFAS and CVD risk (high vs. low PFOA exposure) • RR = 0.80 (95 % CI: 0.66, 0.94) | CVD definition: combined fatal and nonfatal MI, ischemic stroke, and heart failure. EpiHealth study: 2278 subjects (age, 45–75 years) PIVUS study. 1016 subjects (age, 70 years) |
| Ho et al. 2022 Review of 58 studies from the USA, Europe, Asia, and Oceania | | Studies published 2003–2022 | Blood lipids | *Adults* • PFOA and PFOS exposures more likely to be associated with higher LDL-C, HDL-C, and TC. *Children* • PFOA and PFOS exposures more likely to be associated with higher LDL-C and TC. | |
| Liu et al. 2023 Meta-analysis including 29 studies from the USA, Europe, and Asia | | Studies published 1996–2022. | Blood lipids | IQR increase of PFOA associated with • 2.1 mg/dL increase in TC (95 % CI: 1.2, 3.0) • 1.3 mg/dL increase in TG (95 % CI: 0.1, 2.4) • 1.4 mg/dL increase in LDL-C (95 % CI: 0.6, 2.2) IQR increase in PFOS associated with • 2.6 mg/dL increase in TC (95 % CI: 1.5, 3.6) • 1.9 mg/dL increase in LDL-C (95 % CI: 0.9, 3.0) • No association with HDL-C for either. | C8 cohort constituted over half of participants. No indication of possible publication bias of studies including PFOA and PFOS. |
| Pan et al. 2023 Meta-analysis of 13 studies. 81,096 people. | | Studies published 2012–2022 | Hypertension | Associations between PFAS and hypertension • PFOS: OR = 1.19 (95 % CI: 1.06, 1.34) • PFOA: OR = 1.12 (95 % CI: 1.02, 1.23) | PFOS and PFOA associations only in men, not women. |

**Table 5a** (*continued*)

| Study and population | PFAS serum concentration (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| Sakr et al. 2009 4747 workers | Median PFOA: 5.1 ppm-years | Employed 1948–2002 | Ischemic heart disease (IHD) | No statistically significant PFOA association with mortality risk from IHD over a 20-year lag | |
| Winquist and Steenland 2014 32,254 adults. West Virginia. USA | Median PFOA: 26.1 (measured in 2005–2006) | 2008–2011 disease surveys | TC, coronary artery disease, hypertension, | PFOA and Hypercholesterolemia: <br> • HR = 1.24 (95 % CI: 1.15, 1.33) <br> No association between PFOA exposure and hypertension or coronary artery disease, according to authors. | Hypertension and CAD risk sometimes higher for lower exposures than high exposures and p < 0.05 vs. referent. |
| Xiao et al. 2023 Meta-analysis of 15 studies. 69,949 people | | Studies published 2012–2022 | Hypertension | Associations between PFAS and hypertension <br> • PFOS: OR = 1.31 (95 % CI: 1.14, 1.51) <br> • PFOA: OR = 1.16 (95 % CI: 1.07, 1.26) | Considerable heterogeneity among study outcomes. |

CAD, coronary artery disease; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; Q1/2/3/4, quartiles; IQR, interquartile range (Q1-Q3); ng/mL, nanogram(s) per milliliter.

respectively. In the Physicians' Health Study, Morris et al. [82] found increased risk for low fish intake (1 meal/week vs. < 1 meal/week) on myocardial infarction (MI) with no association at higher intake up to ≥ 5 meals/week and no effect by fish type or on stroke. In another analysis of this cohort, Wilk et al. [83] found that higher fish intake was associated with a 30 % lower risk of heart failure. Among Japanese adults, Iso et al. [84] found lower risk for total CHD and MI, but no association with sudden cardiac death for consuming fish 8 times/week (median, 1260 g/week) versus 1 time/week (median, 161 g/week).

Among nurses not diagnosed with diabetes [85] and those having type 2 diabetes [86], but no CVD at baseline, fish intake was associated with a lower risk of CHD incidence. Bernstein et al. found no association with canned tuna, dark fish, or light fish and Hu et al. [86] found progressively lower risk up to intake of fish ≥ 5 times/week (serving size, 3–8 ounces). Similarly, among adults diagnosed with type 2 diabetes, Wallin et al. [87] reported hazard ratios = 0.60 (95 % CI, 0.39, 0.92) for MI and 1.04 (95 % CI, 0.66, 1.64) for stroke for intake of > 3 servings/week vs. ≤ 3 servings/month. The hazard ratios for MI were decreased for fatty, lean, and shellfish, some at p < 0.05.

Fish preparation might play a role in CVD outcomes. For example, Nahab et al. [88] found that intake of fried fish was associated with an increased risk of CVD, while consuming non-fried fish was not. Similarly, Mozaffarian et al. [89,90] found fish consumption to be associated with lower risk of IHD death and incident congestive heart failure among persons consuming more tuna/other fish while fried fish/fish sandwich intake showed increased risk estimates with both health outcomes. A meta-analysis [91] found increased risk from fried fish intake on heart failure (RR=1.37; 95 % CI: 1.20, 1.56 for a monthly increment of six fried fish), although it found no association with total fish intake. More studies are needed that examine health outcomes by fish preparation method.

Type of fish and other factors might play a role as well. For example, Bonaccio et al. [92] found that higher fish intake was associated with 40 % lower risk of CHD and stroke and that the CHD association was confined to fatty fish. Critselis et al. [93] found lower CVD incidence and mortality risk among those who ate a lot of seafood, particularly small fish (e.g., anchovy, sardine, and mackerel). Key et al. [94] found no association between fatal IHD or nonfatal MI and fish intake in a cohort including nine European countries (EPIC). Analyses of different country populations within the EPIC cohort showed lower incidence of ischemic stroke in the Netherlands with lean fish (HR: 0.70, 95 % CI: 0.57–0.86) and fatty fish (HR: 0.63, 95 % CI: 0.39–1.02) and no association with hemorrhagic stroke, CHD, or MI [95]. In Spain, no significant associations were observed between lean fish, fatty fish, and total fish consumption and the risk of total stroke in men or women. However, in men, results revealed an inverse association between lean fish (hazard ratio=0.84; 95 % CI: 0.55, 1.29) and total fish intake (hazard ratio =0.77; 95 % CI: 0.51, 1.16) [96]. In Germany, Kühn et al. [97] found

that fish intake was unrelated to incident MI or stroke.

In addition, eating tuna and dark fish (mackerel, salmon, sardines, bluefish, and swordfish) was not associated with the risk of major CVD among women with low fish intake [98]. Similarly, Wennberg et al. [99] found no associations between fish intake and stroke risk among women (OR 0.50; 95 % CI: 0.24, 1.10), although high intake of lean fish was associated with increased stroke risk in men (OR = 1.80; 95 % CI: 1.00, 3.21) that was driven by men living alone.

Hypertension, a risk factor for CVD, was not associated with fish consumption in whole cohorts, in general [100,101], although Gillum et al. [101] found lower risk among Black women who increased fish intake from < once/week to ≥ once/week (RR = 0.42; 95 % Cl: 0.22, 0.81), but not Black women with high intake at both baseline and follow-up (RR = 0.75; 95 % Cl: 0.45, 1.26).

Lastly, an analysis of 34 meta-analyses of prospective cohort studies [3] found moderate quality evidence for reduced risk associated with a 100 g/d increment in fish intake for CHD (RR = 0.88; 95 % CI: 0.79, 0.99), MI (RR = 0.75; 95 % CI: 0.65, 0.93), stroke (RR = 0.86; 95 % CI: 0.75, 0.99), and heart failure (RR = 0.80; 95 % CI:0.67, 0.95) and low quality evidence for increased risk of heart failure associated with high vs. low intake of fried fish (RR = 1.40; 95 % CI: 1.22, 1.61).

### 3.2.3. Summary of cardiovascular effects

Overall, there is evidence for an association between PFAS exposures and increased TC and LDL-C, but there is no consistent evidence of association with increased risk of CVD. Associations varied by study and endpoint among neutral, beneficial, and adverse associations. In contrast, fish intake was generally associated with neutral or favorable cardiovascular outcomes. However, there were adverse associations in a few studies with intake of fried fish and lean fish, which require further investigation into type of fish, preparation method, and contaminant profiles.

### 3.3. Immune effects

The candidate RfDs for this endpoint are based on PFAS-associated reduced antibody concentrations to diphtheria/tetanus following vaccination in children. The following sections discuss studies of PFAS and antibody responses after vaccination in addition to a survey of immune outcomes associated with fish intake.

### 3.3.1. PFAS studies

EPA relied on the studies by Budtz-Jørgensen and Grandjean [102], Timmermann et al. [103], and Zhang et al. [104] to develop candidate reference doses for the associations between PFAS exposure and antibodies to infectious agents after vaccination. Table 6a presents studies on PFAS exposure and immune outcomes. Budtz-Jorgensen and Grandjean [102] derived benchmark doses for several PFAS by

**Table 5b**

Cardiovascular outcomes and fish consumption.

