

DWQI
New Jersey Department of Environmental Protection

5 February, 2018

Sent by email to watersupply@dep.nj.gov

Dear Sir/Madam,

Re: Health-based Maximum Contaminant Level Support Document: Perfluorooctane Sulfonate (PFOS)
PFOS

I wish to respond to the Drinking Water Quality Institute request for public input on the perfluorooctane sulfonate (PFOS) document (http://www.nj.gov/dep/watersupply/g_boards_dwqi.html). My main concern is that the very comprehensive Report summarizes much of the epidemiological evidence but in its conclusions completely ignores the human data when establishing a limit for PFOS in drinking water. While I understand that there is a regulatory tradition of relying on experimental toxicology information, it is inappropriate to ignore substantive evidence on human toxicity. My estimate is that the proposed limit for PFOS is 100-fold too high and therefore far from protective of human health.

My background for submitting these comments: I am a physician and environmental epidemiologist who, during the last ten years, has studied human exposures to PFOS and other PFASs regarding their possible adverse effects, most studies involving large groups of children. My findings have been published in JAMA and several other peer-reviewed scientific journals.

I am an Adjunct Professor of Environmental Health at the Harvard T.H. Chan School of Public Health in Boston, and I also serve as Professor and Chair, Environmental Medicine, University of Southern Denmark. The PubMed database lists close to 500 of my publications, and the National Institutes of Health has supported my research continuously during the last 20 years. As joint PI representing Harvard University, I received in 2017 a Superfund Center grant (of about \$8 million total for 5 years) to conduct research on perfluorinated compounds. I became a Fellow of the American Association for the Advancement of Science in 1994, received the Bernardino Ramazzini Award from the Collegium Ramazzini in 2015, and was awarded the John R. Goldsmith Award from the International Society for Environmental Epidemiology in 2016. As Member of the Panel on Contaminants (2003-2009) of the European Food Safety Authority (EFSA), I co-authored the "Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts" in 2008.¹ (This opinion reflected the information available at the time and in accordance with EFSA traditions. As this report is now severely outdated, a revised opinion is scheduled for publication in the very near future). I have served for more than 30 years as the Adviser on Toxicology at the Danish National Board of Health. I have also served as member of several Task Groups at the International Agency for Research on Cancer, in part as chairman or subgroup chair, and as member of WHO's European Advisory Committee on Health Research. I currently serve as member of the European Environment Agency's Scientific Committee.

In the following, I shall relate my comments as a university researcher who has been supported by public funds only, and on the basis of my own research and on my evaluation of the research findings in general. None of my comments necessarily reflect the opinions of the above agencies or institutions.

As described by the DWQI Report, PFOS is a highly persistent chemical in the environment and has been disseminated globally. Known for many decades, PFOS is slightly water soluble and has a low vapor pressure, both of which are important properties that lead to environmental dissemination and retention in the human body. The elimination half-life in humans is several years, though some species are capable of excreting the substance more readily, thus complicating the reliance on rodent species in toxicology models. We have shown that PFOS passes the placental barrier and that cord blood contains almost as much PFOS as the maternal blood.² Most recently, we have shown that PFOS is excreted by the mother in milk during breastfeeding, thus causing the serum-PFOS concentration to increase substantially in breast-fed infants.³ I note that most of the epidemiological evidence has not focused on exposures during infancy, although early postnatal development must be considered a highly vulnerable period that must be taken into regard when determining exposure limits.⁴

The DWQI Report summarizes the major adverse effects that have been documented in laboratory animals and also reported in humans. The effects include carcinogenicity, liver function abnormalities and elevated serum lipids, immunotoxicity, endocrine disruption (including delayed breast development), and reproductive toxicity. In the below text, I refer only to publications that are of particular relevance or not cited by the Report.

In regard to carcinogenicity, less evidence is available on PFOS than on PFOA, but the absence of evidence is of course not evidence that a cancer risk is absent. The risk assessment for cancer carried out by the NJ Subcommittee relies on experimental animal evidence and appears to be appropriate, except that it does not consider any increased vulnerability during early development.

