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Dr. Suhair Shallal, Designated Federal Officer (DFO)
Science Advisory Board
Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Mail Code: 1400R

Submitted via email: shallal.suhair@epa.gov

Re: Comments on Meeting Materials for Public Meetings of the Science Advisory Board Per- and Polyfluoroalkyl Substances (PFAS) Review Panel

The 3M Company ("3M") appreciates the opportunity to provide written comments on the meeting materials published in advance of the Environmental Protection Agency ("EPA" or the "Agency") Science Advisory Board's ("SAB") public meetings to review data and analysis prepared by EPA as it considers setting Maximum Contaminant Level Goals ("MCLGs") and National Primary Drinking Water Regulations ("NPDWR") for Perfluorooctanoic Acid ("PFOA") and Perfluorooctanesulfonic Acid ("PFOS").

As an initial matter, the extremely abbreviated timeframe provided for public input on thousands of pages of highly technical reports is wholly inadequate. This rushed process is inconsistent with EPA's regular procedure and timeframe for obtaining input from the SAB. It is particularly problematic that the public was provided with only 50 days for review and comment during the November and December holiday period. The inadequate comment period, and SAB and EPA's refusal to extend the period, is a concerning indication that EPA views the SAB review process here as perfunctory and a procedural impediment rather than an opportunity for robust technical input to ensure the agency is using the best available science in reaching its conclusions.

Due to the inadequate comment period and the timing of the same, 3M is not able to provide the full scope of its technical comments here. This document includes certain of 3M's comments on some aspects of the meeting materials, specifically including EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid ("PFOA") in Drinking Water, and EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanesulfonic Acid ("PFOS") in Drinking Water (collectively, the "Draft MCLG Documents").¹ 3M anticipates

¹ 86 Fed. Reg. 62526 (Nov. 10, 2021).

providing further technical input on these documents, as well as technical input on EPA's Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water, and EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS. 3M anticipates supplementing these comments in the coming weeks. 3M currently expects its supplemental comments will include additional technical input on at least the following topics:

- Further comment and analysis of the epidemiology and toxicology literature that report alternative endpoint considerations. Expanded discussion of the animal and human evidence for EPA's candidate Points of Departure ("PODs") and candidate Reference Doses ("RfDs") beyond those selected for the proposed RfDs (i.e., human tetanus and diphtheria vaccine response).
- Further assessment of epidemiology studies that were omitted from the Draft MCLG Documents.
- Additional assessment of single vaccine antibody response as a critical endpoint, e.g., consider the factors that must be taken into account to properly assign associations with PFOA and PFOS.
- Further considerations of norms and variability in responses to vaccines, and public health benefits of incremental changes in vaccine antibody responses.
- Attempt to replicate and further comment on EPA toxicokinetic and benchmark dose modeling.
- Further consideration of the implications for the remarkably low levels proposed in this draft document, including considerations of infectious disease rates compared with nationwide data on PFOA/PFOS serum levels, and the potential consequences for nationwide regulation based on EPA's analysis

3M encourages SAB to consider the information presented in the comments below as well as any supplemental comments when providing EPA with SAB's technical input on the meeting materials. EPA's approach is deeply scientifically flawed, substitutes non-scientific judgments for science, and employs unprecedented approaches to reach an illogical outcome. SAB should make these technical deficiencies clear to EPA in its response and should recommend that the Agency use scientifically sound approaches in considering these important regulatory levels.

EXECUTIVE SUMMARY

The technical comments below identify a series of compounding errors that raise serious concerns regarding the validity of EPA's approach in the Draft MCLG Documents. Perhaps the most significant error is EPA's failure to clearly and appropriately identify the critical endpoint it is trying to protect against. The Draft MCLG Documents indicate that immune effects may be the critical endpoint considered, but EPA does not explain what it means by this. There is no scientific basis to use antibody titers in response to a single vaccine as the critical endpoint. Indeed, we have found no evidence that EPA has ever taken this approach before for any substance and it is out of step with the larger scientific community. In at least one instance, the Draft MCLG Documents suggest that the critical endpoint is actually infectious disease resulting from decreased immune response. But then EPA fails to provide reliable evidence of *any*

relevant infectious disease outcomes, or evidence of overall immunological suppression. EPA's decision to focus on a single anti-vaccine antibody level – which has not been demonstrated to result in a suppressed immune response to infectious disease, and hence cannot appropriately be characterized as an “immunosuppression effect” – as a critical endpoint here is opaque and lacks a foundation in the science.

The human data relied on in the Draft MCLG Documents is a cherry-picked subset of data that focuses on the insular population of the Faroe Islands. These data are problematic for numerous reasons, including they are not applicable to the pediatric population of the United States, reflect a highly atypical antibody response, and are inconsistent with data from other cohorts. Moreover, EPA's use of the cited immunology data is simply wrong. EPA improperly relies only on a purported decrease in antibody titers. Antibody titers measure only one aspect of immune function and cannot be used as a predictor of immune failure or infection. EPA's treatment of a 0.1 IU/ml antibody titer level as a bright line differentiation between protected and unprotected is arbitrary and rests on a fundamental misunderstanding of how diphtheria and tetanus vaccines work. As discussed below, these vaccines are not intended to prevent infection, but rather are designed to neutralize toxoids generated by a diphtheria or tetanus infection. EPA relies on clinically meaningless differences to draw conclusions regarding protection levels for a single vaccine. The combination of unsound and arbitrary assumptions that form the basis of EPA's conclusions in the Draft MCLG Documents should be rejected by SAB in its responsive feedback to EPA.

As further explained in Section II below, the Draft MCLG Documents include a series of additional scientific errors. Those errors include the fact that the animal studies contradict the human immune response cited by EPA. Although not at all clear from the main text of the Draft MCLG Documents, it appears that EPA simply used BMD modeling results reported by Budtz-Jorgensen and Grandjean 2018 without independently evaluating those results. In addition, EPA's application of an uncertainty factor of 10 was either too high or insufficiently explained. Explanation of the cancer slope factor calculations for PFOA are unclear, lacking equations, and include multiple values for purportedly the same cancer slope factor.

These errors are not without consequence. The cumulative result of the errors and omissions in the Draft MCLG Documents could result in the recommendation of an RfD based on an endpoint never before used by EPA that has no clinical meaning and which would be a gross overstatement of the relative toxicity of PFOA and PFOS. The misdirection of resources that is likely to arise out of these erroneous proposed RfD values may have wide-ranging implications. SAB should identify these and other errors for EPA and provide guidance on how to use the best available science to establish meaningful and appropriate RfDs for PFOA and PFOS.

These and other concerns are addressed in more detail in the “Technical Comments” below. 3M strongly encourages SAB to use its independent review process to help EPA recognize and address these serious deficiencies in its technical analyses and help provide more sound alternative methodologies and frameworks for deriving these important regulatory limits.

TECHNICAL COMMENTS

Given the extremely limited comment period and the complex nature² of the meeting materials published by EPA, the comments below are focused on the Draft MCLG Documents. Although not specifically addressed, much of the information provided herein may also be applicable to the other two technical documents prepared by EPA in advance of the SAB meetings. As discussed above, 3M anticipates providing additional feedback in the coming weeks.

I. EPA'S SELECTION OF CRITICAL ENDPOINTS IS UNCLEAR AND NOT GROUNDED IN SCIENCE.

3M is particularly concerned by EPA's failure to clearly and appropriately identify the critical human health endpoint it is trying to protect against in developing the Draft MCLG Documents. The implication from the documents is that EPA viewed immune effects as the critical endpoint driving the analysis, but EPA has never used a specific antibody titer to a single vaccine, without increased risk to the infectious disease to which the titer is to protect in this manner before. A review of EPA's Risk assessment information system³ indicates that, while human data has been used for the critical effect for a number of compounds, vaccine response has not been used before for reference dose ("RfD") development. This novelty alone demands close scrutiny and even a high level review, limited by the short timeframe provided, has revealed serious errors that the SAB should identify and help EPA address. Moreover, even if the critical endpoint EPA is using is actually infectious disease resulting from decreased immune response, which the Draft MCLG Documents vaguely imply, there is no reliable evidence given of *any* relevant infectious disease outcomes, nor is there evidence of overall immunological suppression. Such an approach also fundamentally misunderstands how the diphtheria and tetanus vaccines work. EPA erroneously states that "[t]hough decreases in anti-tetanus [anti-diphtheria] antibody concentrations are not in themselves an adverse effect, they do prevent against tetanus [diphtheria] infection"⁴ Neither anti-tetanus nor anti-diphtheria antibodies protect against infection. They are antitoxin antibodies that protect against tetanus or diphtheria toxoids. EPA's opaque discussions of the critical endpoint it used, combined with misunderstandings and misapplications of immune response data that do not rely on best available science, should be emphasized by SAB in its feedback to EPA.

More broadly, the SAB should consider whether the novel use of vaccine antibody response, pertaining to only two specific endpoints, tetanus and diphtheria, as the 'critical endpoint' for regulatory purposes is appropriate. As discussed in detail below, antibody responses to administration of a vaccine are highly dependent on many factors that must be taken into consideration to equate a particular antibody titer to protection from the agents in particular and more broadly to immune status. These include:

² The complexity of the Draft MCLG Documents should not be confused for accuracy. Indeed, as discussed below, much of EPA's analysis is opaque and in many cases, simply does not add up upon closer inspection. 3M anticipates being able to provide further detail with adequate time to fully respond to the Agency's materials.

³ https://rais.ornl.gov/cgi-bin/tools/TOX_search.

⁴ PFOA draft p. 340, PFOS draft at p. 310.

- Measurement and testing protocols e.g., timing of measurement post administration, assay methods and validation/consistency.
- The human subject's individual factors, some known and some unknown, e.g., age, method of administration, diet, body mass, disease/immune status, household factors that influence immune factors, genetics, and others.
- Significance of the ranges of antibodies measured and identifiable levels that associate protection with measured levels - the FDA level of 0.1 is merely a guidance level without intention or support for being a bright line to define vaccine effectiveness; small and inconsistent variations are not meaningful.

EPA failed to account for these factors in developing the Draft MCLG Documents. The SAB should also recommend that EPA consider the lack of consistent evidence of responses to vaccines in general and the lack of confirmatory evidence to demonstrate immune system challenges or infectious disease consequence, which if confirmed would require evidence for designation of 'critical endpoint' for either rather than for the single, or limited, measurement of antibodies as a response to a vaccine.

A. EPA's Unprecedented Use of a 5% BMR for Vaccine Antitoxin Antibodies as the POD Is Scientifically Inappropriate.

EPA's novel choice of a 5% decrease in tetanus anti-toxin antibody for PFOA and diphtheria anti-toxin antibody for PFOS as the benchmark responses on which to base the respective MCLGs is not based on best available science and is inappropriate. EPA justifies this choice by contending that a 5% decrease may result in clinically significant effects as a sizable portion of the population may have antibody concentrations close to 0.1 IU/ml and excess PFOA or PFOS exposures may decrease these levels below 0.1 IU/ml – a level EPA cites as the protection threshold. As EPA states:

For tetanus and diphtheria, a clinically significant decrease would be a decrease that brought a person's antibody concentration below a level thought to provide protection. If a person had a concentration of 0.1 IU/ml but a 5% decrease brought their concentration below 0.1 IU/ml, that would be clinically significant. Depending on the population, there might be a large number of persons (30-40%) with antibody concentrations close to 0.1 IU/ml.⁵

There are several profound problems with this reasoning. First, antibody titers measure only one aspect of immune function. Immunity also depends significantly on other physiological factors, including cellular-mediated immune response that are not captured by a simple titer measurement. Nor was cellular-mediated immune measured in the Faroese cohort studies. Thus, a 5% antibody titer decrease cannot be used *a priori* as a predictor of immune failure as EPA

⁵ EPA. External Peer Review Draft – Proposed Approaches to the Derivation of a Draft Maximum Containment Level Goal for Perfluorooctanoic Acid (PFOA) CASRN 335-67-1) in Drinking Water ("Draft PFOA MCLG Approach"), at 340, December 2021; EPA. External Peer Review Draft – Proposed Approaches to the Derivation of a Draft Maximum Containment Level Goal for Perfluorooctane Sulfonic Acid (PFOS) CASRN 1763-23-1) in Drinking Water ("Draft PFOS MCLG Approach") at 310, December 2021. At one point (PFOA draft at p. 340), EPA also references 0.15 IU/ml as the protection level for tetanus but provides no reference for this.

purports to do. Second, there is no bright line antibody titer cut-off between protected and unprotected and the EPA's treatment of 0.1 IU/ml as such is immunologically flawed and leads to inappropriate conclusions.⁶ Protection occurs along a gradient, one aspect of which is the antibody titer. A 5% change, particularly around the 0.1 IU/ml level, is *de minimus* and likely within the intra-assay variability of the antibody assay, *i.e.*, unmeasurable. Immunologically, having a 5% lower antibody level has no clinical or biological significance in terms of response to the toxins involved and, similarly, in terms of response to infection. EPA apparently deems a 5% decrease as biologically significant by positing that a significant portion of the population might have an antibody titer between 0.1 and 0.1053 IU/ml where a 5% decrease would lead to titers between 0.095 and 0.099 IU/ml, which then strictly fall below the EPA-presumed "protection" level. These small differences are clinically meaningless and cannot be used to predict immunity. Third, 0.1 IU/ml is not a universally recognized level of protection as EPA seems to presume. The World Health Organization ("WHO") uses 0.01 IU/ml as the protection level for diphtheria and also for tetanus, when using a modified ELISA or bead-based immunofluorescence assay to measure titers.⁷ By deeming only persons above 0.1 IU/ml as protected, EPA and the Faroese studies on which it relies overstate the subjects who would fall below protective levels resulting from a 5% decrease. Moreover, a 5% change at the 0.01 IU/ml level is even smaller than a respective change around the 0.1 IU/ml level, which is already clinically and biologically meaningless. A 5% decrease in an antibody response cannot be used as a surrogate for a meaningful biologic effect and EPA should not use it as the benchmark response.

B. Faroe Islands Population Data Are Not Appropriate For Quantitative Use in Deriving Generally Applicable PFOA and PFOS MCLGs

EPA's unprecedented critical endpoint approach is predicated primarily upon several Faroe population studies. But these studies show highly atypical antibody responses, precluding the generalizability of the results. For example, notwithstanding receiving three inoculations within the first year after birth, at age five (pre-booster), over 37% of the Faroese cohort members had diphtheria antitoxin antibody levels below 0.1 IU/ml.⁸ Similarly, after receiving the age-five booster, the antibody levels at age 13 were also abnormally low, with nearly 40% of the subjects having levels below 0.1 IU/mL for diphtheria anti-toxin antibodies.⁹ This contrasts with U.S. population data for adolescents (not segregated by vaccine status), where only

⁶ WHO. The Immunological Basis for Immunization Series Module 2: Diphtheria. Update 2009 (noting that "there is no sharply defined level of antitoxin that gives complete protection from diphtheria" and "[o]ther factors may influence vulnerability to diphtheria including the infecting dose and virulence of the diphtheria bacilli, and the general immune status of the person infected. . . ."); WHO. The Immunological Basis for Immunization Series Module 3: Tetanus. Update 2017 (noting a "protective antibody concentration may not be considered a guarantee of immunity under all circumstances.").

⁷ WHO. The Immunological Basis for Immunization Series Module 2: Diphtheria,. Update 2009; WHO The Immunological Basis for Immunization Series Module 3: Tetanus. Update 2017.

⁸ Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, Heilmann C. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012 Jan 25;307(4):391-7.

⁹ Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jørgensen E. Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. Environ Health Perspect. 2017 Jul 26;125(7):077018.

approximately 20% had diphtheria anti-toxin antibody levels less than 0.1 IU/ml, respectively.¹⁰ The proportion of children aged 6 to 11 with titers below this limit was even lower. The atypical immune response seen among the Faroe population makes quantitative use of their antibody titers to derive a 5% benchmark response level applicable to a generalized United States pediatric population inappropriate.