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| *Meta-analyses* | | | | |
| Alhassan et al. 2017<br>  Meta-analysis of 14 intervention trials including 1378 adults | Studies published, 1990–2012 | Blood lipids | Consuming oily fish associated with<br>• TGs (−0.11 mmol/L; 95 % CI: −0.18, −0.04)<br>• HDL-C (0.06 mmol/L; 95 % CI: 0.02, 0.11)<br>No significant effect for total cholesterol, LDL cholesterol, blood pressure, CRP, IL−6, ICAM, insulin, glucose. | Oily fish consumption range: 20–150 g/d |
| Bechthold et al. 2019<br>  Meta-analysis of 47 studies from the USA, Europe, and Japan. | Studies published, 1992–2017 | CHD, stroke, heart failure | Risks associated with fish consumption in the linear dose-response analysis:<br>• CHD: RR = 0.88 (95 % CI: 0.79, 0.99)<br>• Stroke: RR = 0.86 (95 % CI: 0.75, 0.99)<br>• Heart failure: RR = 0.80 (95 % CI: 0.67, 0.95)<br>Risk decreased steadily with any daily fish intake up to > 300 g for CHD and > 100 g for stroke | |
| Chen et al. 2021<br>  Meta-analysis. 17 cohort studies. 672,711 people. | Studies published, 1995–2016 | Stroke | Higher fish consumption<br>• Stroke RR = 0.87 (95 % CI: 0.78, 0.98) | Eating 1000 g fish/mo: Stroke RR=0.93 (95 % CI: 0.83, 0.98) |
| Giosuè et al. 2022<br>  Meta-analysis of 19 studies | Studies published, 1995–2020 | CHD incidence and mortality | Fatty fish (highest vs. lowest intake)<br>• CHD incidence RR = 0.92 (95 % CI: 0.86, 0.97)<br>• CHD mortality RR = 0.83 (95 % CI: 0.70, 0.98)<br>Lean fish and shellfish intake: No association | |
| Hou et al. 2012<br>  Meta-analysis of 5 prospective cohort studies - 5273 cases, 144,917 participants. | Participant follow-up, 1987–2008 | Heart failure | Heart failure risk<br>• High vs. low fish intake: RR = 1.00 (95 % CI: 0.81, 1.24)<br>• Fried fish intake: RR = 1.40 (95 % CI: 1.22, 1.61)<br>• Increment 6 fried fish/mo: RR = 1.37 (95 % CI: 1.20, 1.56) | |
| Jayedi and Shab-Bidar 2020<br>  Review of 34 meta-analyses of 298 prospective observational studies. | Studies published, 2010–2019 | CHD, heart failure, MI, stroke | For each 100-g/d increment in fish consumption:<br>• CHD: RR = 0.88 (95 % CI: 0.79, 0.99)<br>• Heart failure: RR = 0.80 (95 % CI: 0.67, 0.95)<br>• MI: RR = 0.75 (95 % CI: 0.65, 0.93)<br>• Stroke: RR = 0.86 (95 % CI: 0.75, 0.99) | |
| Larsson and Orsini 2011<br>  Meta-analysis of 15 prospective studies. 383,838 people. | Studies published, 1995–2011 | Stroke | Risk of stroke with increment of 3 servings/wk of fish:<br>• RR = 0.94 (95 % CI: 0.89, 0.99) | |
| Zhang et al. 2020<br>  Meta-analysis. 22 studies of CHD incidence and 27 studies of CHD mortality. | Studies published, 1985–2019 | CHD incidence and mortality | Higher fish consumption<br>• CHD incidence (RR= 0.91, 95 % CI: 0.84, 0.97)<br>• CHD mortality (RR = 0.85, 95 % CI: 0.77, 0.94)<br>Each 20 g fish/d intake increment associated with 4 % reduced risk of CHD incidence and mortality. | |
| Zheng et al. 2012<br>  17 cohorts including 315,812 participants. | Studies published, 1985–2010 | CHD mortality | CHD mortality risk (vs. < 3 servings/mo)<br>• 1 serving/wk: RR = 0.84 (95 % CI: 0.75, 0.95),<br>• 2–4 serving/wk: RR = 0.79 (95 % CI: 0.67, 0.92)<br>• > 5 serving/wk: RR = 0.83 (95 % CI: 0.68, 1.01)<br>15 g/d fish intake increment: RR = 0.94 (95 % CI: 0.90, 0.98). | Dietary assessment method, gender, energy adjustment affected results remarkably. |
| *Individual studies* | | | | |
| Amiano et al. 2016<br>  41 020 adults (age, 20–69 y) in the Spanish arm of EPIC. | 1992–1996<br>13.8-year follow-up | Stroke | No significant associations between stroke incidence and lean fish, fatty fish and total fish in men or women. In men, lean fish HR = 0.84 (95 % CI: | |

**Table 5b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| | | | 0.55, 1.29) and "total fish" HR = 0.77 (95 % CI: 0.51, 1.16). | |
| Bernstein et al. 2010<br>  84,136 women (ages, 30–55 y). Nurses' Health Study. USA | 1980–2002 | CHD | Fish intake of 1 serving/d:<br>• CHD incidence RR = 0.8 (95 % CI: 0.7, 1.0)<br>Intake ≤ 3 servings/d associated with lower CHD risk. | Diet data collected every 4 years. |
| Bonaccio et al. 2017<br>  20,969 subjects free from CVD in the Moli-sani study. Italy. | 2005–2010<br>(4.3 year follow-up) | CHD, stroke | Fish intake > 4 times/wk vs. < 2 times/wk:<br>• CHD HR = 0.60 (95 % CI: 0.38, 0.94)<br>• Stroke HR = 0.62 (95 % CI: 0.26, 1.51) | HR reduced only with fatty fish intake. Neutral for dried,/canned/lean fish. |
| Critselis et al. 2023<br>  Prospective cohort study including 2020 healthy adults. Athens, Greece. | 2002–2022 | CVD incidence and mortality | Marine fish and seafood intake (> 2 servings/wk vs. ≤ 2 servings/wk)<br>• CVD incidence: HR = 0.76 (95 % CI: 0.56, 1.02)<br>• CVD mortality: HR = 0.26 (95 % CI: 0.11, 0.58)<br>Small fish consumption<br>• CVD incidence: HR = 0.76 (95 % CI: 0.48, 1.19)<br>• CVD mortality: HR = 0.24 (95 % CI: 0.06, 0.99) | Small fish included anchovy, sardine, and mackerel. |
| Gillum et al. 2001<br>  5394 adults (age, 25–74 y). NHANES Epidemiologic Follow-up Study (NHEFS). USA | 1971–1975<br>10-year follow-up | Hypertension | Hypertension and fish intake<br>• Black women who increased fish intake<br>  o  RR = 0.42 (95 % CI: 0.22, 0.81).<br>• Black women with high intake at baseline and follow-up<br>  o  RR = 0.75 (95 % CI: 0.45, 1.26).<br>• White people: No association. | |
| Hengeveld et al. 2018<br>  34,033 participants, (age, 20–70 y), from EPIC-Netherlands cohort. | 1993–1997 | CHD, MI, stroke | Ischemic stroke incidence (eating ≥ 1 portion/wk of fish)<br>• lean fish (HR = 0.70; 95 % CI: 0.57, 0.86)<br>• fatty fish (HR = 0.63; 95 % CI: 0.39, 1.02)<br>No association with hemorrhagic stroke/CHD/MI | Portion = 100 g |
| Hu et al. 2003<br>  5103 female nurses diagnosed with type 2 diabetes but free of CVD at baseline | 1980 – 1996 | CHD | CHD and fish intake vs. < 1 serving/mo:<br>• RR = 0.70 (95 % CI: 0.48, 1.03) (1–3/mo)<br>• RR = 0.64 (95 % CI: 0.42, 0.99) (2–4/wk)<br>• RR = 0.36 (95 % CI: 0.20, 0.66) (≥ 5/wk) | CHD (CHD deaths and nonfatal myocardial infarction) |
| Iso et al. 2006<br>  41,578 adults (age, 40–59 y) free of CVD and cancer diagnosis. Japan. | 1990–1992<br>followed to 2001 | CHD, MI, sudden cardiac death | Highest (8x/wk, median=180 g/d) vs. lowest (1x/wk, median=23 g/d) quintile of fish intake<br>• Total CHD HR = 0.63 (95 % CI: 0.38, 1.04)<br>• Definite MI HR = 0.44 (95 % CI: 0.24, 0.81)<br>• Sudden cardiac death HR = 1.14 (95 % CI: 0.36, 3.63) | Nonfatal coronary events HR=0.43 (95 % CI: 0.23, 0.81)<br>Fatal coronary events HR=1.08 (95 % CI: 0.42, 2.76) |
| Key et al. 2019<br>  409,885 men and women in the European Prospective Investigation Into Cancer and Nutrition (EPIC) | 1992–2000<br>12.6 years of follow-up | IHD, MI | Fatal IHD or first nonfatal MI<br>• White fish intake: No association.<br>• Fatty fish intake: Q5 vs. Q1 HR = 0.92 (95 % CI: 0.86, 0.99) | |
| Kühn et al. 2013<br>  48,315 participants (ages, 35–65 y) in German arm of EPIC. | 1994–1998 | MI, stroke | Fish intake and CVD outcomes:<br>• Incident MI: HR = 0.84 (95 % CI: 0.66, 1.08)<br>• Stroke: HR = 0.96 (95 % CI: 0.73, 1.26) | |
| Mohan et al. 2021<br>  147, 645 adults from the Prospective Urban Rural Epidemiology (PURE) study + 43,413 patients having CVD or diabetes from three multinational randomized clinical trials. 21 countries. Five continents. | Through July 31, 2019 | CVD, blood lipids | Major CVD risk with fish intake.<br>175 - < 350 g/wk of fish vs. ≤ 50 g/month<br>• For existing CVD or at high-risk (HR = 0.86; 95 % CI: 0.80, 0.92)<br>• Without CVD (HR = 0.94; 95 % CI: 0.88, 1.04)<br>Fish with higher ω−3 fatty acids: HR = 0.94 (95 % CI: 0.92, 0.97) per 5-g increment of intake. | LDL-C and fasting glucose increased with increased fish intake. Triglycerides decreased. HDL-C unchanged.<br>Fish low in ω −3 or shellfish: No association. |

