3M Comments on ANNEX XV PFOA Restriction Report

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3M Comments on the ANNEX XV PFOA Restriction Report, 11 June, 2015

Executive Summary

Annex XV Restriction Report (herein referred to as "Restriction Report") proposes a restriction use on perfluorooctanoic acid (PFOA), PFOA salts and PFOA-related substances using a single threshold of 2 ppb. In this multi-disciplinary framework, human health hazard and risk characterization is a subcategory that incorporated both non-human (laboratory animal) and human health data in its assessment.

The Restriction Report calculated derived no-effect levels (DNELs) for 3 non-human (toxicology) and 2 human (epidemiology) study outcomes. These were: reduced mice pup weight (Lau et al. 2006); reduced neonatal survival in mice (Abbott et al. 2007); delayed mammary gland development in mice (Macon et al. 2011); increased total cholesterol and LDL in human serum (Steenland et al. 2009); and reduced birth weight in human offspring (Fei et al. 2007). A range of Risk Characterization Ratios (RCR), defined as exposure/DNEL, was derived for both workers (fluorochemical workers and professional ski waxers) and the general population (adults and children). The Restriction Report concluded "*there are strong indications that the risk is not controlled and actions are needed for both workers and general populations*."

Upon reviewing the human health assessment in this Restriction Report, 3M respectfully disagrees with its conclusion. The DNELs and RCRs are not scientifically justifiable based on the evidence chosen in this Restriction Report for (1) delayed mammary gland development in mice (Macon et al. 2011), (2) increased total cholesterol and LDL in human serum (Steenland et al. 2009), and (3) a reduction in birth weight in humans (Fei et al. 2007). 3M's review of the Restriction Report revealed a superficial analysis of both the toxicological and epidemiological literature regarding these three outcomes. *We do not believe the DNELs and RCRs cited in the Restriction Report are appropriate for human risk assessment.* Specifically:

- There is strong scientific evidence against using the mouse mammary gland data reported by Macon et al. on the basis of flawed study design, use of subjective endpoints, and erroneous data interpretation. In addition, inconsistent mouse mammary gland data reported by others reflected questionable biological significance of this endpoint.
- The use of data from Steenland et al. to infer a causal association between exposure to PFOA and increased total cholesterol: a) disregarded the fact that a cross-sectional study cannot be used to infer temporality; b) discounted viable alternative explanations; c) dismissed the lack of risk for heart disease and stroke observed in several occupational and community studies; and d) did not take into account the striking contrary evidence of hypolipidemia reported in a Phase I human clinical trial with high administered dosages of PFOA that is consistent with the animal evidence.
- The Restriction Report erroneously assumed PFOA reduces birth weight based on findings from Fei et al. and a meta-analysis (Johnson et al. 2014; Lam et al. 2014) neither of which adjusted for the major confounding effect of the glomerular filtration rate as recently demonstrated in a published PBPK model by Verner et al. (2015).

In conclusion, this assessment appears to be a biased presentation that omits contradictory evidence, excludes important data which leads to alternative conclusions, and, *most importantly*, fails to critically synthesize the literature in the weight of the evidence review for these three health-based outcomes.

Reported herein are 3M's detailed comments regarding the (1) Non-human Health Data (toxicology) and (2) Human Health Data (epidemiology). Each of these sections are sub-divided into a) overall comments, and b) specific comments according to Section B.5 of the Restriction Report.

Overall Comments

Non-human Health Data

For laboratory studies, although several long-term toxicity studies are available on PFOA in rodents and non-human primates, the ANNEX XV Restriction Report (herein referred to as "Restriction Report") relied solely on the developmental effects in rodent as the sensitive endpoints for its risk assessment. Among the three developmental studies selected for risk evaluation: Abbott et al. (2007), Lau et al. (2006), and Macon et al. (2011), only the latter study by Macon et al. (2011), which reported delayed mammary gland development in mice, contributed to risk characterization ratios (RCRs) that were greater than 1 and was subsequently identified as the critical key study to support the Restriction Report's risk characterization.

The risk assessment approach taken by the Restriction Report was not a comprehensive evaluation of the mammary gland development literature as many other studies were not properly reviewed or cited (Albrecht et al. 2013, Tucker et al. 2014, White et al. 2007, White et al. 2009, White et al. 2011, Yang et al. 2009, Zhao et al. 2010, Butenhoff et al. 2004, Biegel et al. 2001; Butenhoff et al. 2012, Butenhoff et al. 2002). Therefore, a re-evaluation of this weight-of-evidence is necessary for this Restriction Report to have scientific value.

Guidance from ECHA (Chapter R.8: Characterisation of dose [concentration]-response for human health, Version 2.1, November 2012) clearly states:

> "*The choice of key studies and derivation of DN(M)ELs will depend on expert judgement, including the use of a weight of evidence approach.*"
>
> *"A weight-of-evidence approach should be used in assessing the level of consistency of the total data base and especially of the starting point (e.g. N(L)OAEL, BMDL) to be brought forward to the risk characterisation. This approach requires a critical evaluation of the entire body of available data for consistency and biological plausibility."*
>
> *"Secondly, the hazard data should be assessed for the reliability and consistency across different studies and endpoints and taking into account the quality of the testing method, size and power of the study design, biological plausibility, dose-response relationship and statistical association (adequacy of the database).*"

The Restriction Report did not consider any other studies that have investigated the effects of PFOA on the developing mammary glands in mice as a consequence of exposure during either the *in utero* or postnatal/peripubertal window (Albrecht et al. 2013, Tucker et al. 2014, White et al. 2007, White et al. 2009, White et al. 2011, Yang et al. 2009, Zhao et al. 2010). *Importantly*, these studies either found *no effect, inhibition, or stimulation* of mammary gland development and function. Upon review of those studies, it becomes evident that the effects of PFOA on mammary gland development *cannot be consistently* described and quantified in mouse models. Therefore, despite that the availability of a large body of data on the mammary gland development in mice, the use of a single study to support risk characterization ratio derivation did not meet ECHA's own guidance.

The fact that the proposed Restriction Report failed to identify a concordance among all the available data on mammary gland development in mice brings into question the biological significance of this phenotype and its relevance to human health. This is important because the quality of the dataset chosen was not robust and the mode of action on mouse mammary gland development and its biological plausibility (to human) is not understood. Furthermore, the key non-human toxicological study chosen in this Restriction Report (Macon et al. 2011) had numerous scientific reasons why it is not an appropriate study to support risk assessment decision with its study design flaws and inadequate supporting evidence to substantiate a true effect (see Specific Comments section for details).

Specific Comments (Section by Section)

Non-human Health Data

B.5.1.9 Toxicity for Reproduction

We respectfully disagree that there is sufficient evidence to warrant classification of PFOA into Category 1B for developmental effects. The effects cited in support of the classification (increased pup mortality, decreased pup body weight, and delayed sexual maturation) occurred at maternally toxic dose levels. Developmental studies in rats and rabbits have not shown effects (Lau et al. 2004). The weight of evidence suggests that the current classification is scientifically unsupported.

B.5.1.9.1 Developmental toxicity

In non-human data, decreased fetal growth (pup body weight) is the developmental effect cited by the Restriction Report. As stated above, this occurred at maternally toxic dose levels and in itself is an indirect effect to maternal effects.

B.5.1.9.1.1 Non-Human information

In addition to the classic developmental studies described here, several toxicological studies have focused and investigated the effects of PFOA on the developing mammary glands in mice as a consequence of exposure during either the in utero or postnatal/peripubertal window. These studies were briefly discussed in the Restriction Report. Upon reviewing, it becomes apparent that these studies either found no effect, inhibition, or stimulation of mammary gland development and function (Albrecht et al. 2013, Tucker et al. 2014, White et al. 2007, White et al. 2009, White et al. 2011, Yang et al. 2009, Zhao et al. 2010). The results and findings were notably different and often reflect experimental designs, measures and assessments that lack considerable scientific rigor.

Rather than evaluating other endpoints that have been consistently seen in laboratory animals upon exposure to PFOA, the Restriction Report simply deferred to findings from the study by Macon et al. (2011) for describing effects of PFOA on the developing mammary glands and for the subsequent DNEL derivation. Using the weight-of-evidence approach set by ECHA, the inconsistencies of these (mammary gland) data do not meet the data robustness criteria. In addition, the integrity of the subsequent risk evaluation process appears to have relied upon poor data quality.

Outlined below are a number of specific concerns that warrant careful consideration before using data from Macon et al. (2011) for risk characterization.

(1). Study design & outcome

Macon et al. examined the effects of exposure to various ammonium PFOA (APFO) concentrations during gestation on mammary gland development in progeny born to CD-1 mice. A subset of females was dosed with APFO during almost the entire gestation when they received either 0 (vehicle control, DI water), 0.3, 1, or 3 mg/kg/day APFO from gestational day (GD) 1 – 17. In a separate study, other gestating females received APFO from GD10-17, at levels of 0 (vehicle control, DI water), 0.01, 0.1, or 1 mg/kg/day APFO. Treatment with APFO, a known agonist for xenosensor nuclear receptors such PPARα and CAR/PXR (Elcombe et al. 2010) resulted in increased liver weight in all offspring from

dams exposed to APFO from GD 1 – 17, and also in offspring from dams exposed to 1 mg/kg/day APFO from GD 10 – 17. Even though hepatic hypertrophy appeared to be dose-dependent, the authors concluded there was "*significantly stunted mammary epithelial growth*" concomitant with fewer terminal end buds (TEBs) for all offspring and that a no observable adverse effect level for delayed mammary gland development could not be established.

(2). Inadequate animal acclimation procedure

The pregnant mice used by Macon et al. were only acclimated to the new environment for *one* day between the time of arrival and the administration of APFO at the study facility. Given the fact that these mice were newly impregnated and had gone through various physical and environmental stresses in less than a week (*e.g.*, co-habituation with a male, mating, becoming pregnant, transportation-induced physiological changes, adaptation to a new vivarium with inherent differences in environmental conditions), it is hard to imagine that these mice did not experience undue stress between the day of arrival and the start of the study. For reasons such as these, many institutions require a minimum acclimation period for mice of 3 - 5 days prior to the initiation of any experimentation on animals (ILAR 1996).

A further reason for concern with maternal stress arises when the authors stated that 15% of females were not pregnant "*as expected*". The rationale for this so-called expectation was not explained or justified. Given that such rates of loss were stated to be unrelated to PFOA exposure, this outcome further suggested that dams were stressed. Macon et al. did not provide any indication of what treatment groups these losses occurred in or to what extent. They described that n=13 pregnant dams were assigned to each treatment group, yet went on to say that 15% of dams were not pregnant; thus, group sizes of n=13 could not have been realized in the final study.

On a related note, dams in the full-gestation study were transported around day 0 of gestation, whereas dams in the latter study (late gestation exposure) were transported around day 8 of gestation. Thus, while all females experienced the same aforementioned short (1 day) acclimation period, this stressful experience was superimposed on different stages of fetal development, which may confound any extension of results.

(3). Maternal health

Guidance from the European Union, Section 3.7.2.4.1. states:

> *"Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms."*

Therefore, it is important to be able to differentiate whether the developmental effects associated with APFO occurred in the presence or absence of marked maternal toxicity. The fact that Macon et al. did not provide any body weight data for the pregnant dams is unusual and disconcerting. Body weight data is an easy and objective clinical endpoint to measure and it is often the primary clinical index used to ascertain the well-being of an animal, especially those that are pregnant. In addition to lack of maternal body weight data, no data were provided for maternal liver weight or maternal PFOA concentrations.

(4). Litter handling / Sample selection bias

In their study, Macon et al. took newborn pups on postnatal day (PND) 1 and randomly distributed them with pups from other dams in the same treatment group. This allocation resulted in unequal numbers of pups per litter, with 7 – 9 pups per litter (4 – 7 females per litter).

A well-planned developmental study would have attempted to cull and reach equal number of pups (per litter) with natural dams when possible. The authors stated they mixed pups and litters to be consistent with the approach used in their previous study (White et al. 2007). It was not clear why pups were randomly distributed to different dams because the instinctive and protective nature of a lactating dam (i.e., sensory recognition) can compromise the quality of the care for, and even the survival of, the foster pups. This oversight may be the reason why there were unequal numbers of pups per litter.

This oversight in experimental design may also be the reason why there were insufficient control female pups survived until PND 63 for sampling. Based on the experimental description, given that n = 13 dams (a seemingly sufficient number) was assigned to each treatment group, approximately n = 11 dams would have been expected to produce litters (with the expected 15% parturition loss). Given that the litter sizes were normalized to 4 - 7 females/litter, there should have been approximately 44 - 77 female pups available for necropsy across the 7 different postnatal ages (PND 7, 14, 21, 28, 42, 63 and 84) with a minimum of 6 female pups or more per postnatal time point for evaluation. It was not clear nor discussed by the authors as to why there were insufficient control female pups on PND 63. This not only raises the question as to the cause(s) and occurrence of postnatal death in the control group, it also reflected a poor study design and a lack of knowledge in animal handling.

