October 14, 2015

VIA EMAIL AND OVERNIGHT MAIL

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Re: 2015 DART Prioritization (PFOS)

Dear Dr. Gold, DARTIC Members, and Dr. Sandy:

I am pleased to submit the attached comments of 3M Company on the proposed prioritization of perfluorooctane sulfonate (“PFOS”).

Up until its voluntary phase-out of PFOS and related chemistries, 3M Company was the only known manufacturer of PFOS and PFOS precursors in the United States. 3M has worked closely with US EPA on regulatory measures restricting these chemicals’ manufacture, import and use. Over the years, the company also has invested substantial resources to understand the effects of these chemicals on human health. The attached comments reflect the in-depth analysis of these chemicals by the company’s experts. In sum, 3M Company’s experience, expertise and product stewardship of these chemicals are valuable assets that can support the efforts of the Developmental and Reproductive Toxicant Identification Committee (the “DARTIC”) and OEHHA in this proceeding.

3M Company also separately is submitting comments on the proposed prioritization of perfluorooctanoic acid (“PFOA”). Although some of 3M’s comments on these two chemicals (including this cover letter) overlap, we believe it is appropriate to submit separate comments on these two separate chemicals to avoid their inadvertent conflation.

We understand the prioritization process to embody a somewhat qualitative approach to ascertaining whether a particular chemical should undergo the next regulatory step, OEHHA’s resource-intensive process of developing hazard identification materials. The goal of the
prioritization process is to focus the DARTIC’s efforts on “chemicals that may pose significant hazards to Californians.” Among the factors to be considered are the potential for exposure to the chemical and the overall evidence of a causal relationship between exposures to the chemical and the health effects of concern under Proposition 65.

As discussed in more detail in the attached comments, PFOS should not be designated as a high priority for further evaluation under Proposition 65 because:

- 3M Company was the only known manufacturer of PFOS and PFOS precursors in the United States. The company initiated a voluntary phase-out of these chemicals in 2000.
- Since 2002, US EPA has imposed strong restrictions on the manufacture, import and use of PFOS and PFOS precursors pursuant to its Significant New Use Rule authority under the Toxic Substances Control Act.
- There is an unmistakable downward trend in residues of PFOS in human blood since 2000, reflecting the results of 3M’s voluntary phase-out and US EPA’s restrictions.
- The overall weight of the evidence of reproductive toxicity, particularly when measured against Proposition 65 listing criteria and combined with existing ample margin of safety, does not warrant the extensive resources necessary for the preparation of hazard identification materials.

The well-documented diminishing exposures to this chemical, alone, warrant a finding that it should not be designated as high priority. For this and further reasons detailed in the attached comments, we respectfully submit that prioritizing PFOS will not achieve the process’ goal of focusing the DARTIC’s efforts on chemicals that may pose significant hazards to Californians.

We trust that the enclosed comments will be helpful to the DARTIC and OEHHA’s evaluation of this chemical for prioritization.

Very truly yours,

Ann G. Grimaldi

Encl.

cc: Carol Monahan-Cummings, OEHHA Chief Counsel (via email)

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Recommendation Regarding
Prioritization of

PFOS

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October 14, 2015
**EXECUTIVE SUMMARY**

The Office of Environmental Health Hazard Assessment (“OEHHA”) of the California Environmental Protection Agency is soliciting public comments on five chemicals proposed for the Developmental and Reproductive Toxicant Identification Committee (“DARTIC”) consideration as candidates for potential listing as reproductive and developmental toxicants for purposes of Proposition 65. Among the five chemicals proposed in their August 2015 Notice, perfluorooctane sulfonate (“PFOS”) had 15 analytical epidemiologic studies considered as having “adequate quality reporting an association between exposure to the chemical and increased risk of adverse developmental or reproductive outcomes plus additional studies in humans and animals” and was recommended to be considered in the prioritization.