There could be several reasons for the poor antibody response seen among the Faroese children, all of which argue against EPA's quantitative use of these cohorts. First, the Faroese cohorts utilized a four-vaccine administration schedule, starting with three doses spaced at 3, 5, and 12 months followed by a booster administered at age 5, which was not the standard in the United States at the time. Furthermore, the current routine schedule for administering DTaP to children in the United States calls for five shots: a 3-dose series at age 2, 4, and 6 months, followed by boosters at age 15–18 months and 4–6 years.¹¹ Accordingly, the immune response from the Faroe population has limited applicability to children in the United States either in the past or currently.

Second, vaccine response is highly dependent on administration technique, with optimum results achieved via intramuscular injections into the deltoid or the anterolateral aspects of the thigh. Injection into subcutaneous fat by going more medial in the thigh, using a shorter needle, or administering in the buttocks will lead to significantly lower seroconversion rates and poor overall antibody response.¹² The overall lower antibody levels in the Faroese cohort may be due to suboptimal vaccine administration technique. Furthermore, the administration of the vaccine in more outlying islands and rural areas where dietary differences are expected to lead to higher PFAS levels (discussed below) would have likely been given by different health care providers than those on the main island. Less optimal vaccination techniques by some rural health care providers could skew the results and account for the apparent inverse associations between some antibody and PFAS levels.

Third, the Faroese population is a unique, relatively insular society, with a high level of inbreeding, particularly in rural areas.¹³ Dietary differences between rural and urban areas are also significant, with seafood being the main source of food in poorer, rural areas. The suboptimal antibody response seen in the population may well be the result of these unique population features and make generalizing the results to a United States pediatric population inappropriate.

Finally, the poor overall immune responses exhibited in the Faroese cohorts compared with the United States adolescent population cannot be explained by higher PFAS exposures

¹⁰ McQuillan GM, Kruszon-Moran D, Deforest A, Chu SY, Wharton M. Serologic immunity to diphtheria and tetanus in the United States. *Ann Intern Med.* 2002 May 7;136(9):660-6.

¹¹ Centers for Disease Control and Prevention, Recommended child and adolescent immunization schedules for ages 18 or younger, United States, 2021, available at <https://www.cdc.gov/vaccines/schedules/hcp/imz/child-indications.html#note-dtap>.

¹² Zuckerman JN. The importance of injecting vaccines into muscle. Different patients need different needle sizes. *BMJ.* 2000 Nov 18;321(7271):1237-8.

¹³ Binzer S, Imrell K, Binzer M, Kyvik KO, Hillert J, Stenager E. High inbreeding in the Faroe Islands does not appear to constitute a risk factor for multiple sclerosis. *Mult Scler.* 2015 Jul;21(8):996-1002 (noting the average level of relatedness observed in the Faroe Islands is to the degree of second cousins).

among the Faroe population. The mean maternal, five-year-old, seven-year-old, and thirteen-year-old PFOA and PFOS serum concentrations in the Faroese cohorts are lower than mean background PFOS/PFOA exposures reported in the United States from the 1988-1994 timeframe, when the antibody data reported by McQuillan discussed above were collected, as well as the 1999-2000 timeframe as reported in the first NHANES general population PFOA/PFOS analysis.¹⁴

Genetic, geographic, and dietary differences within the Faroese population are also important confounders that were not thoroughly assessed by the authors in evaluating correlations between PFAS and the toxoid antibody responses, making quantitative use of the data inappropriate. Immune response is modified by innumerable individual factors that can never be completely controlled for in observational studies. When associations are observed between PFOA or PFOS and toxoid antibody responses among the many comparisons conducted in Faroe studies, the magnitude of those associations are at most modest, making it difficult to distinguish any true relationships from residual confounding “noise.” Approximately 42% of the Faroe Island population lives in metropolitan areas, with Torshavn on the island of Streymoy being the largest.¹⁵ The remaining population majority is rural, generally poorer, and less genetically diverse than the urban population. The rural population also consumes more seafood, including marine mammals, than the urban population, which can be a source of PFAS, making rural residence have a potential impact on PFAS levels.¹⁶ The urban population, with lower PFAS levels, is also expected to contain more recent emigrants, be more outbred, and thus have a different genetic makeup than the rural population. Genetics is a key component of the immune response due to highly variable immune response genes.¹⁷ Rural residents are expected to have had a different network of routine health care providers who administered vaccines as noted above. If rural study participants, who have higher PFAS concentrations as a result of their predominant seafood diets, have less than optimal vaccine administration or if urban participants are on average better vaccine responders due to increased genetic diversity, it could lead to the observed inverse associations between PFOA or PFOS and toxoid antibody responses. The inability to completely control for the many potential confounding factors could explain the

¹⁴ Olsen GW, Huang HY, Helzlsouer KJ, Hansen KJ, Butenhoff JL, Mandel JH. Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ Health Perspect.* 2005 May;113(5):539-45 (reporting geometric mean PFOS and PFOA concentrations of 33.3 ppb and 5.5 ppb from general population samples taken in 1989 from the Washington County, Maryland area). See also Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2021. Volume 1 NHANES 1999-2010 (reporting geometric mean PFOS and PFOA concentrations of 30.4 ppb and 5.12 ppb, respectively, in the U.S. general population and 29.1 ppb and 5.46 ppb, respectively, among adolescents 12-19 in 1999-2000 – the first year of general population analysis).

¹⁵ World Bank, Urban Population (% of total population) – Faroe Islands, United Nations Population Division. World Urbanization Prospects: 2018 Revision, available at [Urban population \(% of total population\) - Faroe Islands Data \(worldbank.org\)](https://data.worldbank.org/Urban-population); United States Central Intelligence Agency, The World Factbook – Faroe Islands, last updated Dec. 14, 2021, available at [Faroe Islands - The World Factbook \(cia.gov\)](https://www.cia.gov/library/publications/the-world-factbook/docs/00ad_0000.html).

¹⁶ See Timmermann CAG, Pedersen HS, Budtz-Jørgensen E, Bjerregaard P, Oulhote Y, Weihe P, Nielsen F, Grandjean P. Environmental chemical exposures among Greenlandic children in relation to diet and residence. *Int J Circumpolar Health.* 2019 Dec;78(1):1642090 (noting in a similar north Atlantic location and population that area of residence and marine diet are significant predictors of PFAS concentrations).

¹⁷ Mangino M, Roederer M, Beddall MH, Nestle FO, Spector TD. Innate and adaptive immune traits are differentially affected by genetic and environmental factors. *Nat Commun.* 2017 Jan 5;8:13850.

modest associations found in the Faroese studies in whole, or in part, and makes quantitative use of the Faroese cohorts wholly inappropriate for deriving MCLGs.

II. EPA’S ANALYSIS IN THE DRAFT MCLG DOCUMENTS IS FUNDAMENTALLY FLAWED

EPA’s flawed approach using a novel critical endpoint is compounded by numerous additional analytical errors. Some of the obvious errors identified during 3M’s high-level review are described in turn below. Additionally, many of the critical aspects in the Draft MCLG Documents lack adequate transparency in the methods and/or decision process used by EPA, including but not limited to benchmark dose modeling, use of pharmacokinetic model-derived internal dose-metrics when measured values are available, and cancer slope derivation and reporting. Additional analytical analysis is needed, as well as likely more disclosure from EPA, to truly understand and provide necessary input on the Agency’s approach here.

A. EPA’s Literature Review Omits Numerous Relevant Epidemiological Studies Addressing Immunotoxicity.

To understand the potential immunotoxicity of PFOA and PFOS, EPA should consider all of the available epidemiological studies of PFOA and PFOS in association with immune outcomes. EPA’s literature review, however, omits a substantial proportion of the approximately 100 published epidemiological studies on this topic. Even for the more focused endpoints of antibody-mediated immunity and infection (grouped by EPA as “immunosuppression effects”),¹⁸ EPA omits ten relevant epidemiological studies published in 2020 or earlier, as well as seven additional studies published in 2021. These omitted publications include six studies of antibody-mediated immunity (Granum et al. 2013, Looker et al. 2014, Kielsen et al. 2016, Stein et al. 2016b, Shih et al. 2021, Timmermann et al. 2022); nine studies of infectious disease outcomes other than COVID-19 (Leonard et al. 2008, Fei et al. 2010, Okada et al. 2012, Kishi et al. 2013, Huang et al. 2020, Bulka et al. 2021; including one update of a study considered by EPA (Dalsager et al. 2021) and two studies that also evaluated antibody levels (Granum et al. 2013, Looker et al. 2014)); and four studies of COVID-19 outcomes (Grandjean et al. 2020, Catelan et al. 2021, Ji et al. 2021, Nielsen and Jöud 2021). SAB should recommend that EPA broaden its literature review to include recent studies to ensure the Agency does not turn a blind eye to recent developments.

Among the 17 omitted studies, eight are prospective cohort studies (two from the same study population (Okada et al. 2012, Kishi et al. 2013)) with individual-level serum or plasma PFOA, PFOS, and other PFAS, highly complete follow-up for validated health outcomes, and statistical adjustment for multiple confounders (Fei et al. 2010, Okada et al. 2012, Granum et al. 2013, Kishi et al. 2013, Huang et al. 2020, Dalsager et al. 2021, Shih et al. 2021, Timmermann et al. 2022). Another is a retrospective cohort mortality study with no information on individual-level PFOA exposure and minimal adjustment for confounders, but with the advantage of taking place in an occupational setting with high average exposure levels (Leonard et al. 2008). Likewise, Olsen et al. 2001 retrospectively reviewed administrative employee health data to evaluate episodes of care among workers with occupational-level exposures to PFOS. Two of

¹⁸ Draft PFOA MCLG Approach, p. 150; Draft PFOS MCLG Approach, p. 136.

these study populations were investigated in studies that were included in EPA’s review (Granum et al. 2013, Dalsager et al. 2021); however, the included studies addressed somewhat different endpoints and age groups, making their results non-duplicative (Dalsager et al. 2016, Impinen et al. 2019). EPA’s omission of these studies thus leaves important gaps in the Agency’s presentation of the context of the epidemiological literature on potential “immunosuppression effects” of PFOA and PFOS. The exclusion of these studies also impairs EPA’s ability to evaluate the consistency of findings on its selected critical effect (i.e., decreased serum anti-tetanus antibody levels in children) across studies, as well as the consistency of these data with results on other specific antibody levels and clinical infectious disease endpoints.¹⁹

B. EPA’s Reliance on Decreased Serum Anti-Tetanus Antibody Levels in Children (PFOA), and Serum Anti-Diphtheria Levels in Children (PFOS), Ignores Inconsistent Results Across and Within Epidemiological Studies.

EPA’s reliance on observed associations of PFOA with decreased serum anti-tetanus antibody levels and PFOS with decreased serum anti-diphtheria antibody levels among children in the Faroe Islands (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a, Grandjean et al. 2017b) ignores inconsistent results presented in other epidemiological studies. EPA should not be considering the Faroe Islands data in isolation. Instead, like all scientific results, they must be interpreted in the context of other, related findings within and among independent study populations. In particular, comparisons with other relevant results should be made to evaluate whether the overall data set is consistent and coherent, and thus supportive of the validity of the potential critical effect.

Combining the studies EPA identified and as well as those not identified by EPA in the Agency’s Draft MCLG Documents, 16 publications from 14 independent study populations addressed the relationship between PFOA and/or PFOS and antibody levels (Grandjean et al. 2012, Granum et al. 2013, Looker et al. 2014, Mogensen et al. 2015, Kielsen et al. 2016, Stein et al. 2016a, Stein et al. 2016b, Grandjean et al. 2017a, Grandjean et al. 2017b, Pilkerton et al. 2018, Zeng et al. 2019, Abraham et al. 2020, Timmermann et al. 2020, Zeng et al. 2020, Shih et al. 2021, Timmermann et al. 2022). EPA refers to Grandjean et al. (2012, 2017a, 2017b) and Mogensen et al. (2015) as being “three studies,”²⁰ but in fact these represent *two* independent study populations, since three of these publications are based on the same cohort (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a), and one reports results for a non-overlapping cohort, as well as the two cohorts combined (Grandjean et al. 2017b).

A review of the design and results of the broader body of scientific literature, briefly summarized in 3M’s Appendix A, Table 1 (below),²¹ reveals several overarching points. First,

¹⁹ While the remaining eight omitted studies have methodological weaknesses such as an ecological (Catelan et al. 2021, Nielsen and Jöud 2021), cross-sectional (Looker et al. 2014, Kielsen et al. 2016, Stein et al. 2016b, Grandjean et al. 2020, Bulka et al. 2021), or retrospective case-control study design (in this instance, prone to reverse causation) (Ji et al. 2021), EPA’s failure to consider them at all demonstrates an inadequate literature review. EPA’s literature review protocols require the Agency to review all relevant studies before evaluating the data. <https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf>.

²⁰ Draft PFOA MCLG Approach, p. 151; Draft PFOS MCLG Approach, p. 137.

²¹ Tables are intended to provide a broad overview of the design and results of epidemiological studies. Details on quantitative results and study methods are not described.

reported associations of PFOA and PFOS with specific antibody levels are diverse, including inverse, positive, and null associations, with no clear direction of association overall. Thus, any effect of PFOA or PFOS on antibody levels, if causal, is not global for all antibodies. Second, few studies reported results for any given antibody type. Several antibodies (e.g., anti-hepatitis A virus, anti-coxsackievirus A 16, anti-influenza A/H3N2) were measured in only one study each, and the most commonly studied antibodies (anti-diphtheria and anti-tetanus) were measured in eight and nine publications (six and seven separate study populations), respectively. Thus, the body of epidemiological literature on any given PFAS-antibody association is relatively sparse. Third, every study reported some apparent associations (inverse and/or positive) and some null results, sometimes for the same PFAS-antibody combination in different study subgroups. Thus, focusing only on inverse associations (i.e., between higher PFOA or PFOS levels and lower antibody levels) overlooks numerous other results that are relevant to the assessment of potential effects on antibody-mediated immunity.

Results for anti-tetanus antibody levels in particular are available from seven study populations (five of children and two of adults, although one of the adult studies measured PFAS exposure during childhood) (Grandjean et al. 2012, Granum et al. 2013, Mogensen et al. 2015, Kielsen et al. 2016, Grandjean et al. 2017a, Grandjean et al. 2017b, Abraham et al. 2020, Shih et al. 2021, Timmermann et al. 2022). This body of evidence enables an assessment of the consistency of findings for anti-tetanus antibodies across studies. As summarized briefly in Table 2, associations between PFOA and lower anti-tetanus antibody levels in at least some of the many comparisons were observed in 1) a prospective cohort study of children born in the Faroe Islands in 1997–2000 (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a); 2) a second prospective cohort study of Faroe Islands children born in 2007–2009 (Grandjean et al. 2017b); and 3) a cross-sectional study of one-year-old infants in Germany (Abraham et al. 2020). In contrast, no association between PFOA and anti-tetanus antibody levels was observed in 1) a prospective cohort study of children in Norway (Granum et al. 2013); 2) a prospective cohort study of children in Greenland (Timmermann et al. 2022); 3) a prospective cohort study of adults followed since birth in the Faroe Islands in 1986–1987 (Shih et al. 2021); and 4) an exploratory cross-sectional study of adults in Denmark (Kielsen et al. 2016).

For PFOS, an association with lower anti-tetanus antibody levels was observed only in the 2007–2009 Faroe Islands birth cohort (Grandjean et al. 2017b). In the 1997–2000 Faroe Islands birth cohort, prospective analyses showed either no association between PFOS and anti-tetanus antibodies or an association with higher anti-tetanus antibody titers (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a). Otherwise, besides the 1) 1997–2000 Faroe Islands birth cohort, no association between PFOS and anti-tetanus antibody level was found in the 2) German (Abraham et al. 2020), 3) Norwegian (Granum et al. 2013), 4) Greenland (Timmermann et al. 2022), 5) 1986–1987 Faroe Islands (Shih et al. 2021), and 6) Danish studies (Kielsen et al. 2016).

