Comments of The 3M Company (3M) on
Draft NTP Technical Report No. 598 on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd: Sprague Dawley® SD® Rats)

The 3M Company (3M) appreciates this opportunity to provide the enclosed comments to the Draft National Toxicology Program (NTP) Technical Report No. 598 on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) (PFOA). As a science-based company, 3M encourages NTP to use the best available science when assessing this chemical and the referenced studies. As our comments reflect, 3M has substantial experience and expertise regarding PFOA, PFOS and other PFAS, informed in part by the fact that 3M scientists are authors or contributors to many of the studies cited in the references listed in the draft Technical Report. As a result of this expertise, 3M has significant concerns with the draft Technical Report and the underling toxicology and carcinogenesis studies under the NTP's consideration.

3M's specific comments on the draft NTP Technical Report are grouped according to the page and section numbers of that Report to which the comments refer.

Abstract:

- The abstract should clarify what the toxicities were for F_1 male rats in Study 1 which resulted in early termination.
- For contextual purpose, the abstract should also include what the plasma PFOA concentration measurements were in conjunction with the key findings, including neoplasms.
- The abstract as well as the main text of the draft report need to provide a basis in terms of how an effect was called out even though it was not statistically significant (i.e., occurrence of pancreatic lesions in female rats). The readers would appreciate consistency as well as clarification for such determination.
- It will be helpful for the abstract to summarize and justify its final conclusion with the findings for female rats, given the uncertainties of the findings were either statistically insignificant or not observed in another 2-year carcinogenicity study by Butenhoff et al. 2012 (Toxicology 298 1-13).
- There is no mentioning of the lack of testicular Leydig cell tumor findings in this study with exposure to dietary PFOA – this should be clearly discussed in the abstract as well as in the conclusion of this draft report.
- For contextual purpose, the abstract should include a short paragraph comparing the present neoplastic findings relative to the two previous published carcinogenicity studies, which the two previous studies reported increased incidence of testicular Leydig cell tumors while the current study did not. This will add a broader perspective on the potential carcinogenicity of PFOA in Sprague Dawley rats.

Page 2:

Experimental Animals:

- Please revise the citation source for the ATRSDR reference on provisional PFOA in terms of 78 ppt (78 ng/L) for adults and 21 ppt (21 ng/L) for children in the drinking water. It should be https://www.atsdr.cdc.gov/pfas/mrl_pfas.html; not the draft toxicology profile.
- It should be noted the current tolerable weekly intake for PFOA released by the European Food Safety Authority is provisional.
- In the following statement where the draft report stated: "The notable sex difference of PFOA elimination in rats is reduced by castration of males, which increases clearance of PFOA, with evidence suggesting changes in organic anion transporter (OAT) 2 and OAT3 as the mechanism. This mechanism is assumed to apply to the other PFAS that display similar sex differences." This is not true, given that PFAS is a large class of chemicals and even the ones with sex difference in terms of serum elimination, they do not share the same propensity for OAT2 and OAT3 profiles.

Humans:

- Estimation of half-lives should have measures of precision reported (e.g., 95% confidence limits, as they exist in these papers). Per references 32 and 33 cited in this draft document, the 95% CI are included below for your reference:
  - Reference #32: Olsen et al. Environ Health Perspect 2007
    Arithmetic Mean: 3.8 years (95% CI 3.1 - 4.4)
    Geometric Mean: 3.5 years (95% CI 3.0 - 4.4)

  - Reference #33: Li et al. Occ Environ Med 2018
    Arithmetic Mean: 2.7 years (95% CI 2.5 – 2.9)

    Note in Reference #33:
    Arithmetic Mean for Males 2.8 years (95% CI 2.4 – 3.4)
    Arithmetic Mean) for Females 2.4 years (95% CI 2.0 – 3.0)

- Should also consider citing Bartell et al. 2010 Environ Health Perspect 118 222-228
  Arithmetic Mean 2.3 years (95% CI 2.1 – 2.4). Note: This narrow 95% CI was influenced by a minimum 12-month measurements of PFOA after the installation of GAC filters in the water distribution system.