The purpose of Proposition 65 (the Act) is to protect Californians from exposure to reproductive toxicants (and carcinogens) through the discharge prohibition and warning requirement that the Act imposes. PFOS is perfluoroalkyl sulfonate for which 3M announced the phase out in 2000. Given that the serum concentrations of PFOS in the U.S. general population have been steadily declining in the last decade, and PFOS can no longer be manufactured, imported or used without EPA permission in the United States, it does not warrant priority attention from OEHHA and the DARTIC. We respectfully submit that further review of PFOS is not necessary to accomplish the goals of the Act, and would unnecessarily divert the OEHHA’s and DARTIC’s valuable resources that otherwise could be invested in other efforts where more meaningful public benefit would result, for the following reasons:

1. **The Absence of Production of PFOS in the United States.** Manufacturing and importation of PFOS into the United States ceased in 2002 (except for a small number of critical applications with limited exposure potential) under restrictions imposed by the United States Environmental Protection Agency (“US EPA”).

2. **Declining Residues in Human Blood.** There is an unmistakable downward trend in the levels of PFOS found in the U.S. general population in the last 15 years. Based on CDC’s National Health and Nutrition Examination Survey (NHANES) data, mean blood levels of PFOS in the general population have declined by approximately 80% since 1999-2000.

3. **Absence of Data That Would Support Reproductive Toxicity.** Our review on the data identified in the Prioritization Notice, and other data that were omitted, are discussed in detail below.

   (i) The reported epidemiological associations between PFOS and reproductive toxicity in humans is likely confounded the underlying pharmacokinetics of PFOS; and

   (ii) Developmental observations reported in the laboratory rodents for PFOS were primarily mediated by maternal effects (developmental effects in offspring associated with PFOS-effected maternal animals). In addition, rodents may not the most appropriate for the hazard assessments of PFOS due to demonstrated differences in mode of action data.
4. **An Ample Margin of Safety.** Even if PFOS was a strong candidate for listing (which is not supported by the data), the levels of PFOS causing a potential in reproductive / developmental toxicity in rats are *two to three orders of magnitude* higher than the levels experienced by the general population, demonstrating an ample margin of safety.

In summary, the above four points lead to the reasonable conclusion that PFOS *should not be assigned a high priority* for review by OEHHA. In the detail that we provide below, we address each of the above points, including the issues related to exposure and the human and animal data relevant to the potential reproductive toxicity of PFOS.
DISCUSSION

1. The Absence of Production of PFOS in the United States

3M was the only known manufacturer of PFOS and PFOS-precursor products in the United States. From the early 1960s until 2000, these materials were used in an increasingly wide variety of consumer and industrial products. In 1998, 3M scientists reported to the US Environmental Protection Agency (EPA) that had identified PFOS in the blood of the general population (at levels measured in parts per billion, ng/mL). In May 2000, 3M announced that it was voluntarily phasing out of production of PFOS and products that could degrade or metabolize to PFOS.

After 3M ceased the manufacture of PFOS, the US EPA promulgated federal regulations that prevent other manufacturers (as well as 3M) by law from manufacturing or importing PFOS or PFOS precursors, subject to a handful of very narrow critical use exceptions with limited exposure potential approved by EPA. See 40 Code of Federal Regulations §721.9582, listing several hundred PFOS precursors that cannot be manufactured or imported without EPA permission, and the permissible uses approved by EPA via its Significant New User Rule (SNUR). EPA’s rules allowed the continuation of a few specifically limited, highly technical uses of these chemicals for which no alternatives were available, and which were characterized by very low volume, low exposure and low releases. Any other uses of these chemicals would require prior notice to and review by the Agency.

2. Residual Levels of PFOS in Blood in the United States General Population Have Declined and Continue to Consistently Decline Since Production Ceased.