Results for anti-diphtheria antibody levels in association with PFOA and PFOS are available from all but one of the study populations that evaluated anti-tetanus antibody levels, again allowing for an evaluation of consistency across studies (Grandjean et al. 2012, Mogensen et al. 2015, Kielsen et al. 2016, Grandjean et al. 2017a, Grandjean et al. 2017b, Abraham et al. 2020, Shih et al. 2021, Timmermann et al. 2022). As broadly summarized in 3M’s Appendix A,

Table 2, associations between PFOS and lower anti-tetanus antibody levels were reported in two prospective cohort studies of children born in the Faroe Islands in 1997–2000 (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a) and 2007–2009 (Grandjean et al. 2017b); and 3) the small cross-sectional study of adults in Denmark (Kielsen et al. 2016). In contrast, no association between PFOS and anti-diphtheria antibody levels was found in 1) the prospective cohort study of Faroe Islands adults followed from birth in 1986–1987 to age 28 years (Shih et al. 2021); or 2) the cross-sectional study of one-year-old infants in Germany (Abraham et al. 2020). In addition, 3) the prospective cohort study of children in Greenland found no association of maternal or child PFOS with continuous anti-diphtheria antibody levels, but a positive association of child PFOS with a greater risk of having an anti-diphtheria antibody concentration < 0.1 IU/mL (Timmermann et al. 2022)

For PFOA and anti-diphtheria antibody levels, some inverse associations (although not all associations tested) were reported in 1) the 1997–2000 Faroe Islands prospective birth cohort (Grandjean et al. 2012, Mogensen et al. 2015, Grandjean et al. 2017a); 2) the 2007–2009 Faroe Islands prospective birth cohort (Grandjean et al. 2017b); and 3) the cross-sectional study of German infants (Abraham et al. 2020). However, no association between PFOA and anti-diphtheria antibody levels was found in 1) the prospective cohort study of children in Greenland (Timmermann et al. 2022); 2) the 1986–1987 Faroe Islands prospective birth cohort (Shih et al. 2021); and 3) the small cross-sectional study of Danish adults (Kielsen et al. 2016).

Even within the combined Faroe Islands birth cohorts that EPA ultimately selected as the critical study for PFOA and PFOS, results were inconsistent (Grandjean et al. 2012, Grandjean et al. 2017a, Grandjean et al. 2017b). With respect to anti-tetanus antibody levels, for instance, in the 1997–2000 birth cohort, maternal prenatal serum levels of PFOA and PFOS were not associated with lower titers in their children at ages 5 and 7 years (on the contrary, maternal PFOS was associated with higher anti-tetanus antibodies in 7-year-olds) (Grandjean et al. 2012). Prospectively collected child serum PFOA in this cohort was associated with lower anti-tetanus antibody levels at 7 years, but not 13 years (Grandjean et al. 2012), and associations with anti-tetanus antibody levels at 5 years varied depending on whether PFOA was measured at birth, 3 months, or 18 months (no association) or 6 or 12 months (inverse association) (Grandjean et al. 2017b).

Internally inconsistent results were also observed for anti-diphtheria antibody levels in the Faroe Islands birth cohorts (Grandjean et al. 2012, Grandjean et al. 2017a, Grandjean et al. 2017b). For example, in the 1997–2000 birth cohort, maternal prenatal serum levels of PFOS were associated with lower anti-diphtheria antibodies at age 5 years, but not 7 years, whereas the opposite age-specific pattern was observed for PFOA (Grandjean et al. 2012). In the 2007–2009 cohort, PFOS measured at birth and 3, 6, 12, 18, and 60 months was not associated with anti-diphtheria antibody titers at 5 years; instead, where associations with PFOS were detected (with PFOS measured at birth and 3 months, but not later), they were only in the 1997–2000 cohort or the combined cohorts (Grandjean et al. 2017b). In contrast, the only observed associations between PFOA and anti-diphtheria antibodies (with PFOA measured at birth, but not at 3, 6, 12, 18, or 60 months) were seen in the 2007–2009 cohort or the combined cohorts.

In summary, although a superficial review may suggest some “consistent” associations of PFOA or PFOS with poorer antibody-mediated immunity based on selected results,²² closer inspection reveals considerable within- and between-study heterogeneity in observed associations by vaccine type, PFAS type, timing and dose of PFAS exposure, age group, and other factors. Data on any specific association, such as between PFOA or PFOS and anti-tetanus antibody levels, as evaluated in seven study populations, are currently insufficient to determine whether the heterogeneity is due to chance, bias, confounding, or real differences in antigen-specific immune responses, PFOA or PFOS dose, participant characteristics, or study setting. Thus, besides selecting critical effects that are not established as causal, EPA ignores substantial unexplained inconsistency and variability in the observed association. Before relying on isolated results from a singular study for its risk assessment, EPA should seek a better understanding and explanation of why results differ among studies, including across the three Faroe Islands birth cohorts, as well as within studies.

C. EPA’s Reliance on Decreased Serum Anti-Tetanus and Anti-Diphtheria Antibody Levels in Children as the Critical Effects for PFOA and PFOS, Respectively, Ignores Mostly Null Findings for Clinical Infectious Disease Outcomes.

Although serum diphtheria and tetanus antitoxin levels of at least 0.1 international units (IU) are sometimes referenced as “protective,” levels as far as 10 times lower—that is, 0.01 IU/mL—still confer some degree of protection (Food and Drug Administration 1985) and are cited by WHO as protective levels. . Given that incremental changes in specific antibody levels may or may not translate to overt differences in antibody-mediated immunity to infectious agents, a full interpretation of the epidemiological database on EPA’s selected critical effects also requires consideration of related findings on the association between PFOA and/or PFOS and clinical infectious disease endpoints. From a clinical perspective, susceptibility to infection is a leading indicator of immune function; indeed, nearly all of the 10 cardinal warning signs of primary immunodeficiency relate to the frequency and severity of recent infections (Jeffrey Modell Foundation 2016). If such clinically recognizable abnormalities are not observed, then immunodeficiency cannot be presumed to exist. Thus, from a clinical immunologist’s point of view, proper interpretation of laboratory test results, such as specific antibody levels, requires a consideration of whether such results predict disease in the form of infection. If not, then “at best time and money are wasted, and at worst a patient is informed erroneously that he or she is sick or will get sick when this is not true, thereby breaking the rule of ‘*primum non nocere*’ – above all do no harm” (Chang et al. 2016).

Combining the studies identified and not identified by EPA in the Agency’s literature search, 21 publications from 16 independent study populations²³ addressed the relationships of PFOA and PFOS with various infectious disease outcomes (Leonard et al. 2008, Fei et al. 2010, Okada et al. 2012, Granum et al. 2013, Kishi et al. 2013, Looker et al. 2014, Dalsager et al. 2016, Goudarzi et al. 2017, Impinen et al. 2018, Impinen et al. 2019, Manzano-Salgado et al. 2019,

²² See e.g., Draft PFOA MCLG Approach, pp. 166–167; Draft PFOS MCLG Approach, pp. 156–157.

²³ Five pairs of studies originated from the same underlying cohort: (Okada et al. 2012, Kishi et al. 2013), (Granum et al. 2013, Impinen et al. 2019), (Dalsager et al. 2016, Dalsager et al. 2021), (Goudarzi et al. 2017, Ait Bamai et al. 2020), (Impinen et al. 2018, Kvale et al. 2020).

Abraham et al. 2020, Ait Bamai et al. 2020, Grandjean et al. 2020, Huang et al. 2020, Kvalem et al. 2020, Bulka et al. 2021, Catelan et al. 2021, Dalsager et al. 2021, Ji et al. 2021, Nielsen and Jöud 2021). See 3M's Appendix A, Table 3.

As summarized broadly in Table 3, these studies showed no apparent pattern of association between PFOA or PFOS and risk of overt infectious diseases, with scattered positive, inverse, and null associations. Focusing on the higher-quality prospective cohort studies, omitting one study with duplicative results (Okada et al. 2012) and retaining studies with non-duplicative results from the same cohort, reported findings remained inconsistent across 12 studies from eight separate study populations (Fei et al. 2010, Granum et al. 2013, Kishi et al. 2013, Dalsager et al. 2016, Goudarzi et al. 2017, Impinen et al. 2018, Impinen et al. 2019, Manzano-Salgado et al. 2019, Ait Bamai et al. 2020, Huang et al. 2020, Kvalem et al. 2020, Dalsager et al. 2021). The majority of associations tested were weak in magnitude and statistically null, and associations detected between PFOA and/or PFOS and specific types or groups of infection (e.g., upper or lower respiratory tract infections, gastroenteritis, or otitis media/ear infection) were not consistently detected within or across studies. Only one prospective cohort study tested associations with a vaccine-preventable infection, namely, chicken pox, which exhibited no association with PFOA or PFOS among 7-year-olds in Hokkaido, Japan (Ait Bamai et al. 2020).

If PFOA or PFOS exposures were having clinically meaningful effects on immune function as argued by EPA based on the hypothesis generating work of Grandjean et al. and others, one would predict the occurrence of primary and secondary immune effects, at a minimum, among the most highly exposed populations. For example, individuals with true immune deficiency exhibit clear increased risks to chronic respiratory diseases, such as COPD, brought on by immune system dysfunction.²⁴ Yet, the available studies show no indication of this in cohorts more highly exposed to PFAS.

Steenland et al. studied the incidence of disease among DuPont workers exposed to PFOA.²⁵ As of 2005, they had a median serum concentration of 115 ppb, compared with general population levels of 4 ppb at that time. Steenland et al. report no significant associations with COPD, which might be expected if workers had immune system dysfunction conferring susceptibility to respiratory infections. Likewise, Leonard et al. 2008 did not find any excess mortality due to infectious disease among DuPont workers.

In 2001, 3M conducted a study of its Decatur, Alabama workforce, who was more highly exposed to POSF-derived chemistries, including PFOS, by evaluating episodes of care²⁶ obtained

²⁴ Berger M, Geng B, Cameron DW, Murphy LM, Schulman ES. Primary immune deficiency diseases as unrecognized causes of chronic respiratory disease. *Respir Med.* 2017 Nov;132:181-188; Bhat TA, Panzica L, Kalathil SG, Thanavala Y. Immune Dysfunction in Patients with Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc.* 2015;12 Suppl 2(Suppl 2):S169-S175.

²⁵ Steenland K, Zhao L, Winquist A. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occup Environ Med.* 2015 May;72(5):373-80.

²⁶ An episode of care is a unique metric that is not directly comparable to other standard epidemiologic endpoints as it may include incident cases, prevalent cases, and tentatively diagnosed cases that are the routine consequence of the differential diagnoses that individuals may undergo in the course of disease diagnosis, treatment and management. The episode of care concept can be a useful screening method for the potential risk of diseases and/or

from administrative health data from 3M workers extending from 1993 to 1998.²⁷ Evaluating episodes of care is a particularly useful screening method for the potential risk of diseases and/or conditions when there are two study populations from the same company, covered by the same medical plan, who live within the same community undergoing comparable local/regional medical care, and who differ primarily only in their workplace exposure. This scenario existed with the employees at the 3M Decatur manufacturing site which had two separate neighboring manufacturing plants: fluorochemical and film production.

PFOS exposures in the fluorochemical cohort were significantly higher than the general population background-level PFOS exposures that were at issue in the Faroe Islands cohorts. For example, mean serum PFOS levels for Decatur fluorochemical production employees participating in a 1995 medical surveillance assessment were 2,400 ppb with levels extending up to and in excess of 12,000 ppb.²⁸ This contrasts with the Faroe Islands cohorts, whose highest mean PFOS levels were 27 ppb in maternal serum at birth and were as low as 6.7 ppb in children by age 13. In the adjacent 3M film plant, where fluorochemicals were not significantly used, PFOS exposures were shown to be substantially less based on a random sample of employees at these two adjacent plants at the Decatur site.²⁹

The primary analysis in the study generated risk ratio episodes of care by dividing the observed to expected³⁰ episodes of care experienced among 652 fluorochemical production plant employees by the observed to expected episodes of care experienced among 659 film plant employees. Further, subgroup analyses included comparing only fluorochemical production plant employees who never worked in the film plant to film plant employees who never worked in the fluorochemical production plant as well as comparing the highest-exposed (those working in high-exposure jobs) and longest-exposed (those having at least 10 years employment in the fluorochemical plant prior to the study period) fluorochemical plant workers to the

conditions where such an assessment would be impractical to conduct through formal investigations involving comprehensive medical record reviews.

²⁷ Olsen GW, Berlew MS, Hocking BB, Skratt JC, Burris JM, Mandel JH. An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. Final Report. May 18, 2001. EPA Doc. No. AR-226-1030a021. US Environmental Protection Agency, Washington DC.

²⁸Olsen GW, Burris JM, Mandel JH, Zobel LR. An epidemiologic investigation of clinical chemistries, hematology and hormones in relation to serum levels of perfluorooctane sulfonate in male fluorochemical production employees. April 22, 1998. EPA Docket No. AR-226-0030. US Environmental Protection Agency, Washington DC.

²⁹Final Report. Fluorochemical exposure assessment of Decatur chemical and film plant employees. August 11, 1999. EPA Docket No. AR-226-0950. US Environmental Protection Agency, Washington DC. See also Olsen GW, Logan PW, Hansen KJ, Simpson CA, Burris JM, Burlew MM, Vorarath PP, Venkateswarlu P, Schumpert JC, Mandel JH. An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. *AIHA J* (Fairfax, Va). 2003 Sep-Oct;64(5):651-9. Overall, mean PFOS serum levels for fluorochemical plant employees were approximately an order of magnitude higher than film plant employees. Exposure contrasts would be even larger for comparisons between highly exposed fluorochemical plant workers and the lowest exposed film plant workers.

³⁰ The expected number of episodes of care for the fluorochemical and film plant populations was calculated from the health claims experience of the larger 3M U.S. manufacturing population. In this way, the observed to expected ratios for the fluorochemical and film plants were each standardized for proper comparison to each other. Details on the methods and statistical analyses can be found in Olsen GW, Burlew MM, Hocking BB, Skratt JC, Burris JM, Mandel JH. An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. Final report. May 18, 2001. EPA Docket No. AR-226-1030a021. US Environmental Protection Agency, Washington DC. Portions of this report were published as Olsen et al. 2004 *J Occup Environ Med* 46 837-846.

corresponding lowest-exposed workers from the film plant. None of these comparative analyses showed a significant difference in the risk ratios of episodes of care for either infectious disease or respiratory infections between chemical plant and film plant workers.³¹ If PFOS led to clinical immune deficiency, one would expect to see evidence of increased infections among the more highly exposed 3M fluorochemical production workers when compared to the film plant workers.

Moreover, EPA's analysis in the Draft MCLG Documents is inconsistent with three studies (two of which were omitted from the Agency's evaluation of "immunosuppression effects") that evaluated both specific antibody levels and infections simultaneously in the same population (Granum et al. 2013, Looker et al. 2014, Abraham et al. 2020). These studies generally indicated that even in the presence of some associations with lower antibody levels, no impact on actual infectious outcomes may be observed. Specifically, a cross-sectional study of one-year-old infants in Germany detected inverse associations between PFOA and anti-*Haemophilus influenza* type B, anti-tetanus, and anti-diphtheria antibody levels, but no associations between PFOA and any infections or surrogates of infection evaluated, including month of first infection, total number of infections, number of infections with fever, three-day fever, number of antibiotic treatments, ever use of antibiotics, otitis media (ever or number of episodes), pneumonia (ever or number of episodes), diarrhea (ever or number of episodes), varicella, napkin candidiasis, and oral candidiasis (Abraham et al. 2020). PFOS was not associated with any specific antibody levels or infections in this study. Another cross-sectional study conducted among adults in the Mid-Ohio River Valley found inverse associations between PFOA and post-vaccination anti-influenza A/H3N2 antibody levels (but not post-vaccination anti-influenza type B or A/H1N1 antibody levels), yet in the same study population, PFOA was not associated with self-reported "flu" infection, common cold, or number of colds in the past year, and PFOS was not associated with any of these outcomes (Looker et al. 2014). The third study, a small prospective cohort of up to 93 young children in Denmark, yielded a mixed pattern of findings, with inverse associations of PFOA and PFOS with antibody levels against rubella, but not measles, *Haemophilus influenzae* type B, or tetanus; and positive associations of PFOA with a greater number of episodes of common cold and gastroenteritis, but not ever having common cold or gastroenteritis, and no association of PFOS with any of these infectious outcomes (Granum et al. 2013).