Endocrine disruption and reproductive toxicity has been documented in substantial detail in mouse studies. As an indication of endocrine disruption, studies at NIEHS have shown delayed breast development at elevated exposure to perfluorinated compounds.⁵ Although this evidence mainly relates to PFOA, human studies show that the duration of breastfeeding is significantly shorter in women with higher serum-PFOS concentrations.⁶ In our study, a doubling in the serum-PFOS concentration was linked to a decrease in breastfeeding duration by about 6 weeks – a very substantial and statistically significant decrease. Supporting findings were published from a U.S. cohort,⁷ thus suggesting that this association is of concern at current PFOS exposure levels. In a report that will be published in *PLoS Medicine* in late February, we show that baseline serum-PFOS concentrations predict the body weight increase following a six-month calorie-restriction diet.⁸ The results also showed that the metabolic rate was inversely associated with the PFOS concentration. Similar results were obtained for PFOA. In another study, also in press, we show that elevated serum-PFOS concentrations in serum obtained from American nurses in the late 1990s were associated with the risk of developing type 2 diabetes in subsequent years.⁹ Given the paucity of experimental toxicology findings exploring these high relevant outcomes, reliance on human data is crucial. Likewise, subfecundity¹⁰ has been reported at higher

serum-PFOS concentrations in populations with background exposures. Again, this outcome is of major public health relevance, but may not be appropriately disclosed in animal models applied so far.

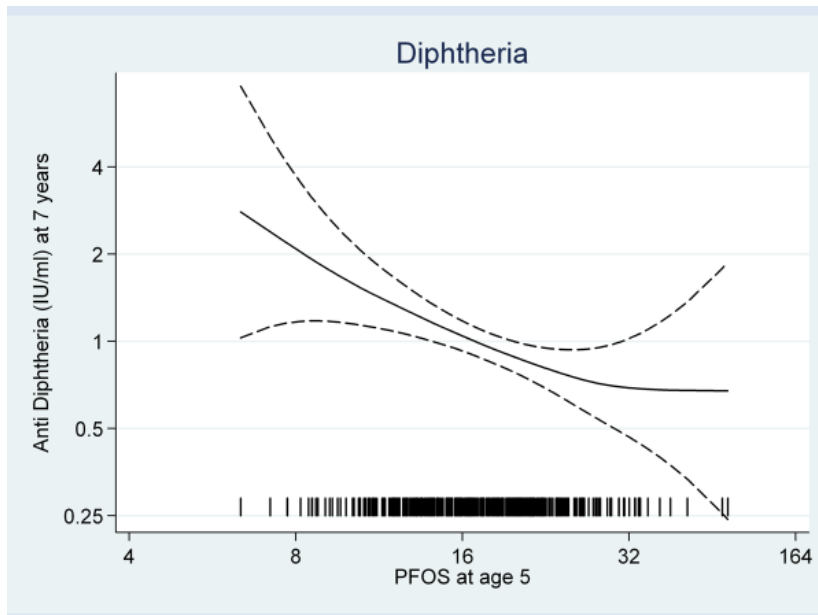
I shall now focus on PFOS immunotoxicity, as this effect has been well documented and may well represent the critical adverse effect in humans, on which risk assessment should focus. PFOS-induced immune deficiency has been reported in mice,¹¹ and increased mortality from virus infection has also been documented.¹² In Rhesus monkeys, fairly crude outcomes, such as decreased spleen and thymus weights, lowered total immunoglobulin, and decreased leukocyte counts, were documented in an unpublished monkey study commissioned by a PFC producer 40 years ago. Based on the experimental and epidemiological evidence, the NTP recently concluded that PFOS must be “presumed to be an immune hazard to humans...,” a conclusion that relied in part upon a “high level of evidence...from animal studies.”¹³

The early findings in mice spurred an interest in pursuing the antibody response outcomes in epidemiological studies. In fact, an international working group had recommended this approach as a valid and clinically relevant methodology in human immunotoxicological research.¹⁴ The advantages include the fact that a vaccination constitutes a natural and highly feasible experiment of antigen exposure, where the same dose of antigen is applied at the same age, so that the antibody response can be ascertained by a routine assay and where the outcome is of clinical relevance. We have therefore carried out extensive studies of children exposed to PFOS and related compounds. Our findings show an inverse association of serum-PFOS concentrations with the response to booster vaccination in children and adults,¹⁵⁻¹⁷ thus suggesting a deficit in the B cell reactivation by T cells in the germinal centers, thereby resulting in B cells becoming less effective with respect to antibody production. These findings are supported by *in vitro* studies using human white cells,¹⁸ although experimental studies have not yet revealed the detailed mechanisms.¹³

The adaptive immune system is at first dominated by Th2 responses; Th1 responses mature during infancy to allow proper responses to infections and routine immunizations.¹⁹ Allergy and asthma are characterized by a Th2-biased immune response, and increased odds of asthma in children were reported at elevated PFAS exposures,²⁰ although this finding has not been replicated.²¹ The lack of clear evidence on PFAS-associated allergy may in part be due to uncontrolled and variable allergen exposures and the absence of well-defined outcome variables comparable to the vaccine-induced antibodies used to assess Th1 activity. Also, previous vaccination with attenuated virus plays an important role.²² I also note that breastfeeding is generally considered advantageous for the child’s immune system development,²³ although the evidence is somewhat equivocal, perhaps because very few studies have taken into regard the inverse effects of immunotoxicants present in human milk.²⁴ Our studies of PFAS-exposed children show no clear benefit of breastfeeding, perhaps as a result of human milk acting as a vehicle for immunotoxicants that counteract any benefits.