(5). Mammary gland biology end points

a. Subjective scoring system for mammary gland development

The methods used by Macon et al. for assessing mammary gland development in offspring were performed *subjectively* on whole mounts using a categorical scale of 1 – 4 (1 = poor development and 4 = best development). In using this approach, the authors attempted to describe many different variables within the mammary glands as a single value rather than scoring or quantifying each variable. It is critical to recognize that the mammary glands undergo several developmental processes at once (i.e., ductal growth, branching, alveolar budding) and each of these landmark events must be quantified individually. Also, each of these processes is sensitive to different developmental and reproductive cues, and any comparisons of mammary gland development need to take the accompanying biology into account, such as age, metabolic bodyweight, stage of estrous cycle, and onset of ovarian function. It is worth noting that Macon et al. did not provide any information regarding stage of the estrous cycle, sex hormone concentrations, or histology of the reproductive organs. These baseline facts should have been adequately established to allow for a proper overall assessment.

What is most disconcerting is Macon et al. combined a subjective assessment of each variable within the mammary glands (i.e., ductal growth, branching, and alveolar budding) and integrated them into a single score that was not generated mathematically. The relative contribution or weighting of each variable in the final subjective score was never defined. The statement "*It should be noted that statistical differences found in a single quantitative endpoint did not necessarily determine aberrant development; rather, all quantitative and qualitative*

measurements were collectively utilized to determine overall developmental mammary gland scores" reflected the fact that an undefined method was employed to generate their final scores for mammary gland development.

There are several significant limitations to this subjective scoring approach. A subjective scoring system precludes repetition by other laboratories, even those skilled in the art of mammary gland biology, given that the precise nature of the categorical scale is never documented. Moreover, even within the same laboratory there appears to be inconsistent definition and use of this scoring system. In their study, Macon et al. used a scale where 1 = "*poor development*" and 4 = "*best development*" that they described as being similar to methods described by other laboratories (Hilakivi-Clarke et al. 1997a, Hilakivi-Clarke et al. 1997b, Welsch et al. 1988). It is interesting to note that 2 of the 3 referenced papers (Hilakivi-Clarke et al. 1997b, Welsch et al. 1988) used rats (not mice) as the test subjects. In addition, the development of the mammary glands in rats is considerably different from that in mice, thereby raising questions as to how the scale was developed or implemented. The remaining referenced study also used CD-1 mice (Hilakivi-Clarke et al. 1997a), but that paper did not provide sufficient information that would enable replication of their scoring method.

Macon et al. described that "*Scores were based on qualitative and quantitative histological characteristics of each developmental time point, including, but not limited to, lateral and longitudinal epithelial growth, change in epithelial growth, appearance of budding from the ductal tree, branching density, and number of differentiating duct ends (Hilakivi-Clarke et al. 1997a). Where applicable, at a given time point, mammary glands from both studies were compared on the microscope to ensure consistency in the scoring scale between studies*". It is unclear what other variables contributed to the subjective score given the results were not limited to those variables detailed above.

By contrast, in a similar study from the same research group, White et al. (2011) used a scale where 4 = "*excellent development/structure*" and 1 = "*poor development/structure*". The number of primary ducts and large secondary ducts, lateral side branching, appearance of budding from the ductal tree, and longitudinal outgrowth were assessed. Thus, in two studies from the same laboratory (Macon et al. and White et al.) published in the same year, there was variation between the scoring criteria and strategies used. Likewise, in both cases, it was not clear whether "*best development/structure*" and "*excellent development/structure*" scores are synonymous, and whether a score of 4.0 represents that of an average control gland for a given age, which one might expect. In another instance, while Macon et al. reported the control mammary glands at PND21 with average scores of 3.3 (see Table 1, Macon et al. 2011) and 3.4 (see Supplemental Table 3, Macon et al. 2011), a recent study from the same laboratory, control glands from CD-1 mice at PND21 received a mean developmental score of 2.9 (Tucker et al, 2014). Even though the exact measures used to compute this score was not documented, it did appear that a score of 4.0 was realizable for control glands, as occurred at PND 84 (Macon et al. 2011).

Similarly, there appeared to be considerable variation among the population of CD-1 mice in this laboratory at PND21. The only data that were found to be statistically different at the 0.01 mg/kg dose at PND 21 was the value for this subjective developmental score (see Table 4,

Macon et al. 2011). Even at 0.1 mg/kg, statistical differences were only detected for this subjective score and another quantitative measure of terminal end bud number. The ductal tree in the mammary glands of control females at PND 21 had only outgrown a few millimeters (see Figure 1A, Macon et al. 2011). By contrast, in comparable control CD-1 females at PND 21 in a recent paper from the same laboratory, the mammary ducts at PND21 have already reached the supramammary lymph node (see Figure 2A, Tucker et al. 2014), although again, no quantification of mammary growth was performed in that study. This difference is on the order of several millimeters, which relative to the size of the ductal tree at PND21, is substantial. *This dramatic difference in mammary gland development within the control population of CD-1 mice from this facility raises concern about how the mammary gland is being used as a toxicological end point*.

Another consideration that warrants further evaluation concerns the incorrect statistical inferences that have been made in analyzing the subjective mammary gland scores. By using a subjective scale, Macon et al. utilized a categorical method to generate their data. In performing their statistics by analysis of variance they assumed (incorrectly) that their mammary development scoring system increased linearly and/or with consistent increments. This assumption and statistical test is fundamentally invalid, further calling into question any conclusion about the low dose effects reported by Macon et al.

b. Lactation performance of dams as a critical variable

Another important consideration for this study and any study of gestational exposure concerns the consequences for the dam as she goes on to rear offspring. Specifically, the process of lactation is sensitive to a number of factors that can impact a dam's ability to provide milk to her offspring, thereby suppressing their development and that of their organs, including the mammary glands. Two processes that are most susceptible to such exposures are: 1) functional development of the dam's mammary glands during pregnancy in readiness for lactation; and 2) dam's ability to metabolically adapt to the massive nutrient demands of milk synthesis and secretion.

Studies by the same research group had suggested that exposure of pregnant mice to PFOA impaired the ability of the maternal mammary glands to undergo full growth and functional differentiation (White et al. 2007; White et al. 2009). In these studies, dams exposed to 5 mg/kg PFOA during gestation weaned pups at PND20 that were 33% lighter in bodyweight than controls (White et al. 2007), while White et al (2009) also found reductions in weaned bodyweight following *in utero* plus lactational exposure to 3 mg/kg PFOA. It is unclear why Macon et al. did not find this same effect on progeny bodyweight at the 3 mg/kg dose.

Regardless, one must consider the potential for one or more aspects of pre-weaning development to be disrupted as a result of impacts on the lactational capacity of the exposed dams. A point that is relevant to the findings by Macon et al. is that growth of the mammary glands in female mice offspring before the onset of allometric growth at puberty is isometric – that is, mammary gland development is proportional to body size when it is expressed as a function of their "metabolic bodyweight" (typically considered to equal $BW^{0.66\text{-}0.75}$). Hence, any measure of

mammary gland development should be expressed relative to metabolic bodyweight, not merely total bodyweight. This type of correction cannot be performed for subjective scores.

A parallel consideration that must be taken into account is the energy expenditure by dams when litter size varies as it did in this study. Each unit volume of milk secreted contains considerable energy derived from the dam's reserves and from her nutrient intake. This point is relevant when considering the work by Macon et al. given that litter size varied. For example, in full-gestation exposure study the authors state they balanced litters to 10 pups despite not being able to realize a 50/50 male/female target ratio. In late-gestation exposure study the authors declared they had litter sizes ranging from 7-9 pups. A difference in litter size such as this can dramatically affect maternal performance – a dam feeding 7 pups expends considerably less energy for milk production than a dam feeding 10 pups in a litter. In turn, these differences in metabolic state of the dam can have major ramifications for milk yield and quality that can then go on to affect many aspects of pup growth and development.

(6). Data presentation bias

The authors provide a few quantitative measures in terms of mammary gland measurement for a subset of the study samples (from the late gestation study; see Table 1, Macon et al. 2011) although it is not clear (or explained) why no similar data were shown for the mice from full-gestation study (subjective scores only, supplemental data Table 3). Thus, one must consider that the data set, as presented, is incomplete. It should be noted that Macon et al. disregarded significant outliers without explanation.

Regarding the mammary gland assessments for the full gestation study, Macon et al. stated that there were histological characteristics similar to previous findings; unfortunately they did not show any histological data at all.

The representative mammary glands presented in Figure 1 from Macon et al. (2011) did not align well with the author's claims. Macon et al. stated that mammary glands from the 0.3 and 1.0 mg/kg treatment groups were less developed, however the variation was substantial and much of this could be explained by variables such as individual differences, stage of estrous cycles, or lack of, for that matter. In particular, it should be noted that being an outbred strain, CD-1 mice have more inherent variation within their phenotypes. Macon et al. also emphasized that in the mature mouse mammary gland "*….in the adult mouse at PND 84, there are no TEBs*". However, there did not appear to be any visual differences in the distribution of TEBs presented as the examples in histological sections of the mammary glands for PND 84 between control (Figure 1D) and female pups from 0.3 mg/kg (Figure 1E) and 1 mg/kg (Figure 1F) dose groups. This raises the question whether the qualitative scores used by Macon et al. have a strong foundation based on histological analyses. The Restriction Report should re-examine the histology data presented by Macon et al.

Regarding the late gestation study, the authors reported reduced elongation at PND 14 by 14.4 and 37% in the 0.1 and 1 mg/kg doses (see Table 1, Macon et al. 2011), whereas in Figure 4 the most pronounced reduction is at PND 21. This figure would have benefited from counting number of ductal branches. The authors show reduced TEB number at PND 21 in Table 1; it is unclear however where the other quantitative data for the rest of the experiment are.

Conclusion

The study by Macon et al. (2011) was flawed in several important aspects of study design and had numerous instances of inappropriate data interpretation. The authors failed to consider all aspects of biology and rather than scope out the best objective endpoints for the assessment, the study gave very few quantitative measures. The authors had attributed various phenotypic consequences (i.e., reduction in mammary gland development) to the direct effects of PFOA. Alternative interpretations suggest that PFOA may be affecting mammary gland function in the lactating dams. Without any supporting evidence for maternal well-being, the data presented by Macon et al. are built on a great deal of speculation with a lack of definitive reproductive data combined with a lack of quantitative mammary gland analysis.

Subsequent to this report, a follow-up manuscript was recently published by the same group (Macon et al. 2014, doi: 10.1093/toxsci/kfu253) in which they examined gene expression within the mammary glands of mice exposed to PFOA. However, this publication was later *retracted* due to erroneous data presentation.

In conclusion, 3M believes there are important technical reasons to question the study data by Macon et al. (2011). The fact that the effects of PFOA on mammary gland development cannot be consistently described and quantified in all mouse models brings into question the biological significance of this phenotype as described, and its relevance to human health is unclear.

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Zhao Y, Tan YS, Haslam SZ, et al. 2010. Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57Bl/6 mice. Toxicological Sciences 115:214-224.

Overall Comments

Human Health Data

According to the Annex XV Restriction Report (herein referred to as "Restriction Report"), the human health hazard assessment focused on the assertion that "*PFOA is toxic for reproduction levels and that it affects human cholesterol levels*." The use of the word "*affects*" implies a causal association in humans.

While it is recognized there have been several epidemiological studies (the great majority cross-sectional) that have reported positive associations between serum PFOA concentrations and increased cholesterol, these studies, whether individually or collectively, are unable to establish methodologically a causal association. Nevertheless, this Restriction Report chose one of these cross-sectional studies (Steenland et al. 2009) to estimate an effect by calculating DNELs for workers and the general population under the pretense of "*increased total cholesterol and LDL*." The Restriction Report did not rule out whether this study and other cross-sectional studies like it, are actually observing associations that are the consequence of chance, bias, or confounding. For example, the Restriction Report chose not to consider the distinct possibility that the association with PFOA at low serum concentrations (Steenland 2009 and other studies) and "*increased total cholesterol and LDL*" may be the consequence of a saturated response reflecting physiological conditions or unknown confounding factors including binding to lipoproteins or a relationship with bile acids. The Restriction Report acknowledged but did not consider contradictory evidence that multiple worker and community studies have not observed an increased risk for heart disease, stroke, or hypertension that would be expected in populations with hypercholesterolemia. The Restriction Report did not cite the findings from a Phase I clinical trial, where patients received high dosages of PFOA (up to 1200 mg weekly for six weeks), and reported *lowered* LDL levels, a finding that is *consistent* with the hypolipidemic response in animal studies**.** In not considering the data from this Phase I clinical trial, the Restriction Report avoided having to ask itself the troubling question as to how exposure to PFOA can result in *hypolipidemia* with high administered dosages in a Phase I clinical trial and also cause the effect of *hypercholesterolemia* in humans with serum PFOA concentrations that are orders of magnitude lower.