Page 3:

- Under Toxicity – Humans: The NTP report inappropriately cites attributions to the C8 Science Panel. The expert panel (reference 46 which are the probable link statements from the C8 Science Panel), which made "probable link" findings based on a standard articulated in a litigation settlement agreement, did not find a probable link with liver "damage". In other words, the C8 Science Panel did not conclude a probable link with increased liver enzymes or liver disease with community or worker exposure to PFOA. Nor did this C8 Science Panel (reference 46) conclude there was a probable link with immune effects in this mid-Ohio Valley community.

- Under Reproductive and Developmental Toxicity - Humans: The association of *low* (emphasis added) birth weight and PFOA exposure is not a situation of reverse causality as there is no association reported between PFOA and *low* birth weight. The term *low* birth weight refers to an absolute weight of <2500 g regardless of gestational age. Small for gestational age (SGA) refers to newborns whose birth weight is less than the 10th percentile for gestational age. Stein et al. (2009 Am J Epidemiol 170 837-846) reported there were no associations with low birth weight exposure to PFOA in the mid-Ohio Valley community. The association in multiple studies of the general population has been with "lower" birth weight and maternal or cord blood measurements of PFOA. ATSDR (draft 2018) in their Toxicology Profile for Perfluoroalkyls referred to lower birthweight as "Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight (PFOA, PFOS)."

  NTP cites meta-analyses by Johnson et al. (reference 49) and Negri (reference et al 50) but does not acknowledge the sentinel work done by Verner et al. (2015 Environ Health Perspect 123 1317-1324) who evaluated the potential confounding by the glomerular filtration (GFR) rate using a PBPK model. Verner et al. showed the potential confounding by GFR likely occurred when maternal PFOA measurements were taken after the 1st trimester. This confounding is likely the consequence of plasma volume expansion that occurs during the first half of gestation and would be greater in mothers of larger fetuses. The recent meta-analysis (reference 51, Steenland et al. 2019 Epidemiology) found associations with lower birth weight associated with maternal measurements of PFOA measured in second or third trimester but essentially no association with lower birthweight when such maternal PFOA measurements occurred in first trimester; thus, minimizing any confounding by GFR if maternal PFOA was measured in the first trimester.

  Although reference 52 (Rappazzo et al.) discusses an association between PFOA and age at menarche and also adverse renal function in children, this review paper

by Rappazzo et al. (despite it being a "systematic review") failed to cite the PBPK modelling by Wu et al. (2015 Environ Int 82 61-68) that showed pharmacokinetics, rather than a toxic effect of PFOA or PFOS, partially explained the relationship between these perfluoroalkyls and age at menarche. The review by Rappazzo et al. also failed to adequately understand the association with renal function as this was explained in the paper by Watkins et al. (cited with reference 52) conducted by C8 Science Panel researchers. It showed the cross-sectional association between eGFR and serum PFOA observed in this and prior studies was likely a consequence of, rather than a cause of, decreased kidney function. This was further confirmed by the results from Dhingra et al. (2017 Environ Health Perspect 125 416-421), also conducted by the C8 Science Panel, who reported reduced kidney function is likely the cause rather than the result of increased measured serum PFOA. These results suggested to the C8 Science Panel investigators that researchers should be cautious about using measured perfluoroalkyls in cross-sectional studies. As a consequence of the misinterpretation by Rappozzo et al, NTP may wish to cite individual studies rather than the inaccurate conclusions from the Rappozo et al review.

