

DWQI

10 November 2016

New Jersey Department of Environmental Protection

Sent by email to watersupply@dep.nj.gov

Dear Sir/Madam,

Re: Health Effects Subcommittee Report: Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA)

I wish to respond to the Drinking Water Quality Institute request for public input for perfluorooctanoic acid (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 ignores the human data when establishing a limit for PFOA in drinking water. Thus, I estimate that the proposed limit for PFOA is approximately 50-fold too high.

My background for submitting these comments: I am a physician and environmental epidemiologist who has studied human exposures to PFOA and other PFASs in regard to possible adverse effects in 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 430 of my publications, and the National Institutes of Health has supported my research continuously during the last 20 years. 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 earlier this year. 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 As this report is now severely outdated, a revised opinion is scheduled for publication next year). 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. I am currently a member of the European Environment Agency's Scientific Committee and of WHO's European Advisory Committee on Health Research. In the following, I shall relate my comments as a university researcher who has been supported by public funds only. None of my comments necessarily reflect the opinions of the above agencies or institutions.

As described by the DWQI Report, PFOA is a highly persistent chemical in the environment and has been disseminated globally. Known for many decades, PFOA is slightly water soluble and has a low vapor

pressure, both of which are important properties in regard to its dissemination and retention in the human body. The elimination half-time in humans is several years, though some species are capable of excreting the substance more readily, thus complicating the use of some species in toxicology models. We have shown that PFOA passes the placental barrier and that cord blood contains almost as much PFOA as the maternal blood.² Most recently, we have shown that PFOA is excreted by the mother in milk during breastfeeding, thus causing the serum-PFOA 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, the sites affected include the liver, pancreas, and testicles. I note that the U.S. EPA's Science Advisory Board considers PFOA "likely to be carcinogenic to humans," while IARC has concluded that PFOA is "possibly carcinogenic to humans." 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 take into account 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 PFOA exposure.⁵ These results correspond to our recent finding that the duration of breastfeeding is shorter in women with higher serum-PFOA concentrations.⁶ In our study, a doubling in the serum-PFOA concentration was linked to a decrease in breastfeeding duration by about 6 weeks – a very substantial and statistically significant decrease. Parallel findings were published from a U.S. cohort,⁷ thus suggesting that this association is of concern at current exposure levels. Among related effects, decreased birth weight and miscarriage have been reported in several studies⁸ that suggest that PFOA may have multiple toxicological targets.

PFOA immunotoxicity has been reported in mice and Rhesus monkeys, and it has been shown in *in vitro* studies of human leukocytes.⁹ Most outcomes are fairly crude, such as decreased spleen and thymus weights, lowered total immunoglobulin, and decreased leukocyte counts. Such measures were also used in an unpublished monkey study commissioned by a PFOA producer almost 40 years ago. Of particular interest are the more recent findings of decreased antibody responses after PFOA exposure.^{9,10} Based on this evidence, the NTP recently concluded that PFOA 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."¹¹

These findings spurred an interest in pursuing the antibody response outcome in epidemiological studies. In fact, an international working group had recommended this approach to immunotoxicological research in humans.¹² 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 at exposure, 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 PFOA and related compounds. Our findings and those reported by other colleagues show an inverse association of serum-PFOA concentrations with the response to booster vaccination in children and adults,¹³⁻¹⁵ thus suggesting a deficit in the reactivation by T cells of B 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,¹⁶ but the mechanisms are unclear at present.¹¹