According to the National Health and Nutrition Examination Survey (United States Centers for Disease Control (CDC) National Center for Environmental Health), which is a nationally representative sample of the U.S. population (noninstitutionalized), the concentration of PFOS in the serum (blood) of the general population has declined by approximately eighty percent since 2000 as reported for the geometric mean and the 95th percentile. This decline in PFOS is observed across males and females (see Figures 1A and 1B), age (see Figures 2A and 2B), and ethnicity/race (see Figures 3A and 3B). Data obtained for these figures can be found in the Fourth National Report on Human Exposure to Environmental Chemicals (See http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf, accessed October 9, 2015). The 80% decline in the geometric mean in the general population between 2000 and 2012, given the absence of production activities in the United States, is reasonably consistent with a published geometric mean serum elimination “half-life” of 4.8 years in retired occupationally exposed 3M production workers (Olsen et al. 2007).
Other cross-sectional biomonitoring studies, including analyses from six American Red Cross blood donation centers that have been collected in 1999-2000 (Olsen et al. 2003), 2006 (Olsen et al. 2008), and 2010 (Olsen et al. 2012). One of these blood donation centers is the American Red Cross Southern California Region located in Los Angeles. Biomonitoring California has presented geometric mean results of several studies that they are conducting since 2010. However, all of these Biomonitoring California studies began collecting samples in 2010 or later so trend data are not available at this time. The largest biomonitoring study, the California Teachers Study (CTS), reported a geometric mean serum PFOS concentration of 6.85 ng/mL and a 95th percentile of 20.6 ng/mL for serum samples collected between 2011-2013 from 856 primarily white women (See http://www.biomonitoring.ca.gov/sites/default/files/downloads/California_Teachers_Study_PFCs_07112013_1.pdf, accessed October 9, 2015). These CTS findings are similar to the female PFOS serum data for NHANES in the 2011-2012 time period (see Figure 1A and 1B).

Given the absence of PFOS production since 2002 and subsequent US EPA regulatory decisions (see 1, vide supra), it is anticipated PFOS serum concentrations will continue to decline. Biomonitoring data for the 2013-2014 period is anticipated to be released by NHANES within two years. The American Red Cross blood donor study is currently analyzing blood samples collected in July 2015 from the same six donation centers in their previous studies conducted in 2000, 2006, and 2010 (Olsen et al. 2003; 2007b; 2010). Ongoing Biomonitoring California study findings can be found on its website (See http://www.biomonitoring.ca.gov, accessed October 12, 2015).

3. Absence of Data That Would Support Reproductive Toxicity.

The Prioritization Process requires the OEHHA staff to screen chemicals for reproductive effects based on human epidemiological and laboratory experimental data. Although the prioritization process evaluates chemicals in a somewhat qualitative manner, the evaluation of studies against Proposition 65 listing criteria is a useful measure of how a chemical should be prioritized. In these comments we discuss numerous studies not identified in OEHHA’s Prioritization Notice. We submit that current data would not support a listing decision. This conclusion, combined with the cessation of PFOS production and importation, as well as the steep decline in blood serum levels, warrant a finding that PFOS should not be designated as a high priority chemical.

A reproductive toxicant is defined by the State as follows:

- A chemical is deemed “known to the State to cause reproductive toxicity” if “it has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity.”

- For definition purpose, “reproductive toxicity” includes developmental toxicity, female reproductive toxicity, and male reproductive toxicity and it is recommended that “a weight-of-evidence approach” be used when evaluating the available data.

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1 Cal. Health & Safety Code § 25249.8(b). (emphasis added)

According to OEHHA, it requires a “causal relationship between the chemical and reproductive toxicity in the human data.”

Or, it requires “studies in experimental animals indicate that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible.”

In the section titled “Human Data” below, relevant and controversial epidemiological studies that address human reproductive data are discussed in detail and the weight-of-evidence do not support a causal relationship between PFOS and reproductive toxicity in humans. In the section titled “Animal (mammalian) Data” below, several comprehensive reproductive and developmental toxicity studies are also reviewed. These studies provide strong evidence that many of the developmental outcomes reported in laboratory rodents are the consequence of maternal effects. In addition to the fact that these effects occurred at serum PFOS concentrations that are several orders of magnitude higher than general population, mode-of-action data further suggest that rodents may not be the most appropriate species for the hazard assessment of PFOS toxicity in humans.