Page 4:

The draft NTP Technical Report states that "*Epidemiological studies provide evidence to suggest a link between PFOA exposure and immunomodulation*" and cites four epidemiologic studies (Granum et al. 2013 J Immunotoxicol 10 373-379; Mogensen et al. 2015 Environ Health 14 47; Stein et al. 2016 Pediatric Res 79 348-357; Looker et al. 2014 Toxicol Sci 138 76-88) in support of this statement. [The NTP also cites a consensus statement (Grandjean et al. 2015 Endocrinology 156 3408-3415) on developmental exposure to environmental stressors that did not specifically address PFOA (or any PFAS substance) and immunotoxicity]. NTP claims that these studies demonstrate an association between elevated serum PFOA concentrations and reduced antibody response to vaccines. 3M respectively disagrees with this conclusion for the following reasons:

- NTPs review of the epidemiology literature is outdated and fails to accurately reflect the inconsistencies both within and across all studies. The draft NTP Technical Report sites only 4 epidemiology studies. However, to date, there are 11 published studies that have examined PFOA exposure and antibody responses to vaccines (Grandjean et al. 2012 JAMA 307 391-397; Grandjean et al. 2017 Environ Health Perspect 125 077018; Granum et al. 2013 J Immunotoxicol 10 373-379; Mogensen et al. 2015 Environ Health 14 47; Stein et al. 2016 Pediatric Res 79 348-357; Looker et al. 2014 Toxicol Sci 138 76-88; Kielsen et al. 2016 J Immunotoxicol 13 270-273; Stein et al. 2016 Environ Res 149 171-178; Pilkerton et al. 2018 PLoS ONE 13 e0203330; Zeng et al. 2019 Sci Tot Environ 663 60-67; Grandjean et al. 2017 J Immunotoxicol 14 188-195). Existing epidemiologic studies have measured antibody responses to 10 distinct vaccines (e.g., tetanus, diphtheria, rubella,

measles, mumps, influenza A (H1N1), influenza A (H3N2), influenza B, enterovirus and coxsackievirus). It is inappropriate to interpret antibody responses to distinct vaccines as a single health outcome (i.e. reduced vaccine response). The NTP has acknowledged that there are differences in immune response across vaccines, and stated that "*The strength of an antibody response in terms of antibody level and length of time that an elevated/effective antibody response is maintained is known to differ across vaccines*" (NTP 2016, https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf). Granum et al (2013), also concluded that "*different vaccines may stimulate different components of the immune system, which can explain the vaccine-dependent differences in the effect of PFAS exposure*". Therefore, observed changes in antibody response to a particular vaccine should not be interpreted as consistent with changes in the antibody response to another vaccine.

- Epidemiologic studies do not provide consistent evidence of a significant association between PFOA exposure and decreased vaccine responses. Mostly null findings have been reported across all studies and results are inconsistent by vaccine type. For example, among the 6 existing studies (Grandjean et al. 2012 JAMA 307 391-397; Grandjean et al. 2017a Environ Health Perspect 125 077018; Grandjean et al. 2017b J Immunotoxicol 14 188-195; Granum et al. 2013 J Immunotoxicol 10 373-379; Mogensen et al. 2015 Environ Health 14 47; Kielsen et al. 2016 J Immunotoxicol 13 270-273) that have examined antibody responses to the tetanus vaccine (the most commonly studied vaccine type) relative to serum PFOA levels, 3 studies reported a significant decrease in antibody levels (Grandjean et al., 2012; 2017b; Mogensen et al., 2015). The other 3 studies did not observe a significant decrease in tetanus antibody levels. It is worth noting that inconsistent results were reported within the study by Mogensen et al (2015) which observed a significant decrease in tetanus antibody levels when using structural equation modeling, but did not observe an association when linear regression models were used.

- Small changes in antibody response do not necessarily translate to an increased risk of infectious disease. Several epidemiologic studies (Dalsager et al. 2016 Environ Int 96 58-64; Fei and Olsen 2010 Am J Epidemiol 171 131-132; Impinen et al. 2018 Environ Res 160 518-523; Okada et al. 2012 Environ Res 112 118-125; Granum et al. 2013 J Immunotoxicol 10 373-379; Looker et al. 2014 Toxicol Sci 138 76-88; Stein et al. 2016 Environ Res 149 171-178) have examined PFOA and PFOS levels and infectious disease outcomes (i.e., occurrence of common colds and otitis media, symptoms of infections, mortality from infectious and parasitic diseases and hospitalizations from infectious diseases). Across all reported measures, mostly null associations between PFOA levels and increased risk of infectious disease outcomes have been observed. Further, the National Toxicology Program concluded that there is low confidence that exposure to PFOA is associated with

increased incidence of infectious disease (or lower ability to resist or respond to infectious disease) (NTP, 2016).