From our study published in JAMA,¹⁶ I would like to emphasize that many children at age 7 years (two years after the age-5 diphtheria and tetanus vaccination booster) had an antibody against diphtheria and/or tetanus below the clinically protective level of 0.1 IU/mL. This means that the children had no long-term protection against the diseases despite a total of four vaccinations. We calculated the odds

ratios (ORs) for a doubling in the child's age-5 serum-PFOS concentration as a predictor of having an antibody concentration below 0.1 IU/mL at age 7 years. The ORs for tetanus and diphtheria were 2.38 and 2.61, both of borderline statistical significance. When looking at the antibody concentration before the age-5 booster, a doubling in the prenatal PFOS exposure showed an OR of 2.48 for diphtheria, which was highly significant, although not for tetanus. When we used a structural equation model that allowed us to combine the two serum-PFOS measurements at ages 5 and 7 years, we found that a doubled serum concentration of PFOS, combined with PFOA and PFHxS, was associated with an approximate decrease by 50% of the overall vaccine antibody concentration.²⁵ We have recently shown that mutual adjustment of PFOA and PFOS results only in minor changes of the results, thus suggesting that, while humans are exposed to both compounds, PFOA immunotoxicity cannot explain the immunotoxic effects associated with PFOS, and *vice versa*.²⁶ Likewise, adjustment for the elevated PCB exposure in the Faroes did not materially affect the calculations.¹⁶



The plot on the left shows the correlation between the age-5 serum-PFOS concentrations and the age-7 anti-diphtheria antibody concentration in the birth cohort described in the JAMA article.¹⁶

These findings support the notion that PFOS has an independent immunotoxic effect, which is in accordance with the data from the animal experiments referred to above and reviewed by NTP. Still, the human evidence reviewed relies on serum-PFOS measured at two postnatal ages, thus not taking into account the possible effects of immunotoxicity occurring during potentially more vulnerable ages in early postnatal life (i.e., infancy). The reported associations may therefore underestimate the toxicity at younger ages. In our most recent study of a younger Faroese birth cohort, we modeled serum-PFOS concentrations during infancy from the prenatal exposure level and information on the duration of breastfeeding.¹⁷ In the absence of blood samples, this calculation provides a reasonable estimate of the changing exposures. Our results showed a clear tendency that serum-PFOS at age 3 months was a much stronger indicator of vaccine antibody concentrations at age 5 years than was the calculated PFOS concentration at ages 6 and 12 months. Again, these results are crucial for prudent risk assessment, as they refer to vulnerable human populations and to exposure settings that are not easily modeled in laboratory animal studies.

From its review of the human evidence, which includes several other studies in addition to ours, the NTP concluded that PFOS is “presumed to be an immune hazard to humans...” while taking into regard a “moderate level of evidence from studies in humans.”¹³ This conclusion refers to the fact that exposures to PFOS often correlate with exposures to other PFASs, so that epidemiological studies, in contrast to experimental studies, cannot easily attribute associations to particular PFASs. Nonetheless, as indicated above, limited human evidence is available on the adverse effects of PFOS alone, as most exposures involve PFAS mixtures that include PFOS.

The question has been raised whether our use of antibody responses to vaccinations is appropriate for establishing exposure limits to prevent adverse effects. One could argue that changes in antibody concentrations are subclinical and of questionable relevance to long-term health. On the other hand, this routine outcome reflects immune functions that may well be of relevance to resistance toward infections and to other immune-associated abnormalities. As already outlined, antibody concentrations pose substantial advantages in epidemiological research, and they constitute a well-established indicator of complex immune functions. Deviations in this immune function biomarker at the individual level may then be linked to important shifts in the prevalence of related diseases at the population level – changes that would be apparent only in large prospective studies. Calculations have shown decreases in antibody concentrations of up to about 50% at a doubled PFAS exposure within the range of background exposures. Such decreases are not trivial, and effects of this magnitude would otherwise be expected only with exposures to such factors as ionizing radiation and cytostatic cancer drugs.