**Human Data:**

Several epidemiologic associations that have been reported are likely confounded by the underlying pharmacokinetics of PFOS as related to the physiology and/or pathology of the outcomes studied.

Longnecker (2006) commented the advent of modern analytical chemistry not only enabled lower concentrations of environmental chemicals to be biomonitored but also allowed for a great proportion of the variation measured could be accounted for by differences in subjects’ metabolism and excretion. The low concentrations measured may be a reflection of the byproduct of the underlying pharmacokinetics, systems biology, and pathogenesis. Several of the epidemiologic associations that have been identified as statistically significant findings in the OEHHA epidemiologic screen process may be confounded by the underlying pharmacokinetics of PFOS as related to the pathophysiology of these outcomes. This includes epidemiologic associations related to PFOS and time to pregnancy (subfecundity), birth weight, delayed menarche, decreased breast feeding duration, early onset menopause, and endometriosis.

As stated in the OEHHA criteria for recommending chemicals for listing, sufficient evidence in humans to list as “known to the state to cause reproductive toxicity” requires epidemiological studies to be scientifically valid according to generally accepted principles, provide convincing evidence to support a causal relationship between exposure and the developmental or reproductive effect in question which requires accurate exposure and toxicity endpoint classification and proper control of confounding factors, bias and effect modifiers.

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3 Cal. Code Regs., tit.27, §25306(g)(1).
4 Cal. Code Regs., tit.27, §25306(g)(2).
We illustrate three examples that OEHHA will encounter as it ascertains whether there was “proper control of confounding factors.” These examples are: a) PFOS and time to pregnancy; b) PFOS and birth weight; and c) PFOS and delayed onset to menarche.

a). Time to Pregnancy

The initial investigation that suggested an association between PFOS and increased infertility and decreased fecundability was a study of the Danish National Birth Cohort (DNBC) (Fei et al. 2009). The DNBC is a nationwide follow-up study of approximately 100,000 children and their mothers. Pregnant women in their first trimester were recruited through their physicians. Fei et al. randomly selected 1400 women from all participants (n = 43,045) who gave birth to a single live born child without congenital malformation and who participated in a set of 4 telephone interviews, including questions regarding the length of time required to have achieved a planned successful pregnancy. Blood samples (weeks 4 – 14 of pregnancy) were used to measure PFOS among the 1240 women who met this definition. Infertility was defined as reporting a time to pregnancy (TTP) > 12 months or infertility treatments for this current pregnancy. Fecundity odds ratios (FORs) were calculated that measured the odds of a successful conception for women who had higher levels of PFOS compared with the reference level within a given calendar month, given that pregnancy was not achieved in the prior month. FORs < 1 indicate decreased fecundity and a longer TTP.

Among the 1240 women with planned pregnancy, their mean PFOS concentration was 35.5 ng/mL. The mean PFOS concentrations by time to pregnancy (number of participants in parentheses) were 34.6 ng/mL at < 6 months (n = 861), 36.6 ng/mL at 6 – 12 months (n = 191), and 38.3 ng/mL at >12 months (n = 188), respectively.

Provided in Table 1 are the odds ratios for infertility and fecundability from the Fei et al. (2009) study for PFOS. These odds ratios were adjusted for maternal age at delivery, parity, pre-pregnancy BMI, maternal SES, alcohol consumption before pregnancy, paternal age, and paternal education. There were statistically significant trends for infertility and fecundability with PFOS. Fei et al. acknowledged that the exposure time window of interest was at the start of pregnancy planning but their exposure data for PFOS were measured at 4 – 14 weeks gestation and would have been rather stable over pregnancy due to the long elimination rate for PFOS in humans (Olsen et al. 2007). Fei et al. suggested exposure to PFOS at levels found in the general population may increase TTP and could explain some of the fertility differences among different populations developed countries.