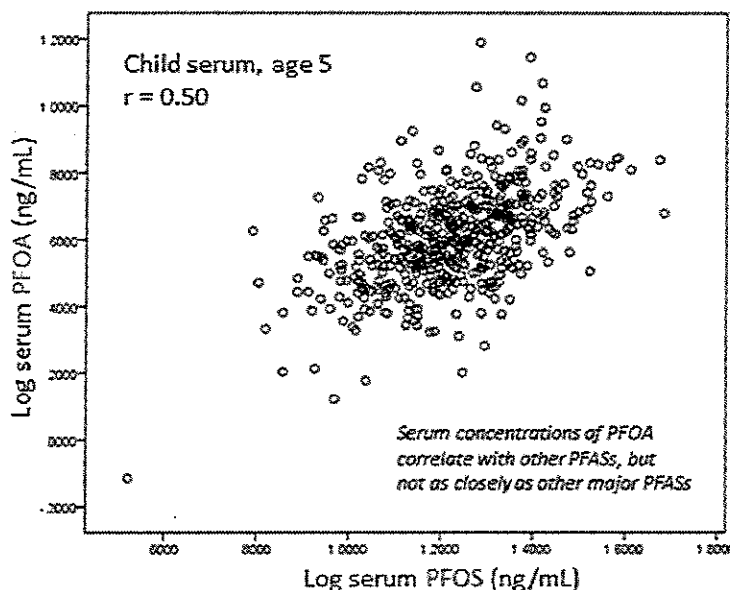
The adaptive immune system is at first dominated by Th2 responses; Th1 responses mature during infancy to allow proper responses to infections and routine immunizations.¹⁷ Allergy and asthma are characterized by a Th2-biased immune response, and increased odds of asthma in children were reported at elevated PFAS exposures,¹⁸ although this finding has not been replicated.¹⁹ Our own study²⁰ suggested that serum-PFAS concentrations at age 5 years were associated with increased odds of asthma only among the children who had not yet been vaccinated against measles, mumps, and rubella (MMR), while the association was reversed among MMR-vaccinated children. While inhibition of antibody responses, perhaps associated with increased risk of allergy development, could represent a change in the Th1/Th2 balance,²¹ the relative role of the immune system components is complex. 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. I also note that breastfeeding is generally considered advantageous for the child's immune system development,²² although the evidence is somewhat equivocal, perhaps because very few studies have taken into regard the inverse effects of immunotoxicants present in human milk.²³ 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,¹⁵ I would like to emphasize that several 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-PFOA concentration as a predictor of having an antibody concentration below 0.1 IU/mL at age 7 years. The ORs for tetanus was 4.2 (95% CI, 1.5-11.4) and for diphtheria 3.3 (95% CI, 1.4-5.5). Both were significant at a *p* level <0.01. PFOS showed lower ORs (marginal significance), and other PFASs had ORs that were below 2 and non-significant. Our regression analyses also showed PFOA to be a strong predictor of lowered antibody concentrations. When we used a structural equation model that allowed us to combine the two serum-PFOA measurements at ages 5 and 7 years, we found that a doubled serum-PFOA concentration was associated with a change in the age-7 antibody concentration of -38.2% (95% CI: -56.1; -13.0) for tetanus and -34.7% (95% CI, -52.5; -10.2) for diphtheria. When we adjusted for the other PFASs, the regression coefficients were -29.6% and -26.9%, respectively, i.e., virtually unchanged.²⁴ Likewise, adjustment for the elevated PCB exposure in the Faroes did not materially affect the calculations.

These findings support the notion that PFOA has an independent immunotoxic effect, which is in accordance with the data from the animal experiments referred to above. Still, the human evidence relies on serum-PFOA measured at two postnatal ages and does not take into account the possible effects of immunotoxicity occurring during potentially more vulnerable ages in early postnatal life (i.e. infancy). Thus, the reported associations may underestimate the toxicity at younger ages.

From its review of the human evidence, which includes several other studies in addition to ours, the NTP concluded that PFOA is “presumed to be an immune hazard to humans...” while taking into regard a “moderate level of evidence from studies in humans.”¹¹ This conclusion refers to the fact that exposures to PFOA 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 PFOA alone, as most exposures involve PFAS mixtures that include PFOA. As reviewed in the DWQI Report, workers exposed mainly to PFOA showed an increased prevalence of ulcerative colitis and rheumatoid arthritis that was significantly associated with the exposure, and another study likewise linked ulcerative colitis to water-PFOA exposure. These findings likely reflect immune system dysfunctions and therefore complement our findings in regard to antibody responses associated with serum-PFOA concentrations.

Our observation that PFOA effects may be distinguished from effects of other PFASs probably relates to the fact that PFOA in the Faroese population correlated less closely with the co-exposures, thereby allowing mutual adjustment. The below (unpublished) plot shows the correlation of age-5 serum concentrations in the birth cohort described in the JAMA article.