**Table 5b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| Morris et al. 1995<br>Prospective cohort study of 21,185 US male physicians.<br>Physicians' Health Study. USA | 1982–1988<br>4 years follow-up | MI | Fish intake ≥ 1 meal/wk vs. < 1 meal/wk<br>• Total MI: RR = 1.5 (95 % CI: 1.1, 2.1)<br>  o RR = 1.3 for 2–4 meals/wk<br>  o RR = 0.9 for ≥ 5 meals/wk | No exposure-related trend |
| Mozaffarian et al. 2003<br>Population-based prospective cohort study. 3910 adults, ≥ 65 years. The Cardiovascular Health Study. USA | 1989–1990<br>+ 9 years follow-up | Total IHD death | Fish intake ≥ 3 times/wk vs. < 1 time/mo<br>Tuna/other fish<br>• HR = 0.47 (95 % CI: 0.27, 0.82)<br>Eating fried fish/fish sandwiches<br>• HR = 1.37 (95 % CI: 0.48, 3.90) | |
| Mozaffarian et al. 2005<br>Population-based prospective cohort study. 4738 adults, ≥ 65 years. The Cardiovascular Health Study. USA | 1989–1990<br>+ 12 years follow-up | CHF | Fish intake 1–2 times/wk vs. < 1 time/mo<br>Tuna/other broiled or baked fish<br>• HR = 0.80 (95 % CI: 0.64, 0.99)<br>Fried fish<br>• HR = 1.35 (95 % CI: 1.12, 1.62) | |
| Nahab et al. 2016<br>16,479 participants. REasons for Geographic And Racial Differences in Stroke (REGARDS) study, USA | 2003–2007 | CVD | Incident CVD risk (≥ 2 servings/wk vs. < 1 serving/mo)<br>• Fried fish: HR = 1.63 (95 % CI: 1.11, 2.40)<br>• Not fried: HR = 1.09 (95 % CI: 0.78, 1.52) | |
| Qin et al. 2018<br>Meta-analysis of five prospective studies from Europe | Studies published, 2006–2017 | Stroke | Highest vs. lowest category of fish intake stroke risk<br>• Fatty fish: RR = 0.88 (95 % CI: 0.74, 1.04)<br>• Lean fish: RR = 0.81 (95 % CI: 0.67, 0.99) | |
| Rhee et al. 2016<br>38,392 women (≥ 45 years).<br>Women's Health Study. USA. | 1993–2014 | CVD | Tuna and dark fish intake not associated with risk of incident major CVD (MI, stroke, CV death). | Most cases ate ≤ 1 serving dark fish/week. |
| Virtanen et al. 2008<br>40,230 male health professionals (age, 40–75 y). Health Professionals Follow-Up Study (HPFS). USA. | 1986 start with 18 years of follow-up | CVD | 1–4 servings/wk vs. < 1 serving/mo<br>Lower total CVD (stroke/MI)<br>• E.g., RR = 0.85 (95 % CI: 0.73, 0.99) for 2–4 servings/wk. | Assessed lifestyle and other risk factors every 2 y and diet every 4 y. |
| Wallin et al. 2018<br>2225 adults diagnosed with type 2 diabetes (45–84 years). Swedish Mammography Cohort; Cohort of Swedish Men. | 1998 – 2012 | MI, stroke | Intake of "total fish" > 3 servings/wk vs. ≤ 3 servings/mo.<br>• MI HR = 0.60 (95 % CI: 0.39, 0.92)<br>• Stroke HR = 1.04 (95 % CI: 0.66, 1.64)<br>HRs for MI all < 1 for fatty, lean, and shellfish. | |
| Ward et al. 2020<br>197,761 adults. Million Veteran Program cohort study (mean age: 66 years, 92 % men). USA | 2011–2017.<br>Median follow-up of ~3 years | Nonfatal CAD/stroke | Fish intake 2–4 servings/wk, vs. < 1 serving/mo<br>• CAD: OR = 1.02 (95 % CI: 0.93, 1.11)<br>• Ischemic stroke: OR = 0.96 (95 % CI: 0.86, 1.07) | |
| Wennberg et al. 2016<br>735 incident stroke cases and 2698 controls. Northern Sweden Health and Disease Study. | 1987–2007 | Stroke | No associations with total/fatty fish intake.<br>Lean fish intake (> 2x/wk vs. < 1x/mo):<br>• Men: OR = 1.80 (95 % CI: 1.00, 3.21)<br>  o Driven by men living alone<br>• Women: OR = 0.50 (95 % CI: 0.24, 1.10) | Authors: Increased risk in men may be due to chance or confounding. |
| Wilk et al. 2012<br>1576 men. Nested case-control study. Physicians' Health Study | 1982 | Heart failure | Lower risk of heart failure with fish intake ≥ 1 time/mo<br>• E.g., ≥ 2 times/wk, RR = 0.72 (95 % CI: 0.54, 0.96) | Similar risk magnitude for all intake amounts |
| Zhang et al. 2021<br>462,155 participants in the UK Biobank cohort | 2006–2010 enrollment.<br>11.2 y follow-up | CVD | Fish intake ≥ 3 meals/week vs. < 1 meal/week:<br>• CVD HR = 0.92 (95 % CI: 0.89, 0.96) | |

CAD, coronary artery disease; CHD, coronary heart disease; CHF, congestive heart failure; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; IHD, ischemic heart disease; LDL-C, low density lipoprotein cholesterol; MI, myocardial infarction; TG, triglycerides

analyzing data from two Faroe Islands cohorts (Cohort 3 and Cohort 5) that assessed maternal and child PFAS exposures and child serum concentrations of IgG antibodies against tetanus and diphtheria. Faroese children receive vaccinations against diphtheria and tetanus at ages 3 months, 5 months, and 12 months and a booster at age 5 years. The authors reported inverse associations between 1) maternal exposure to PFOS/PFOA and diphtheria/tetanus antibody concentrations at 5 years and 2) serum concentrations of PFOS/PFOA at age 5 years and antibody concentrations at age 7 years. EPA relied on the latter association of PFOS/PFOA at age 5 years with anti-tetanus antibody concentrations at

**Table 6a**

Immune outcomes and PFAS.

| Study and population | PFAS serum concentration (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| *RfD candidate studies (or their cohorts)* | | | | | |
| Grandjean et al. 2012<br>587 consecutive singleton births in the Faroe Islands | **Cohort 3**<br>Geometric mean:<br>*Maternal*<br>PFOS: 27.3<br>PFOA: 3.20<br>*5 years*<br>PFOS: 16.7<br>PFOA: 4.06 | Born<br>1997–2000 | *Diphtheria/tetanus serum antibody concentrations after vaccination*<br>*in relation to doubling serum PFAS concentration* | *Diphtheria* antibody *% change*<br>PFOS<br>• Maternal PFOS vs. pre-booster antibody at 5 y (−38.6 %; 95 % CI:−54.7, −16.9)<br>• 5-y PFOS vs. antibody at 7 y (−27.6 %; 95 % CI:−45.8, −3.3)<br>PFOA<br>• Maternal PFOA (−22.8 %; 95 % CI: −39.4, −1.7) and 5 y PFOA (−25.2 %; 95 % CI: −42.9, −2.0) vs. antibodies at 7 y<br>*Tetanus* antibody *% change*<br>PFOS<br>• Maternal PFOS vs. antibody at 7 y (35.3 %; 95 % CI: −3.9, 90.6), and when adjusted for 5 y antibody (33.1 %; 95 % CI: 1.5, 74.6)<br>• PFOS at age 5 y vs. antibody at age 5 y post-booster (−28.5 %; 95 % CI: −45.5, −6.1)<br>• Other PFOS-antibody associations not at $p < 0.05$.<br>PFOA<br>• 5-y PFOA vs. antibodies at 7 y (−35.8 %; 95 % CI: −51.9, −14.2)<br>• Other PFOA associations equally negative or positive but not significant at $p < 0.05$ | Antibody differences ranged down to −59 % when reduced and up to 35 % when increased.<br>Maternal blood sampled in late pregnancy. |
| Grandjean et al. 2017<br>490 members of a birth cohort at the National Hospital in Torshavn, Faroe Islands | **Cohort 5**<br>Median:<br>18 mo<br>• PFOS: 7.1<br>• PFOA: 2.8<br>5 y<br>• PFOS: 4.7<br>• PFOA: 2.2 | Born<br>2007–2009 | *Diphtheria/tetanus serum antibody concentrations after vaccination*<br>*in relation to doubling serum PFAS concentration* | Antibody (age 5 y, pre-booster) vs. PFAS (maternal, 1.5 years, 5 years).<br>Diphtheria antibody<br>• PFOA at birth only (−18.93 %; 95 % CI: −33.16, −1.66)<br>• Other associations showed antibodies decrease or increase for both PFOS/PFOA<br>Tetanus antibody<br>• Antibody decreased with doubling PFOA at birth, 1.5 y, and 5 y (e.g., −22.25 %; 95 % CI: −35.25, –6.63 for PFOA at birth).<br>• No significant associations for PFOS at any age. | Children had clinicals exam and blood samples at ages 18 mo (n=275) and 5 y (n=349).<br>Maternal blood drawn 2 weeks after expected term date.<br>Authors noted higher child serum PFAS (15 %−25 %) for exclusive vs. no breastfeeding. |
| Timmermann et al. 2022<br>338 children, median age 9.9 y (range, 7.1, 12.1). Greenland | Median<br>Maternal<br>PFOS: 19.6<br>PFOA: 2.13<br>Child<br>PFOS: 8.68<br>PFOA: 2.28 | 2012–2015 | *Diphtheria/tetanus serum antibody concentrations after vaccination*<br>*in relation to serum PFAS concentration* | Tetanus and diphtheria antibody not associated with PFAS exposure after adjusting for breastfeeding duration or area of residence. Adjustment for vaccine booster date decreased diphtheria antibody with a 1 ng/mL increase in PFOS (−9 %; 95 % CI: −16, −2) and PFHxS (−78 %; 95 % CI: −25, −94). No significant change in tetanus. | Vaccination records: n = 163.<br>Maternal blood analyses: not available for 112 children. |
| Zhang et al. 2023<br>819 persons (ages 12–19 y). NHANES. USA | Geometric means<br>PFOS: 12.4<br>PFOA: 3.3 | 2003–2004 and 2009–2010. | *Rubella/mumps serum antibody concentrations after vaccination*<br>*in relation to a 2.7-fold increase in serum PFAS concentration* | % difference in antibody concentration among those with lower serum folate<br>Rubella<br>• PFOS: −11 % (95 % CI: −18.1, −3.3)<br>• PFOA: −10.9 % (95 % CI: −19.3, −1.6)<br>Mumps | Findings driven by 2003–2004 cohort when PFAS in serum was higher.<br>Folate associated with better immune outcomes and lower PFAS in serum. |