In sum, the absence of clinical immunosuppression along with inconsistent findings both within and across the epidemiologic studies, do not support an association between serum PFOA and decreased antibody responses to vaccines in humans.

Page 7:

- Given the low pKa of (free) perflurooctanoic acid, the report needs to clarify why the ammonium salt of potassium salt of PFOA was not sought in the preparation of the dietary administration. What was the resulting pH of the diet? Did the incorporation of free acid potentially affect the overall study quality because free acid was being used?
- The report needs to clarify why two different diets were used in the study (NIH-07 and NTP-2000)? What was the rationale? The pros and cons of one diet over the other?

Page 8:

- In terms of the storage condition for the prepared diets, why was NIH-07 diet refrigerated and why was the NTP-2000 diet stored at room temperature?
- Did the different storage condition attribute to any bias in study outcomes?
- According to the draft report, the sources of time-pregnant Hsd: Sprague Dawley female rats were from two separate locations (Madison WI or Indianapolis, IN). For clarification, it will be helpful to identify the specific source of animals for each study. Furthermore, was there any information on the background genetic drift between the two locations for the same stock of rats? Were the rats from the same breeding colony?

Page 9:

- In Table 1 and Table 2, it will be helpful for the readers if the study duration (both interim and terminal) were added; specifically, with the corresponding sexes.
- Under the section for Study 1, were 4 days adequate for acclimation for time-pregnant rats prior to test material administration?
- The second paragraph under Study 1 was for the observation of sentinel animals – the report should just state so; as well as clarify what the purpose of sentinel animals was.

Page 10:

- Similar to Study 1, in this section where Study 2 was described, were 4 days adequate for acclimation for time-pregnant rats prior to test material

administration? Also, please be specific and clear about the purpose and the use of sentinel animals.

- At the bottom of this page, it stated that "On PND 4, dams with unacceptable litters from the 0 ppm (n = 5) and 300 ppm (n = 4) groups were selected for biological sampling." What were the conditions and how were "unacceptable litters" determined? Please address.

Page 21:

- The report stated that "chemical consumption from LD 14–21 was not calculated due to the entire litter eating feed and an accurate assessment could not be made." Why not? The feed consumption can be and should be presented as litter in a typical reproduction study.

Page 23:

- In Table 8, were there other developmental hallmarks that were determined? For example, attainments of sexual maturation and eye opening?

Page 33:

- In the paragraph where liver clinical chemistries were discussed, it is worthwhile to provide the corresponding histological findings which largely mediated these changes.

Page 45:

- As stated earlier, the draft report needs to provide a basis in terms of how an effect was called out even though it was not statistically significant (i.e., occurrence of pancreatic lesions in female rats). The readers would appreciate consistency as well as clarification for such determination.

Page 84:

- First paragraph: it should be noted that the serum PFOA concentrations in the general population of the United States and Western Europe are declining. The draft report needs to reflect this factual data in its interpretation in the first paragraph.
- Second paragraph: it is incorrect to state that "In the current studies, exposure during the perinatal period was up to 300 ppm, which led to plasma concentrations of 74–75 µM in the dam on gestation day (GD) 18 and postnatal day (PND) 4. The similar concentrations at different time periods suggest concentrations were at steady state." This observation reflected the lactation transfer and should not be mistaken as steady state.
- Fourth paragraph: It is not clear what the objective is with the following statement "The rat plasma concentrations were marginally higher than concentrations measured in the NTP 28-day toxicity studies (57 µM) with

administered doses of 100 mg/kg/day via gavage route of exposure." There was no follow-up to this statement.