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. On the other hand, this routine outcome

reflects deviating immune functions that may well be of relevance to other immune-associated abnormalities and diseases. As already outlined, antibody concentrations pose substantial advantages in epidemiological research, and they constitute a well-established indicator of interference with complex immune functions. Deviations in the 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. Some of our calculations have shown decreases in antibody concentrations of up to about 50% at a doubled PFAS exposure. These decreases are not trivial, and effects of such magnitude would otherwise be expected only with exposures to such factors as ionizing radiation and certain 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. In a small group of Norwegian children, a positive association was seen between the maternal serum-PFOA concentration at childbirth and the number of episodes of common cold and gastroenteritis in the children, as assessed by questionnaire.²⁵ In addition, serum-PFOA showed a highly significant inverse association with the anti-rubella antibodies at age 3 years, although three other vaccine antigens did not show a significant association in this small study.

A 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.²⁶ For the number of days with fever >38.5° (101.3° F), comparison of high and low tertiles of maternal pregnancy serum-PFOA concentrations showed an OR for days with fever above the median of 1.97 (95%CI: 1.07, 3.62). Similar tendencies were observed for episodes with nasal discharge and fever as well as for coughing and fever. These observations suggest that our findings in regard to specific antibodies as markers of immune system functions are clinically relevant.

The average serum-PFOA concentration in the Faroese children in the JAMA study was about 4 µg/mL. This level is only slightly higher than current averages in Americans. In the Danish study of PFOA-associated infections, the average level was less than half that level, and even lower in the Norwegian study, thus suggesting that PFOA-associated immunotoxicity may be highly prevalent at exposures similar to those in the U.S. The most recent results from the CDC suggest that serum-PFOA concentrations average about 2 µg/mL and that values above 10 µg/mL are fairly frequent.

As a true threshold may not necessarily be present, the U.S. EPA uses calculations of the mathematically-defined benchmark dose level (BMDL) to derive a reference dose (RfD) that is assumed to be virtually safe, the latter often calculated as one-tenth of the former, as the BMDL is not a threshold, and to take into account differences in 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-PFOA concentration.²⁸ 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 0.3 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 PFOA effects only, the EPA guidelines indicate that an RfD can be estimated as one-tenth of the BMDL, i.e., 0.03 ng/mL, as a virtually safe level resulting from all PFOA exposure sources. If water contributes 20% of the exposure, that would mean that water-PFOA can contribute a long-term dose that corresponds to 0.006 ng/mL serum, or 6 ng/L.

As reviewed by the DWQI Report, long-term ingestion of PFOA in water will result in a serum concentration that is about 100-fold higher. The serum-PFOA that corresponds to the RfD will therefore require that the water-PFOA concentration be kept below 0.3 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.²⁹ 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 PFOA-associated adverse effects. As can be seen, the NJ proposal of 14 ng/L is about 50-fold above the approximate level that is estimated to be necessary to prevent immunotoxic effects in humans. At chronic exposure to a water-PFOA concentration of 14 ng/L will result in a serum-PFOA increase by 1.4 µg/mL (100-fold the water concentration), i.e., an increase by about 70% of the current average serum concentration measured by CDC.

While it may be argued that this evidence may not appropriately represent the toxicity risks associated with PFOA exposures, I note that the DWQI Report also reviewed the studies on PFOA-induced delayed mammary gland development,⁵ where the RfD has been calculated to be 0.8 ng/mL serum. Like the RfD for immunotoxicity, this level is also below the average serum-PFOA concentration in the U.S. general population. Thus, if endocrine disruption or reproductive toxicity rather than immunotoxicity is chosen as the critical effect of PFOA exposure, the proposed water limit of 14 ng/L would also be too high to provide the desired protection of the exposed population.