Besides the generally null findings from epidemiological studies of PFOA and PFOS in association with clinical infectious diseases, population-level data show that despite substantial declines, approaching an 80 percent decline for PFOA and a 90 percent decline for PFOS in the U.S. general population over the past two decades (CDC 2021), incidence rates of tetanus and diphtheria in the U.S. appear not to have changed (World Health Organization 2020). Thus, these ecological data also fail to show any impact of PFOA or PFOS on the occurrence of tetanus and diphtheria.

In summary, most reported associations of PFOA and PFOS with infectious outcomes are null, and the remainder are an inconsistent assortment of positive and inverse associations with no clear pattern by type, timing, or dose of PFAS, type of infection, age or sex, or other study population characteristics. The general lack of associations between PFOA or PFOS and

³¹ See Tables 7, 8, 9, and 10 in Olsen et al. 2001.

infectious outcomes suggests that any effect of PFOA or PFOS on antibody-mediated immunity to certain vaccine antigens (i.e., tetanus toxoid or diphtheria toxoid), if it exists at all, may not impact the immune response to other specific pathogens, or that any effect does not lead to a clinically apparent change in susceptibility to infections in general. At bottom, EPA's Draft MCLG Documents use antibody levels for diphtheria or tetanus toxoids as a critical effect in the face of, at best, extremely limited and contradictory evidence that PFOA or PFOS actually cause clinical adverse immunological outcomes. SAB should recommend that EPA reconsider its analytical approach.

D. The Reference Doses Proposed by EPA's Office of Water for the SAB's Consideration Are Far Lower Than Those It Previously Derived in 2016 and Are Not Supported by the Body of Scientific Literature.

The reference doses proposed by EPA present a stark contrast to its earlier values and those of other federal agencies and states regulatory authorities. As detailed in Tables 1 and 2 below for PFOA and PFOS respectively, EPA's proposed reference doses ("RfDs") are orders of magnitude smaller than those derived by EPA in 2016, ASTDR in 2021 and several states.

Table 1 PFOA Derivations of Reference Levels

	Parameter	Units	USEPA	MNDOH	MADEP	ATSDR	MDHHS	MSAW	NJDWQI	NHDES	CA OEHHA	USEPA 2021
			2016	2018	2019	2021	2019	2019	2016	2019	2019	2021
Dose-Response	Species	-	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Human
	Selected critical endpoint	-	Developmental (reduced ossification, accelerated puberty)	Delayed ossification, accelerated PPS in male offspring, trend for decreased pup body weight, and increased	Developmental (reduced ossification, accelerated puberty)	Skeletal alterations in mice (Koskela et al. 2016)	Neurobehavioral effects (decreased number of inactive periods, altered novelty induced activity), and skeletal alteration such as bone morphology and bone cell differentiation in the femurs and tibias.			Increase in relative liver weight		Decreased serum anti-tetanus antibody concentration in children
	Dose-response POD Modeling Method	-	LOAEL	LOAEL	LOAEL	LOAEL	LOAEL	LOAEL	BMDL	BMDL	LOAEL	BMDL5
	POD Serum	ng/mL	38,000	38,000	38,000	8,290	8,290	8,290	4,350	4,351	970	0.17
Uncertainty Extrapolation	POD Human Equivalent Dose	ng/kg/day	5,300	5,300	5,300	821	1,368	1,163	610	610	136	0.015
	Total Composite (UF _T)	unitless	300	300	1000	300	300	300	300	100	300	10
Reference Value - RfD, MRL, or 'toxicity value'	Serum	ng/mL	130	130.0	38	28	28	28	14.5	43.5	3.2	0.017
	Human Equivalent Dose (RfD)	ng/kg/day	20	18	5.3	3	5	3.9	2.0	6.1	0.45	0.0015
Drinking Water guidance level (DWGL)		ng/L	70	35	20	10.5*	9	8	14	12	2	0.0053**

Notes:

Note for clarity the doses in the above are expressed in units of ng/kg/day rather than the customary mg/kg/day. Italicized values are not presented in the original documents and have been calculated.

*ATSDR does not calculate DW levels, the value shown is calculated using the ratio of ATSDR's MRL to EPA's 2016 RfD on the EPA 2016 DWLG (70ppt).

** Value is the 2016 HA level of 70 ppt scaled by ratio of the 2016 to proposed 2021 RfD (13,333x lower) = 0.0053 ng/L = 5.3 pg/L, parts per quadrillion

Table 2 PFOS Derivations of Reference Levels

			USEPA 2016	MNDOH	MADEP	ATSDR	MDHHS	MSAW	NIDWQI	NHDES	CA OEHA	USEPA 2021
	Parameter	Units	2016	2019	2019	2021	2019	2019	2018	2019	2019	2021
	Species	-	Rat	Mouse	Rat	Rat	Rat	Mouse	Mouse	Mouse	Mouse	Human
	Selected critical endpoint	-	decreased pup body weight	Increased IL-4 and decreased SRBC specific IgM levels	Pup body weight and developmental delays	Delayed eye opening and decreased pup body weight in rats	Delayed eye opening and decreased mean pup body weight	Increase in liver mass and suppression of plaque-forming cell response in mice	Decreased plaque forming cell response	Suppressed immunoglobulin M (IgM) production in male mice	Decreased plaque forming cell response	Decreased serum anti-diphtheria antibody concentration in children.
Dose-Response	Dose-response POD Modeling Method	-	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL	NOAEL	BMDL5
	POD Serum	ng/mL	6,260	2,360	6,260	7,430	7,430	674	674	2,360	674	0.54
	POD Human Equivalent Dose	ng/kg/day	510	307	510	515	515	86.6	55	303	55	0.079
Uncertainty Extrapolation	Total Composite (UF _T)	unitless	30	100	100	300	300	30	30	100	30	10
Reference Value - RfD, MRL, or 'toxicity value'	Serum	ng/mL	209	24	63	25	24.8	22	23	23.6	22	0.054
	Human Equivalent Dose (RfD)	ng/kg/day	20	3.1	5.1	2	2	2.89	1.8	3.0	1.8	0.0079
DW guidance level		ng/L	70	15	20	7*	8	16	13	15	7	0.028**

Notes:

Note for clarity the doses in the above are expressed in units of ng/kg/day rather than the customary mg/kg/day. Italicized values are not presented in the original documents and have been calculated.

*ATSDR does not calculate DW levels, the value shown is calculated using the ratio of ATSDR's MRL to EPA's 2016 RfD on the EPA 2016 DWLG (70 ppt).

** Value is the 2016 HA level of 70 ppt scaled by ratio of the 2016 to proposed 2021 RfD (2,532x lower) = 0.028 ng/L = 28 pg/L, parts per quadrillion

Indeed, EPA's 2021 proposed RfD³² for PFOA is over 13,300 times lower than the Agency's previous value derived in 2016. For PFOS the proposed RfD³³ is 2,532 times lower than the 2016 value. In addition, the reference doses proposed are far lower than those derived by ATSDR and state regulatory authorities.³⁴ Figures 1 and 2 demonstrate these differences.

Figure 1 PFOA RfD HED Values

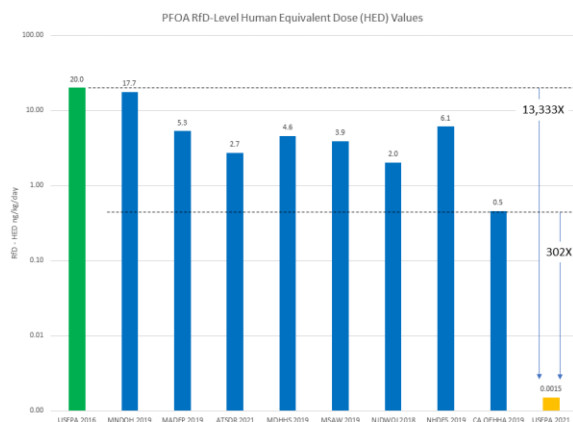
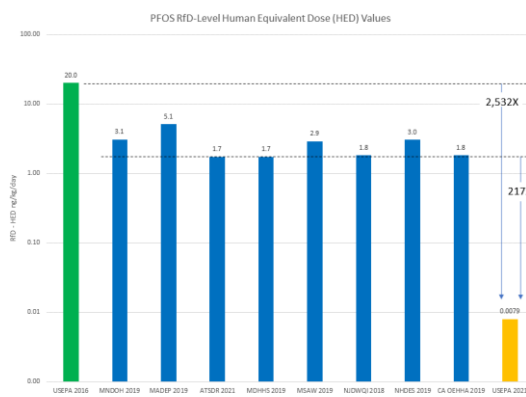


Figure 2 PFOS RfD HED Values



³² Draft PFOA MCLG Approach, p. 340.

³³ Draft PFOS MCLG Approach, p.310.

³⁴ See references at the end for ATSDR and State values presented in these tables.

As part of its review, the SAB should consider PFOA and PFOS's relative toxicity and whether the proposed RfDs are supportable considering there is no reliable evidence of adverse effects in humans or animals at those levels.

E. The blood serum concentrations that correspond to the reference doses are significantly lower than background levels for the US population and lower than European guidance values for serum.

EPA's proposed PODs and RfDs, and analogous "reference" values from ATSDR (MRLs) and states, are human equivalent doses that correspond to internal dose blood serum levels. The blood serum levels that correspond to PODs and RfDs (or RfD-equivalent levels) are shown in Tables 1 and 2 (above) and on Figures 3 and 4 (PODs) and 5 and 6 (RfDs).

Figure 3 PFOA POD Serum Levels

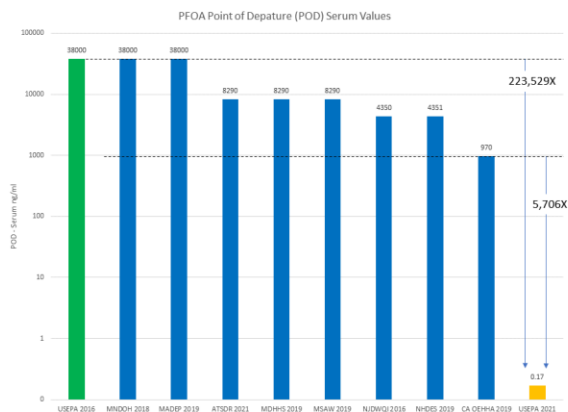


Figure 4 PFOS POD Serum Levels

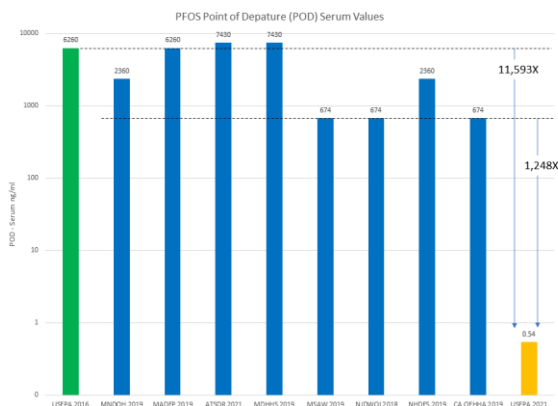


Figure 5 PFOA RfD Serum Levels

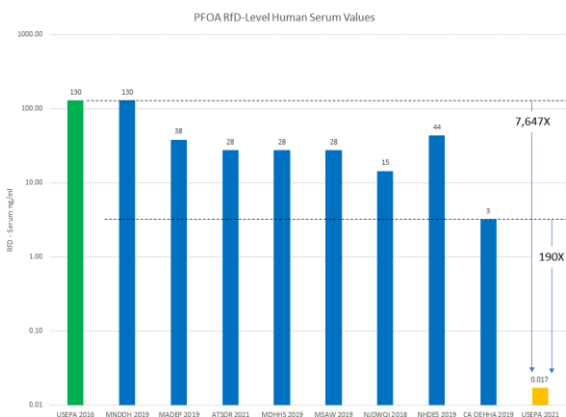
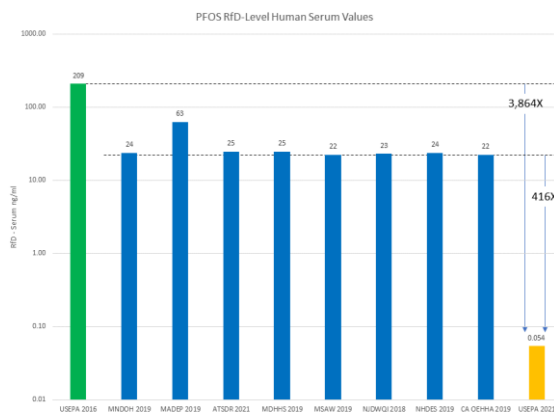
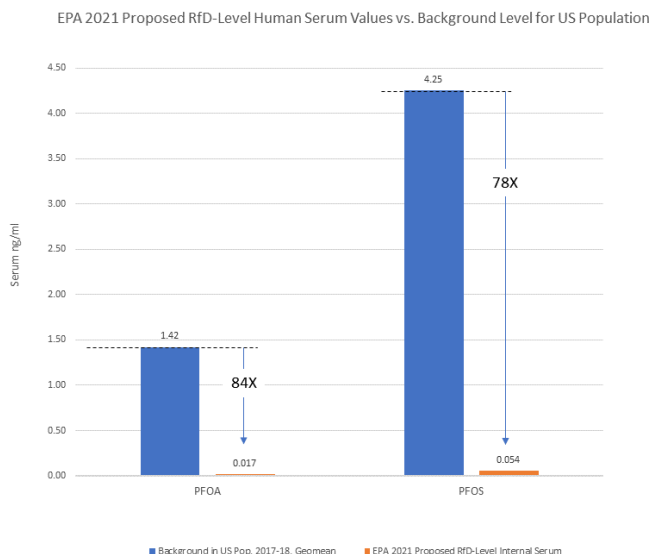


Figure 6 PFOS RfD Serum Levels



The EPA’s 2021 proposed RfD-level serum values, 0.017 ng/ml for PFOA³⁵ and 0.054 ng/ml for PFOS³⁶, are well below background levels present in the US. The latest mean serum concentrations for the US population are 1.42 ng/ml (95% confidence interval 1.33-1.52) for PFOA and 4.25 (3.90-4.62) for PFOS. In both cases the proposed reference serum values are about 80 times lower than the mean background levels in the US population. *See Figure 7.*

Figure 7 Proposed Reference Serum Levels Compared to US Pop. Background



In Europe, the European Food Safety Authority (EFSA) and the Human Biomonitoring Commission of the German Environment Agency (HBM Commission) have derived guidance levels based on serum levels similarly based wholly or in part on human vaccine studies.³⁷ EFSA has derived its tolerable weekly intake (“TWI”) value for food using vaccine studies – specifically the TWI is based on limiting the serum level in humans to 6.9 ng/ml for the sum of four PFAS (PFOA, PFOS, PFHxS and PFNA).³⁸ The HBM Commission has published HBM-I and HBM-II values for PFOA and PFOS. HBM-I values that are deemed safe “concentration of a substance in human biological material below which, according to the current status of assessment, no adverse health effects are to be expected” and HBM-II values that are “concentration[s] of a substance in human biological material which, when exceeded, may lead

³⁵ Draft PFOA MCLG Approach; 1.7×10^{-4} mg/L internal POD for tetanus (Table 21) divided by UF of 10 (Table 22) = 1.7×10^{-5} mg/L = 0.017 µg/L = 0.017 ng/ml.

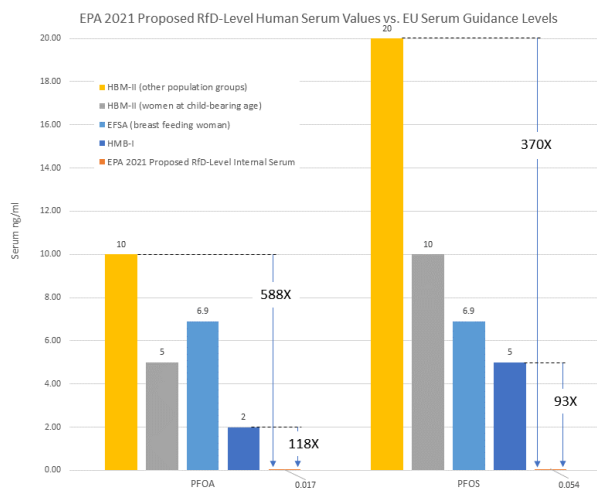
³⁶ Draft PFOS MCLG Approach; 5.4×10^{-4} mg/L internal POD for diphtheria (Table 21) divided by UF of 10 (Table 22) = 5.4×10^{-5} mg/L = 0.054 µg/L = 0.054 ng/ml.