In avoiding to address the above questions, the Restriction Report gave undue credence to the C8 Science Panel probable link opinion regarding hypercholesterolemia. The C8 Science Panel was not a governmental regulatory body held publicly accountable to anyone except to a West Virginia (state) district court and the settling parties in a class action lawsuit. While many of the individual C8 Science Panel studies have been subjected to peer review, their probable link opinions - which stated only if a "*connection*" exists between PFOA and a health outcome in the studied population – have not been. The C8 Science Panel has acknowledged their probable link opinions may not be confirmed with additional research and other associations may have been missed.

The Restriction Report also concluded that PFOA "*reduced birth weight in humans*". Using the Fei et al study (2007), the Restriction Report considered there was a 5.6 g reduction in birth weight caused by a 1 ng/mL increase in maternal plasma PFOA. The Restriction Report supported its selection of the study by Fei et al. (2007) by relying on a meta-analysis of 9 studies (including Fei et al.) conducted by Johnson et al. (2014) that suggested PFOA was a human reproductive toxicant: the meta-analysis estimated a reduction of 18.9 grams in birth weight per 1 ng/mL increase in serum or plasma PFOA.

The above association became questionable when considerable evidence was developed (Morken et al. 2014) that showed a significant association between glomerular filtration rate (GFR) and birth weight, thus making GFR a potential confounder in the association between PFOA and birth weight. Verner et al. (2015) developed a physiologically-based pharmacokinetic model (PBPK) that indeed, demonstrated a substantial proportion of the association between PFOA and birth weight may be attributable to confounding by GFR. Verner et al.

(2015) estimated, in their meta-analysis of 7 (including Fei et al.) of the 9 studies included by Johnson et al. (2014), that the GFR explained up to 50% of the association between PFOA and reduced birth weight. This confounding may be more prevalent in studies with serum PFOA concentrations measured later in pregnancy. Therefore, the Restriction Report must consider the confounding attributable to GFR when studying an association between measured PFOA and birth weight.

3M respectfully disagrees with the Restriction Report's conclusion that PFOA increases total cholesterol and LDL and reduces birth weight in humans. The DNELs and RCRs for "*increased total cholesterol and LDL*" and "*reduced birth weight*" are irrelevant in this Restriction Report because they are based on the faulty assumption that the epidemiologic evidence has shown PFOA to increase total cholesterol and LDL and reduce birth weight. Such an "*affect*" is not established in the scientific literature. The associations published in the literature as cited in the Restriction Report are shown to be either highly inconsistent and/or confounded by underlying physiologic processes.

Provided below are numerous detailed comments, section by section of the Restriction Report, that present 3M's position why the Restriction Report has failed to provide a scientifically defensible review of the epidemiologic literature.

Specific Comments (Section by Section)

Human Health Data

B.5 Human Health

B.5.1 Human health hazard assessment

The Restriction Report states "*the human health hazard assessment in the following sub-chapters focuses on PFOA as toxic for reproduction and that it affects human cholesterol levels*." 3M respectfully disagrees that this Restriction Report has shown PFOA to affect (increase) human cholesterol levels and reduced birth weight. The specific comments that follow, section by section, will detail multiple deficiencies in the arguments made in this report.

B.5.1.6.2 Human information

Probable link reports from C8 Science Panel, based on epidemiological data

The creation of a 3-member C8 Science Panel (Steenland et al. 2014) was the result of a 2004 settlement of a class action lawsuit in a mid-Ohio river community between residents and DuPont through the Woods County circuit court (West Virginia). This legal agreement between the plaintiffs and defendant (DuPont) acknowledged that the C8 Science Panel had to have complete independence from both parties in order to conduct the epidemiological research that would be funded by the defendant through the state district court. Ultimately the C8 Science Panel conducted 12 studies over 5 years and determined probable link judgements for 55 diseases evaluated, including 21 cancers and a number of conditions such as hypertension and hypercholesterolemia (Steenland et al. 2014). A probable link was defined as "given the available scientific evidence, it is more likely than not that among class members a connection exists between PFOA exposure and a particular human disease." See http://www.c8sciencepanel.org/prob_link.html. The word "*connection*" is not part of the epidemiology lexicon. It may best be described as an association (i.e., statistical association). The C8 Science Panel was never charged by the state district court to establish causality, only whether a "*connection*" existed.

The C8 Science Panel considered there were six diseases and conditions that were "*more probably than not linked to PFOA exposure*" in this mid-Ohio river community: kidney cancer, testicular cancer, ulcerative colitis, thyroid disease, hypercholesterolemia, and pregnancy-induced hypertension (Steenland et al. 2014). However, the C8 Science Panel also stated that as more scientific evidence accumulates, "some associations may not be confirmed. Others may be identified that we (the C8 Science Panel) had missed" (Steenland et al. 2014).

Although not acknowledged by the Restriction Report, the C8 Science Panel *was not a public (governmental) body*. Unlike governments that solicit public comments on carcinogenic classifications, exposure guidance, and rule making, the C8 Science Panel conducted their deliberations in private (voting of the 3 members per each disease and health condition to obtain a majority opinion). The C8 Science Panel released their consensus of the six probable link determinations to the settling parties and written conclusions on their website. While many of the individual C8 Science Panel studies have been published in the peer reviewed scientific literature, the probable link determinations were never subjected to an external scientific peer review process – as the court settlement considered the C8 Science Panel to be this review process. The only entities that agreed to abide by the C8 Science Panel's conclusions were the settling parties. The Restriction Report should therefore

be careful to distinguish the acknowledgment of the scientific opinions offered by the C8 Science Panel versus granting a deferential treatment to these viewpoints. Even the C8 Science Panel has acknowledged their probable link determinations may or may not be confirmed with future research.

Probable link reports from C8 Science Panel on elevated cholesterol levels

Contrary to the Restriction Report's writing in the above section, the C8 Science Panel did not gather information on the baseline health status and PFOA exposure from people living near the DuPont Washington Works plant in West Virginia (Steenland et al. 2014). The original baseline survey, referred to as the C8 Health Project, was conducted by Brookmar, Inc. which was created by physicians for the purposes of gathering health and exposure information as approved by the court system. Brookmar, Inc. took approximately one year to complete the task at a cost of about 70 million dollars (Steenland et al. 2014). Each participant was paid $400 for their participation which included a health and exposure (e.g., occupational, residential) questionnaire and collection of a blood sample for analysis of PFOA and other perfluoroalkyls. A total of 69,030 individuals participated in the C8 Health Project of which 54,457 were >= 20 years of age. The Department of Community Medicine at the University of West Virginia agreed to be the data hosting site that managed the C8 Health Project database. This C8 Health Project cross-sectional database was then used by the C8 Science Panel in their analysis of PFOA measurements and serum cholesterol levels in children (Frisbee et al., 2010) and adults (Steenland et al. 2009).

As reported in both of these cross-sectional studies (Frisbee et al. 2010; Steenland et al. 2009), a positive association between measured serum concentrations of PFOA and total cholesterol was observed. As noted by the Restriction Report, the association among adults was "*steepest*" among those with lower PFOA concentrations. Figure A is from the Steenland et al. (2009) study of 46,294 adult residents 18 years or older that showed this "steep" association with rising cholesterol levels that was observed below 50 ng/mL PFOA. Similar curves were displayed by Steenland et al. (2009) for LDL and triglycerides but not HDL. However, it is incorrect to infer such steepness is "*indicating a low dose effect*." No cross-sectional study can describe an effect because of its inability to separate the temporality between exposure and response. It only describes a cross-sectional study relationship between serum cholesterol and measured serum PFOA levels in these adults. The measured serum PFOA levels shown on the x-axis of Figure A is not a "dose" (e.g., mg/kg/d).

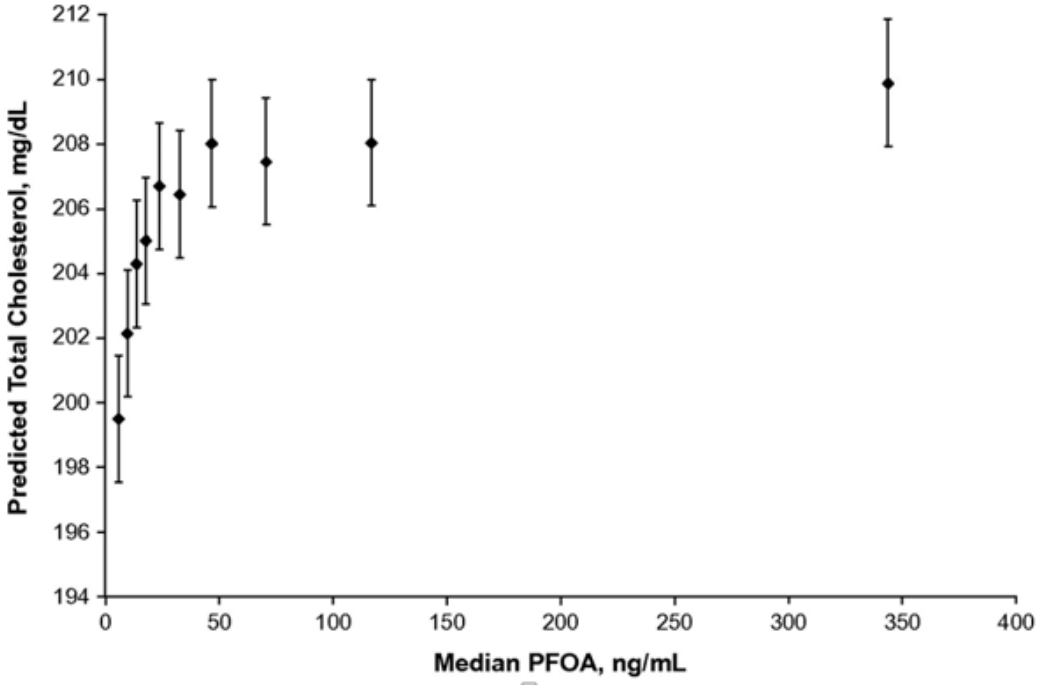

Figure A. From Steenland et al. Am J Epidemiol 2009;170:1268-1278. Total cholesterol by decile of perfluorooctanoic acid (PFOA), with 95% confidence intervals, Ohio and West Virginia, 2005–2006. The model was adjusted for covariates. The *x*-axis uses medians of PFOA deciles. Predicted cholesterol levels are based on an "average" subject with the following characteristics: age 60–69 years, male gender, not taking cholesterol-lowering medication, never smoked, high school diploma, does not participate in regular exercise, does not drink alcohol, and body mass index between 24 and 27 kg/m^2. Confidence intervals are based on the predicted population mean given those covariate levels. Study population = C8 Health Project (cross-sectional).

Also, Steenland et al. calculated, by quartiles (not deciles as shown in Figure A), the odds ratios for high cholesterol (defined in adults as >= 240 mg/dL). These odds ratios were 1.00 (referent), 1.21, 1.31, and 1.40 that Steenland et al. suggested was an upward trend in the magnitude of the risk from the 2nd through 4th quartiles of PFOA concentrations.

For reasons that have not been discussed by the C8 Science Panel, a nearly identical "*steep*" curve (including confidence intervals) was published between their cross-sectional analysis of measurements of serum PFOA and uric acid in this adult C8 Health Project population (Figure B) (Steenland et al. 2010a) as was seen with total cholesterol and PFOA (Figure A) (Steenland et al. 2009). Similar to the hypercholesterolemia analysis provided by Steenland et al. (2009), Steenland et al. (2010a) calculated the odds ratios by quartile for hyperuricemia (> 6.0 mg/dL for women, 6.8 mg/dL for men) by quintile of PFOA: 1.00 (referent), 1.33, 1.35, 1.47, and 1.47 with the same plateauing of risks as seen with hypercholesterolemia.