Based on their review of the Fei et al. (2009) data, Olsen et al. (2009) discussed that parity is both an outcome of fecundity and is associated with perfluoroalkyl concentrations. Because perfluoroalkyl levels would be lower after a pregnancy, a longer interval between births would result in more time for a woman to absorb concentrations that could replace the loss incurred from the birth. In other words, there would be a longer time for reaccumulation to occur. Women who begin with comparable perfluoroalkyl concentrations and equal parity may have different perfluoroalkyl concentrations at their next birth based on the time elapsed between births (which includes the time required to become pregnant). Olsen et al. surmised
if all else is equal, those women with longer TTP will have longer intervals of time between births and so may have higher perfluoroalkyl levels prior to the next pregnancy. This would result in an association between perfluoroalkyl concentrations and TTP but the direction of the causality would be backwards (i.e., reverse causation).

Whitworth et al. (2012) elaborated upon this hypothesis in a case-control study of women who originated from the Norwegian Mother and Child Cohort (MoBa) Study. Eligibility in their study was similar to Fei et al. (2009). Women were restricted to those who delivered a live-born child and provided a plasma sample around 17 weeks of gestation. Subfecund cases (n = 416) were defined as TTP > 12 months. Controls (n = 494) were defined as TTP ≤ 12 months. Median PFOS concentrations were 14 ng/mL for cases and 13 ng/mL for controls. Whitworth et al. (2012) stratified their results by parity (nulliparous vs. parous). Parity was not considered a potential confounder because it is influenced by a woman’s underlying fecundability. Among parous women, the interval between the 2 most recent pregnancies, the number of previous pregnancies, and the duration of breast-feeding were examined for their influence on measured levels of PFOS.

Among parous women, Whitworth et al. (2012) reported odds ratios for TTP of similar magnitude as Fei et al. for PFOS (Table 1). However, among nulliparous women, they reported odds ratios for TTP below null and the trend appeared to decrease with increasing PFOS. In additional analyses, Whitworth et al. concluded that due to the pharmacokinetics of perfluoroalkyls during pregnancy, delivery, and lactation, associations between PFOS and subfecundity may be produced when a causal association does not exist. They recommended studying nulliparous women regarding the potential reproductive toxicity of PFOS.

Because PFOS was not measured at the beginning of the time to pregnancy interval but after a pregnancy had been achieved, Fei et al. (2012) acknowledged in a commentary that TTP could have potentially influenced the measurement of PFOS in their data. Fei et al. then reanalyzed their data by stratifying on parity and still found an association with infertility and fecundability with PFOS.

In a third analysis of the DNBC data, Bach et al. (2015) analyzed a second (new) participant subsample of the DNBC that differed somewhat in methodology, including covariates, from the original study as published by Fei et al. (2009). In this second sample, there were 65% fewer subjects (n = 440) than the original study described above. Median PFOS serum concentrations were slightly less at 27.9 ng/mL. Unlike the first analysis (Fei et al. 2009), and its reanalysis (Fei et al. 2012), Bach et al. (2015) did not observe an association between PFOS and infertility or fecundability (Table 1).

Other studies (see Table 1) have been published including relatively small prospective cohort studies by Vestergaard et al. (2012) and Buck Louis et al. (2013). Neither have shown an association between TTP and PFOS. Nor have associations been reported between TTP and PFOS by Jørgensen et al. (2014). Vélez et al. (2015) recently reported on a data set from the Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. Information on TTP and maternal blood concentrations was collected during the first trimester of pregnancy (6 to < 14 weeks) of the current pregnancy. A total of 1,625 subjects...
were included in this analysis. Concentrations were log-transformed and divided by their SDs. The geometric mean PFOS concentration was 4.59 ng/mL. The adjusted odds ratio for fecundability was 0.96 (95% CI 0.91 – 1.02).