³⁷ Even EFSA’s TWI values were extremely conservative. 3M provided extensive comments to EFSA on its guidance and will provide such comments to SAB and EPA upon request.

³⁸ EFSA. 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). doi: 10.2903/j.efsa.2020.6223. Adopted: 9 July 2020.

to health impairment which is considered as relevant to affected individuals.”^{39,40,41} These values are at least about 100x higher than those proposed by EPA. See Figure 8.

Figure 8 Proposed Reference Serum Levels vs EU Serum Guidance



F. EPA’s benchmark dose analysis is flawed.

EPA used the benchmark dose levels (“BMDLs”) as the basis of the points of departure (“PODs”) in the Draft MCLG Documents. The BMDLs were then fed into the pharmacokinetic (“PK”) model, which was developed by EPA. The BMDLs underlying the PK model were flawed. For both PFOA and PFOS, EPA states a 5% benchmark response (“BMR”) was used for the immune effects in children for reduced antibody concentrations for diphtheria and tetanus, and that a BMR of one standard deviation was used for the immune effects of decreased plaque forming cell response to SRBC (PFOS)⁴² and reduced IgM response (PFOA).⁴³ While the BMR of one standard deviation is consistent with EPA BMD technical guidance, a BMR of 10% is generally recommended by EPA for dichotomous data.⁴⁴ The PFOA draft document specifically

³⁹ HBM I values for Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS) in blood plasma. Statement of the German Human Biomonitoring Commission (HBM Commission). Bundesgesundheitsbl 2016 · 59:1364. DOI 10.1007/s00103-016-2437-1. Translation of HBM-I-Werte für Perfluorooctansäure (PFOA) und Perfluorooctansulfonsäure (PFOS) in Blutplasma. Stellungnahme der Kommission Human-Biomonitoring des Umweltbundesamtes. (DOI 10.1007/s00103-016-2434-4).

⁴⁰ HBM-II values for perfluorooctanoic acid (PFOA) and perfluorooctane- sulfonic acid (PFOS) in blood plasma. Statement by the Human Biomonitoring Commission of the German Environment Agency. Translation of the German version in Bundesgesundheitsbl 2020 · 63:356–360 [https:// doi.org/ 10.1007/ s00103- 020- 03101-2](https://doi.org/10.1007/s00103-020-03101-2).

⁴¹ The authors of HBM II have acknowledged various limitations in the analysis. “The HBM-II values were chosen from the range of POD values by expert assessment, considering the uncertainties and the specifics of certain target groups. However, this value cannot be used to quantify, with sufficient certainty, an individual’s risk of suffering health impairment as a result of her/his internal exposure to PFOA or PFOS.” Schumann et al. 2021 Regul Toxicol Pharmacol 121 104868.

⁴² Draft PFOS MCLG Approach, p. 293 and Table 16.

⁴³ Draft PFOA MCLG Approach, p. 321 and Table 16.

⁴⁴ Benchmark Dose Technical Guidance. EPA/100/R-12/001 June 2012.

states: “When severe or frank effects are modeled, a lower BMR can be adopted. For example, developmental effects are frequently serious effects and BMDs for these effects could employ a 5% BMR....”⁴⁵ In addition, in Table 16 of both draft documents, the Agency states (regarding the 1SD BMR): “No information is readily available that allows for determining a minimally biologically significant response. The BMD Technical Guidance...recommends a BMR based on 1 SD...when biological information is not sufficient to identify the BMR.”

The vaccine studies EPA ultimately selected for derivation of the RfD demonstrated only inconsistent reduced antibody concentrations; they did not demonstrate an increased incidence of infectious disease or fatalities from contracting these diseases. Thus, there is “no information readily available that allows for determining a minimally biologically significant response”, according to Agency guidance. The Agency should reconsider using a BMR of 10% and following their own guidance since the antibody levels EPA considered are not biologically significant. There also seems to be no scientific discussion of what antibody levels would be considered “minimally adverse.”

In the main text of the Draft MCLG Documents, EPA does not clearly state how its BMD results for the immune endpoints were calculated. In reading Appendix B of both documents, it becomes apparent that EPA simply used the BMD modeling results reported by Budtz-Jorgensen and Grandjean 2018.^{46,47,48} Also, it is not stated if or how EPA independently evaluated these results. In fact, Budtz-Jorgensen and Grandjean 2018 did not even use the US EPA’s BMD modeling software for their analyses (they used SAS). EPA should independently evaluate Budtz-Jorgensen’s results, as well as perform BMD analysis using its own guidance and judgment.

G. The Toxicokinetic Modeling Lacks Necessary Detail to Allow Public Comment.

EPA’s toxicokinetic model approach lacks the necessary detail to allow the public to provide adequate input. EPA states in both Draft MCLG Documents that the “large majority of [physiological based pharmacokinetic modeling] PBPK models for PFOA/PFOS are based on the original publications of Loccisano et al...and it was noted during a review of this model’s code that the implementation of protein binding appears to ‘double-count’ the parameter that corresponds to the free fraction of PFOA/PFOS in plasma.”^{49,50} The Agency then summarily dismisses PBPK models and does not give further justification as to why they did not use these models or specifically what they found wrong with these models or any PBPK models that they discuss beyond stating that “due to the previous issues in implementing PBPK models for PFAS, the known issues with the Loccisano model and the models based upon it, we decided that a one-compartment model was the best approach...[as] a one-compartment model is sufficient to predict blood (or serum/plasma concentrations....[t]his makes serum/plasma a good biomarker

⁴⁵ Draft PFOA MCLG Approach, p. 320.

⁴⁶ *Id.*, B-1.

⁴⁷ Draft PFOS MCLG Approach, B-1.

⁴⁸ Budtz-Jorgensen E; Grandjean P. 2018. Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS ONE 13: e0205388.

⁴⁹ Draft PFOA MCLG Approach, p. 332-333.

⁵⁰ Draft PFOS MCLG Approach, p. 303.

for exposure.” EPA should provide detail as to why it believes the Loccisano models and other models developed from them ‘double-count’ and further explain its rationale for not using PBPK models.

For modeling animal PK, EPA chose the compartment model (consisting of 3 compartments) developed by Wambaugh et al. 2013,⁵¹ which appears to be validated using literature data for rats, mice, and monkeys. EPA has not explained, however, why it did not develop or modify an existing physiologically-based model of its own if EPA did not find published models acceptable. EPA’s methods of predicting parameter distributions using a compartmental model are confusing and there is no explanation for why the added complexity is necessary. The Wambaugh model did not originally account for various life stages, so EPA modified it for gestation, lactation, and post-weaning phases. For PFOA, EPA tested this life stage model with one rat study and one mouse study (gestation/lactation).⁵² For PFOS, EPA tested this life stage model with one rat study (gestation/lactation).⁵³ This validation is quite limited and EPA states that “the Wambaugh model was not parameterized for a post-partum infant...”, which implies uncertainty in model predictions yet the model accounts for post-weaning.⁵⁴ There is no analysis detailing whether EPA made any attempt to extend their animal model to humans or why EPA’s animal model was not parameterized for post-partum infants, considering that the selected RfD was based on 5-year-olds. This lack of transparency prevents the public from providing fulsome comments to assist SAB in ensuring EPA is relying on the best available science.

Similarly, for human PK modeling, EPA used the Verner et al. 2016 model, which is a one-compartment model for humans.⁵⁵ EPA made several adjustments to the model, including how the body weight during pregnancy was calculated and updated parameters for some of the partition coefficients (specifically, those for the chemical cord blood: maternal serum and the chemical breastmilk: maternal serum).⁵⁶ This updated model was then used to simulate the HEDs from the animal PODs that were obtained from BMD modeling and to simulate selected human studies. There is no indication that EPA tested this modified model before using it to estimate internal PODs and subsequently HEDs. Considering that this human model was used for derivation of the RfD selected by the EPA, SAB should recommend that EPA validate the model or explain why EPA did not believe the model needed to be validated.

EPA also states that one of the advantages in its choice of a PK model is that a single model structure could be used for all species of interest.⁵⁷ Yet EPA did not do so. SAB should recommend that EPA should provide its rationale for choosing a different model structure for humans than was used for simulating the animal studies. This analytical choice has consequences on EPA’s conclusions. Indeed, the internal dose metrics and the POD_{HED} values

⁵¹ Wambaugh et al. 2013. Dosimetric anchoring of in vivo and in vitro studies for perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci* 136: 308-327.

⁵² Draft PFOA MCLG Approach, p. 329.

⁵³ Draft PFOS MCLG Approach, p. 300.

⁵⁴ Draft PFOA MCLG Approach, p. 328; Draft PFOS MCLG Approach, p. 299.

⁵⁵ Verner et al. 2016. A simple pharmacokinetic model of prenatal and postnatal exposure to perfluoroalkyl substances (PFAS). *Environ Sci Technol* 50: 978-986.

⁵⁶ Draft PFOA MCLG Approach, p. 331-332; Draft PFOS MCLG Approach, p. 301-302.

⁵⁷ Draft PFOA MCLG Approach, p. 323; Draft PFOS MCLG Approach, p. 295.

are much greater when derived from the animal studies than from the human studies.⁵⁸ EPA should explain why these values differ by orders of magnitude and what drove EPA's choices.

Limitations of the human modeling approach are discussed which include uncertainty in the parameters V_d , half-life, and clearance in the human population and how these parameters could be different in children and adults (i.e., even more uncertainty).⁵⁹ EPA states that in the Verner et al. 2016 model, these parameters (V_d , half-life, and thus clearance) were assumed to be constant across ages and sexes; EPA did not state that it did anything differently. Although there is uncertainty about these parameters (also, EPA previously stated in both documents that “the Wambaugh model was not parameterized for a post-partum infant...”), especially in kids, the Agency used this model in order to derive RfDs based on measurements in children (i.e., the Grandjean vaccine studies). This obviously could introduce uncertainty in the internal dose metrics and thus the estimated HEDs. The Agency should explain this rationale.

H. The Application of Uncertainty Factors is Unjustified.

For both PFOA and PFOS, EPA applied a total uncertainty factor (“UF”) of ten times (“10X”) for both immune and developmental endpoints.⁶⁰ This 10X factor (UF_H) was applied to account for variability in responses within the human population, including life stage. EPA states that “the Wambaugh model was not parameterized for a post-partum infant...” and that there is uncertainty around key PK parameters in children. These concerns do not support using the default 10X factor. If EPA believes its models have good predictive ability it should justify its use of the default 10X UF. Alternatively, EPA should explain why it chose to use models that lack the requisite predictive accuracy.

I. The Cancer Slope Factor for PFOA is Unclear.

For the revised (from EPA's 2016 assessment) PFOA cancer slope factor (“CSF”), EPA used both animal studies and a human study, Shearer et al. 2021 (discussing renal cell carcinoma in humans).^{61,62} EPA states that it used the same methods as the draft CalEPA 2021 document to estimate the human CSF.⁶³ However, the methods as presented in the main text are confusing and unclear. EPA should present equations for each step. In addition, EPA discusses that the CSF is calculated as “ CSF_{serum} ” and presents two values in Table 25 (0.01483 per ng/kg/day and 0.0352 per ng/kg/day). Then EPA's Appendix B states that the estimated CSF_{serum} value is per 0.00178 per ng/kg/day. EPA needs to clarify why there are multiple CSF values and how, or whether, those values were used. EPA should also state its derived CSF in the main document text. EPA should also address why the CSFs derived from the human data are much lower than the CSFs derived from the animal studies.

⁵⁸ Draft PFOA MCLG Approach, Table 21; Draft PFOS MCLG Approach, Table 21.

⁵⁹ Draft PFOS MCLG Approach, p. 304; Draft PFOA MCLG Approach, p. 333.

⁶⁰ Draft PFOA MCLG Approach, Table 23; Draft PFOS MCLG Approach, Table 23.

⁶¹ Draft PFOA MCLG Approach, p. 343-345.

⁶² Shearer et al. 2021. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. *J Natl Cancer Inst* 113: 580-587.

⁶³ CalEPA 2021. Public Health Goals: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water (First Public Review Draft ed). California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Branch.

J. The Animal Immune Response Data Selected for POD Modelling for PFOA and PFOS Differ From, and in Some Cases Contradict the Human Vaccine Response Data.

The immune system provides complex and varied mechanisms of responding to exogenous and endogenous triggers. Different immunoglobulin classes have different functions and roles. The primary antibody response is characterized by the abundant generation of IgM antibodies that are not T cell dependent and do not show immunologic memory. In contrast, IgG antibodies predominate as part of the secondary antibody response (Janeway and Travers, 1996). The more mature IgG secondary antibodies, which have far greater affinity for the antigen than do primary response antibodies, are an important indicator of immunological memory. Grandjean et al. (2017) specifically measured secondary antibody responses to vaccination against both tetanus and diphtheria in a cohort of children from the Faroe Islands. These children were vaccinated at 3, 5 and 12 months and a booster was administered at 5 years. Blood sampling was conducted at 13 years of age and serum concentrations of IgG antibodies were again measured.

The animal studies cited by EPA and included for POD derivation focused instead on IgM antibodies and/or evaluated both IgM and IgG antibody responses, but with results that differed from the human studies included for POD derivation.⁶⁴ IgM antibody responses are not indicators of immune memory, the T cell component of the immune response, or the robustness of the immune response in humans. Thus, IgM responses, while employed in the mouse studies, are not evaluated in terms of protection from infection; their primary use is as an indication of early infection prior to the production of IgG antibodies.

For PFOA, DeWitt et al. (2008) evaluated both IgM and IgG antibody responses to injected sheep red blood cells (SRBC) in mice, while Loveless et al. (2008) only measured IgM levels in response to SRBC immunization in both rats and mice. While Grandjean et al. (2017) reported reduced IgG antibody levels in association with increased PFOA serum concentrations, DeWitt et al. (2008) actually found that, in mice, IgG antibody levels were either increased with PFOA exposure or not significantly different from control. Thus, the secondary antibody response in mice did not appear to be adversely affected by PFOA exposure. The inconsistency of these data across species calls into question their relevance to PFOA exposure.

For PFOS, Zhong et al. (2016) reported a decrease in SRBC-specific IgM antibody produced in 4-week-old offspring of mice treated with 1 mg/kg/day (males only) and 5 mg/kg/day (males and females) PFOS (but not 0.1 mg/kg/day) from gestation day (GD) 1 through GD 17. However, this decrease was transient; levels were not decreased at 8 weeks of age for either males or females at any dose. Unlike the secondary antibody IgG antibody levels measured by Grandjean et al. (2012, 2017a,b) in the human studies, Zhong et al. (2016) did not measure IgG levels, nor did the supporting animal studies cited in the EPA PFOS report (Peden-Adams et al. 2008; Keil et al. 2008).⁶⁵

⁶⁴ Draft PFOA MCLG Approach, p. 335; Draft PFOS MCLG Approach, p. 305.

⁶⁵ Draft PFOS MCLG Approach, p. 154.

Thus, the animal toxicology studies do not provide supporting evidence for the human vaccine response studies. The endpoints measured in most animal studies were not equivalent. Further, the reported IgM-related immune effects are generally confined to one particular species of mice, but no effects were observed in rats and no effects in other types of mice. In short, even putting aside that the animal data are not themselves internally consistent, when the same aspects of immune response as measured in the human vaccine studies were assessed, the animal studies provide contradictory results from those reported by Grandjean et al. (2012, 2017a,b).

K. Effects on IgM Levels in Animals Appear to be a Stress-Related Response.

Although both DeWitt et al. (2008) and Loveless et al. (2008) reported significant effects of PFOA exposure on anti-SRBC IgM levels in mice, this response appears to be due to substantial stress in the animals. For example, Loveless et al. (2008) found 14% and 22% body weight reductions in mice with 29 days of gavage dosing at 10 and 30 mg/kg/day, respectively. Thymic and/or splenic atrophy were observed, and these organs substantially decreased in weight at these same doses. No effects on body weight, the spleen or thymus were seen at the next lower dose of 1 mg/kg/day.