**Table 6a** (*continued*)

| Study and population | PFAS serum concentration (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| | | | | • PFOS: −3.6 % (95 % CI: −12.5, 6.2)<br>• PFOA: −14.8 % (95 % CI: −24.5, −3.9)<br>For mumps and rubella, no association in higher folate group. No association with measles by folate concentration.<br>In 2009–2010 cycle, PFOA and PFOS both associated with *higher* antibody concentrations in higher folate exposed group (47 % and 29 %, respectively). | |
| *Meta-analyses, reviews, and relevant studies* | | | | | |
| Crawford et al. 2023<br> Meta-analysis of 14 studies examining associations between serum PFAS and serum antibody concentrations at all ages | | Studies published, 2012–2022 | *Doubling in PFAS serum concentration and percent difference in antibody concentration following a vaccine.* | Difference in natural log of antibody concentration per doubling of serum PFAS<br>PFOS<br>• Diphtheria: −0.12 (−0.23, −0.00) (children)<br>• Rubella: −0.09 (−0.15, −0.03) (all ages)<br>• −0.12 (−0.20, −0.04) (children)<br>• Tetanus: −0.12 (−0.24, −0.00) (children)<br>PFOA<br>• Diphtheria: −0.12 (−0.23, −0.00) (children)<br>• Rubella: −0.09 (−0.17, −0.01) (all ages)<br>• Tetanus: −0.12 (−0.24, −0.00) (children) | Associations assessed for diphtheria, *H. infl* b, *Infl* A H1N1, measles, rubella, and tetanus for children and "all ages". Results not presented were not statistically significant at $p < 0.05$. |
| Granum et al. 2013<br> 99 women. Sub-cohort of Mother and Child Cohort Study. Norway. | Maternal median:<br>PFOS: 5.5<br>PFOA: 1.1 | 2007–2008 | *Rubella/diphtheria/tetanus /measles/influenza serum antibody concentrations after vaccination in relation to serum PFAS concentration* | Rubella antibody in children's serum at age 3 years and the maternal serum PFAS<br>• PFOS: β = −0.08 (95 % CI: −0.14, −0.02)<br>• PFOA: β =−0.40 (95 % CI: −0.64, −0.17)<br>No association with measles/H. infl type b/tetanus vaccines.<br>Maternal PFOA, not PFOS, associated with reported number of child common cold and gastroenteritis episodes. | No associations with reported child allergy- and asthma-related health outcomes. |
| Stein et al. 2016<br> 1191 children.<br> 12–19 y. NHANES. USA. | Geometric means:<br>PFOS: 19.3–29.0<br>PFOA: 3.9–5.4 | 1999–2004 | Measles/mumps/rubella antibodies in relation to doubling PFAS serum concentration | Serum PFAS doubling<br>PFOS<br>• Rubella: −13.3 % (95 % CI: −19.9, −6.2)<br>• Mumps: −5.9 % (95 % CI: −9.9, −1.6).<br>PFOA/PFHxS/PFNA<br>• No association | Higher PFOS less likely associated with allergen sensitization (OR = 0.74; 95 % CI: 0.58, 0.95). |
| Zhang et al. 2022<br> Meta-analysis of 9 studies examining associations between serum PFAS and serum antibody concentrations in children | | Studies published, 2012–2021 | *Diphtheria, H. infl B, measles, mumps, rubella, tetanus antibody concentrations after vaccination in relation to doubling serum PFAS concentration* | Pooled effect estimates<br>PFOS<br>• Tetanus: −10.04 % (95 % CI: −19.12, −0.96)<br>PFOA<br>• Tetanus: −20.11 % (95 % CI: −29.45, −10.77)<br>Diphtheria antibodies were negative even if not statistically significant. | Meta-analyses could not be conducted for antibodies other than tetanus and diphtheria, but study results generally negative. |

Antibody refers to antibody concentration; PFAS refers to PFAS concentration. ng/mL, nanogram(s) per milliliter.

age 7 years to develop candidate RfDs. The data used in this paper were presented in earlier publications [105,106] and are described below.

Among Cohort 3 children born during 1997–2000, Grandjean et al. [105] found decreased diphtheria antibody concentrations at age 7 years in association with doubling of PFOA concentration in both maternal serum (-22.8 %; 95 % CI: −39.4, −1.7) and child serum at 5 years (-25.2 %; 95 % CI: −42.9, −2.0). In addition, doubling of maternal PFOS was inversely related to pre-booster diphtheria antibodies at 5 years (-38.6 %; 95 % CI: −54.7, −16.9) and doubling of PFOS at 5 years to these antibodies at 7 years (-27.6 %; 95 % CI: −45.8, −3.3). For tetanus antibodies and PFOA, the inverse relationship was significant only between doubling exposure at 5 years and antibodies at 7 years (-35.8 %; 95 % CI: −51.9, −14.2). Other associations for tetanus were either inversely or directly proportional to doubling of serum PFAS, but

not at p < 0.05. Alternatively, maternal PFOS was directly proportional to tetanus antibodies at 7 years (33.1 %; 95 % CI: 1.5, 74.6), meaning as PFOS increased in maternal serum, the tetanus antibody concentrations increased at 7 years. Maternal and 5-year-old geometric means for PFOS were 27.3 ng/mL and 16.7 ng/mL, respectively and for PFOA were 3.20 ng/mL and 4.06 ng/mL, respectively.

Cohort 5 (2007–2009) results were presented by Grandjean et al. [106] as associations between doubling of serum PFAS concentrations (maternal, 1.5 years, 5 years) and serum antibody concentrations (5 years, pre-booster). The authors reported inverse associations between serum tetanus antibody concentrations at age 5 years and serum PFOA concentrations at all ages examined and between diphtheria antibody concentrations at 5 years and maternal concentrations. This study also found 15—25 % higher child serum PFAS concentrations with exclusive breastfeeding vs. no breastfeeding.

The second study used by EPA, Timmermann et al. [103] investigated the association between serum PFAS and diphtheria/tetanus antibodies in children ages 7–12 years in Greenland during 2012–2015. In crude analyses, elevated concentrations of PFOS and PFOA were statistically insignificantly (p > 0.05) associated with increased diphtheria and tetanus antibody concentrations and adjusting for duration of breastfeeding did not change the associations. Further adjustment for seven areas of residence resulted in large changes resulting in an inverse association between PFAS and antibody concentrations, although they remained not significant at p < 0.05. When the data were adjusted further for known vaccine booster date (approximately half the cohort) there was some variation, albeit to a smaller extent and the antibody concentration decrease became significant at p < 0.05 for 1 ng/mL increment of PFOS (-9 %; 95 % CI: −2, −16) for diphtheria and not tetanus.

The third study used by EPA, Zhang et al. [104] found lower rubella antibody concentrations in serum for both PFOS and PFOA associated with a 2.7-fold increase in serum PFAS among adolescents from the 2003–2004 and 2009–2010 NHANES cohort cycles. These associations were limited to those who had higher measured folate in their red blood cells (PFOS, −11 %; PFOA, −10.9 %), and not in those having lower folate (PFOS, −5.5 %; PFOA, 3.3 %). For mumps, only PFOA was associated with lower antibody concentrations (-14.8 %) and there was no association with measles antibody for either PFAS. When comparing cohorts, the 2003–2004 cohort, that had higher serum PFAS concentrations, showed significant associations that were not found in the 2009–2010 cohort. In contrast, the later cohort had increased mumps antibodies in association with higher PFOS/PFOA in the higher folate group (PFOS: 29.2 %; PFOA: 47.3 %).

Meta-analyses of studies examining associations between PFAS exposure and vaccine antibody concentrations generally found inverse associations, particularly in children. For example, Zhang et al. [107] found the strongest associations for tetanus for both PFOS (-10.0 %) and PFOA (-20.1 %). The authors noted that associations with diphtheria antibodies were also inverse even if not statistically significant and that not enough studies were available for influenza, measles, mumps, and rubella to make a determination. Similarly, Crawford et al. [108] found decreased serum antibody concentrations for diphtheria, rubella, and tetanus for doubling PFOS and PFOA serum concentrations (difference in natural log of antibody concentration = −0.12, −0.09, −0.12 for PFOS and −0.12, −0.09, −0.12 for PFOA, respectively).

Both EPA and ATSDR concluded that there is good evidence for inverse associations between PFOS/PFOA exposures and the antibody response to vaccines. In addition, EPA noted increased risk of respiratory tract infections in children while ATSDR found that an associated decrease in infectious disease resistance was not found or that results are mixed (Table 3)

Lastly, aside from the ATSDR and EPA weight of evidence evaluations, reviews of the immunotoxicity potential of PFAS [109-112] present various perspectives, including whether or not existing studies are suitable for developing RfDs.