In summary, women with longer TTP will have longer intervals of time between given births and therefore may reaccumulate higher PFOS levels prior to the next pregnancy compared to women with shorter TTP. This would result in longer TTP measurements associated with higher PFOS levels, but the direction of the causality would be backwards; it would be the longer time between births (including the TTP) that resulted in higher PFOS concentrations.

b). Birth weight

Based on a qualitative literature review of PFOS and birth weight, Bach et al. (2015) identified 8 epidemiologic studies (Apelberg et al. 2007; Chen et al. 2012; Darrow et al. 2013; Fei et al. 2007; Hamm et al. 2010; Inoue et al. 2004; Maisonet et al. 2012; Washino et al. 2009) that examined PFOS as a continuous variable. All eight studies were from general populations. Six of these studies reported an association between PFOS and lower birth weight (Apelberg et al. 2007, Chen et al. 2012, Darrow et al. 2013, Fei et al. 2007, Maisonet et al. 2012, Washino et al. 2009) but only three (Washino et al. 2009, Chen et al 2012, Maisonet et al. 2012) were statistically significant. Bach et al. concluded PFOS exposure was associated with decreased average birth weight but the impact on public health was not clear. Bach et al. did not conduct a meta-analysis.

A meta-analysis on prenatal PFOS and birth weight was subsequently performed by Verner et al. (2015) (see Figure 4, Reported Model). They included the same above mentioned studies, except Darrow et al. (2013) and Inoue et al. (2004) but included Whitworth et al. (2012) which Bach et al. (2015) did not. Verner et al. reported a reduced association with birth weight in six of these seven studies. The summary meta-analysis estimate for the seven studies was -5.0 g (95% CI -8.9, -1.1) birth weight per ng/mL increase in PFOS.

However, none of these epidemiologic studies cited above considered the potential confounding that could arise from the glomerular filtration rate (GFR). The maternal GFR increases within one month of conception (Helal et al. 2012) with maternal GFR and renal blood flow increasing by 40 – 65% and 50 - 85%, respectively, during a normal pregnancy. Whitworth et al. (2012) suggested that, because GFR is diminished in lower weight infants, this could lead to less renal elimination of PFOS; thus raising the question whether the epidemiologic studies that assessed a relationship between birth weight and PFOS were confounded by not considering for GFR.
Figure 4 - from Verner et al. 2015, Environ Health Perspect DOI: 10.1289/ehp.1408837.
Difference in birth weight (g) per 1 ng/mL increase in reported and simulated PFOS levels. Simulated model provided the overall maternal (-1.46 g) and cord (-2.72 g) per ng/mL PFOS. The size of the square represents the weight of each study in the calculation of the overall meta-analytic association.
Vesterinen et al. (2015) used the Navigation Guide methodology to offer a systematic analysis of the scientific literature on whether there was an association between GFR and fetal growth. Vesterinen et al. proposed three relationships to consider in assessing fetal growth: (1) fetal growth and GFR; (2) fetal growth and plasma volume expansion (PVE); and PVE and GFR. (See Figure 1 in the Supplement to the Vesterinen et al. paper.) They examined 35 studies through the same Navigation Guide methodology. Vesterinen et al. found consistent evidence of an association among studies reporting the relationship between birth weight and PVE but they found the studies between GFR and birth weight were inconsistent and the majority had small sample sizes (range 9 to 283). They also had low confidence in the studies that examined the relationship between PVE and GFR. Vesterinen et al. concluded “the strength of the evidence of an association between fetal growth and GFR was not classifiable based on the low quality and indeterminate direction of effect of human studies and the small number and size of non-human mammalian studies which were of low quality with indeterminate direction of effect.” Nevertheless, Vesterinen et al. acknowledged “A well-conducted observational human study could increase our confidence in the strength of the association” and because “the review process involved judgments, a different group of researchers at a different time might reach a different conclusion.”