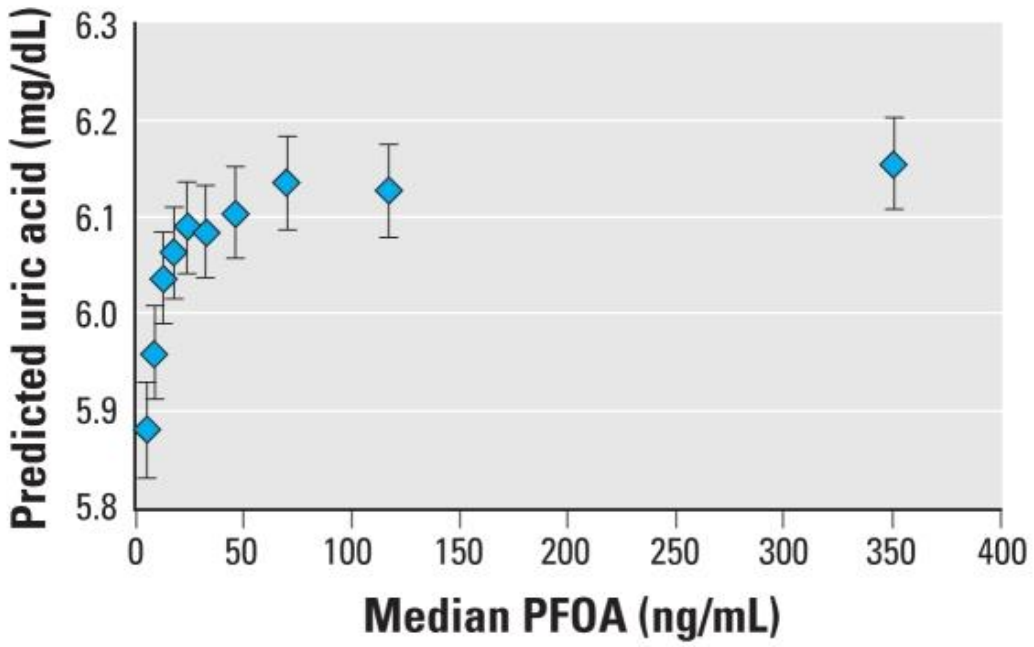

Figure B. From Steenland et al. Environ Health Perspect 2010; 118:229-233. Predicted uric acid with increasing PFOA. Predicted value from regression model for an average participant: 45 years of age, 0.95 mg/dL creatinine, high school education, male, 28.55 kg/m^2 BMI, nonsmoker, nondrinker. Data are population means and 95% CIs.. Study population = C8 Health Project (cross-sectional).

While median PFOA was ≤ 50 ng/mL, both Figure A and Figure B have steep curves in the dependent variable (cholesterol or uric acid), the range of these measurements were quite narrow (199 to 207 mg/dL and 5.85 to 6.15 mg/dL for cholesterol and uric acid, respectively).

Both curves (Figures A and B) could suggest similar selection bias issues that occurred while obtaining subjects with the lower concentrations of PFOA, similar confounding factors that were not considered in the analyses, or saturated responses. Unfortunately, like the C8 Science Panel, the Restriction Report did not comment on these essentially identical curves (Figures A and B).

If each curve is considered separately, possible explanations for the association becomes clearer, especially with uric acid. Both PFOA and uric acid bind to organic anion transporters (e.g., URAT1) in their secretion and reabsorption in the renal proximal tubules (Han et al. 2012). A positive association between PFOA and uric acid in children has also been reported in the NHANES database with lower serum PFOA levels (Geiger et al. 2013) leading Shankar et al. (2011) to suggest that PFOA was associated with chronic kidney disease in the U.S. general population. Chronic kidney disease is defined by a decline in the GFR which also results in an increase in retention of uric acid. This association, between measured PFOA and estimated GFR, was also observed in the C8 Science Panel database of healthy children (Watkins et al. 2013). However, Watkins et al. found no association with kidney function (i.e., GFR) when PFOA was estimated via their historic exposure reconstruction models for PFOA rather than using cross-sectional measurements of PFOA. This led Watkins et al. to suggest that cross-sectional associations between the estimated glomerular filtration rate and serum PFOA

may be "*a consequence of, rather than a cause of, decreased kidney function*." Furthermore, there was not an increased risk in the diagnosis of chronic kidney disease and modeled cumulative PFOA exposure. See C8 Science Panel website http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Kidney_29Oct2012.pdf.

For the question of increased total cholesterol, similar inquiry is required before any causal inference can be considered. Could there be receptors in the gut for PFOA and cholesterol that are saturated at similar concentrations of PFOA? Frisbee et al. (2010) raised this very question of a saturated response from their cross-sectional analysis of the C8 Health Project children data (Figure C). (Note: The odds ratios for 'high cholesterol (defined as ≥ 170 mg/dL) showed a plateaued trend in risk with increasing quintile cutpoints of PFOA exposure: 1.00 (referent), 1.1, 1.2, 1.2, and 1.2.) Specifically, Frisbee et al. wrote "*the nonlinear nature of the observed associations, particularly for PFOA, suggests a possible saturation point in an underlying physiologic mechanism*." It should be noted that Steenland was a co-author of the Frisbee et al. paper. If this is a possible explanation for the children data in Figure C (Frisbee et al. 2010), could it not also be a viable explanation for the adult data displayed in Figure A (Steenland et al. 2009)?

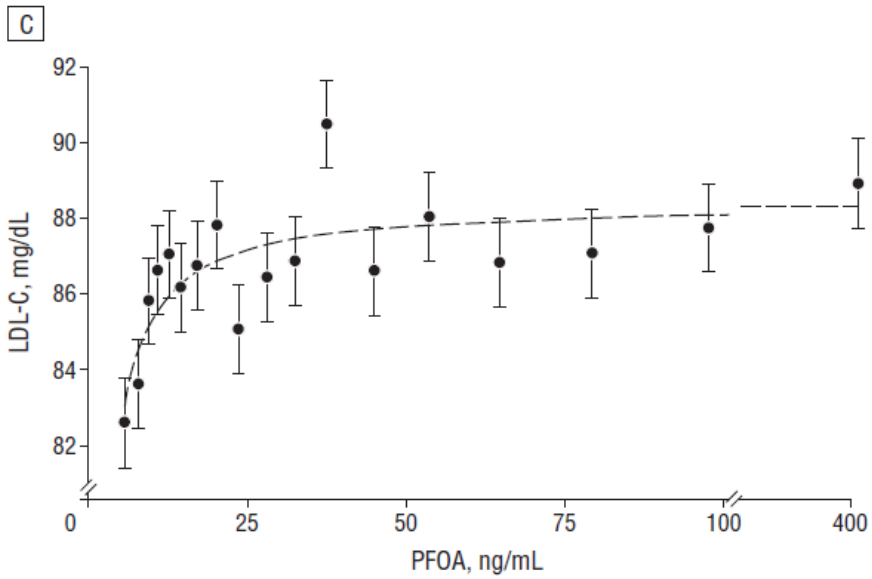

Figure C. From Frisbee et al. Arch Pediatr Adolesc Med 2010;164:860-869. LDL cholesterol (mg/dL) with increasing PFOA (ng/mL) with 95% confidence intervals. Mid-Ohio River Ohio and West Virginia communities, children data, 2005-2006.

Other questions should be asked about the steepness of the cholesterol curve. Does serum PFOA preferentially bind to lipoproteins? Because PFOA binds to serum proteins (*e.g.*, albumin), Butenhoff et al. (2012) questioned whether PFOA distributes into serum lipoprotein fractions that could be evident in populations with minimum exposure to PFOA. Their analysis did not offer much support for such a hypothesis. However, it was quite limited because of its sample size. Even the Restriction Report questioned whether alternative explanations might include high lipid contents that may increase the retention of PFOA in the body. Other thought-provoking questions that require answers are the following: 1) Is there any evidence that this cross-sectional curve, or a possible suggestion of hypercholesterolemia, results in known disease outcomes (coronary artery disease, stroke, and hypertension)? 2) Has anyone attempted to examine this association similar to Watkins et al. (2013) using modeled concentrations of PFOA rather than measured? 3) What about high concentrations of PFOA? 4) At high PFOA dosages, is there any concordance with lipid findings between animal results and humans?

Other reports on elevated cholesterol levels associated with PFOA exposure

As discussed in this section of the Restriction Report, a positive association between low serum concentrations of measured PFOA and cholesterol has been observed in several general populations (*e.g.*, Nelson et al. 2010; Eriksen et al. 2013; Starling et al. 2014), but not always including null findings from Fisher et al. (2013) and Patel et al. (2013), neither cited by the Restriction Reported. The magnitude of effect (if any) observed in these general population studies is not observed in the occupational studies as was well-recognized by Steenland et al. (2010b). As an example of this difference, are the cross-sectional findings of analyses of the general population (NHANES) found in Table 1 (Nelson et al. 2010) and an occupational population found in Table 2 (Olsen and Zobel 2007). Clearly there are several orders of magnitude of differences in PFOA concentrations in these two cross-sectional studies. Yet a positive association between PFOA and cholesterol was only observed among the much lesser exposed general population (NHANES).

Table 1. Distribution of PFOA and cholesterol, persons 20-80 years of age, NHANES, 2003-2004. See Nelson et al. (2010). Environ Health Perspect 118:197-202. See supplemental material.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend
N	223	211	186	240	
PFOA (ng/mL) Median (range)	2.1 (0.1 – 2.7)	3.4 (2.8-3.9)	4.6 (4.0-5.4)	6.9 (5.5-37.3)	
Unadjusted mean values					
Total cholesterol	198.6	201.6	202.0	205.7	
HDL	56.8	54.3	52.7	54.2	
Non-HDL	141.8	147.3	149.4	151.5	
Adjusted mean differences (from reference), 20 – 80 years of age					
Total cholesterol	reference	5.40 (-2.11, 12.92)	7.50 (-3.71, 18.71)	9.76 (-0.23, 19.74)	0.07
HDL	reference	-2.01 (-3.60, -0.42)	-1.61 (-4.62, 1.40)	-1.28 (-3.45, 0.89)	0.34
Non-HDL	reference	7.41 (-0.97, 15.80)	9.11 (-0.97, 15.80)	11.03 (1.20, 20.86)	0.05

Adjusted for age, gender, race/ethnicity, socioeconomic status, saturated fat intake, exercise, time in front of a TV or computer BMI, alcohol consumption, smoking, and parity

Table 2. Adjusted mean for lipid clinical chemistry results, by PFOA decile. Cross-sectional analysis of 506 male perfluorochemical employees not taking cholesterol lowering medications. 3M Company. Antwerp, Decatur, and Cottage Grove locations. See Olsen and Zobel (2007). Int Arch Occup Ind Hyg 81:231-246.

Decile	PFOA (ng/mL) Median[a]	Cholesterol[b,c]	LDL[b,c]	HDL[b,c]	Triglycerides[b,c]
1	60	214 (203-225)	137 (127-147)	50 (46-53)	145 (116-173)
2	200	211 (199-222)	135 (125-145)	51 (48-54)	124 (95-153)
3	360	209 (198-220)	128 (118-138)	51 (48-54)	153 (124-182_
4	540	210 (199-222)	133 (123-143)	50 (46-53)	145 (116-175)
5	910	217 (206-229)	140 (130-150)	48 (45-51)	162 (133-191)
6	1250	218 (206-229)	141 (130-151)	48 (45-51)	160 (131-190)
7	1630	214 (203-225)	133 (123-143)	50 (47-53)	158 (128-187)
8	2180	215 (204-226)	136 (126-146)	47 (44-50)	172 (144-201)
9	2960	221 (210-233)	140 (130-150)	48 (44-52)	165 (135-194)
10	4940	216 (204-227)	133 (123-144)	44 (41-47)	208 (179-238)

a. ng/mL
b. mg/dL
c. Adjusted for age, BMI, alcohol

Adding perplexity to the results from the Nelson et al. (2010) study is the analysis by Patel et al. (2013) who conducted a systemic environmental-wide association study (EWAS) of 4 NHANES databases (1999-2006) on 188 environmental factors and serum lipids. They reported no association between the polyfluorochemicals reported in NHANES (including PFOA) and LDL HDL, and serum triglycerides in their multi-chemical evaluation for the one 2 year cycle they were able to examine (2005-2006). Others have also expressed important general reservations about the NHANES database regarding using individual environmental chemical (biomonitoring) measurements in cross-sectional analyses (LaKind et al. 2012; Sobus et al. 2015).

If a positive association exists between lower PFOA concentrations and cholesterol, that could be due to an unknown saturated response as discussed by Frisbee et al. (2010) (i.e., a non-causal association) and not observed among more highly exposed occupational populations (Olsen and Zobel 2007; Sakr et al. 2007), is there evidence that the opposite effect (hypolipidemia) actually occurs among the highest exposed humans? The answer is "yes".

Findings from a Phase I dose-escalating clinical trial (conducted in Scotland) that administered PFOA (ammonium salt) to 50 cancer patients (primarily solid tumors) who had exhausted standard medical/surgical therapy, showed reductions in LDL at the higher administered dosages of PFOA (Macpherson et al. 2011). The weekly dose of PFOA (ammonium salt) over a six-week time period, escalated in patient groups of 3 in this trial, was from 50 mg/kg to 1200 mg/kg. The 1000 mg/kg/week dose was considered the maximum tolerated dose. Serum PFOA concentrations approached 500,000 ng/mL with these higher doses. The mode of action for lipid-lowering is probably through $PPAR_{alpha}$ activation, which is known to occur in the rodent. However, humans have less $PPAR_{alpha}$ receptors and are considered less responsive (Klauning et al. 2003).