DeWitt et al. (2008) similarly showed significant (6-15%) body weight loss after only 8 days of drinking water exposure at 30 mg/kg/day and a 6% body weight loss after only 15 days of treatment with 15 mg/kg/day. Again, spleen and thymus weights were affected (histopathologic examination was not conducted). Although body weights were not reduced after 15 days of exposure at the next lower doses of 7.5 and 3.75 mg/kg/day, spleen weights were significantly reduced at these doses in the second dose-response study (Study II). Thus, these data are generally consistent with those of Loveless et al. (2008). Importantly, Loveless et al. (2008) showed substantial increases in serum corticosterone levels in mice at the same doses at which IgM levels were reduced. Further, serum corticosterone levels were not affected by PFOA in the rat; nor were IgM levels. Loveless et al. (2008) went on to note that a reduced IgM response in conjunction with increased serum corticosterone levels was consistent with other data reported in the literature by Dracott and Smith (1979) and Pruett et al. (1999).

Similar to the studies with PFOA, Zhong et al. (2016) reported substantially lower body weight (although not statistically significant) at doses associated with alterations in IgM in 4-week-old mice born to dams treated with PFOS during gestation: approximately 9% and 11% decreases for males treated with 1 and 5 mg/kg/day PFOS, respectively. Consistent with the reduced IgM response for females, the overall impact on body weight was also lower for females: approximately 5% and 7% decreases in mice at 1 and 5 mg/kg/day dose levels, respectively. As with the transient nature of the IgG response, body weight differences between groups lessened or disappeared by 8 weeks of age. Zhong et al. did not measure stress hormones in this study, but they noted that their previous research demonstrated increased corticosterone levels in adult mice treated PFOS.

Thus, based on the available data, it cannot be concluded that the reduced IgM responses in mice reported by DeWitt et al. (2008) and Loveless et al. (2008) for PFOA and by Zhong et al. (2016) for PFOS are specifically due to the chemical treatment and not secondary to a

generalized stress response. The inconsistency between rats and mice in the results reported by Loveless et al. (2008) further supports the contention that this is a secondary stress response. In light of this likelihood, a reduced IgM response should not be considered as a potential POD for PFOA.

L. EPA Provides No Justification For Using Pharmacokinetic Modeling to Determine the PFOA Internal Doses From Dewitt et al. (2008) and the PFOS Internal Doses From NTP (2009)

In Table B-16 in Appendix B, internal doses of PFOA in mg/L are shown for each of the doses administered in dose-response Study I from DeWitt et al (2008).⁶⁶ However, these concentrations do not agree with the serum concentrations of PFOA reported by DeWitt et al. (2008) (see table below). Incongruencies also exist regarding the internal doses of PFOA reported for dose response Study II (data not shown herein).⁶⁷ Consequently, it is assumed that EPA used pharmacokinetic modelling to derive the values reported in Appendix B. EPA should either use the internal dose data as reported in the actual study or provide a clear rationale for why pharmacokinetic modelling was used to determine the internal doses.

Table 3

Administered dose (mg/kg/day)	Internal dose (mg/L)		
	From EPA Table B-16	From Table 1 of DeWitt et al. (2008)	
		Day 1 post-dosing	Day 15 post-dosing
0	0	0.05	0.16
3.75	113.4	75	35
7.5	180.9	87	43
15	209.6	128	50
30	242.8	163	53

It is further noted that, in Table B-18 for dose-response Study II, the administered PFOA doses are reported as 0, 3.75, 7.5, 15, and 30 mg/kg/day.⁶⁸ However, the actual doses used in Study II were 0, 0.94, 1.88, 3.75, and 7.5 mg/kg/day. It is unclear if this is merely a typo on EPA's part or if the incorrect external doses were used in the pharmacokinetic modelling. However, because the internal doses calculated in Tables B-16 and B-18 do not match (see below), it is most likely a typo. EPA should further clarify the issue.

⁶⁶ Draft PFOA MCLG Approach, p. B-26.

⁶⁷ *Id.*, p. B-27.

⁶⁸ *Id.*, p. B-27.

Table 4

Administered Dose (mg/kg/day) EPA PFOA Tables B-16 & B-18	Study 1 Internal Dose (mg/L) EPA PFOA Table B-16	Study 2 Internal Dose (mg/L) EPA PFOA Table B-18
0	0	0
3.75	113.4	29.8
7.5	180.9	58.9
15	209.6	113.4
30	242.8	180.9

Similarly, for PFOS, the internal plasma concentrations of PFOS were measured and reported in the NTP (2019) study (see table below), but these do not match the internal doses used by EPA in its benchmark dose modelling. As noted for DeWitt et al. (2008) above, it is assumed that EPA used pharmacokinetic modelling to derive the values reported in the Draft MCLG Document, Appendix B.⁶⁹ EPA should either use the internal dose data as reported in the actual study (as these are a more accurate reflection of the actual internal doses achieved) or provide a clear rationale for why pharmacokinetic modelling was used to determine the internal doses.

Table 5

Administered dose (mg/kg/day)	Internal dose (mg/L)		
	From EPA Table B-47	Plasma concentration from NTP (2019) ⁷⁰	
		Males	Females
0	0	0	0.05
0.312	10.0	23.7	30.5
0.625	20.1	51.6	67.0
1.25	40.1	94.2	135.1
2.5	80.2	173.7	237.5
5	160.4	318.2	413.6

M. The Biological Relevance of the Measured IgM Response From Dose-Response Study II of Dewitt et al. (2008) is Questionable.

The extremely shallow slope of the dose-response curve shown in Figure 9 (an excerpt of Figure B-7 of Appendix B of the Draft MCLG Document for PFOA (copied below) calls into question whether the IgM data from dose-response study II of DeWitt et al. (2008) are appropriate for benchmark dose modelling.⁷¹

⁶⁹ Draft PFOS MCLG Approach, p. B-45.

⁷⁰ From Table PA48 – Summary of Tissue Concentration from the 28-day evaluation of the toxicity (C20617) of perfluorooctane sulfate (PFOS) (1763-23-1) on Harlan Sprague-Dawley rats exposed via gavage, available at: <https://cebs.niehs.nih.gov/cebs/study/002-02656-0003-0000-4>.

⁷¹ Draft PFOA MCLG Approach, p. B-29.

Figure 9: Excerpt of Figure B-7 of Appendix B of the Draft MCLG Document for PFOA

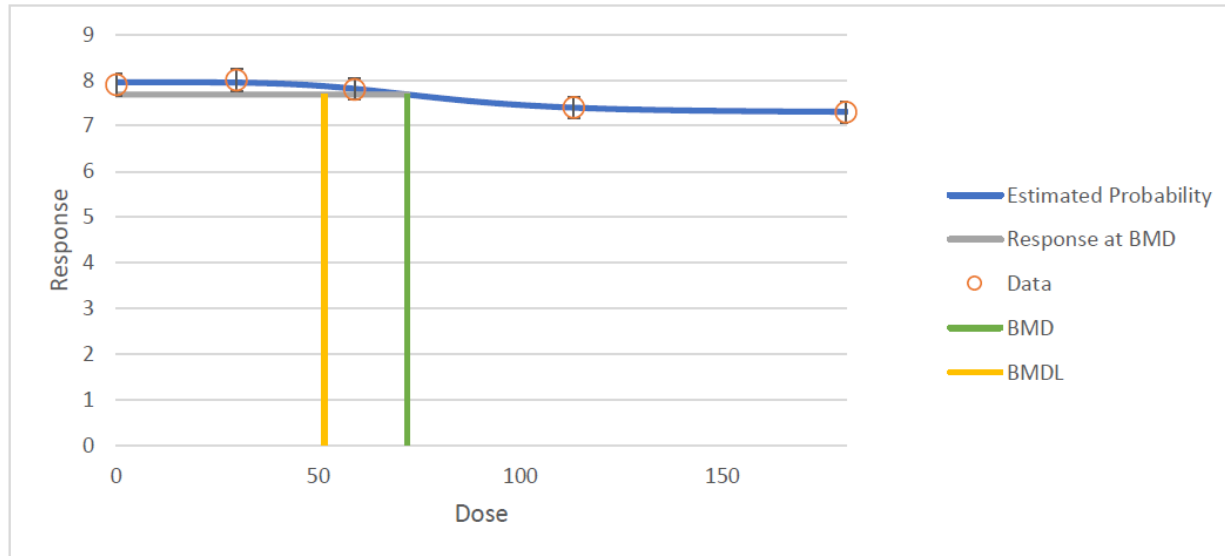


Figure B-7. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA

The control IgM titer response was 7.9 ± 0.3 . The response at 7.5 mg/kg/day ⁷² (the highest dose tested) was 7.3 ± 0.3 (-8% of control).⁷³ Based on the flatness of the dose-response curve and the minimal change measured, it is unclear whether or not the IgM response measured with PFOA treatment in this study is outside the normal range of biological variability.

Table 6

Administered Dose (mg/kg/day)	Study 1 Internal Dose (mg/L) EPA PFOA Table B-16	Study 2 Internal Dose (mg/L) EPA PFOA Table B-18
0		0
3.75	113.4	29.8
7.5	180.9	58.9
15	209.6	113.4
30	242.8	180.9

⁷² Although Table B-18 indicates that the dose was 30 mg/kg/day, the actual top dose in Dose-reponse Study II from DeWitt et al. (2008) was actually 7.5 mg/kg/day.

⁷³ Although Table B-18 indicates that the dose was 30 mg/kg/day (Draft PFOA MCLG Approach, p. B-27), the actual top dose in dose-response Study II from DeWitt et al. (2008) was 7.5 mg/kg/day.

N. EPA May Have Made an Error in Benchmark Dose Modelling of the Data from Loveless et al. (2008).

In Table B-42 in Appendix B, the lowest dose administered to mice in Loveless et al. (2008) is shown as 0.1 mg/kg/day.⁷⁴ This is incorrect. The lowest dose in this study was 0.3 mg/kg/day. Based on the available information, it is unclear whether this is simply a typographic error or if EPA may have used an incorrect input in the internal dose pharmacokinetic modelling and/or benchmark dose modelling.

* * *

3M appreciates the opportunity to provide these technical comments on the meeting materials and encourages SAB to consider the above materials as it provides input to EPA. Thank you for your consideration.

Regards,

Oyebode A. Taiwo, MD, MPH

⁷⁴ Draft PFOA MCLG Approach, p. B-52.

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APPENDIX A – IMMUNOTOXICITY TABLES

Table 1. Overview of studies of PFOA, PFOS, and antibody-mediated immunity

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Abraham 2020	Cross-sectional	Germany	Children	101	PFOA and lower anti- <i>Haemophilus influenza</i> type B IgG level at 1 y; NOAEL at 12.2 µg/L plasma PFOA PFOA and lower anti-tetanus IgG and IgG1 level at 1 y; NOAEL at 16.9 µg/L plasma PFOA PFOA and lower anti-diphtheria IgG level at 1 y; NOAEL at 16.2 µg/L plasma PFOA	PFOS and anti- <i>Haemophilus influenza</i> type B IgG level, anti-tetanus IgG or IgG1 level, or anti-diphtheria IgG level at 1 y
No	Catelan 2021	Ecological	Italy	Adults	563	ΣPFAS and greater risk of COVID-19 mortality	None
Yes	Grandjean 2012	Prospective cohort and cross-sectional	Faroe Islands	Children	587	Maternal PFOA and lower anti-diphtheria antibody level at 7 y (not adj. for 5 y) PFOA at 5 y and lower anti-tetanus antibody level at 7 y (adj./not adj. for 5 y) PFOA at 5 y and lower anti-diphtheria antibody level at 7 y (adj./not adj. for 5 y) Maternal PFOS and greater anti-tetanus antibody level at 7 y (adj. for 5 y) Maternal PFOS and lower anti-diphtheria antibody level at 5 y PFOS at 5 y and lower anti-tetanus antibody level at 5 y PFOS at 5 y and lower anti-diphtheria antibody level at 7 y (not adj. for 5 y)	Maternal PFOA and anti-tetanus antibody level at 5 or 7 y Maternal PFOA and anti-diphtheria antibody level at 5 y PFOA at 5 y and anti-tetanus antibody level at 5 y PFOA at 5 y and anti-diphtheria antibody level at 5 y Maternal PFOS and anti-tetanus antibody level at 5 y Maternal PFOS and anti-diphtheria antibody level at 7 y PFOS at 5 y and anti-tetanus antibody level at 7 y PFOS at 5 y and anti-diphtheria antibody level at 5 y

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Grandjean 2017a	Prospective cohort and cross-sectional	Faroe Islands	Children, adolescents	505	<p>PFOA at 13 y and lower anti-diphtheria antibody level at 13 y (no ER visit/booster)</p> <p>PFOA at 7 y and lower anti-diphtheria antibody level at 7 and 13 y (total, no ER visit/booster, indirect effect (via 7-year antibody))</p> <p>PFOS at 7 y and greater anti-tetanus antibody level at 13 y (no ER visit/booster)</p> <p>PFOS at 7 y and lower anti-diphtheria antibody level at 7 and 13 y (total, no ER visit/booster, total and indirect effects)</p>	<p>PFOA at 7 y and anti-diphtheria antibody level at 13 y</p> <p>PFOA at 13 y and anti-diphtheria antibody level at 13 y (total, no ER visit/booster and no antibody increase)</p> <p>PFOA at 7 or 13 y and anti-tetanus antibody level at 13 y</p> <p>PFOA at 7 y and anti-diphtheria antibody level at 7 and 13 y (no ER visit/booster and no antibody increase)</p> <p>PFOA at 7 y and anti-tetanus antibody level at 7 and 13 y</p> <p>PFOS at 7 or 13 y and anti-diphtheria antibody level at 13 y</p> <p>PFOS at 7 y and anti-tetanus antibody level at 13 y (total, no ER visit/booster and no antibody increase)</p> <p>PFOS at 13 y and anti-tetanus antibody level at 13 y</p> <p>PFOS at 7 y and anti-diphtheria antibody level at 7 and 13 y (no ER visit/booster and no antibody increase)</p> <p>PFOS at 7 y and anti-tetanus antibody level at 7 and 13 y</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Grandjean 2017b	Prospective cohort	Faroe Islands	Children	349	<p>PFOA at birth, 18 m, and 60 m and lower anti-tetanus antibody level at 5 y (2007-2009 cohort, joint cohorts)</p> <p>PFOA at 3 m, 6 m, and 12 m and lower-anti-tetanus antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort, joint cohorts, except at 3 m in 1997-2000 cohort)</p> <p>PFOA at birth and lower anti-diphtheria antibody level at 5 y (2007-2009 cohort, joint cohorts)</p> <p>PFOS at 3 m and lower anti-tetanus antibody level at 5 y, adj. for breastfeeding (joint cohorts)</p> <p>PFOS at 6 m and lower anti-tetanus antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort, joint cohorts)</p> <p>PFOS at birth and lower anti-diphtheria antibody level at 5 y (1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m and lower anti-diphtheria antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort)</p>	<p>PFOA at birth, 18 m, and 60 m and anti-tetanus antibody level at 5 y (1997-2000 cohort)</p> <p>PFOA at 3 m and anti-tetanus antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort)</p> <p>PFOA at birth and anti-diphtheria antibody level at 5 y (1997-2000 cohort)</p> <p>PFOA at 18 m and 60 m and anti-diphtheria antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOA at 3 m, 6 m, and 12 m and anti-diphtheria antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at birth, 18 m, and 60 m and anti-tetanus antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m, 6 m, and 12 m and anti-tetanus antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, at 3 and 12 m in 1997-2000 cohort, at 12 m in joint cohorts)</p> <p>PFOS at birth and anti-diphtheria antibody level at 5 y (2007-2009 cohort)</p> <p>PFOS at 18 m and 60 m and anti-diphtheria antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m, 6 m, and 12 m and anti-diphtheria antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort except at 3 m, joint cohorts)</p>
Yes	Grandjean 2020	Cross-sectional	Denmark	Adults	323	None	<p>PFOA and COVID-19 severity</p> <p>PFOS and COVID-19 severity</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
No	Granum 2013	Prospective cohort	Norway	Children	93	PFOA and lower anti-rubella antibody level at 0-3 y PFOS and lower anti-rubella antibody level at 0-3 y	PFOA and anti-measles antibody level, anti- <i>Haemophilus influenzae</i> type B antibody level, anti-tetanus antibody level at 0-3 y PFOS and anti-measles antibody level, anti- <i>Haemophilus influenzae</i> type B antibody level, anti-tetanus antibody level at 0-3 y
No	Ji 2021	Case-control	China	Adults	160	PFOA and greater risk of COVID-19 PFOS and greater risk of COVID-19	None
No	Kielsen 2016	Cross-sectional	Denmark	Adults	12	PFOS and lower post-vaccination anti-diphtheria antibody increase	PFOA and post-vaccination anti-diphtheria antibody level PFOA and post-vaccination anti-tetanus antibody level PFOS and post-vaccination anti-tetanus antibody level
No	Looker 2014	Cross-sectional	United States	Adults	411	PFOA and lower post-vaccination anti-influenza A/H3N2 antibody increase PFOA and lower odds post-vaccination anti-influenza A/H3N2 seroprotection (titer \geq 1:40)	PFOA and post-vaccination anti-influenza type B antibody level, seroconversion (4-fold titer increase), or seroprotection (titer \geq 1:40) PFOA and post-vaccination anti-influenza A/H1N1 antibody level, seroconversion, or seroprotection PFOA and post-vaccination anti-influenza A/H3N2 seroconversion PFOS and post-vaccination anti-influenza type B antibody level, seroconversion, or seroprotection PFOS and post-vaccination anti-influenza A/H1N1 antibody level, seroconversion, or seroprotection PFOS and post-vaccination anti-influenza A/H3N2 antibody level, seroconversion, or seroprotection