**Table 6b**
Immune outcomes and fish consumption.

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| *Meta-analyses* | | | | |
| Asoudeh et al. 2021 Meta-analysis of seven cohort studies and six case-control studies. | Studies published 1991–2020 | Rheumatoid arthritis (RA) | Highest vs. lowest category of fish intake: <br>• RR = 0.89 (95 % CI: 0.80, 0.99). 100 g/day fish intake increment associated with 15 % lower risk of RA | Lowest RA risk at 20–30 g/day fish intake. |
| Di Giuseppe et al. 2014 Meta-analysis of seven studies | Studies published 1991–2013 | Rheumatoid arthritis | 1–3 servings/wk of fish vs. no intake: <br>• RA RR = 0.76 (95 % CI: 0.57, 1.02). | No effect of 1 serving/wk increment in fish intake on RA. |
| Malmir et al. 2022 Meta-analysis of 31 studies. | Studies published 2005–2018 | Allergic rhinitis, asthma, eczema, food allergy, wheeze | Greater maternal fish intake <br>• allergic rhinitis: OR = 0.91 (95 % CI: 0.75, 1.09) <br>• asthma: OR = 0.99 (95 % CI: 0.89, 1.11) <br>• eczema: OR = 0.93 (95 % CI: 0.84, 1.03) <br>• food allergy: OR = 0.75 (95 % CI: 0.64, 0.88) <br>• wheeze: OR = 0.97 (95 % CI: 0.96, 0.99) | 30 g/wk increment in fish intake during pregnancy associated with 4 % reduced risk of eczema. |
| Papamichael et al. 2018 Meta-analysis of 19 studies conducted in Asia, Europe, and Australia. | Studies published 1992–2016 | Current asthma and wheeze | "all fish" intake vs. no intake <br>• children up to 4.5 years old <br>  o asthma: OR = 0.75 (95 % CI: 0.60, 0.95) <br>  o wheeze: OR = 0.62 (95 % CI: 0.48, 0.80) <br>"fatty fish" intake vs. "no fish" intake <br>• children 8–14 years old <br>  o asthma: OR = 0.35 (95 % CI: 0.18, 0.67) <br>Introducing fish in first 6–9 months and | No association for "all fish" intake on "current asthma" in children aged (2–15 y). No significant association with "current asthma" or "current wheeze" in children ages 6–15 y |

**Table 6b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| | | | regular fish intake (≥ once/wk) decreased risk, prevalence, and asthma symptoms in ages up to 14 y | |
| Rezaeizadeh et al. 2022 Meta-analysis of six case-control studies. | Studies published 1998–2018 | Multiple sclerosis | Cases vs. controls: Multiple sclerosis OR = 0.77 (95 % CI: 0.64, 0.92) | |
| Stratakis et al. 2017 Pooled analysis. 60,774 mother-child pairs from 18 birth cohorts. | Born 1996–2011 | Asthma, allergic rhinitis | Fish intake during pregnancy not associated with offspring wheeze symptoms nor with the risk of child asthma and allergic rhinitis at school age. Results consistent by type of fish and seafood consumption and in sensitivity analyses. | |
| Zhang et al. 2017 Meta-analysis of 19 studies. | Studies published 2003–2013 | Allergic outcomes | Fish intake during first year of life<br>• Eczema: RR = 0.61 (95 % CI: 0.47, 0.80)<br>• Allergic rhinitis: RR = 0.54 (95 % CI: 0.36, 0.81) | |
| ***Individual studies*** | | | | |
| Øien et al. 2019 4264 mother-infant pairs from the Prevention of Allergy among Children in Trondheim (PACT) study. Norway. | 2000–2004 | Asthma, allergic outcomes | Reduced risk of current health outcomes at age 6 y for increased fish consumption at 1 y:<br>• Eczema: OR = 0.72 (95 % CI: 0.56, 0.93)<br>• Asthma: OR = 0.60 (95 % CI: 0.39, 0.91)<br>• Wheeze: OR = 0.66 (95 % CI: 0.50, 0.88)<br>Association driven by lean fish. Oily fish, unrelated. | |
| Takaoka and Norback 2008 153 | 2005–2006 | Asthma, infections, allergy | Fish intake<br>• Resp. infections OR = 0.49 | |

**Table 6b** (*continued*)

| Study and population | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| university students. Japan | | | (95 % CI: 0.28, 0.86)<br>Seafood intake<br>• Pollen allergy OR = 0.66 (95 % CI: 0.44, 0.99) | |
| Talaei et al. 2021 4543 children in the ALSPAC cohort. Sweden. | Born 1994–1996 | Asthma | No association between fish intake at 7 y and incident asthma at 11 y or 14 y (OR = 0.83; 95 % CI: 0.62, 1.13). Having FADS genotype (rs1535): GA/GG genotype was associated with lower asthma risk for highest vs. lowest quartiles of fish intake (OR = 0.59; 95 % CI: 0.37, 0.93). | White fish was 74.6 % of total fish intake followed by tuna (18.4 %). |
| Vu et al. 2022 500,000 adults. UK | 2006–2010 | Respiratory infections | Intake of oily fish (e.g., sardines/salmon/mackerel/herring)<br>• Pneumonia OR = 0.90 (95 % CI: 0.85, 0.94)<br>• COVID−19 OR = 0.98 (95 % CI: 0.90, 1.07) | |

### 3.3.2. Fish consumption studies

Altered immune effects can manifest in autoimmunity (e.g., rheumatoid arthritis, multiple sclerosis), hypersensitivity (e.g., allergies, eczema, asthma, wheeze), or immunosuppression (e.g., infectious disease resistance, altered antibody response). This subsection will focus on studies that evaluated an association between fish intake and immunosuppression to match the PFAS studies used by EPA for RfD derivation. Table 6b presents studies on fish intake and immune outcomes.

In exploring the effect of diet on pneumonia, influenza, and COVID-19 infections, Vu et al. [113] found that intake of oily fish such as sardines, salmon, mackerel, and herring was associated with lower odds of having pneumonia and influenza among adults in the UK Biobank cohort, but no association with COVID-19. Similarly, Takaoka and Norback [114] found that among female university students in Japan, fish intake reduced the odds of developing respiratory infections (OR = 0.49; 95 % CI: 0.28, 0.86).

Although outside the scope of this paper, there is evidence to support decreased risk of autoimmune disease [115–118] and hypersensitivity [119–124] with higher fish intake among adults and children. These studies are summarized in Table 6b.

### 3.3.3. Discussion of immune effects

As summarized above, some studies showed inverse associations between serum PFAS and vaccine antibody concentrations and others did not, yet others showed directly proportional associations. It is

possible that these associations are vaccine- or PFAS exposure-specific. For example, three recent studies [125–127] found no association between PFAS exposure and COVID-19 vaccine antibodies or COVID-19 infection in Sweden, Michigan, and Alabama, respectively as did several studies summarized above for other vaccines. More research into interactions between vaccine type, PFAS concentration, and PFAS species is needed.

Studies used by EPA for developing RfDs showed associations with antibodies against toxoids of diphtheria and tetanus, diseases that have been largely contained globally by vaccines. However, if exposures as prevalent globally as PFAS are estimated to reduce vaccine effectiveness, one might expect to see some increase in disease prevalence, despite the current rarity or changing potency of some of these infections. In the United States, ≤ 3 cases of diphtheria and < 38 cases of tetanus were reported annually during 2012–2022 [128]. The tetanus cases were mostly among those unvaccinated or not up to date on their vaccine shots [129]. The WHO and the Faroe Islands health department have not responded to inquiries about vaccine-preventable disease rates in the Faroe Islands where studies found inverse associations between serum PFAS and vaccine antibodies. Studies in Norway and the United States also showed inverse associations between serum PFAs and rubella vaccine antibody concentrations [104,130,131]. During 2012–2022 < 4 cases and < 10 cases were reported annually for Norway and the United States, respectively [128].

Several factors can affect the immune response, particularly to vaccines. These include sex, gut flora, physical activity patterns, nutrition status (particularly fish), geographic residence, body mass index, income, tap water source, and Th1 phenotype, among others [111]. To illustrate, Migliore et al. [132] reported that low intake of essential nutrients was correlated with poor response to pneumococcal vaccine in children 5–7 years of age and higher intake of dietary fiber, vitamin B1, zinc, iron, and magnesium with adequate response. Wang et al. [133] reviewed animal and human studies suggesting that responses to vaccines, antibody concentrations, B-cell, and T-cell responses depend on the time of day of vaccination. Similarly, Fernandes et al. [134] showed circadian periodicity in the plaque forming cell immunotoxicity assay, an outcome of the PFOS studies in mice used to develop comparison values [135,136]. Moreover, Eliakim et al. [137] showed that BMI can influence IgG antibodies to tetanus vaccine and Ayling et al. [138] found that positive mood, adequate caloric intake, and steps taken per day were positively correlated with H1N1 vaccine IgG concentrations, while negative mood and perceived stress correlated negatively.

Further, common non-PFAS toxins have been associated with altered vaccine responses. For example, among Bangladeshi children aged 5 years, vaccinated against diphtheria and tetanus, Welch et al. [139] found that blood lead during pregnancy was directly proportional to vaccine response in children and arsenic in water drank during pregnancy was inversely proportional. Zheng et al. [140] reviewed additional studies of the effect of heavy metals exposure on vaccine response and immunoglobulin titers. Similarly, Heilmann et al. [141,142] have also shown associations between doubling of child serum PCB and maternal milk PCB and decreased diphtheria and tetanus vaccine response in children.