Another question to be addressed by those who would argue a "*low dose effect of PFOA with increased cholesterol*" is whether there is an increased risk of heart disease or stroke. The answer appears to be "No". Although the Restriction Report suggested as such, actual data were not presented. This omission is rectified in these comments. Whether it was an occupational cohort mortality study of the DuPont Washington Works plant that used PFOA as a processing aid in the polymerization of tetrafluoroethylene (Table 3), a disease incidence cohort study of the same DuPont population (Table 4), or a cohort study of the mid-Ohio river valley community whose water contained PFOA from the environmental emissions of the DuPont plant (Tables 5 and 6), increased risks for coronary artery disease, stroke, and hypertension have not been observed. Furthermore, an independent (not from the C8 Science Panel) group of investigators (Raleigh et al. 2014) examined the 3M Cottage Grove cohort that actually manufactured PFOA (ammonium salt) (this study was also not cited in the Restriction Report) and did not observe increased risks in these manufacturing workers for mortality from coronary heart disease or stroke (Table 7). All of these studies used internal referent groups to avoid the healthy worker effect in their analyses.

Table 3. Standardized Mortality Ratios (95% confidence intervals) for ischemic heart disease and stroke. DuPont Washington Works plant. Reference = other DuPont workers in the Appalachian region. See Steenland and Woskie. (2012). Am J Epidemiol 176:909-917.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Ischemic Heart Disease				
No lag*	1.07 (0.85-1.32)	1.02 (0.80-1.28)	0.87 (0.67-1.11)	0.93 (0.86-1.09)
(N = 287 deaths)				
10 year lag**	0.95 (0.76-1.18)	1.01 (0.79-1.27)	0.93 (0.71-1.20)	0.59 (0.93-1.20)
(N = 273 deaths)				
20 year lag***	1.00 (0.79-1.24)	0.92 (0.70-1.18)	1.05 (0.79-1.37)	0.89 (0.65-1.18)
(N=243 deaths)				
Stroke				
No lag*	0.63 (0.85 (1.32)	0.78 (0.39-1.39)	1.34 (0.82-2.07)	0.69 (0.32-1.31)
(N = 50 deaths)				
10 and 20 year lags not provided				

*Modeled cumulative PFOA by quartile: 0 - <ppm-years; 904 – 1520 ppm-years; 1,520 - <2700 ppm-years; >-2700 ppm-years where ppm = 1000 ng/mL

**Modeled cumulative PFOA by quartile: 0 - <798 ppm-years; 798 - < 1379 ppm-years; 1379 - <2384 ppm-years; >=2384 ppm-years

***Modeled cumulative PFOA by quartile: 0 –<515 ppm-years; 515 – 1057 ppm-years; 1057 - <1819 ppm-years; >=1819 ppm-years

Table 4. Disease-specific relative risks (95% confidence interval) from Cox regression models of a cohort incidence study of the DuPont Washington Works plant and modeled PFOA exposure. See Steenland et al. J Occup Environ Med 72:373-380

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value Trend
Hypertension medications (N = 1430 cases)					
No lag	1.00	0.95 (0.81-1.11)	0.97 (0.82-1.15)	1.04 (0.87-1.25)	0.99
10 year lag	1.00	0.95 (0.81-0.98)	0.91 (0.75-1.09)	0.95 (0.77-1.16)	0.12
Coronary artery disease (N = 380 cases)					
No lag	1.00	1.03 (0.71-1.49)	1.23 (0.84-1.78)	1.03 (0.70-1.52)	0.39
10 year lag	1.00	1.20 (0.82-1.75)	1.06 (0.71-1.58)	0.93 (0.61-1.41)	0.99
Stroke (N = 108 cases)					
No lag	1.00	2.63 (1.06-6.59)	2.13 (0.83-6.44)	2.07 (0.81-5.29)	0.17
10 year lag	1.00	1.48 (0.56-3.69)	1.53 (0.60-3.89)	1.33 (0.51-3.43)	0.22

*Quartile cut-offs for the no lag analysis were (ng/mL-year): 3030; 6160; 11420

*Quartile cut-offs for the 10-year lag analysis were (ng/mL-year): 800; 3440; 7040

Table 5. Hazard ratios (95% confidence interval) for hypertension and coronary artery disease by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Winquist and Steenland (2014) Environ Health Perspect 122:1299-1305.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Hypertension*					
	1.00	1.10 (1.02-1.19)	1.10 (1.02-1.18)	1.05 (0.97-1.12)	0.98 (0.91-1.06)
Coronary artery disease**					
	1.00	1.26 (1.10-1.45)	1.17 (1.02-1.35)	0.99 (0.86-1.14)	1.07 (0.93-1.23)

* Modeled cumulative PFOA ng/mL per year by quintile: <111; 111- <191; 191 - <471; 471 - 2763; >=2763

**Modeled cumulative PFOA ng/mL per year by quintile: <147; 147 - <248; 248 - <717; 717 - <5058; >= 5058

Table 6. Hazard ratios (95% confidence interval) for stroke by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Simpson et al. (2014) Environ Res 127:22-28.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Stroke					
Retrospective model*					
(825 cases)	1.00	1.39 (1.11-1.76)	1.36 (1.08-1.71)	1.45 (1.15-1.82)	1.13 (0.90-1.44)
Prospective model**					
(252 cases)	1.00	1.07 (0.73-1.59)	1.07 (0.72-1.58)	1.18 (0.79-1.75)	0.87 (0.58-1.30)

* Modeled cumulative PFOA ng/ml per year by quintile: <178; 178 - <319; 319 - <912; 912 - <4490; >=4490

** Modeled cumulative PFOA ng/ml per year by quintile: <244; 244 - <460; 460 - <1240; 1240 - <5500; >=5500

Table 7. Hazard ratios (95% confidence intervals) for ischemic heart disease and cerebrovascular disease (stroke) by PFOA (ammonium salt) quartiles of exposures to a non-exposed referent plant population. 3M Cottage Grove manufacturing plant. See Raleigh et al. (2014). Occup Environ Med 71:500-506.

	Non PFOA Production Plant	PFOA Manufacturing Plant			
Disease	Reference*	Quartile 1 (95%CI)**	Quartile 2	Quartile 3	Quartile 4
Ischemic Heart N = 444 deaths (reference)	1.00	0.93 (0.73-1.18)	0.87 (0.66-1.13)	0.88 (0.57-1.53)	0.89 (0.66-1.21)
N = 248 deaths (PFOA plant)					
Stroke N = 112 deaths (reference)	1.00	0.57 (0.32-1.02)	0.70 (0.39-1.24)	0.93 (0.57-1.53)	0.98 (0.53-1.81)
N = 57 deaths (PFOA plant)					

*Reference: Non PFOA 3M manufacturing plant located in same Minneapolis-St. Paul metropolitan area

**Quartile cutpoints were ($\mu g/m^3$ years): 2.9×10^{-5}; 1.5×10^{-4}; 7.9×10^{-4}

If no associations were observed with heart disease, stroke, or hypertension with cumulative exposure to PFOA, what was the basis for the probable link of hypercholesterolemia declared by the C8 Science Panel? In large part, it was the study by Winquist and Steenland (2014) who modeled PFOA exposure and subjects' responses to a survey questionnaire that asked "*did your physician ever diagnose you*" as having "*high cholesterol*". These surveys were conducted between 2008 – 2011 in the mid-Ohio river community. Age at having recalled "*first physician diagnosis*" was used as the age of onset for hypercholesterolemia. Participants were only included in the analysis if they reported current prescription medication use in the same survey. Winquist and Steenland (2014) wrote in their community/worker cohort study that they did not observe a continued increased risk in hypercholesterolemia with modeled cumulative concentrations of PFOA past the 2nd quintile analysis for all subjects (Table 8). The most pronounced trend for hypercholesterolemia (as defined above) was for men 40-59 years, yet, it too, had hazard ratios that essentially did not change past the second quintile 2 (Table 8 and Figure D). Winquist and Steenland (2014) did not report other hazard ratios but did offer a graphical display of these hazard ratios (see Figure D). The variability of these hazard ratios by age and sex is quite apparent in Figure D.

Table 8. Hazard ratios (95% confidence interval) for high cholesterol) by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Winquist and Steenland (2014) Environ Health Perspect 122:1299-1305.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Hypercholesterolemia*					
All subjects	1.00	1.24 (1.15-1.33)	1.17 (1.09-1.26)	1.19 (1.11-1.27)	1.19 (1.11-1.28)
Men 40-59 yrs	1.00	1.38 (1.21-1.56)	1.32 (1.17-1.50)	1.31 (1.16-1.48)	1.44 (1.28-1.62)

* Modeled cumulative PFOA ng/ml per year by quintile: <142; 142 - <234; 234 - <630; 630 - <3579; >=3569

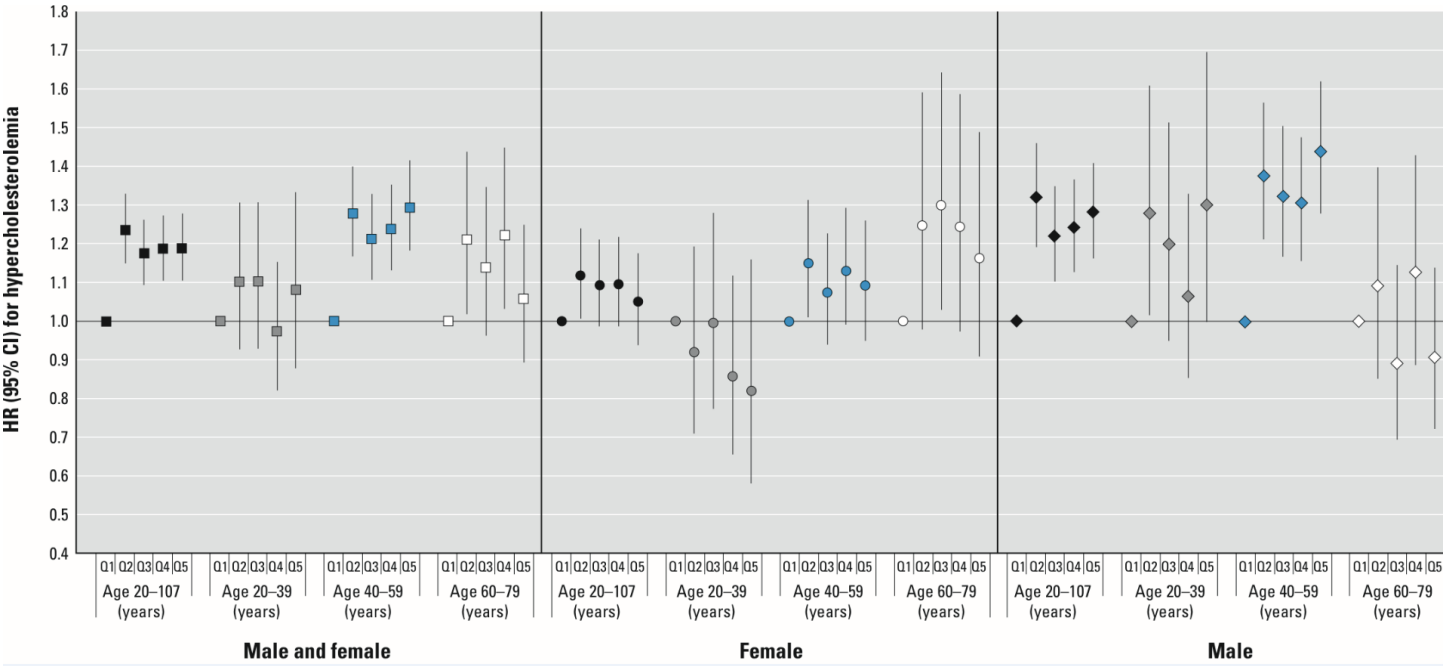

Figure D. From Winquist and Steenland Environ Health Perspect 2014;122:1299-1305.
Hazard Ratios and 95% CIs for hypercholesterolemia in the primary retrospective analysis for the combined cohorts, cumulative exposure. Quintile (Q) cut points (μg/mL per year) were < 0.142, 0.142 to < 0.234, 0.234 to < 0.630, 0.630 to < 3.579, ≥ 3.579. The analysis included 9,653 cases of self-reported hypercholesterolemia with medication. Models were stratified by single-year birth year and were either stratified by sex or controlled for sex and the interaction between sex and age. Models also controlled for years of schooling (not time-varying; < 12 years, high school diploma/GED, some college, or ≥ bachelor's degree), race (white vs. nonwhite or missing), smoking (time-varying; current, former, none), smoking duration (time-varying), smoking pack-years (time-varying linear term created by multiplying the self-reported number of packs smoked per day by the smoking duration to that point), regular alcohol consumption (time-varying; current, former, none), BMI (at time of first study survey; underweight, normal, overweight, obese), and self-reported type 2 diabetes (time-varying according to reported age at diagnosis).

