

A Brief Synopsis of 3M Legacy PFAS Health Science Research

(By [REDACTED], 3M Company Legacy PFAS Research Program, 18 Jan 2019)

3M phased out the production of the legacy PFAS chemicals starting in the early 2000's; however, 3M is still dedicated to understanding the science for health-related queries through its own research programs, for both toxicology and epidemiology disciplines, with collaborative efforts from expert researchers in academic institutions, federal governments, and contract research labs.

There is increased scientific debate whether serum cholesterol levels are altered in humans with exposure to environmental levels of PFOA and PFOS. In laboratory animal studies, reduced serum total cholesterol (mediated by nuclear receptor PPAR α) is typically observed with high doses of PFOA or PFOS. Depending on the animal model used, the corresponding serum concentration of PFOA or PFOS where lipid reduction occurred typically were at several orders of magnitude higher than levels measured in the general population. These observations are in direct contrast with several cross-sectional human epidemiological studies (e.g., CDC NHANES survey data) which reported positive associations with non-HDL cholesterol. With the most recent provisional TWIs published by EFSA CONTAM where benchmark response models of these (cross-sectional) human epidemiological data were used in deriving TWIs for PFOA and PFOS, it is worth noting that:

- In these cross-sectional studies, serum PFOS and PFOA concentrations are several orders of magnitude less than the animal studies; and, in the US and Europe, the levels of PFOS and PFOA have been declining in the general population since 2000's (Olsen et al. 2017 Environ Res 157 87-95).
- The association became much weaker or absent when the serum PFOA and PFOS concentrations reached 25 - 50 ng/mL or higher. In other word, there was not a consistent exposure-response as exposure (serum PFOA / PFOS concentrations) increased from general population with ambient exposure, to exposed community with water exposure, to occupationally-exposed workers.

While mechanistic studies in animals have consistently demonstrated that PFOA and PFOS lower blood lipid, there has not been any biological (mechanistic) explanation demonstrating why/how environmental exposure to low levels of PFOA and PFOS in humans can be attributed to higher cholesterol values.

3M has been working with [REDACTED] at TNO Biosciences (Leiden, The Netherlands) using humanized Apo*E3.Leiden.CETP mice to study lipid metabolism and PFAS. This mouse model mimics human lipoprotein metabolism and is widely used in human atherosclerosis research. In the most recent research, three different PFOA exposure scenarios were incorporated with these mice and evaluated for their lipid profiles over time (total cholesterol, triglyceride, VLDL, lipase activities, hepatic lipid uptake and synthesis *de novo*, and gene transcripts in liver and intestines). At the end of 4 – 6 weeks exposure, the mean plasma PFOA approximated 50 ng/mL, 1500 ng/mL, and 144,000 ng/mL (representing general / community population level, production workers' level, and toxicological level, respectively). Exposures to PFOA at the two lower doses did not affect the lipid metabolism in these mice. Only the highest PFOA dose group did, which is consistent with previous animal data. NextSeq gene analysis on both liver and intestine tissues for all dose groups showed only the high dose PFOA group had effects on the hepatic lipid transport and synthesis but these effects were not observed with the two lower dose groups. The study data from these humanized mice did not support a causal explanation for the association observed between serum lipid and low PFOA levels in humans. This

work has now been accepted for publication in Toxicological Sciences (<https://doi.org/10.1093/toxsci/kfz015>).

There are also other studies related to cholesterol and PFOA / PFOS that may be of additional interest; and, one or more 3M coauthors contributed to these work:

- (1). With PFOA, 3M had the opportunity to sub-license a 3+3 dose escalation phase 1 clinical trial data with ammonium PFOA among 49 human subjects (Convertino et al. 2018 Toxicol Sci 163 293-306). Weekly dosages between 50 mg and 1200 mg ammonium PFOA were administered for six weeks. Using generalized estimating equations, a probabilistic analysis using probability distribution functions at various PFOA concentrations, and a 2-compartmental pharmacokinetic/pharmacodynamic model, there was strong evidence of a reduction (not increase) in serum total cholesterol and LDL cholesterol (not HDL cholesterol) with a definitive transition in the shape of the probability distribution functions around 175,000 – 230,000 ng/mL PFOA. Convertino et al. discussed the fact that these findings are similar to animal (rodent) models but at higher concentrations of administered PFOA dosages which is likely the consequence of the rodent being the more sensitive species to the ligand binding effects of PFOA on PPAR(alpha), and possibly other nuclear receptors.
- (2). With PFOS, 3M also conducted a detailed clinical study with a cohort of 36 cynomolgus monkeys (n=18/sex). The monkeys extensively for 42 – 105 days prior to any PFOS dose was given (to obtain their baseline clinical chemistry profiles) and they were treated with up to 3 doses of PFOS that resulted in the highest PFOS concentrations up to 165000 ng/mL. There were no substantive changes in most of the clinical chemistry parameters (including thyroid) evaluated except a slight decrease in HDL cholesterol where the lower-bound 5th percentile benchmark concentrations were determined at 74,000 and 76,000 ng/mL for male and female monkeys respectively (Chang et al. 2017 Toxicol Sci 156 387-401).
- (3). Among the cross-sectional studies, there are inconsistent associations being reported for cholesterol and dietary factors (e.g., red meat, fish, saturated fat). However, toxicological research shows that dietary modification can artificially create a positive correlation between blood lipids and PFOA levels in Apo*E3.Leiden.CETP mice (Chang et al. 2012 The Toxicologist 126 403).
- (4). 3M has also investigated whether lipoproteins may preferentially bind with PFOA and PFOS, but found little evidence to support this hypothesis with a single donor sample. (Butenhoff et al. 2012 Toxicol Letters 210 360-365).

There is now substantive contrary evidence based on data originating from both experimental studies at higher dosages/concentrations conducted in animals (primates and rodents) and humans on the effects of PFOA / PFOS and serum lipid. Therefore, if an advisory or regulatory assessment is to use cross-sectional data, it infers a causal association and as such, the mode of action should be identified; and whenever possible, the actual mechanism. To our best knowledge, a mode-of-action regarding low PFOA / PFOS concentration and higher cholesterol has not been demonstrated in humans.

Also, there is a quantitative bias associated with epidemiological data when it comes to background levels of PFOA / PFOS in the general population. In examining this issue, 3M has sponsored several research studies using physiologically-based pharmacokinetic (PBPK) models through internationally recognized experts in this modelling approach (), and others such as physician-epidemiologist (). The improvement in the modern analytical capability

has evolved greatly in the past decade and it obviously has allowed for the measurement of PFAS at extremely low levels (ng/mL or lower). Because most PFASs are highly protein-bound, and, as such, any physiological or pathological processes that could affect blood flow could potentially confound the concentrations of PFASs being measured in the blood, especially at low levels when measured near the time of an adverse health diagnosis. This has also been referred to in the literature as “reverse causation”. In epidemiological studies where the PFASs are being detected near the lower limit of detection, associations between health outcomes in relation to such very low levels of concentrations to PFASs are susceptible to be confounded by study subjects’ metabolic status around clearance mechanisms (e.g., glomerular filtration, pregnancy, lactation, and menstruation). To evaluate this, PBPK models have been constructed to evaluate the epidemiological data for this potential confounding. This includes examination of PFOA/PFOS and birth weight (Verner et al. 2015 Environ Health Perspect 123 1317-1324) whose results were recently confirmed by Steenland et al. in their meta-analysis published and available on-line in Epidemiology (<https://doi.org/10.1097/EDE.0000000000000903>). Other health outcomes that have been examined by PBPK models include PFOA/PFOS and delayed menarche (Wu et al. 2015 Environ Int 82 61-68), PFOA/PFOS and early onset of menopause (Ruark et al. 2017 Environ Int 99 245-254), and PFOA/PFOS and endometriosis (Ngueta et al. 2017 Environ Int 104 118-121). 3M is currently assessing whether the cholesterol association observed at general population PFOA/PFOS concentrations may be a consequence of one or more physiological phenomenon.

Lastly, ATSDR released their third draft PFAS toxicology profile in June, 2018. Among it, MRLs for several PFAS were derived in this draft profile, including PFOA, PFOS, and PFHxS. Should you be interested, please see <https://www.regulations.gov/document?D=ATSDR-2015-0004-0063> for 3M’s submitted comments to ATSDR during the public consultation period.

Thank you for your enthused interests in our research. We appreciate this opportunity to provide you with our data and we look forward to answering any questions or comments that you might have.

Kind regards,

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