Mendivil [143] reviewed the potential health benefits of consuming fish and marine ω-3 fatty acids and found support for benefits on intestinal bacteria, anti-inflammatory mediators, and reduction in chronic inflammatory conditions, partially mediated via fatty acids serving as substrates for synthesis of proresolving lipids, like resolvins, protectins, and maresins, that promote resolution of inflammation, repair, and healing. Moreover, the review indicated that melatonin in fish can have a role in T-cell differentiation and prevention of inflammatory joint disease pathogenesis. In support, West et al. [144] found supplementation of infants with Lactobacillus F19 bacteria associated with enhanced diphtheria antibody concentrations and a review by Whelan et al. [145] suggests that marine-derived ω-3 polyunsaturated fatty acid (PUFA) supplementation may benefit patients that are lower antibody

responders to vaccines by enhancing B-cell activation and antibody production.

Lastly, risk assessors and communicators should be informed of dose and effect for some of these outcomes. For example, ATSDR [11] found suggestive evidence of increased odds of ulcerative colitis with PFOA exposure based on studies by Steenland et al. [146,147]. These studies showed increased odds for PFOA and ulcerative colitis in the combined community/worker cohort (OR = 2.86; 95 % CI: 1.65, 4.96) and worker cohort (OR = 6.57; 95 % CI: 1.47–29.40) when the fourth quartile of PFOA exposure was compared to the first quartile of PFOA exposure. Median serum PFOA was 24 ng/mL (IQR: 12–59) in the community cohort (n = 28,541), 113 ng/mL (IQR: 56–256) in the worker cohort (n=3713), and 26 ng/mL (IQR: 13–68) with cohorts combined. A recent study of small populations from Sweden [148] reported no effect of PFOS or PFOA exposure on ulcerative colitis morbidity or mortality (range of medians over sampling periods – PFOS: 142–271 ng/mL; PFOA: 9–16 ng/mL). The 2017–2018 NHANES PFOA geometric mean serum concentration in adults is 1.42 ng/mL and it might be lower in the next NHANES cycle [149] which suggests that most current exposures in the United States and elsewhere are much lower than those associated with increased risk of ulcerative colitis.

### 3.3.4. Summary of immune effects

Overall, the lower antibody concentrations are evident in several studies with higher PFOS and PFOA exposures and outcomes varied within and across studies, by vaccine type and potentially exposure concentration. However, there were inconsistencies and a lack of good evidence for increased risk of infectious diseases. Studies that examined the association between fish intake and immunity are limited, but generally showed potentially favorable outcomes on autoimmunity, hypersensitivity, and immunosuppression. There is a need for studies to investigate the interaction between PFAS exposure and fish intake on immune outcomes, particularly immunosuppression.

### 3.4. Liver effects

The candidate RfDs for this endpoint are based on increments in ALT associated with PFAS exposure. Increased ALT and other liver enzymes can indicate liver damage. The following sections explore changes in liver enzymes and liver disease in association with PFAS exposure and fish intake.

### 3.4.1. PFAS studies

EPA relied on the studies by Gallo et al. [150], Darrow et al. [151], and Nian et al. [152] for associations between PFAS and liver function indicators in serum. These include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT), among others.

In the C8 study cohort, Gallo et al. [150] found elevated ALT associated with each natural log unit (ln) increase in PFOA (OR = 1.10; 95 % CI: 1.07, 1.13) and ln-PFOS (OR = 1.13; 95 % CI: 1.07, 1.18). The study median serum concentration was 28.0 ng/mL for PFOA and 20.3 ng/mL for PFOS. In a follow-up study of the same C8 cohort Darrow et al. [151] found that an increase from the first to the fifth quintile of modeled cumulative PFOA exposure was associated with a 6 % increase in ALT. The authors reported a lack of evidence of PFAS exposure effect on all liver disease, enlarged liver, fatty liver, and cirrhosis. The study population lived in West Virginia, close to a chemical plant that used PFOA in the manufacture of fluoropolymers. Study participants who had ever worked in the plant had a median serum PFOA concentration of 93 ng/mL and those who had never worked at the plant a median of 15 ng/mL. Among adults in Shenyang, China, Nian et al. [152] found a 7.4 % higher serum ALT concentration for each ln-unit increase in PFOA and a 4.1 % higher serum ALT concentration for each 1 ln-unit increase in PFOS. AST and GGT increased as well. Median total serum concentration of PFOA was 6.2 ng/mL and of PFOS was 24.2 ng/mL. A

**Table 7a**
Liver outcomes and PFAS.

| Study and population | PFAS serum concentration (ng/mL) | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|---|
| *RfD candidate studies* | | | | | |
| Darrow et al. 2016 30,000 residential/ worker cohort. C8 study. West Virginia. USA | Median PFOA: Ever worked at plant: 93.3 Never worked at plant: 14.8 | 2005–2006 | ALT, liver disease, enlarged liver, fatty liver, cirrhosis | An increase from first to fifth quintile of cumulative PFOA exposure associated with 6 % increase in ALT. No evidence of cumulative exposure effect on all liver disease, enlarged liver, fatty liver, or cirrhosis. | PFOA exposure steadily increased with income. 1892 workers at the plant and 28,831 residents who never worked at the plant. |
| Gallo et al. 2012 47,092 adults from the C8 study cohort. USA | Median: PFOA: 28.0 PFOS: 20.3 | 2005–2006 | ALT, bilirubin | 1 ln-PFOA and 1 ln-PFOS association with increased ALT • PFOS: OR = 1.13 (95 % CI: 1.07, 1.18) • PFOA: OR = 1.10 (95 % CI: 1.07, 1.13) Increased direct bilirubin with PFOS increase • OR = 1.11 (95 % CI: 0.96, 1.28) | |
| Nian et al. 2019 1605 adults. Shenyang, China. | Median: PFOA: 6.19 PFOS: 24.22 | 2015–2016 | ALT, AST, GGT | 1 ln-unit increase in PFAS vs. serum ALT increase • Total PFOS: 4.1 % (95 % CI: 0.6, 7.7) • Total PFOA: 7.4 % (95 % CI: 3.9, 11.0) | AST (~3 %) and GGT (~8 %) also increased with PFOA/PFOS |
| *Meta-analyses and other relevant studies* | | | | | |
| Cheng et al. 2023 1150 adults. NHANES population. USA. | Median: PFOS: 4.60 PFOA: 1.47 | 2017–2018 | NAFLD, liver pathology indicators | No PFAS effect on NAFLD. Increase in liver fibrosis indicator, FIB−4, in relation to PFOS (effect estimate = 0.07; 95 % CI: 0.01, 0.13). | Other indicators of liver fibrosis not associated with PFAS exposure. |
| Costello et al. 2022 Meta-analysis of 24 studies. | | Exposures assessed 1951–2016 | Liver enzymes | ALT increased for higher PFOA/PFOS/PFNA exposures. AST and GGT increased with higher PFOA exposure. | |
| Limei et al. 2023 3464 persons. NHANES cohort. USA. | Median: PFOS: 8.65 PFOA: 2.37 | 2005–2018 | NAFLD | Increased risk of NAFLD for all PFAS in women, but not in men. Lower risk for normal/underweight, increased risk for overweight (for PFOA and a composite of 4 PFASs), but not in obese people except for PFNA. | |
| Momo et al. 2024 10,234 adults. NHANES population. USA | Decline of the mean from 2003 to 4–2017-8: PFOS: 26.1–7.2 PFOA: 4.5–1.7 ΣPFAS: 35.4–11.3 (PFOS/PFOA/PFHxS/PFNA) | 2003–2018 | NAFLD, liver enzymes | Liver enzymes generally elevated with ΣPFAS and select individual PFAS. Odds for NAFLD hepatic steatosis index and fatty liver index decreased with higher ΣPFAS (statistical significance varied by quartile and PFAS species). No associations with NAFLD Transient Elastography with Controlled Attenuation Parameter | Later years showed lower odds of NAFLD for PFAS exposures as compared to earlier years despite growing prevalence of NAFLD with time. Study excluded heavy drinkers. |
| Zhang et al. 2023 1135 people. NHANES. USA | Geometric mean: PFOS: 4.6 PFOA: 1.49 | 2017–2018 | Fatty liver disease, liver enzymes | Fatty liver disease odds among heavy drinkers per log standard deviation: • PFOS OR = 1.47 (95 % CI: 0.84, 2.57) • PFOA OR = 1.79 (95 % CI: 1.07, 2.99) • PFHxS OR = 2.06 (95 % CI: 1.17, 3.65), • ΣPFAS OR = 2.12 (95 % CI: 0.99, 4.54) Some, but not all PFAS associated with higher AST, GGT, total bilirubin, and albumin. | No PFAS-associated fatty liver disease risk among non- or light drinkers. Fatty liver disease association might be driven by those on a high fat diet or obese as shown by an analysis on PFHxS. |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; NAFLD, nonalcoholic fatty liver disease; ng/mL, nanogram(s) per milliliter.

meta-analysis by Costello et al. [153] confirmed ALT elevation with higher PFOA and PFOS exposures. Table 7a presents studies on PFAS exposure and hepatic outcomes.