The most pronounced trend for hazard ratios for hypercholesterolemia (as defined above) in the Winquist and Steenland (2014) study appeared when the modeled PFOA exposure analyses ended in 1987 (see Winquist and Steenland, 2014 supplement). These hazards diminished after 1987; however, it should be noted that a reduction in the modeled serum PFOA concentration estimates did not begin until 2001 (see supplement to Winquist and Steenland, 2014). Also, there were no increased trends for hypercholesterolemia in a prospective analysis through 2011.

In addition, the risk for taking medications to treat hypercholesterolemia was not observed with cumulative PFOA concentrations in the DuPont workforce as recently reported by Steenland et al. (2015) (Table 9). This latter study was published after the release of the Restriction Report.

Table 9. Disease-specific relative risk for employees taking high cholesterol medications (95% confidence interval) from Cox regression models of a cohort incidence study of the DuPont Washington Works plant and modeled PFOA exposure. See Steenland et al. (2015) J Occup Environ Med 72:373-380.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value Trend
High Cholesterol medications (N = 1298 cases)					
No lag*	1.00	1.11 (0.94-1.30)	1.06 (0.89-1.27)	1.05 (0.87-1.27)	0.50
10 year lag**	1.00	0.93 (0.79-1.10)	1.01 (0.84-1.22)	0.96 (0.78-1.18)	0.48

*Quartile cut-offs for the no lag analysis were (ng/mL-year): 3030; 6160; 11420

**Quartile cut-offs for the 10-year lag analysis were (ng/mL-year): 800; 3440; 7040

The Restriction Report stated the paper by Fitz-Simon et al. (2013) "*strengthens the hypothesis of a probable link and a causal effect between an increase in PFOA and higher cholesterol*." However, the Restriction Report offered no critique of this paper. Nor did the Restriction Report cite the papers that were subsequently published in response to Fitz-Simon et al. by Burstyn (2013) and in response Fletcher et al. (2013) by Vanden Heuvel (2014). Fitz-Simon et al. reported that of the thousands of adults who participated in the C8 Health Project in 2005-2006, 560 were re-measured for serum PFOA serum concentrations as well as blood lipids in a one-time follow-up examination 4.4 years later in 2010. None of these adults stated they were on cholesterol-lowering medications during this time period. The geometric mean PFOA concentrations in these adults decreased from 74.8 ng/mL to 30.8 ng/mL primarily due to water filtration that was introduced in 2006 – 2008. Among the 560 individuals during these same two measurements (baseline and follow-up in parentheses), their geometric mean total cholesterol (192.5 vs 192.8 mg/dL), LDL cholesterol (107.7 vs. 109.2 mg/dL), HDL cholesterol (48.6 vs 47.2 mg/dL), and triglycerides (144.1 vs. 146.9 mg/dL) were *unchanged* from a clinical perspective despite the 58.8 percent decline in PFOA concentrations, but the variability of measurements were large among the individuals. Fitz-Simon et al. (2013) suggested a "*tendency for greater decreases in LDL to occur with individuals who had greater declines of PFOA*." Using a model that adjusted for age, sex, the time interval between measurements, and fasting status, Fitz-Simon et al. statistically modeled, for a person with a 50% decline in PFOA, that this individual would have had a predicted decline in LDL of 3.6% (95% CI 1.5 to 5.7%). Therefore, Fitz-Simon et al. concluded the opposite was possible (i.e., an increase in PFOA may have been associated with an increase in LDL).

If the association between PFOA and increased cholesterol is causal and not related to chance, confounding, or bias, this would still have been unlikely to result in any clinical implications, as argued by Burstyn (2013) (not cited in the Restriction Report). Burstyn (2013) suggested that even after doubling PFOA concentrations, 97.5% of LDL values for most people, who had an initial healthy (normative range) level at baseline, would have still remained within the normal reference range for LDL. Burstyn also argued that the observed change in LDL mirrored that of NHANES data over the time period (approximately 3 percent decline of median LDL) and furthermore the average in LDL was 5 to 10 percent higher than these 560 adults. While acknowledging that the observed changes were very small, Fitz-Simon et al. (2013) counter argued that NHANES did not exclude individuals on cholesterol lowering medications and thus such a comparison was not entirely appropriate. However, Fitz-Simon et al. did not consider life-style behavioral changes that might have occurred in this mid-Ohio river valley population due to the intense scrutiny the population underwent (i.e., medical testing) in 2005-2006 that might have led to improvements in medical tests independent of their declining PFOA concentrations. Also, the 560 individuals studied by Fitz-Simons et al. were a highly self-selected population from the original C8 Health project. These 560 individuals were *only one percent* of the original C8 Health Project population studied. Whether such a small self-selected subsample of the original population can be used to infer causality remains untested.

Finally, it should be noted that the associations reported by Fitz-Simon et al. were observed for log transformations of PFOA and cholesterol. As Fitz-Simon stated in their supplemental material to their paper, *"although the logarithmic transformation gave more credence to the assumptions of the linear regression model, this does not necessarily imply an underlying logarithmic association."* When Fitz-Simons et al. fitted models for the untransformed differences, adjusted for possible time-varying confounders, they reported no associations were observed for cholesterol, LDL, HDL, triglycerides when regressed on tertiles of PFOA (see eAPPENDIX to Fitz-Simon et al. paper) as shown in Figure E. When Fitz-Simon et al. analyzed PFOA as a continuous untransformed measurement (per 1 ng/mL), there were no statistically significant differences via regression models with lipid measurements: LDL estimate -0.10 (95% CI -1.86, 1.67); total cholesterol -0.72 (95% CI -2.34, 0.90); HDL 0.11 (95% CI -0.39, 0.61); and triglycerides -0.27 95% CI 5.97, 5.43).

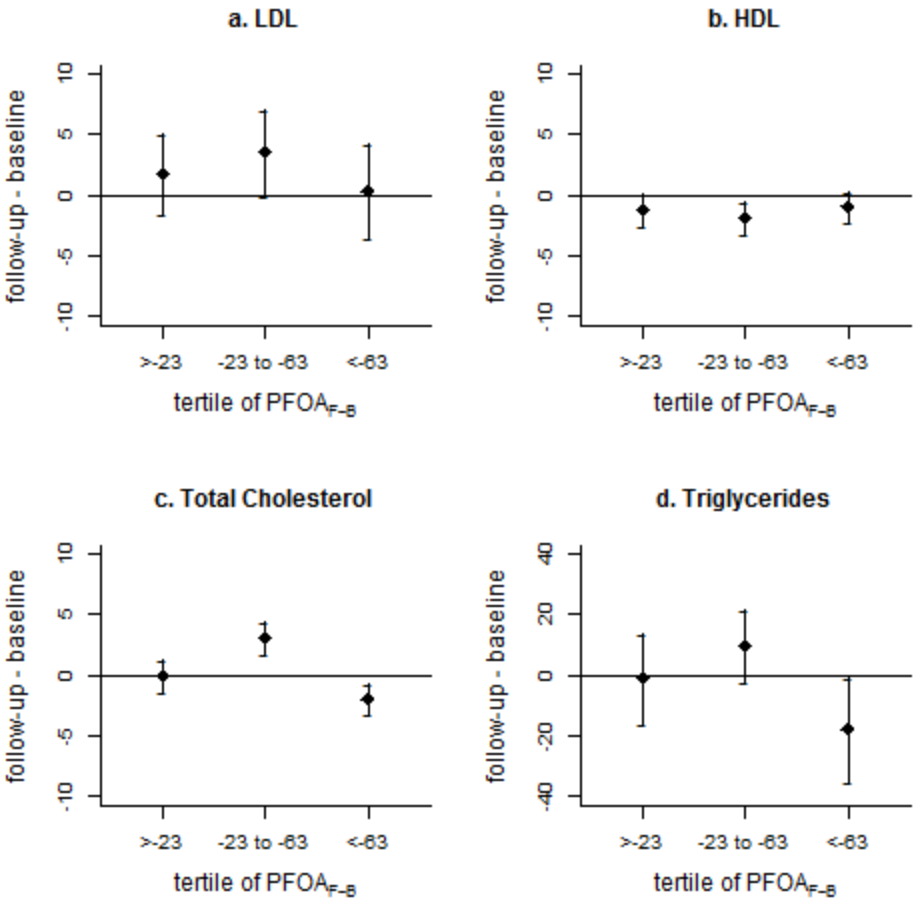

Figure E. From Fitz-Simon et al. Epidemiology 2013;24:569-576 (eAPPENDIX). Mean follow-up – baseline (F-B) difference (95% confidence interval) in each lipid measurement (mg/dL), by tertile of decrease in PFOA (ng/mL).

The Restriction Report chose to cite the paper by Fletcher et al. (2013) that suggested there were gene expression changes in 290 C8 Science Panel participants that was consistent with a "*hypercholesterolemic environment*." Specifically, Fletcher et al. reported an inverse association between serum PFOA and whole blood expression level with NR1H2, MNPC1 and ABCG1 genes which are involved in cholesterol transport. When analyzed by sex, there was reduction in the levels of mRNAs involved in cholesterol transport in men (APC1, ABCG1, and PPARA) and women (NR1H2) gene expression. Fletcher et al. acknowledged their study limitations included their small sample size and examining whole blood rather than gene expression in target organs with more direct relevance to lipid synthesis and metabolism (not possible, of course) and that they tested only a small subset of genes that can influence cholesterol metabolism. In fact, this point was made much more clear by Vanden Heuvel (2014) (not cited in the Restriction Report) who presented convincing evidence that the only conclusion from Fletcher et al. was their thought provoking nature but that it fell short of evidence for a "*hypercholesterolemic environment*" promoted by exposure to PFOA. Vanden Heuvel

summarized his findings in a figure (see Figure 1 to Vanden Heuvel 2014 paper) that showed 67 major genes involved in the regulation of cholesterol and cholesterol ester concentrations and the transcription factors that regulate their expression. Fletcher et al. only reported on 11 of the 67 genes depicted in the Vanden Heuvel figure of which half were actually involved with reverse cholesterol transport and would therefore result in lipid lowering (not increasing) phenotype responses.

The Restriction Report did not cite another C8 Science Panel paper (Watkins et al. 2014) of the same C8 Short Term Follow-up study discussed above (Fletcher et al. 2013.) Watkins et al. contacted 1199 C8 Health Project participants (approximately two percent) for a short term follow-up study in 2010. A total of 973 individuals completed a telephone interview, 755 provided a blood sample, and 685 had sufficient DNA for LINE-1 methylation analysis. LINE-1 (long interspersed nuclear element 1) is a group of retrotransposon sequences that are highly methylated. Methylation of CpG regions suppresses expression of related genes (demethylation increases gene expression). According to Watkins et al. (2014), hypomethylation of LINE-1 elements is associated with ischemic heart disease, stroke, and hypertension, increased LDL and decreased HDL cholesterol. Similar to the gene expression study (see Fletcher et al. 2013), Watkins et al. regressed the 50% change in PFOA concentrations between 2005/2006 and 2010 with % LINE-1 methylation measured in 2010. There were no measurements taken in 2005-2006. LINE-1 methylation was not significantly associated with PFOA in any analysis.

Two other smaller longitudinal studies are inconsistent with the longitudinal assessment of Fitz-Simon et al. (2013). Both of these were occupational studies. Sakr et al. (2007) reported a 1000 ng/mL increase in PFOA resulted in only a 1 mg/dL increase in total cholesterol among 454 DuPont workers. The Restriction Report did not cite another longitudinal study which did not observe an association among demolition workers although the number of workers (approximately 175) was not large and interval of time period followed (6 months) was relatively short (Olsen et al. 2014). However, the Olsen et al. longitudinal study did not support the magnitude of effect reported by Nelson et al. (2010) in their cross-sectional analysis of the NHANES database.

The Restriction Report cited the Shankar et al. study as evidence that "*PFOA levels in serum have been positively associated with self-reported cardiovascular disease (CVD) in an adult US population.*" Shankar et al. (2012) results were from NHANES (Table 10). Shankar et al. defined CVD as whether the NHANES participant was ever told by a physician that they had coronary heart disease, heart attack, or stroke. Shankar et al. combined NHANES data in the 1999-2000 and 2003-2004 time periods. The Restriction Report did not cite, however, an almost identical study by Melzer et al. (2010) that examined NHANES data in the 1999-2000, 2003-2004, and 2005-2006 time periods. Melzer et al. examined whether the NHANES participants were ever told, by a physician, that they had ischemic heart disease (coronary artery disease, angina, and/or heart attack). Substantively different results were published between Shankar et al. (2012) and Melzer et al. (2010). Whereas Shankar et al. reported a positive association between PFOA and CVD (Table 10), Melzer et al. did not (Table 11). Why the difference between the two studies of the same database (NHANES)? It appears the only data analysis difference was that Shankar et al. included a question about stroke in their CVD definition and Melzer included 33% more population. Both studies adjusted for many of the same factors except Shankar et al. included hypertension (absent or present), diabetes (absent or present) and serum total cholesterol (mg/dL). Of course, none of the previously described occupational and community/worker studies showed an increased risk for coronary artery disease, hypertension or stroke with cumulative exposure to PFOA (Table 3 through Table 9).