In children, a relevant outcome that may be the result of poor antibody responses is the frequency of infections. Although infectious disease during childhood is often associated with housing conditions, daycare, the presence of siblings at home and other factors that may be difficult to adjust for in statistical analyses, two studies have examined this possible connection. First, in a small group of Norwegian children, a positive association was seen between the maternal serum-PFOS concentration at childbirth and the number of episodes of common cold and gastroenteritis in the children, as assessed by questionnaire.²⁷

A more recent study of 359 Danish children aged 1-3 years obtained information from the mother on the presence of fever and symptoms in the child every two weeks for one year via text messages.²⁸ The mother’s early-pregnancy serum-PFOS concentration was a strong predictor of the child’s incidence of infections, where a PFOS in the high tertile compared to the low tertile was associated with an increased proportion of days with fever (IRR: 1.65 (95% CI: 1.24, 2.18), p for trend <0.001). Further, higher PFOS concentrations were associated with increased numbers of episodes of co-occurrence of fever and coughing and fever and nasal discharge during the one-year study period. These observations suggest that our findings in regard to specific antibodies as markers of immune system functions are clinically relevant. Again, these findings document the public health implications of PFOS exposures in the general population, the plausibility of which are demonstrated by experimental toxicology reports.

As a true threshold may not necessarily be present, the U.S. EPA relies on the calculation of the mathematically-defined benchmark dose level (BMDL) as a basis for deriving a reference dose (RfD) that is assumed to be virtually safe. As a default, the RfD is calculated as one-tenth of the BMDL, given that

the BMDL is not a threshold and refers to an average degree of vulnerability. (When the RfD is expressed in terms of the serum concentration, it is sometimes called the Target Human Serum Level.) Dealing with human populations where an unexposed control group is not present, we have used the recommended statistical method²⁷ to calculate a BMDL for the serum-PFOS concentration as a predictor of immune deficiency.²⁹ Using a default linear dose-effect curve and a benchmark response of 5% (meaning a 5% decrease in the antibody level), we found the BMDL to be approximately 1.3 ng/mL. Modeling other curve shapes is possible; a logarithmic curve shape fits the data better and results in a lower BMDL. Analysis of pooled data may result in higher BMDL results due to the decreased uncertainty at a larger number of observations. The calculated BMDL should therefore be considered an approximate level.

Assuming that this calculation reflects the PFOS effects only, as our most recent calculations suggest, the EPA guidelines indicate that an RfD can be estimated as one-tenth of the BMDL, i.e., 0.13 ng/mL, as a virtually safe level resulting from all PFOS exposure sources. It is my opinion, as based on my experience and expertise, that a safe water-PFOS limit must secure that human serum-PFOS levels are kept below this Target Human Serum Level. I note that the DWQI report has calculated a Target Human Serum Level of 23 ng/mL from animal toxicology studies. This very substantial difference clearly reflects that the DWQI relies on experimental studies using animals that are much more resistant to PFOS than humans, where exposures do not reflect the most vulnerable developmental windows, and where the outcomes chosen do not properly reflect the adverse effects that are of critical importance in humans.

From the Target Human Serum Level derived from animal studies, the DWQI Report recommends a water-PFOS limit of 12 ng/L. Considering the fact that this level is approximately 175-fold greater than the level calculated from human studies, a protective water limit would then be about 0.07 ng/L.

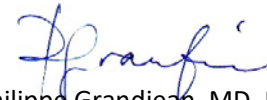
We have previously highlighted the fact that current limits for PFASs in drinking water greatly exceed our estimate of the concentrations necessary to prevent PFAS-associated immunotoxicity.³⁰ The calculations above are not meant to constitute the exact calculations to be used in a formal risk assessment document, but the approximate magnitude of the epidemiology-based RfD illustrates the consequence of ignoring human data on PFOS-associated adverse effects.

In conclusion, while I understand that the DWQI must primarily rely on experimental toxicology data, I am surprised that the DWQI has disregarded the extensive epidemiological evidence when estimating safe exposure levels for PFOA in drinking water. The differences between species in regard to PFOS toxicokinetics and toxicity are well established, and the above calculations clearly show that these differences have not been appropriately taken into account. In addition, developmental exposure likely represents the main risk to humans, and the DWQI to some extent ignores this consideration. Likewise, the reliance on fairly crude outcomes in toxicology studies fails to acknowledge the importance of less serious outcomes, such as vulnerability to infectious disease, metabolic abnormalities, or subfecundity. Similar concerns were recently raised in a more general sense by scientists from the U.S. EPA,³¹ who concluded that “to protect public health more effectively, future risk assessments will need to use the full range of available data, draw on innovative methods to integrate diverse data streams, and consider health endpoints that also reflect the range of subtle effects and morbidities observed in human populations.”

For these reasons, I conclude that prudent risk assessment for PFOS should take into regard both animal data and human data, especially in the present context where a water limit relying on animal data alone appears to be at least 100-fold above the limit that would result if relying on human data.

The key references are referred to by numbers in the above text and are listed below.

I hope that these comments may be of use to the DWQI. Should questions arise, I am of course willing to provide further information or clarification.



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