In exploring the effect of PFAS exposure on liver disease, Momo et al. [154] confirmed the increased liver enzyme findings in an NHANES population of 10,234 adults (2003–2018) that excluded heavy alcohol drinkers; however, there were inverse associations between "total PFAS" exposures (PFOS/PFOA/PFHxS/PFNA) and both nonalcoholic fatty liver disease (NAFLD) hepatic steatosis index and NAFLD fatty liver index (statistical significance varied by PFAS species and quartile of exposure). In addition, later years (lower PFAS exposures) showed generally lower odds of NAFLD in association with PFAS exposures as compared to earlier years, despite a growing prevalence of NAFLD in the dataset. In a smaller NHANES subset (1135 adults, 2017–2018), Zhang et al. [155] found elevated FLD odds in heavy alcohol drinkers but not light drinkers, exposed to higher PFOA and PFHxS (also with PFOS, although p > 0.05). A sub-analysis for PFHxS showed that the elevated odds might

be a function of obese status or high fatty foods consumption. This might signal an interaction between PFAS, heavy alcohol intake, obesity, diet, or other factors as was observed by Choi et al. [156] for mercury exposure and alcohol intake. This also raises the question of whether pathological changes in the liver affect PFAS distribution between liver and blood. Limei et al. [157] found increased relative risks of NAFLD for higher exposure to all PFAS examined in women, but not in men, for the 2005–2018 NHANES cohort (3464 persons) (e.g., PFOS: 1.85; 95 % CI: 1.09, 3.14 and PFOA: 1.92; 95 % CI: 1.21, 3.04 for Q4 vs. Q1). Stratifying by BMI for both sexes showed a lower risk for normal/underweight people, increased risk for overweight people (for PFOA and a composite of four PFAS), but no change in risk in obese people (except for an increased risk with PFNA). Also, Cheng et al. [158] analyzed the NHANES data for 1150 adults (2017–2018) and found no PFAS effect on NAFLD, but PFOS was associated with a small increase in a liver fibrosis indicator, FIB-4, calculated by a formula that includes platelet count, ALT, AST, and age (effect estimate = 0.07; 95 % CI: 0.01, 0.13). Other

**Table 7b**

Liver outcomes and fish consumption.

| Study | Years | Endpoint | Outcome | Notes |
|---|---|---|---|---|
| Choi et al. 2017<br>508 adults. South Korea | 2010–2015 | Liver enzymes | Fish intake associated with lower GGT (p=0.007). (15 % for Q1 vs. Q4)<br>AST and ALT slightly lower but not significant at $p < 0.05$ (4 % for Q1 vs. Q4). | GGT increased 11.0 % (95 % CI: 4.5, 18.0) in women and 8.1 % (95 % CI: −0.5, 17.4) in men per doubling of Hg conc. AST and ALT not associated with Hg. |
| He et al. 2022<br>Randomized trial of 34 patients having NAFLD/hepatic steatosis. | 2019–2020 | Hepatic steatosis | Hepatic steatosis decreased with dietary intervention of freshwater fish (−4.89 %) | Baseline fish intake: 89.7 g<br>Intervention fish intake: 344.1 g |
| Parker et al. 2012<br>Meta-analysis of studies investigating effect of omega−3 PUFA on liver fat | Studies published 2004–2010 | Hepatic steatosis, liver enzymes | PUFA supplementation effect sizes<br>• Liver fat: −0.97 (95 % CI: −0.58, −1.35)<br>• AST: −0.97 (95 % CI: −0.13, −1.82)<br>• ALT: 0.56 (95 % CI: −1.16, 0.03) | |
| St Jules et al. 2013<br>223 children (age, 6–18 y) from the Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC) trial. USA | 2005–2007 | Liver pathology, liver enzymes | Lack of fish intake associated with greater portal (p = 0.03) and lobular (p = 0.09) inflammation.<br>Higher fish and ω−3 fatty acid intake associated with lower ALT (p > 0.05). | |
| Svensson et al. 1994<br>43 males with varying levels of fish intake. Sweden | Not available | Liver enzymes | No association with liver enzymes at $p < 0.05$, although ALT and AST appeared elevated with higher fish intake. | |
| Tan and Shin 2022<br>43,655 adults. South Korea. | 2004–2016 | Fatty liver index (FLI) NAFLD | Oily fish intake vs. NAFLD incidence<br>• Men: No associations<br><br>• Women: Inverse association | Lowest to highest quartiles of fish intake:<br>• Liver function enzymes increased 2 %–3 %<br>• Triglycerides decreased |
| Wang et al. 2023<br>1862 participants (average age 61 years) from Guangzhou Biobank Cohort Study. China | 2009–2010 | NAFLD | Fatty fish intake ≥ 3 servings/wk vs. none<br>• NAFLD: OR = 1.64 (95 % CI: 1.12, 2.39)<br>"Other fish", much more consumed than fatty fish, and evenly distributed among groups was not associated | One fish serving = 50 g<br>Consumption of red meat, poultry, and processed meats not associated with NAFLD. |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; NAFLD, nonalcoholic fatty liver disease; PUFA, polyunsaturated fatty acids; Q1/2/3/4, quartiles; Hg, mercury.

liver fibrosis indicators were not associated with PFAS exposure.

Lastly, EPA and ATSDR both found evidence for small or inconsistent increases in serum liver enzymes with higher PFOS/PFOA exposures and both similarly noted limited availability or consistency among studies examining associations between PFAS exposure and risk of liver disease (Table 3).

*3.4.2. Fish consumption studies*

Among studies that examined the association between fish intake and liver function and disease, Tan and Shin [159] found no association between NAFLD risk and oily fish intake in men, although there was an inverse trend (p < 0.05) among women. Similarly, He et al. [160] saw a 5 % reduction in hepatic steatosis following an 84-day intervention with dietary freshwater fish in 34 patients having NAFLD. Among children (6–18 years), St Jules et al. [161] reported that lack of fish intake was associated with greater portal (p=0.03) and lobular inflammation (p=0.09). In contrast, in a cross-sectional study in China (2009–2010), Wang et al. [162] found that fatty fish intake ≥ 150 g/week vs. no intake had higher odds of NAFLD (OR = 1.64; 95 % CI: 1.12, 2.39). However, consumption of ≥ 550 g/week of either "other fish" or "red meat" or ≥ 150 g/week of processed meat, showed no association with NAFLD in this study.

Lastly, in a meta-analysis, Parker et al. [163] showed beneficial changes in liver fat (effect size = −0.97; 95 % CI: −0.58, −1.35) and liver enzyme concentrations (AST; p = 0.02 and ALT; p = 0.06) with PUFA supplementation. In individual studies, Choi et al. [156] reported lower GGT (p = 0.007) with higher fish intake and Svensson et al. [164] found no association. In contrast, [159] found liver enzymes (AST and ALT, but not GGT) increased by 2–3 % from lowest to highest quartile of oily fish intake (10.5 g/week to 98.1 g/week) in men, but not women. Table 7b presents studies on fish intake and hepatic outcomes.

*3.4.3. Summary of liver effects*

Overall, higher PFOS and PFOA exposures were associated with small increases in ALT and other liver enzyme concentrations, although there was a lack of good evidence for increased risk of liver disease with some studies showing reduced risk and some showing increased risk in smaller groups, including heavy alcohol drinkers. More studies are needed to explore PFAS/liver disease outcomes with attention to interactions by sex, diet, and lifestyle factors. Studies examining fish intake effects on liver enzymes and disease are few but lean towards beneficial effects on liver outcomes. However, more studies are needed in that area.

**4. Discussion**

Studies used by EPA to develop RfDs for PFOS/PFOA show small decrements in BW among babies of exposed mothers; small increases in serum TC without good evidence of increased CVD risk; lower vaccine antibody concentrations with inconsistencies and a lack of good evidence for increased risk of infection; and small increases in ALT, and in some cases, other liver enzymes, in the absence of good evidence for increased liver diseases. In comparison, fish intake was generally associated with 1) higher BW and lower odds of adverse birth outcomes, although intake of fish known to be high in persistent organic pollutants and methylmercury sometimes had the opposite effect; 2) no association or lower risk of CVD, although fried fish intake was associated with adverse outcomes; 3) some desirable outcomes on autoimmune and hypersensitive endpoints, with scant evidence of favorable effects on resisting infectious diseases; and 4) scant evidence of favorable liver function indicators.

The EPA RfDs are based on changes in BW, serum cholesterol, liver enzymes, and vaccine antibody concentrations and more work is needed to determine the clinical relevance of these findings, which come mainly

**Table 8**

Change in PFAS geometric mean concentrations (ng/mL) (95 % confidence interval) in NHANES, 1999–2018.

|  | Total population (> 12 years) | | | Adolescents (12–19 years) | | |
| --- | --- | --- | --- | --- | --- | --- |
|  | 1999–2000 | 2017–2018 | % change | 1999–2000 | 2017–2018 | % change |
| PFOS | 30.3 (27.1–33.9) | 4.25 (3.90–4.62) | −86 | 29.0 (26.1–32.3) | 2.68 (2.31–3.12) | −91 |
| PFOA | 5.22 (4.72–5.78) | 1.42 (1.33–1.52) | −73 | 5.50 (5.00–6.05) | 1.18 (1.06–1.31) | −79 |
| PFHxS | 2.15 (1.92–2.41) | 1.08 (1.00–1.18) | −50 | 2.67 (2.11–3.39) | 0.87 (0.73–1.02) | −68 |
| PFNA | 0.55 (0.45–0.66) | 0.41 (0.36–0.46) | −25 | 0.47 (0.40–0.55) | 0.35 (0.29–0.42) | −26 |

from cross-sectional studies. Given the large declines in recent decades in select PFAS exposures in people in the United States (Table 8) and other populations, it should be possible to investigate any decrease in load of associated diseases. It is also possible to conduct a benefit/risk evaluation for PFAS and fish intake that considers and improves on those undertaken by several groups [1,165,166].

*4.1. Exposure considerations*

Most of the PFAS health studies that EPA used to develop RfDs were conducted during periods or in locations where there were higher average exposures to the PFAS most frequently detected in humans (e.g., PFOS, PFOA, PFHxS, PFNA), although other studies, not all used by EPA, had exposures comparable to the latest NHANES population (2017–2018). PFAS concentrations declined considerably in the NHANES population from 1999–2000 to 2017–2018 (Table 8) CDC [149]. Exposures to these PFAS are likely lower now in the United States, six years after the last NHANES cohort. Elsewhere, Dassuncao et al. [167] showed a 14.4 % annual decline in the sum of 19 PFAS in Faroese children (ages 5–13 years) between 1993 and 2012. Declines in population exposures have also been noted in several countries [168, 169]. Some countries, however, do not necessarily have comparable restrictions/agreements on releases of PFOA/PFOS that the United States has enjoyed for two decades [170]. More work is needed to identify population exposures worldwide in addition to PFAS exposure contributions from water, food, personal products, and other sources.