Table 10. Associations between presence of cardiovascular disease (CVD) and peripheral artery disease (PAD) and serum PFOA. NHANES, 1999-2000 and 2003-2004. See Shankar et al. Arch Intern Med (2012) 172:1397-1403.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value trend
PFOA ng/mL					
Women	<2.9	2.9-3.9	4.0-5.6	>5.6	
Men	<3.9	3.0-4.3	4.4-6.1	>6.1	
Cholesterol Mean (mg/dL) (SE)	206 (4.4)	212 (3.5)	212 (2.7)	218 (2.4)	0.11
Adj Odds Ratio CVD (95% CI)	1.00 (reference)	1.58 (0.80-3.12)	1.77 (1.04-3.02)	2.01 (1.12-3.60)	0.01
Adj Odds Ratio PAD (peripheral artery disease) (95% CI)	1.00 (reference)	0.75 (0.37-1.52)	1.18(0.47-2.96)	1.78(1.03-3.08)	0.04

*see text for adjustments.

Table 11. Association between serum PFOA concentrations (ng/mL) and self-reported prevalence odds ratio for ischemic heart disease. NHANES, 1999-2000, 2003-2004, and 2005-2006. See Melzer et al. (2010). Environ Health Perspect 118:686-692.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
PFOA ng/mL				
Women (mean)	1.71	3.32	4.79	9.47
Men (mean)	2.47	4.42	6.12	10.39
Adj Odds Ratio Ischemic Heart Disease (95% CI)	1.00 (reference)	0.95 (0.59-1.51)	1.02 (0.65-1.61)	1.08 (0.70-1.69)

*see text for adjustments.

B.5.1.6.3 Summary and discussion of repeated dose toxicity

The C8 Science Panel was created as a process to reach a legal agreement between two settling parties of a class action lawsuit. The C8 Science Panel was not a governmental agency subject to legislative oversight or overall public accountability. The 3-member C8 Science Panel probable links opinion is just that – their scientific opinion – to the settling parties of this lawsuit. The C8 Science Panel has gone on record to state "*that as more scientific evidence accumulates, some associations may not be confirmed. Others may be identified that we (the C8 Science Panel) had missed*." Therefore, although the C8 Science Panel's research studies should be evaluated in this Restriction Report, it is critical that their probable link statements not be accepted as causal conclusions.

The Restriction Report wrote in this section that "there is a trend that low exposed (human) populations show a greater trend in cholesterol unit change in PFOA than high exposed workers. This would indicate a low dose effect." 3M disagrees. If anything, the associations observed at low concentrations is likely more indicative of a saturated response (as mentioned by Frisbee et al. 2010). The one study (Steenland et al. 2009) used by the Restriction Report in their DNEL calculations for "*increased total cholesterol and LDL in human serum*" cannot indicate a low dose effect. It simply illustrates an association between measured serum PFOA and cholesterol levels. At best, it appears to also suggest a possible threshold – that is identical to that of uric acid – where the association disappears (above 50 ng/mL PFOA). If the Restriction Report suggests "*a low dose effect*", then there needs to be more scientific evidence as to why this does not indicate the possibility of a saturated transporter or the consequence of other confounding factors (*e.g.*, binding to lipoproteins based on large studies).

There is no evidence to suggest, as written in this section, that "*a possible low chronic increase in cholesterol may increase the risk of atherosclerosis and eventually the risk of heart disease, pregnancy induced hypertension or preeclampsia due to the fact that the exposure is of a chronic nature combined with the long half-life of PFOA in humans.*" This speculation does not belong in this Restriction Report. If anything suggests a causal interpretation in the literature, it is the hypolipidemic response (lowered LDL values) that was reported in the Phase I clinical trial of cancer patients administered high dosages of PFOA ammonium salt (up to 1200 mg weekly). In this regard, the Restriction Report was silent. This reduction in LDL likely reflects the $PPAR_{alpha}$ expression in humans (not the consequence of the lack of $PPAR_{alpha}$ expression for the "positive associations" reported elsewhere). Finally, 3M strongly encourages the Restriction Report to incorporate the Vanden Heuvel (2014) constructive technical critique of the Fletcher et al. (2013) premise of a "*hypercholesterolemic environment*."

B.5.1.8 Carcinogenicity

The Restriction Report did not cite the updated 3M cohort (mortality and cancer incidence) study of PFOA (ammonium salt) that was manufactured at the 3M Cottage Grove plant (Raleigh et al. 2014). This plant population has not been shown to be at risk for being diagnosed with kidney cancer incidence or mortality. There were too few testicular cancer cases for an exposure response trend to be calculated in this study. Unlike the DuPont Washington Works plant where PFOA was used as a processing aid in the polymerization of tetrafluoroethylene (TFE), the electrochemical cell fluorination manufacture of PFOA (ammonium salt) at this 3M Cottage Grove plant was manufactured in near isolation of any exposure to TFE. This is important as Steenland et al. (2012) did not consider TFE as a confounding exposure in their cohort mortality analysis of the

DuPont workforce. TFE exposure to rats has produced renal cancer. A multi-company, multi-location (sites in U.S. and Europe) cohort mortality study of TFE production workers was unable to disentangle an association between kidney cancer with PFOA or TFE exposure (Consonni et al. 2012). The largest of these fluoropolymer plants in the Consonni et al. study was the DuPont Washington Works plant.

The Restriction Report did not mention the International Agency for Research on Cancer (IARC) Monograph 110 deliberations (June 2014) PFOA was categorized as a 2B human carcinogen – "*possibly carcinogenic to humans*." According to IARC, a 2B classification is unable to rule out chance, bias or confounding with reasonable confidence. Approximately 290 chemicals and physical agents have been classified as possible (2B) by IARC (Benbrahim-Tallaa et al. 2014). The Restriction Report needs to better address the methodological limitations that were used in the epidemiology studies as was aptly critiqued by Chang et al. (2014) in their review of cancer epidemiology of PFOA.

B.5.1.9 Toxicity for reproduction

B. 5.1.9.1.2 Human information

As was briefly described in this section of the Restriction Report, a set of 4 papers was published in Environmental Health Perspectives in October 2014 whose purpose was to conduct a comprehensive and transparent assessment on the nonhuman mammalian and human evidence of whether fetal growth, in particular birth weight at term, was inversely associated with exposure to PFOA or its salts (Woodruff and Sutton 2014; Koustas et al. 2014; Johnson et al. 2014; Lam et al. 2014).

These investigators used a Navigation Guide methodology to conduct a "*rigorous approach to research synthesis that has been developed to reduce bias and maximize transparency in the evaluation of environmental health information*" (Woodruff and Sutton 2014). The evaluation process involved three major steps: 1) specify the study question; 2) select the evidence; and 3) rate the quality and strength of the evidence according to consistent criteria, and performing appropriate statistical analyses (e.g., meta-analyses). For each systematic review of the nonhuman mammalian and human data, the strength of the evidence was defined as either sufficient, limited, inadequate, or lack of evidence of toxicity, similar to those used by the IARC. Integration of each separate rating for nonhuman and human data resulted in an overall final strength of evidence rating.

Koustas et al. (2014) addressed the question of whether PFOA or its salts affected fetal growth in animals. They initially reviewed 21 toxicology studies relevant to the question. They determined only a subset of the data, 8 mouse gavage data sets from 7 studies, could be combined for their meta-analysis of birth weight in relation to PFOA doses administered. Only the low PFOA doses were considered in their meta-analysis in order to minimize adverse impacts from higher administered doses in these studies. The mouse species was chosen, as compared to the rat, due to its longer half-life of PFOA and lack of pharmacokinetic differences between the sexes. The meta-analysis estimate of PFOA calculated from these 8 data sets was a change in mean pup birth weight of -0.023 g (95% CI -0.029, 0.016) per 1-unit increase in dose (mg/kg body weight per day). Koustas et al. summarized the strength of evidence across the 8 mouse gavage data sets as "*sufficient evidence of toxicity*" based on their *a priori* definition of "*one or more well-designed, well-conducted studies*" and the conclusion is unlikely to be strongly affected by findings from future studies.

Similarly, Johnson et al. (2014) reported the systematic review of the human evidence by identifying 18 epidemiologic studies of which 9 data sets were considered combinable in a meta-analysis for birth weight and PFOA exposure. These 9 data sets represented 4,149 births. Reviewing each study for risk of bias (recruitment

strategy, blinding, exposure assessment, confounding, incomplete outcome data, selective outcome reporting, conflict of interest, and other bias), Johnson et al. rated the quality of evidence across the studies as moderate. Their meta-analysis of these 9 data sets reported an estimate of -18.9 grams (95% CI -29.8, -7.9) birth weight per ng/mL increase in serum/plasma PFOA (Figure F). Johnson et al. summarized the strength of human evidence as "*sufficient evidence of toxicity*" which was defined as a positive (in this case inverse) relationship defined between birth weight and PFOA exposure and that "*chance, bias and confounding ruled out with reasonable confidence*."

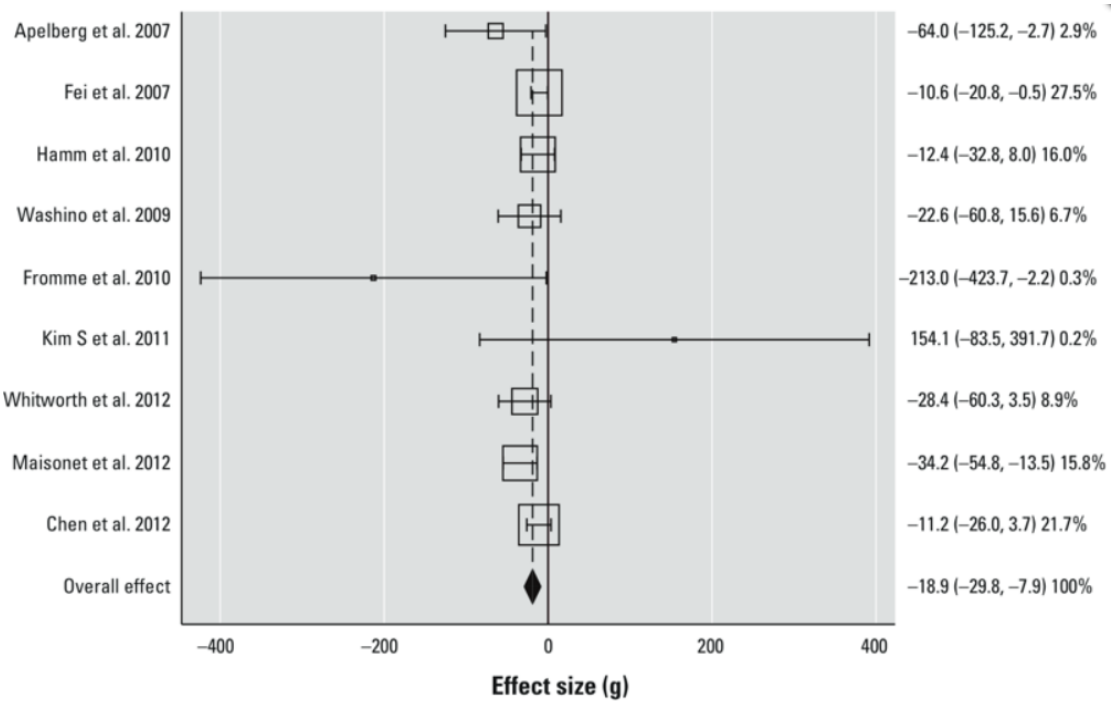

Figure F. From Johnson et al. Environ Health Perspect 2014;122:1028-1039. Results of meta-analysis for birth weight (n = 9 studies, 4,149 births) shown as effect estimates [change in birth weight in grams per nanogram of PFOA per milliliter of serum or plasma (95% CIs)]. The percentages are weightings of the individual studies in the meta-analysis according to the inverse of the variance, and the sizes of the boxes are scaled accordingly. The dashed line indicates the overall effect estimate derived from the DerSimonian-Laird random effects meta-analysis, and the diamond indicates the 95% CI of the overall effect estimate. Heterogeneity statistics: Cochran's Q = 12.92; p = 0.12; I^2 = 38%. Estimates were adjusted as follows: Apelberg et al. (2007): maternal age and gestational age; Fei et al. (2007): maternal age, gestational age, quadratic gestational age, infant sex, socio-occupational status, parity, smoking, prepregnancy body mass index, and gestational week at blood draw; Hamm et al. (2010): maternal age, gestational age, race, gravidity, maternal prepregnancy weight, maternal height, smoking status, and infant sex; Washino et al. (2009): maternal age and gestational age; Fromme et al. (2010): unadjusted; Kim S et al. (2011): maternal age, gestational age, and parity; Whitworth et al. (2012): maternal age, gestational age, prepregnancy body mass index, and parity; Maisonet et al. (2012): smoking, prepregnancy body mass index, previous live birth, and gestational age; Chen et al. (2012): maternal age and gestational age.