Page 85:

- Second paragraph: what kind of "liver toxicity" did the male rats exhibit in Study 1? Please specify for clarity.
- Third paragraph:
    - Increases in liver biomarkers should not be interpreted as "hepatocellular injury". In most cases these mild changes reflected adaptive liver hypertrophy.
    - In the sentence where bile acid / cholestasis was discussed, the report should emphasize that it was a possibility (based on increased plasma bile acid from the 16-week interim study male rats) rather than a consistent observation (absent from the 2-year study). In addition, there was no pathological evidence to suggest the occurrence of cholestasis.
- Fourth paragraph: it will be beneficial for this report to expand the discussion on the possible causes for the absence of testicular Leydig cell tumor in male rats. It will be helpful to put the plasma PFOA concentration in context, as well as the resulting aromatase activities.

Page 87:

- Fourth full paragraph: it will be helpful for this report to expand the discussion on the observation of pancreatic cell tumor in female rats, even though it was not observed in a previous study by Butenhoff et al. 2012 (Toxicology 298 1-13).

Page 88:

- First full paragraph: it will be helpful for this report to expand the discussion on the observation of possible uterus finding in female rats, even though it was not statistically significant and it was not observed in a previous study by Butenhoff et al. 2012 (Toxicology 298 1-13).
- NTP only cited Shankar et al. (reference 135) as reporting an association with chronic kidney disease in humans when they (Shankar et al.) conducted a cross-sectional analysis of the NHANES database (1999-2000; 2003-2008 cycles). NTP failed to cite several more pertinent studies related to chronic kidney disease and exposure to PFOA. While the following is not a comprehensive review, it does demonstrate the need for NTP to provide a much more exhaustive review in their discussion section on chronic kidney disease. Two cohort studies of considerably higher exposed occupational workers reported inconsistent associations with mortality from chronic kidney disease and PFOA (Steenland and Woskie 2012 Am J Epidemiol 176 909-917; Raleigh et al. 2014 Occup Environ Med 71 500-506). A prospective study of highly exposed occupational workers with chronic kidney disease incidence (Steenland et al.

2015 Occcup Environ Med 72 366-372) did not report an association with chronic kidney disease and PFOA. As discussed earlier, NTP should be very careful with reviewing the evidence of chronic kidney disease, as was expressed by Watkins et al. (2013 Environ Health Perspect 121 625-630) and Dhingra et al. 2017 (Environ Health Perspect 125 416-421) in their evaluation of chronic kidney disease by only using serum concentrations of PFOA (not a modelled estimate) as measurements of PFOA may reflect the consequence of the disease, and not the cause, when citing cross-sectional studies such as Shankar et al (reference 135) with chronic kidney disease. Finally, NTP should acknowledge that the C8 Science Panel did not declare a probable link for chronic kidney disease in large part due to the findings from their longitudinal community worker study by Dhingra et al. (2016 Environ Res 145 85-92). Of this 32,254 community and worker cohort, Dhingra et al (2016). reported there were 397 medically confirmed cases of chronic kidney disease (187 diabetics, 110 non-diabetics). Modeled cumulative PFOA exposure was used as the exposure metric as has been described elsewhere by Shin et al. (2011 Environ Health Perspect 119 1760-1765). For the full cohort that included the 397 cases and 27,843 non-cases hazard ratio for chronic kidney disease per modeled cumulative exposure quintile, Dhingra et al. (2016) reported hazard ratios (HR) per quintile of 1.00 (reference), 1.26 (95% CI 0.90, 1.75), 1.12 (95% CI 0.80,1.55), 1.12 (0.81,1.56), and 1.24 (0.88,1.75) with a log (cum exp) trend test = 0.80. Furthermore, Dhingra et al. (2016) did not find significant trend tests for HR of chronic kidney disease and PFOA whether they examined only the non-diabetic population, conducted only a prospective study, examined different lag analyses or modeled year-specific PFOA serum concentration quintiles. Thus, on page 88, NTP should greatly expand its review of chronic kidney disease and potential exposure to PFOA. To only cite Shankar et al. (reference 135), as evidence for chronic kidney disease with exposure to PFOA, is highly misleading.

Page 89 (conclusions)

- There is no mention of the lack of testicular Leydig cell tumor findings in this study with exposure to dietary PFOA – this should be clearly discussed in this conclusion of this draft report.