*4.2. Fish monitoring*

Many studies exploring associations between fish intake and health outcomes were conducted during periods when populations, particularly in the United States, had higher PFAS body loads. Therefore, it is reasonable to assume that the outcomes of these studies, particularly those with favorable health outcomes, occurred despite these higher body loads. Moreover, there is some evidence that PFOS/PFOA concentrations in fish have decreased, particularly in freshwater fish in some locations in the United States. For example, Stahl et al. [17] measured PFAS in freshwater fish filet in the National Rivers and Streams Assessment during 2013–2014 (1416 fish) and 2018–2019 (1109 fish), maintaining half the sampling locations between periods. For PFAS with frequent detections, there was a decreasing trend in concentration over time (e.g., median PFOS decreased from 6.49 ng/g to 3.1 ng/g, wet weight). When comparing PFOS median concentrations over time in the same species, more recently collected channel catfish had approximately a third of the concentration, smallmouth bass slightly decreased, and PFAS in largemouth bass were essentially unchanged, despite overlapping IQRs. Similarly, in an assessment of PFAS from filets of fish collected from the Delaware river, MacGillivray et al. [171] reported steady decreases in PFOS from 2004 to 2018 in channel catfish, white perch, white sucker, and smallmouth bass. Other PFAS, when found, either decreased or were unchanged. Moreover, Newsted et al. [172] also found decreases in PFAS measured in filets of fish collected from a 33-mile stretch of the Upper Mississippi River between 2009 and 2013 (e.g., PFOS decreased by 65, 76, 50, and 44 % for bluegill, freshwater drum, white bass, and smallmouth bass, respectively, between periods).

Further, monitoring by Young et al. [15] of 20 PFAS in highly consumed seafood market products showed the highest PFAS concentrations in crab and clam (dominated by PFOA), mostly imported from Indonesia and China, followed by cod, tuna, pollock, tilapia, salmon, and shrimp, although concentrations were generally lower than those found in freshwater fish from the United States. Longer chain PFAS (e.g., PFUdA, PFDoA, PFTrDA) were detected in most samples. The authors indicated that the trends observed in this study are comparable to those in the literature where benthic organisms tend to have the highest PFAS concentrations, followed by lean fish, fatty fish, and aquaculture. This calls for an increased role for FDA in working with importers and producers to reduce PFAS in seafood where needed. When compared to the fish monitoring studies mentioned above, the findings by Young et al. indicate generally lower PFAS concentrations in marine vs freshwater fish and this is supported by findings from Ruffle et al. [16].

There is a need for more monitoring for PFAS of finfish, shellfish, and marine mammals, including organs, to supplement previous and ongoing fish monitoring efforts. This is essential to better characterize exposure, and subsequently risk. Liver, blood, and other organs are more likely to have higher concentrations of PFAS than filets [173,174]. Also, there is evidence that fish cooking and processing can modify the concentration of PFAS in fish, and potentially exposure to consumers, in either direction depending on method [175,176]. More work is needed in this field that includes effects of fish cleaning, processing, and cooking method on exposure.

Lastly, in a cross-sectional analysis, Christensen et al. [177] analyzed data from NHANES cohorts spanning 2007–2014 and found that higher finfish and shellfish intake in the past month was associated with higher PFAS concentrations in serum than in those who reported eating finfish and shellfish less frequently. For example, PFOA/PFOS increased approximately 3–8 % with finfish intake and 7–20 % with shellfish intake. Among the PFAS measured, serum PFUdA increased the most for finfish/shellfish by 30–40 %, although it accounts for relatively low exposure in the NHANES population (e.g., 2011–2018 median = 0.1 ng/mL) [149]. Similarly, PFAS exposure to infants increased with breastfeeding (e.g., 29 % increase in infant PFOS serum per month of breastfeeding) [178]. Despite this considerable PFAS exposure to infants through breastfeeding, the CDC and ATSDR find that the benefits outweigh the risks [179].

*4.3. Fish consumption recommendations*

Fish consumption recommendations typically account for the most susceptible populations and therefore are designed to be protective of the entire population. In the case of methylmercury, the contaminant driving the risk for most fish advisories, the most susceptible populations tend to be the fetus (via maternal exposure) and younger children for neurodevelopmental endpoints. Recommendations exist for older people as well but might not necessarily suit every person. There is a role for health agencies and health care providers to educate each other and collaborate on advising people while considering health history, diet, physical activity, mental health, age, child-bearing plans, current PFAS exposures, and PFAS in consumed fish when known, among other factors. Underlying health conditions, however, do not necessarily suggest limiting fish intake, which can have a net benefit on health outcomes. One example is CVD, that is the leading cause of

morbidity and mortality in the United States and for which fish intake has been shown to have favorable outcomes in many studies. So far, PFOS and PFOA exposures have been mainly associated with small changes in blood lipids, including increased TC and HDL-C. In many cases, eating more fish is good advice.

Risk assessors and risk managers have a responsibility to critically appraise the science and weigh the benefits and risks for the respective population(s). For example, as evaluated by ATSDR and EPA (see Table 3) the evidence is mainly for small changes in biomarkers and birthweight while the evidence is inconsistent or lacking for associations between PFOS/PFOA exposure and increased risk of disease at current exposures for most of the United Stated population. For developing fish intake recommendations, because of the benefits associated with eating fish, risk assessors and policy makers might develop RfDs for fish consumption that are based on increased risk of diseases or conditions (e.g., cardiovascular, infectious) in either human or animal studies of PFAS health effects after a weight of evidence evaluation. In addition, a reverse uncertainty factor (or benefit factor) might be applied that accounts for the benefits of eating fish at the expense of contaminant exposures. This benefit factor would be in addition to the uncertainty factors applied in deriving the RfD and would typically allow for higher fish intake. Collaboration is needed to explore the magnitude of this factor and when it would be applied.

The utility of advice concerning changes to fish intake as an intervention should be weighed in terms of effect, particularly in the short-term (e.g., during pregnancy). Many PFAS, including PFOS/PFOA have half-lives in humans in the order of years. Any short-term change might deprive a person of much needed nutrients while not appreciably affecting exposure. In addition, any resulting health benefit might be insignificant, given previous body load and relatively small magnitude of PFAS-associated adverse outcomes in epidemiology studies.

### 4.4. Communication and perspective

Removing or minimizing intake of fish and other seafood can result in loss of health benefit. Moreover, it can sow anxiety and fear from foods for which the risk is extremely low or not fully characterized and the alternative to these foods might be less nutritious or of unknown safety. Rather than restricting fish intake, recommendations should lean towards fish consumption of species not known to be highly contaminated, as part of a diet rich in other nutritious foods such as eggs, fruits, vegetables, whole grains, and legumes, with lower consumption of highly processed food, cured meats, and foods with high sugar or salt content. Further, risk assessors and managers need to understand the magnitude of risk associated with health endpoints associated with PFAS and the balance of benefits and risks in order to provide recommendations that allow fish eaters to make the right choices, including consuming fish without unfounded concern. Lastly, there should be awareness that the benefits surrounding fish are not limited to eating fish – the recreational, traditional, cultural, and spiritual benefits are considerable.

### 4.5. Recommendations for additional studies

Ultimately, studies are needed that adjust for fish consumption when looking at contaminant health effects. Gennings et al. [55] adjusted for fish intake and showed a resulting beneficial effects on BW that contrasted with the PFOS effect, even if the authors did not account for all fish types or fish preparation method. The other study that adjusted for fish intake did not find associations between maternal PFAS exposure either before or after adjustment [180]. Stewards of epidemiology cohorts that track fish intake and contaminant health effects might consider analyzing the effect of fish intake, including by fish type, on health outcomes. Conducting these analyses or incorporating them in new studies could inform fish consumption recommendations when resources and study participant consent allow. Concurrently, it is important to conduct more monitoring of seafood for PFAS.

## 5. Conclusions

Fish intake studies, by default, account for the effects of contaminants, although they do not always account for variation among fish species or contaminant content. Some of these studies reviewed above showed effects by fish type and, in some cases, those were related to preparation method or contaminants like PCBs and mercury. Intake of fish not known to be highly contaminated appears to have generally favorable or neutral effects on all endpoints surveyed in this paper. In some cases, high intake was associated with adverse outcomes and requires further investigation to determine causes. Given the review and analysis in this paper, for much of the United States and many world populations, PFAS and other contaminants do not appear to pose net risk, at least of birth outcomes or CVD, from fish intake in the range of 200–500 g/week and many studies support a safe margin or benefit at higher fish intake. This fish intake estimate is based on dose response graphs available in fish intake health effects meta-analyses [27,74,181]. This is in line with several national fish intake recommendations citing health benefits, including cardiovascular and developmental benefits [182,183]. For PFAS, this is supported by several observations: 1) PFAS concentrations in the general population have dwindled appreciably over the last two decades in the United States and elsewhere; 2) there is some indication that there are lower PFAS concentrations in some fish than previous years; 3) studies that reported associations between PFAS exposure and health effects or biomarkers sometimes had populations with average exposures higher than most current exposures in the United States and elsewhere; 4) the studies used by EPA to derive RfDs have small effect magnitudes or do not have clear evidence for association with health conditions; 5) studies of fish intake, regardless of contaminant profiles, often show neutral or favorable health outcomes; and 6) the RfDs/ comparison values developed by the EPA and others do not consider the benefits of fish consumption.

### CRediT authorship contribution statement

**Ali Hamade:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ali Hamade is an associate editor for Toxicology Reports. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The opinions and analyses in this paper are those of the author and do not necessarily represent the position of the Oregon Health Authority or the State of Oregon.

### Data Availability

No data was used for the research described in the article.

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