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Mogensen 2015	Prospective cohort	Faroe Islands	Children	459	PFOA at 7 y or 5 and 7 y and lower anti-diphtheria antibody level at 7 y PFOA at 5 and 7 y and lower anti-tetanus antibody level at 7 y PFOS at 7 y or 5 and 7 y and lower anti-diphtheria antibody level at 7 y	PFOA at 7 y and anti-tetanus antibody level at 7 y PFOS at 7 y or 5 and 7 y and anti-tetanus antibody level at 7 y
No	Nielsen 2021	Ecological	Sweden	Adults	898	ΣPFAS and greater risk of COVID-19	None
Yes	Pilkerton 2018	Cross-sectional	United States	Children, adults	1,196 children 1,193 adults	PFOA and lower anti-rubella antibody level (19-60 y total, 19-60 y men) PFOA × sex and anti-rubella antibody level (19-60 y) PFOS and lower anti-rubella antibody level (19-60 y total)	PFOA and anti-rubella antibody level (12-18 y, 19-60 y women) PFOA × sex interaction and anti-rubella antibody level (12-18 y) PFOA × ethnicity interaction and anti-rubella antibody level (12-18 y, 19-60 y) PFOS and anti-rubella antibody level (12-18y, 19-60 y women, 19-60 y men)) PFOS × sex interaction and anti-rubella antibody level (12-18 y, 19-60 y) PFOS × ethnicity interaction and anti-rubella antibody level (12-18 y, 19-60 y)

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
No	Shih 2021	Prospective cohort	Faroe Islands	Adults	399	<p>PFOA at birth and lower anti-hepatitis A virus antibody level at 28 y (women)</p> <p>PFOA at birth and greater anti-hepatitis A virus antibody level at 28 y (men)</p> <p>PFOA at 14 y and lower anti-hepatitis A virus antibody level at 28 y (men)</p> <p>PFOS at birth and lower anti-hepatitis A virus antibody level at 28 y (women)</p> <p>PFOS at birth and greater anti-hepatitis A virus antibody level at 28 y (men)</p> <p>PFOS at 7 y and greater anti-hepatitis A virus antibody level at 28 y (women)</p> <p>PFOS at 7 y and greater anti-hepatitis B surface antibody level at 28 y (women)</p>	<p>PFOA at birth (all), 7 y (all, women, men), 14 y (all, women), 22 y (all, women, men), or 28 y (all, women, men) and anti-hepatitis A virus antibody level at 28 y</p> <p>PFOA at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-hepatitis A virus antibody level at 28 y</p> <p>PFOA at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-diphtheria antibody level at 28 y</p> <p>PFOA at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-tetanus antibody level at 28 y</p> <p>PFOS at birth (all), 7 y (all, men), 14 y (all, women, men), 22 y (all, women, men), or 28 y (all, women, men) and anti-hepatitis A virus antibody level at 28 y (all, women, men)</p> <p>PFOS at birth (all, women, men), 7 y (all, men), 14 y (all, women, men), 22 y (all, women, men), or 28 y (all, women, men) and anti-hepatitis A virus antibody level at 28 y</p> <p>PFOS at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-diphtheria antibody level at 28 y</p> <p>PFOS at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-tetanus antibody level at 28 y</p>
Yes	Stein 2016a	Cross-sectional	United States	Adults	78	<p>PFOS and greater odds of seroconversion to FluMist vaccine (anti-influenza A/H1N1) measured by hemagglutinin inhibition (low baseline antibodies; tertile 2, not 3)</p>	<p>PFOA and seroconversion to FluMist vaccine (anti-influenza A/H1N1) measured by hemagglutinin inhibition or by immunohistochemistry</p> <p>PFOS and seroconversion to FluMist vaccine (anti-influenza A/H1N1) measured by hemagglutinin inhibition (total population) or by immunohistochemistry</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Timmermann 2020	Randomized controlled trial (prospective cohort)	Guinea-Bissau	Children	237	PFOA and lower measles antibody titer at 4-7 m, no vaccination (excl. outliers) PFOA and lower measles antibody titer at 2 y, 1 vaccination (excl. outliers) PFOS and lower measles antibody titer at 4-7 m, no vaccination (excl. outliers) PFOS and lower measles antibody titer at 9 m, no vaccination (full dataset, excl. outliers) PFOS and lower measles antibody titer at 9 m, 1 vaccination (full dataset, excl. outliers) PFOS and lower measles antibody titer at 2 y, 1 vaccination (excl. outliers) PFOS × sex and measles antibody titer at 2 y, 1 vaccination (inverse for girls, null for boys)	PFOA and measles antibody titer at 4-7 m, no vaccination (full dataset) PFOA and measles antibody titer at 9 m, no vaccination (full dataset, excl. outliers) PFOA and measles antibody titer at 9 m, 1 vaccination (full dataset, excl. outliers) PFOA and measles antibody titer at 2 y, 1 vaccination (full dataset) PFOA and measles antibody titer at 2 y, 2 vaccinations (full dataset, excl. outliers) PFOS and measles antibody titer at 4-7 m, no vaccination (full dataset) PFOS and measles antibody titer at 2 y, 1 vaccination (full dataset) PFOS and measles antibody titer at 2 y, 2 vaccinations (full dataset, excl. outliers)
No	Timmermann 2022	Prospective cohort and cross-sectional	Greenland	Children	314	PFOS and greater risk of anti-diphtheria antibody level < 0.1 IU/mL at 7-12 y	PFOA (maternal or child) and anti-tetanus antibody level at 7-12 y PFOA (maternal or child) and anti-diphtheria antibody level or < 0.1 IU/mL at 7-12 y PFOS (maternal or child) and anti-tetanus antibody level at 7-12 y PFOS (maternal or child) and anti-diphtheria antibody level at 7-12 y
Yes	Zeng 2019	Prospective cohort and cross-sectional	China	Children	201	ΣPFOA and lower anti-coxsackievirus A 16 level at birth ΣPFOA and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m ΣPFOA and lower anti-enterovirus 71 levels at birth and at 3 m ΣPFOA and greater risk of anti-enterovirus 71 below protective level at birth and at 3 m	ΣPFOA and anti-coxsackievirus A 16 level at 3 m n-PFOA and anti-coxsackievirus A 16 level at 3 m ΣPFOS and anti-coxsackievirus A 16 level at 3 m n-PFOS and anti-coxsackievirus A 16 level at 3 m Br-PFOS and anti-coxsackievirus A 16 level at 3 m

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
						n-PFOA and lower anti-coxsackievirus A 16 level at birth n-PFOA and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m n-PFOA and lower anti-enterovirus 71 levels at birth and at 3 m n-PFOA and greater risk of anti-enterovirus 71 below protective level at birth and at 3 m ΣPFOS and lower anti-coxsackievirus A 16 level at birth ΣPFOS and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m ΣPFOS and lower anti-enterovirus 71 levels at birth and at 3 m ΣPFOS and greater risk of anti-enterovirus 71 below protective level at birth and at 3 m n-PFOS and lower anti-coxsackievirus A 16 level at birth n-PFOS and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m n-PFOS and lower anti-enterovirus 71 levels at birth and at 3 m n-PFOS and greater risk of anti-enterovirus 71 below protective level at birth and at 3 m Br-PFOS and lower anti-coxsackievirus A 16 level at birth Br-PFOS and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m Br-PFOS and lower anti-enterovirus 71 levels at birth and at 3 m Br-PFOS and greater risk of anti-enterovirus 71 below protective level at 3 m	Br-PFOS and anti-enterovirus 71 below protective level at birth 1m-PFOS and anti-coxsackievirus A 16 levels at birth and at 3 m 1m-PFOS and anti-coxsackievirus A 16 below protective level at birth and at 3 m 1m-PFOS and anti-enterovirus 71 below protective level at birth Σ3m-, 4m-, 5m-PFOS and anti-coxsackievirus A 16 level at 3 m Σ3m-, 4m-, 5m-PFOS and anti-coxsackievirus A 16 below protective level at birth Σ3m-, 4m-, 5m-PFOS and anti-enterovirus 71 below protective level at birth iso-PFOS and anti-coxsackievirus A 16 level at 3 m iso-PFOS and anti-enterovirus 71 below protective level at birth (girls)

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
						<p>1m-PFOS and greater risk of anti-enterovirus 71 below protective level at 3 m</p> <p>1m-PFOS and lower anti-enterovirus 71 levels at birth and at 3 m</p> <p>Σ3m-, 4m-, 5m-PFOS and lower anti-coxsackievirus A 16 level at birth</p> <p>Σ3m-, 4m-, 5m-PFOS and greater risk of anti-coxsackievirus A 16 below protective level at 3 m</p> <p>Σ3m-, 4m-, 5m-PFOS and lower anti-enterovirus 71 levels at birth and at 3 m</p> <p>Σ3m-, 4m-, 5m-PFOS and greater risk of anti-enterovirus 71 below protective level at 3 m</p> <p>iso-PFOS and lower anti-coxsackievirus A 16 level at birth</p> <p>iso-PFOS and greater risk of anti-coxsackievirus A 16 below protective level at birth and at 3 m</p> <p>iso-PFOS and lower anti-enterovirus 71 levels at birth and at 3 m</p> <p>iso-PFOS and greater risk of anti-enterovirus 71 below protective level at birth (all, boys) and at 3 m</p> <p>(Sex-stratified results shown only where PFAS × sex interaction $p < 0.10$)</p>	

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Zeng 2020	Cross-sectional	China	Adults	605	<p>PFOA and greater risk of hepatitis B surface antibody seronegativity</p> <p>n-PFOS and lower serum hepatitis B surface antibody titer</p> <p>n-PFOS and greater risk of hepatitis B surface antibody seronegativity</p> <p>Br-PFOS and greater risk of hepatitis B surface antibody seronegativity</p>	<p>PFOA and serum hepatitis B surface antibody titer</p> <p>Br-PFOS and serum hepatitis B surface antibody titer</p>

Table 2. Overview of studies of PFOA, PFOS, and anti-tetanus or anti-diphtheria antibody levels

In EPA?	Reference	Design	Country	Age Group	Subjects (Max)	Associations	Null Results	
Yes	Abraham 2020	Cross-sectional	Germany	Children	101	PFOA and lower anti-tetanus IgG and IgG1 level at 1 y; NOAEL at 16.9 µg/L plasma PFOA PFOA and lower anti-diphtheria IgG level at 1 y; NOAEL at 16.2 µg/L plasma PFOA	PFOS and anti-tetanus IgG or IgG1 level or anti-diphtheria IgG level at 1 y	
Yes	Grandjean 2012	Prospective cohort and cross-sectional	Faroe Islands	Children	587	Maternal PFOA and lower anti-diphtheria antibody level at 7 y (not adj. for 5 y) PFOA at 5 y and lower anti-tetanus antibody level at 7 y (adj./not adj. for 5 y) PFOA at 5 y and lower anti-diphtheria antibody level at 7 y (adj./not adj. for 5 y) Maternal PFOS and greater anti-tetanus antibody level at 7 y (adj. for 5 y) Maternal PFOS and lower anti-diphtheria antibody level at 5 y PFOS at 5 y and lower anti-tetanus antibody level at 5 y PFOS at 5 y and lower anti-diphtheria antibody level at 7 y (not adj. for 5 y)	Maternal PFOA and anti-tetanus antibody level at 5 or 7 y Maternal PFOA and anti-diphtheria antibody level at 5 y PFOA at 5 y and anti-tetanus antibody level at 5 y PFOA at 5 y and anti-diphtheria antibody level at 5 y Maternal PFOS and anti-tetanus antibody level at 5 y Maternal PFOS and anti-diphtheria antibody level at 7 y PFOS at 5 y and anti-tetanus antibody level at 7 y PFOS at 5 y and anti-diphtheria antibody level at 5 y	

In EPA?	Reference	Design	Country	Age Group	Subjects (Max)	Associations	Null Results	
Yes	Grandjean 2017a	Prospective cohort and cross-sectional	Faroe Islands	Children	505	<p>PFOA at 13 y and lower anti-diphtheria antibody level at 13 y (no ER visit/booster)</p> <p>PFOA at 7 y and lower anti-diphtheria antibody level at 7 and 13 y (total, no ER visit/booster, indirect effect (via 7-year antibody))</p> <p>PFOS at 7 y and greater anti-tetanus antibody level at 13 y (no ER visit/booster)</p> <p>PFOS at 7 y and lower anti-diphtheria antibody level at 7 and 13 y (total, no ER visit/booster, total and indirect effects)</p>	<p>PFOA at 7 y and anti-diphtheria antibody level at 13 y</p> <p>PFOA at 13 y and anti-diphtheria antibody level at 13 y (total, no ER visit/booster and no antibody increase)</p> <p>PFOA at 7 or 13 y and anti-tetanus antibody level at 13 y</p> <p>PFOA at 7 y and anti-diphtheria antibody level at 7 and 13 y (no ER visit/booster and no antibody increase)</p> <p>PFOA at 7 y and anti-tetanus antibody level at 7 and 13 y</p> <p>PFOS at 7 or 13 y and anti-diphtheria antibody level at 13 y</p> <p>PFOS at 7 y and anti-tetanus antibody level at 13 y (total, no ER visit/booster and no antibody increase)</p> <p>PFOS at 13 y and anti-tetanus antibody level at 13 y</p> <p>PFOS at 7 y and anti-diphtheria antibody level at 7 and 13 y (no ER visit/booster and no antibody increase)</p> <p>PFOS at 7 y and anti-tetanus antibody level at 7 and 13 y</p>	