Lam et al. (2014) concluded this Navigation Guide methodology by integrating the strength of the nonhuman mammalian and human ratings. They reached the final conclusion that PFOA is "*known to be toxic*" to human

reproduction and development based on sufficient evidence of decreased fetal growth in both nonhuman mammalian and human species.

However, *a critically important confounder, glomerular filtration rate (GFR),* was not addressed in the meta-analysis by Johnson et al. (2014). GFR is an important confounding variable because it is related to the elimination of PFOA and is positively associated with birth weight. While acknowledging the possibility of this confounding factor, Lam et al. believed their conclusion was not undermined for two reasons: 1) it was not relevant to the nonhuman mammalian data; and 2) their systematic review of the literature for this relation between birth weight and maternal glomerular filtration rate provided insufficient evidence to support this hypothesis. However, it was not transparent in the Lam et al. paper how they systematically reviewed this literature regarding a GFR and birth weight association, except that they provided a list of 8 papers in a supplement to their paper regarding fetal growth and glomerular filtration rate.

Unlike Lam et al. (2014), Verner et al. (2015) found there is a relationship between GFR and birth weight as seen with larger studies, including Morken et al. (2014), Akahori et al. (2012), Laughon et al. (2009), and Knopp et al. (1985). It should be noted that Lam et al. examined these same studies as Verner et al., except for the more recently published Morken et al. study described below.

Morken et al. (2014) is the largest study that addressed the question of whether GFR during pregnancy may affect infant birth weight. Morken et al. analyzed a subset of 953 women (470 with and 483 without preeclampsia) from the Norwegian Mother and Child Cohort study (MoBa). They estimated GFR in the second trimester using three methods: Cockroft and Gault (CG), MDRD, and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). The difference in infant weight for each mL/min increase in the three eGFRs was: GC-formula, 0.73 g ($p < 0.05$); for the MDRD formula, 0.83g ($p < 0.05$) and for the CKD-EPI formula 0.04 g, (not significant). Stratifying the results for women with and without preeclampsia showed statistically homogeneous results for women with and without but the findings were only statistically significant with the preeclampsia group. Unlike normal pregnancies, preeclampsia-related pregnancies have increased percentages of small-for-gestational-age infants which increased the total cohort's study power to detect an association between GFR during pregnancy and infant birth weight. Morken et al. concluded their study was the first to estimate the relationship, albeit modest (partial correlation coefficient 0.07) between GFR and birth weight.

Acknowledging the above GFR birth weight relationship and the recommendation of others (Bach et al. 2015) to incorporate physiologically-based pharmacokinetic models (PBPK) in the epidemiological investigations of PFOA, Verner et al. (2015) constructed a PBPK model that addressed the question of how much of the epidemiologic associations reported between prenatal PFOA exposure and birth weight might be attributable to confounding by GFR. [Note: this important paper was published after the Restriction Report was released for public comments.] Verner et al. were affiliated with the Hamner Institute for Health Sciences and the National Institute of Environmental Health Sciences (US NIEHS). [Note: the Restriction Report erroneously attributed NIEHS affiliation to the Lam et al. meta-analysis investigators (see B.5.1.9.1).] Verner et al. modified an existing PBPK model of pregnancy and lactation and PFASs (Loccisano et al. 2013) to address maternal GFR. Exposure to PFOA considered a multi-compartmental model that examined blood flow rates in and out of compartments, tissue volume, and tissue:blood partition. Verner et al. assumed the initial plasma PFOA, prior to input, was at steady state. The GFR and birth weight relationship was parameterized in the PBPK models by a summary coefficient provided by 3 studies (Morken et al. 2014; Dunlop 1981; Gibson 1973) using an inverse-variance weighted average. The PBPK model incorporated over 40 parameters that could vary within a population (see the supplement to Verner et al. for the PBPK model details and code). Using linear regression

analyses, Verner et al. reported that the association between simulated maternal and cord plasma PFOA levels and birth weight appeared after the third month of pregnancy and was strongest at delivery (Figure G). The association between simulated PFOA levels (per 1 ng/mL increase) and birth weight was comparable for cord plasma [-7.13 g birth weight (95% CI -8.5, -5.8)] and maternal plasma at term [-7.92 g birth weight (95% CI -9.4, -6.4)]. Using a shorter estimate of the mean half-life of 2.3 years for PFOA (Bartell et al. 2010) instead of 3.8 years (Olsen et al. 2007), the strength of the association between simulated cord plasma and birth weight was increased by 14% (-8.1 g, 95% CI -9.4, -6.8). Stronger associations were obtained with lower mean initial plasma PFOA levels and lower standard deviations, and with higher GFR-birth weight coefficients. These changes were additive. For example, in their sensitivity analyses, Verner et al. found a lower PFOA mean (multiplier = 0.5) and a stronger beta for the GFR-birth weight association (multiplier = 2) resulted in a 23.3 g (95% CI -26.0, -20.6) decrease in birth weight per ng/mL increase in simulated cord plasma levels. Conversely a higher PFOA mean multiplier (multiplier – 2) and a weaker beta for the GFR-birth weight association (multiplier = 0.5) resulted in a 2.4 g decrease (95% CI -3.1, -1.8) in birth weight per ng/mL increase in simulated cord plasma.

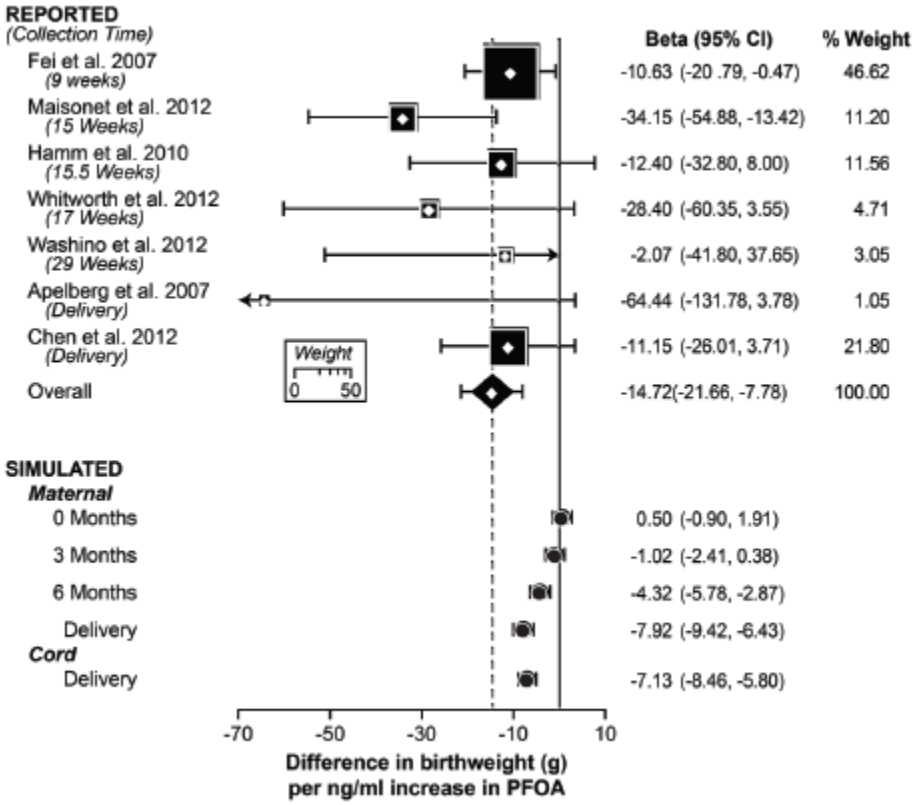

Figure G. From Verner et al. Environmental Health Perspectives 2015;doi.org/10.1289/ehp.1408837. Difference in birth weight (g) per 1 ng/mL increase in reported and simulated PFOA. In the Reported section, the size of the square represents the weight of each study in the calculation of the overall meta-analytic association. The heterogeneity chi-square for the PFOA meta-analysis was 7.4 (not statistically significant).

Verner et al. then conducted a meta-analysis of seven epidemiologic studies (Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2007; Hamm et al. 2009; Maisonet et al. 2012; Washino et al. 2008; Whitworth et al. 2012). All of these studies were included by Johnson et al. 2014 in their meta-analysis of 9 studies (Figure G). In their meta-analyses of these 7 studies, Verner et al., calculated a summary meta-analysis of a 14.7 g (95% CI -21.7, -7.8) reduction in birth weight for a 1 ng/mL increase in PFOA (Figure G). (Note: As discussed above, Johnson et al. (2014) had estimated a summary meta-analysis coefficient of -18.9 g decrease for 1 ng/mL increase in PFOA based on 9 studies.)

Based on their PBPK simulations (Figure G), Verner et al. concluded epidemiologic studies of prenatal PFOA and birth weight may have *a substantial portion of the association attributable to confounding by GFR.* This confounding may be more important in studies with PFOA measurements made later in pregnancy.

B.5.1.9.1.3 Summary and discussion of developmental effects

The Restriction Report concluded "*taken together, the results suggest that PFOA exposure may reduce foetal growth both in animals and humans.*"

There is now substantive evidence that studies on maternal and cord plasma PFOA and birth weight are likely confounded by GFR. To date, none of these epidemiologic studies had adjusted for GFR. Therefore, a DNEL based on epidemiological data (Fei et al. 2007) between PFOA measurements and birth weight, not adjusted for GFR, is biased. Because Fei et al. measured PFOA during gestational weeks 4 to 14, this study, according to the simulation findings by Verner et al., may have a less confounded PFOA and birth weight association. However, as Verner et al. has exquisitely shown in their sensitivity analyses, it is difficult to estimate any PFOA and birth weight association without taking into account initial plasma concentrations and the GFR-birth weight relationship, as both can have an additive effect. DNEL and RCR calculations, such as that employed in this Restriction Report that rely solely on the Fei et al. study findings as presented, are incapable of reflecting the complex physiological nature of pregnancy, including the GFR, that can, and does, confound an association between PFOA maternal or cord measurements and birth weight. Finally, the Restriction Report discounted the C8 Science Panel's conclusion that found no probable link between PFOA and lower birth weight because it relied on retrospective modelling of exposure rather than measured PFOA. Verner et al., however, noted this exposure modelling as a strength (similar to the Watkins et al. (2013) analysis of chronic kidney disease discussed earlier) as these studies (Savitz et al. 2012a; 2012b) could not be confounded by GFR. Neither Savitz et al. study suggested an association between prenatal PFOA exposure and birth weight.

In conclusion, the Restriction Report needs to address GFR as a substantial confounder (but not necessarily the "sole driver" (Verner et al. 2013)) of the association between prenatal PFOA exposure and birth weight.

B.5.1.10 Other effects

This section appears to be a haphazard review of very few toxicology and epidemiology studies with many pertinent references omitted by the Restriction Report. For example, in this extremely brief summary, the Restriction Report commented on the study by Halldorsson et al. (2012) who conducted a 20-year follow-up of the anthropometry of the offspring of 665 Danish women who were pregnant in 1988-1989. Halldorsson et al. analyzed these women's 3rd trimester archived serum samples for PFOA. Halldorsson et al. suggested the measured PFOA was positively associated with the daughters being overweight or obese 20 years after the pregnancy, no associations were observed with the sons. The Restriction Report, however, did not cite studies by Andersen et al. (2013), Barry et al. (2014), and the recently published Høyer et al. (2015) who did not observe overweight and obesity associations with PFOA in either children or adults.

B.5.1.11 Derivation of DNEL(s)/DMEL(s)

3M respectfully disagrees with the Restriction Report's conclusion that PFOA increases total cholesterol and LDL and reduces birth weight in humans. The DNELs and RCRs are based on the faulty assumption that the epidemiologic evidence showed PFOA to increase total cholesterol and LDL (Steenland et al. 2009) and reduce birth weight (Fei et al. 2007). Such an "*affect*", however, is clearly not established in the scientific literature.

Conclusion

The associations published in the literature are shown to be highly inconsistent and/or confounded by underlying physiologic processes. 3M also re-emphasizes its position that this health risk assessment was a biased presentation of the literature reflected by omission of contradictory evidence, excluded important data leading to alternative conclusions, and, *most importantly*, failed to offer a critical synthesis of the literature in the weight of the evidence review for these health-based outcomes.

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