In conclusion, I am surprised that the DWQI has disregarded the extensive epidemiological evidence when estimating safe exposure levels for PFOA in drinking water. As already thoroughly documented in the DWQI Report, evidence from experimental animal studies clearly shows that PFOA can cause serious adverse effects; some of these adverse effects were documented at serum concentrations that are only slightly greater than those observed in epidemiological studies. However, species differences occur in regard to PFOA toxicokinetics, and it may be inappropriate or misleading to rely on findings in experimental animal studies only, and it is necessary to consider the epidemiological evidence available. While humans are rarely exposed to PFOA alone, the data suggest that PFOA is associated with adverse effects that cannot easily be explained by other exposures. I note that, while PFOA (in NTP's words) is "presumed to constitute an immunotoxic risk", as is PFOS, other environmental exposures are not known for sure to be immunotoxic to humans at the levels observed, and they may therefore not be true confounders. For these reasons, I conclude that prudent risk assessment should take into regard

both animal data and human data, especially in the present context where a water limit relying on animal data alone is approximately 50-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.



Philippe Grandjean, MD, DMSC

Harvard T.H. Chan School of Public Health



References

- 1 European Food Safety Authority. Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. The EFSA Journal 653, 1-131 (2008).
- 2 Needham, L. L. et al. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45, 1121-1126 (2011).
- 3 Mogensen, U. B. et al. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environ Sci Technol 49, 10466-10473 (2015).
- 4 Grandjean, P. et al. Life-Long Implications of Developmental Exposure to Environmental Stressors: New Perspectives. Endocrinology 156, 3408-3415 (2015).
- 5 White, S. S. et al. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicol Sci 96, 133-144 (2007).
- 6 Timmermann, C. A. et al. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reprod Toxicol, doi:10.1016/j.reprotox.2016.07.010 (2016).
- 7 Romano, M. E. et al. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. Environ Res 149, 239-246 (2016).
- 8 Jensen, T. K. et al. Association between Perfluorinated Compound Exposure and Miscarriage in Danish Pregnant Women. PLoS One 10, e0123496 (2015).
- 9 DeWitt, J. C. et al. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol Pathol 40, 300-311 (2012).
- 10 DeWitt, J. C. et al. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARalpha and T- and B-cell targeting. J Immunotoxicol 13, 38-45 (2016).
- 11 National Toxicology Program. Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS), URL: <http://ntp.niehs.nih.gov/pubhealth/hat/noms/pfoa/index.html> (2016).
- 12 van Loveren, H. et al. Report of the Bilthoven Symposium: Advancement of Epidemiological Studies in Assessing the Human Health Effects of Immunotoxic Agents in the Environment and the Workplace. Biomarkers 4, 135-157 (1999).
- 13 Looker, C. et al. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci 138, 76-88 (2014).

- 14 Kielsen, K. et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J Immunotoxicol*, 13, 270-3 (2016).
- 15 Grandjean, P. et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* 307, 391-397 (2012).
- 16 Corsini, E. et al. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol* 258, 248-255 (2012).
- 17 Romagnani, S. T cell subpopulations. *Chem Immunol Allergy* 100, 155-164 (2014).
- 18 Dong, G. H. et al. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. *Environ Health Perspect* 121, 507-513 (2013).
- 19 Humblet, O. et al. Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). *Environ Health Perspect* 122, 1129-1133 (2014).
- 20 Timmermann, C. A. et al. Asthma and allergy in children with and without prior measles, mumps, and rubella vaccination. *Pediatr Allergy Immunol* 26, 742-749 (2015).
- 21 Dong, G. H. et al. Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* 85, 1235-1244 (2011).
- 22 Kramer, M. S. et al. Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 335, 815 (2007).
- 23 Grandjean, P. et al. Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. *Environ Health Perspect* 118, 1429-1433 (2010).
- 24 Mogensen, U. B. et al. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environ Health* 14, 47 (2015).
- 25 Granum, B. et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol* 10, 373-379 (2013).
- 26 Dalsager, L. et al. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4 years among 359 children in the Odense Child Cohort. *Environ Internat* 96, 58-64 (2016).
- 27 EFSA Scientific Committee. Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. *The EFSA Journal* 1150, 1-72 (2009).
- 28 Grandjean, P. & Budtz-Jørgensen, E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. *Environ Health* 12, 35 (2013).
- 29 Grandjean, P. & Clapp, R. Perfluorinated Alkyl Substances: Emerging Insights Into Health Risks. *New Solut* 25, 147-163 (2015).