In EPA?	Reference	Design	Country	Age Group	Subjects (Max)	Associations	Null Results	
Yes	Grandjean 2017b	Prospective cohort	Faroe Islands	Children	349	<p>PFOA at birth, 18 m, and 60 m and lower anti-tetanus antibody level at 5 y (2007-2009 cohort, joint cohorts)</p> <p>PFOA at 3 m, 6 m, and 12 m and lower-anti-tetanus antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort, joint cohorts, except at 3 m in 1997-2000 cohort)</p> <p>PFOA at birth and lower anti-diphtheria antibody level at 5 y (2007-2009 cohort, joint cohorts)</p> <p>PFOS at 3 m and lower anti-tetanus antibody level at 5 y, adj. for breastfeeding (joint cohorts)</p> <p>PFOS at 6 m and lower anti-tetanus antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort, joint cohorts)</p> <p>PFOS at birth and lower anti-diphtheria antibody level at 5 y (1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m and lower anti-diphtheria antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort)</p>	<p>PFOA at birth, 18 m, and 60 m and anti-tetanus antibody level at 5 y (1997-2000 cohort)</p> <p>PFOA at 3 m and anti-tetanus antibody level at 5 y, adj. for breastfeeding (1997-2000 cohort)</p> <p>PFOA at birth and anti-diphtheria antibody level at 5 y (1997-2000 cohort)</p> <p>PFOA at 18 m and 60 m and anti-diphtheria antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOA at 3 m, 6 m, and 12 m and anti-diphtheria antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at birth, 18 m, and 60 m and anti-tetanus antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m, 6 m, and 12 m and anti-tetanus antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, at 3 and 12 m in 1997-2000 cohort, at 12 m in joint cohorts)</p> <p>PFOS at birth and anti-diphtheria antibody level at 5 y (2007-2009 cohort)</p> <p>PFOS at 18 m and 60 m and anti-diphtheria antibody level at 5 y (2007-2009 cohort, 1997-2000 cohort, joint cohorts)</p> <p>PFOS at 3 m, 6 m, and 12 m and anti-diphtheria antibody level at 5 y, adj. for breastfeeding (2007-2009 cohort, 1997-2000 cohort except at 3 m, joint cohorts)</p>	
No	Granum 2013	Prospective cohort	Norway	Children	93	None	<p>PFOA and anti-tetanus antibody level at 0-3 y</p> <p>PFOS and anti-tetanus antibody level at 0-3 y</p>	

In EPA?	Reference	Design	Country	Age Group	Subjects (Max)	Associations	Null Results	
No	Kielsen 2016	Cross-sectional	Denmark	Adults	12	PFOS and lower post-vaccination anti-diphtheria antibody increase	PFOA and post-vaccination anti-diphtheria antibody level PFOA and post-vaccination anti-tetanus antibody level PFOS and post-vaccination anti-tetanus antibody level	
Yes	Mogensen 2015	Prospective cohort	Faroe Islands	Children	459	PFOA at 7 y or 5 and 7 y and lower anti-diphtheria antibody level at 7 y PFOA at 5 and 7 y and lower anti-tetanus antibody level at 7 y PFOS at 7 y or 5 and 7 y and lower anti-diphtheria antibody level at 7 y	PFOA at 7 y and anti-tetanus antibody level at 7 y PFOS at 7 y or 5 and 7 y and anti-tetanus antibody level at 7 y	
No	Shih 2021	Prospective cohort	Faroe Islands	Adults	281	None	PFOA at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-diphtheria antibody level at 28 y PFOA at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-tetanus antibody level at 28 y PFOS at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-diphtheria antibody level at 28 y PFOS at birth, 7 y, 14 y, 22 y, or 28 y (all, women, men) and anti-tetanus antibody level at 28 y	
No	Timmermann 2022	Prospective cohort and cross-sectional	Greenland	Children	314	PFOS and greater risk of anti-diphtheria antibody level < 0.1 IU/mL at 7-12 y	PFOA (maternal or child) and anti-tetanus antibody level at 7-12 y PFOA (maternal or child) and anti-diphtheria antibody level or < 0.1 IU/mL at 7-12 y PFOS (maternal or child) and anti-tetanus antibody level at 7-12 y PFOS (maternal or child) and anti-diphtheria antibody level at 7-12 y	None

Table 3. Overview of epidemiological studies of PFOA, PFOS, and infections

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Abraham 2020	Cross-sectional	Germany	Children	101	None	<p>PFOA and month of first infection in first year, total infections in first year, number of infections with fever, number of antibiotic treatments, antibiotic treatment ever, number of otitis media episodes, otitis media infections ever, 3-day fever ever, number of pneumonia episodes, number of diarrhea episodes, diarrhea ever, varicella ever, napkin candidiasis ever, oral candidiasis ever at 1 year</p> <p>PFOS and month of first infection in first year, total infections in first year, number of infections with fever, number of antibiotic treatments, antibiotic treatment ever, number of otitis media episodes, otitis media infections ever, 3-day fever ever, number of pneumonia episodes, number of diarrhea episodes, diarrhea ever, varicella ever, napkin candidiasis ever, oral candidiasis ever at 1 year</p>
Yes	Ait Bamai 2020	Prospective cohort	Japan	Children	2,689	<p>PFOA and greater risk of pneumonia at 7 y (total, with siblings)</p> <p>PFOA and greater risk of respiratory syncytial virus at 7 y (without siblings)</p> <p>PFOS and lower risk of respiratory syncytial virus at 7 y (total, with siblings)</p>	<p>PFOA and rhino-conjunctivitis, chicken pox (total, without siblings, with siblings), otitis media (total, without siblings, with siblings), pneumonia (without siblings), respiratory syncytial virus (total, with siblings) at 7 y</p> <p>PFOS and rhino-conjunctivitis, chicken pox (total, without siblings, with siblings), otitis media (total, without siblings, with siblings), pneumonia (total, without siblings, with siblings), respiratory syncytial virus (without siblings) at 7 y</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
No	Bulka 2021	Cross-sectional	United States	Children, adults	8,778	<p>PFOA and greater total pathogen burden (adolescents, adults)</p> <p>PFOA and greater risk of persistent herpes simplex virus 1 infection (adults)</p> <p>PFOA and greater risk of persistent herpes simplex virus 2 infection (adults)</p> <p>PFOS and greater total pathogen burden (adolescents, adults)</p> <p>PFOS and greater risk of persistent herpes simplex virus 1 infection (adults)</p> <p>PFOS and greater risk of persistent <i>Toxocara</i> spp infection (adults)</p>	<p>PFOA and persistent infection with cytomegalovirus, Epstein-Barr virus, herpes simplex 1 virus, <i>Toxoplasma gondii</i>, or <i>Toxocara</i> spp. (adolescents)</p> <p>PFOA and persistent infection with cytomegalovirus, hepatitis C virus, hepatitis E virus, <i>Toxoplasma gondii</i>, or <i>Toxocara</i> spp. (adults)</p> <p>PFOS and persistent infection with cytomegalovirus, Epstein-Barr virus, herpes simplex 1 virus, <i>Toxoplasma gondii</i>, or <i>Toxocara</i> spp. (adolescents)</p> <p>PFOS and persistent infection with cytomegalovirus, hepatitis C virus, hepatitis E virus, herpes simplex 2 virus, or <i>Toxoplasma gondii</i> (adults)</p>
Yes	Dalsager 2016	Prospective cohort	Denmark	Children	346	<p>PFOA and greater proportion of days with fever at 1-4 y</p> <p>PFOA and greater number of episodes of co-occurrence of fever and nasal discharge at 1-4 y (medium, not high PFOA)</p> <p>PFOS and greater number and proportion of days with fever at 1-4 y</p>	<p>PFOA and number of days with fever at 1-4 y</p> <p>PFOA and proportion or number of days with cough, nasal discharge, diarrhea, or vomiting at 1-4 y</p> <p>PFOA and number of episodes of co-occurrence of fever and coughing at 1-4 y</p> <p>PFOS and proportion or number of days with cough, nasal discharge, diarrhea, or vomiting at 1-4 y</p> <p>PFOS and number of episodes of co-occurrence of fever and coughing or fever and nasal discharge at 1-4 y</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
No	Dalsager 2021	Prospective cohort	Denmark	Children	1,503	<p>PFOA and greater risk of hospitalization for lower respiratory tract infection at 0-4 y</p> <p>PFOS and greater risk of hospitalization for any infection at 0-4 y</p> <p>PFOS and greater risk of hospitalization for lower respiratory tract infection at 0-4 y</p>	<p>PFOA and hospitalization for any infection at 0-4 y</p> <p>PFOA and hospitalization for upper respiratory tract infection at 0-4 y</p> <p>PFOA and hospitalization for gastrointestinal infection at 0-4 y</p> <p>PFOA and hospitalization for other infection at 0-4 y</p> <p>PFOS and hospitalization for upper respiratory tract infection at 0-4 y</p> <p>PFOS and hospitalization for gastrointestinal infection at 0-4 y</p> <p>PFOS and hospitalization for other infection at 0-4 y</p>
No	Fei 2010	Prospective cohort	Denmark	Children	1,400	<p>PFOA and lower risk of hospitalization for infectious diseases (0–10 y (quartile 2 only), 0–<1 y (quartile 2 only), 1–<2 y (quartile 2 only), and 2–<4 y (quartile 3 only); boys; multiparous mothers)</p> <p>PFOA and greater risk of hospitalization for infectious diseases (girls)</p> <p>PFOS and lower risk of hospitalization for infectious diseases (0–<1 y; boys (quartile 3 only))</p> <p>PFOS and greater risk of hospitalization for infectious diseases (≥4 y (quartile 2 only); girls)</p>	<p>PFOA and hospitalization for infectious diseases (≥4 y; primiparous mothers)</p> <p>PFOS and hospitalization for infectious diseases (0–10 y, 1–<2 y, 2–<4 y; primiparous and multiparous mothers)</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Goudarzi 2017	Prospective cohort	Japan	Children	1,558	PFOS and greater risk of total infectious diseases (otitis media, pneumonia, respiratory syncytial virus, and/or varicella) at 4 y (total, boys quartile 4 no trend, girls)	PFOA and total infectious diseases (otitis media, pneumonia, respiratory syncytial virus, and/or varicella) at 4 y
No	Granum 2013	Prospective cohort	Norway	Children	93	PFOA and greater number of episodes of common cold at 0-3 and 3 y PFOA and greater number of episodes of gastroenteritis at 0-3 y	PFOA and ever common cold or ever gastroenteritis at 0-3 y and 3 y PFOS and number of episodes of common cold, ever common cold, number of episodes of gastroenteritis, ever gastroenteritis at 0-3 y and 3 y
No	Huang 2020	Prospective cohort	China	Children	344	None	PFOA and recurrent respiratory tract infections up to 5 years PFOA and number of respiratory tract infections up to 5 y (or in any year up to 5) PFOS and recurrent respiratory tract infections up to 5 years PFOS and number of respiratory tract infections up to 5 y (or in any year up to 5)
Yes	Impinen 2018	Prospective cohort	Norway	Children	641	PFOA and greater number of lower respiratory tract infection episodes from 0-10 y PFOS and greater number of lower respiratory tract infection episodes from 0-10 y	PFOA and number of common cold episodes from 0-2 y PFOA and rhinitis current or ever at 10 y, rhinoconjunctivitis ever at 10 y, rhinitis ever and sIgE > 0.35 at 10 y PFOS and number of common cold episodes from 0-2 y PFOS and rhinitis current or ever at 10 y, rhinoconjunctivitis ever at 10 y, rhinitis ever and sIgE > 0.35 at 10 y

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Impinen 2019	Prospective cohort	Norway	Children	1,207 at 3 y 921 at 7 y	<p>PFOA and lower risk of common cold at 0-3 y (all, girls)</p> <p>PFOA and greater risk of bronchitis/pneumonia at 0-3 y (all, girls)</p> <p>PFOA and greater risk of throat infection with streptococcus at 0-3 y (boys)</p> <p>PFOA and greater risk of pseudocroup at 0-3 y (all)</p> <p>PFOA and lower risk of ear infection at 0-3 y (girls)</p> <p>PFOA and greater risk of diarrhea/gastric flu at 6-7 y (all, girls, boys)</p> <p>PFOA and lower risk of urinary tract infection at 0-3 y (all, girls)</p> <p>PFOS and lower risk of common cold at 0-3 y (all, girls)</p> <p>PFOS and greater risk of bronchitis/pneumonia at 0-3 y (all)</p> <p>PFOS and lower risk of ear infection at 0-3 y (all, girls)</p> <p>PFOS and greater risk of diarrhea/gastric flu at 6-7 y (boys)</p> <p>PFOS and lower risk of urinary tract infection at 0-3 y (all, girls)</p>	<p>PFOA (boys) and common cold at 0-3 y</p> <p>PFOA (boys) and bronchitis/pneumonia at 0-3 y</p> <p>PFOA and bronchitis/pneumonia at 6-7 y</p> <p>PFOA (all, girls) and throat infection with streptococcus at 0-3 y</p> <p>PFOA and other throat infections at 0-3 y</p> <p>PFOA (girls, boys) and pseudocroup at 0-3 y</p> <p>PFOA (all, boys) and ear infection at 0-3 y</p> <p>PFOA and ear infection at 6-7 y</p> <p>PFOA and diarrhea/gastric flu at 0-3 y</p> <p>PFOA (boys) and urinary tract infection at 0-3 y</p> <p>PFOA and urinary tract infection at 6-7 y</p> <p>PFOS (boys) and common cold at 0-3 y</p> <p>PFOS (girls, boys) and bronchitis/pneumonia at 0-3 y</p> <p>PFOS and bronchitis/pneumonia at 6-7 y</p> <p>PFOS and throat infection with streptococcus at 0-3 y</p> <p>PFOS and other throat infections at 0-3 y</p> <p>PFOS and pseudocroup at 0-3 y</p> <p>PFOS (boys) and ear infection at 0-3 y</p> <p>PFOS and ear infection at 6-7 y</p> <p>PFOS and diarrhea/gastric flu at 0-3 y</p> <p>PFOS (all, girls) and diarrhea/gastric flu at 6-7 y</p> <p>PFOS (boys) and urinary tract infection at 0-3 y</p> <p>PFOS and urinary tract infection at 6-7 y</p>
No	Kishi 2013	Prospective cohort	Japan	Children	514	None	<p>PFOA and otitis media at 18 months</p> <p>PFOS and otitis media at 18 months</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Kvalem 2020	Prospective cohort and cross-sectional	Norway	Children	378	<p>PFOA and greater risk of rhinitis in last 12 m at 16 y (total)</p> <p>PFOA and lower risk of ≥ 3 common colds in last 12 m at 16 y (total)</p> <p>PFOA and greater risk of lower respiratory tract infection at 10-16 y (total, girls)</p> <p>PFOS and lower risk of 1-2 or ≥ 3 common colds in last 12 m at 16 y (total, boys)</p> <p>PFOS and greater risk of lower respiratory tract infection at 10-16 y (total, girls, boys)</p>	<p>PFOA and rhinitis in last 12 m at 10 y (total, girls, boys) or 16 y (girls, boys)</p> <p>PFOA and common colds at 10-16 y (total, girls, boys) or in last 12 m at 16 y (girls, boys)</p> <p>PFOA and lower respiratory tract infection at 10-16 y (boys) or in last 12 m at 16 y (total, girls, boys)</p> <p>PFOS and rhinitis in last 12 m at 10 y (total, girls, boys) or 16 y (total, girls, boys)</p> <p>PFOS and common colds at 10-16 y (total, girls, boys) or in last 12 m at 16 y (girls)</p> <p>PFOS and lower respiratory tract infection in last 12 m at 16 y (total, girls, boys)</p>
No	Leonard 2008	Retrospective cohort	United States	Adults	6,027	PFOA and lower risk of mortality from infectious and parasitic diseases (vs. US)	PFOA and mortality from infectious and parasitic diseases (vs. West Virginia or DuPont Region 1)
No	Looker 2014	Cross-sectional	United States	Adults	411	None	<p>PFOA and self-reported "flu" infection in last 12 months</p> <p>PFOA and self-reported cold in last 12 months</p> <p>PFOA and self-reported cold or "flu" in last 12 months</p> <p>PFOA and number of colds reported in last 12 months</p> <p>PFOS and self-reported "flu" infection in last 12 months</p> <p>PFOS and self-reported cold in last 12 months</p> <p>PFOS and self-reported cold or "flu" in last 12 months</p> <p>PFOS and number of colds reported in last 12 months</p>

In EPA?	Reference	Study Design	Country	Age Group	Subjects (Max)	Associations	Null Results
Yes	Manzano-Salgado 2019	Prospective cohort	Spain	Children	1,188	PFOS × study site and risk of lower respiratory tract infection at 1.5-7 y (inverse in Valencia only)	<p>PFOA and lower respiratory tract infection at 1.5-7 y (total, girls, boys), 1.5 y, 4 y, and 7 y</p> <p>PFOS and lower respiratory tract infection at 1.5-7 y (total, girls, boys), 1.5 y, 4 y, and 7 y</p>
No	Okada 2012	Prospective cohort	Japan	Children	343	None	<p>PFOA and otitis media at 18 months</p> <p>PFOS and otitis media at 18 months</p>