Verner et al. (2015) had the distinct advantage of having a one critically-important paper (Morken et al. 2014) that was not available to Vesterinen et al. (i.e., not yet published) and they concluded “there is reason to believe a true association exists between maternal GFR during pregnancy and birth weight.” Morken et al. examined a sub-cohort of 953 women (470 women with and 483 women without preeclampsia) in the Norwegian Mother and Child Cohort (MoBa). The sample size represented 29 more subjects than the combined total (n = 924) from the 13 “small sample studies that were available to Lam et al. at the time of their review (as discussed above). Morken et al. found a statistically significant association between maternal GFR in the second trimester and infant birth with using two different formulas in the total cohort, but not with a third estimated GFR formula. The inclusion of women with preeclampsia in this study increased the study power because it increased the proportion of small-for-gestational age infants in the analysis.

Upon their review of the literature and concluding there was likely a true association between maternal GFR and birth weight, Verner et al. (2015) then modified an existing physiologically based pharmacokinetic model (PBPK) of pregnancy and lactation and PFOS (Loccisano et al. 2012; Loccisano et al. 2013) to address how much of the PFOS-birth weight association might be attributable to GFR. They compared a simulated estimates from their PBPK model to those from their meta-analysis of 7 epidemiologic studies (see Figure 4, Reported Model). Using Monte Carlo and sensitivity analyses, Verner et al. reported the association between maternal plasma levels (per 1 ng/mL increase) and birth weight was strongest at birth as the association between simulated cord plasma levels and birth weight was -2.7 g (95% CI -3.4, -2.0) per ng/mL increase PFOS compared to 1.5 g (95% CI -1.8, -1.1) based on maternal plasma levels (see Figure 4, Simulated Model).
Verner et al. concluded a substantial proportion of the association between prenatal PFOS and birth weight may be attributable to confounding by GFR. Also, Verner et al. concluded epidemiologic studies that measured PFOS early in pregnancy may have been less confounded by GFR than those who measured PFOS late in pregnancy.

In summary, epidemiological associations between maternal PFOS and birth weight are confounded by GFR.

c). Delayed age at menarche

Based on the cross-sectional C8 Health Project data obtained in 2005-2006, Lopez-Espinosa et al. (2011) categorized 3,067 boys and 2,931 girls aged 8 – 18 years as whether they have reached puberty based on sex steroid hormone levels or onset of menarche. Using total testosterone (> 50 ng/dL) or free testosterone (> 5 ng/dL) in boys and self-reported menarche and estradiol >20 pg/mL in girls as markers of puberty, PFOS (girls only) was associated with median delays of three to six months based on quartile analyses. The authors acknowledged that clearance may have an explanatory role, as an earlier menarche would result in behavioral and physiologic changes, including menstrual blood loss, which may result in lower PFOS levels. Increase in body mass with the onset of male puberty could also be a factor in this association.

The delayed menarche association reported by Lopez-Espinosa et al. for PFOS was not observed in two longitudinal studies (Christensen et al. 2011; Kristensen et al. 2013). Christensen et al. (2011) conducted a nested case-control study within a cohort of approximately 14,000 pregnant women in 1991-1992. Cases were defined as female offspring who self-reported menarche before 11.5 years (n = 218). Median PFOS concentrations for females who reported an early menarche was 19.5 ng/mL compared to 20.0 ng/mL for those who did not report an early menarche (n = 230). The adjusted odds ratios was 0.83 (95% CI 0.56 – 1.23) for reporting an earlier age at menarche. Kristensen et al. examined the recalled age of menarche among 343 daughters aged 20 years whose mothers had an archived blood sample measured while at pregnancy week 30. Median maternal PFOS was 21.1 ng/mL. Mean age of menarche was 13.2 years. Daughters exposed to PFOS in utero were 1.5 months later in age (95% CI -2.5, 5.4) at menarche among the highest exposed group (maternal PFOS level 23.6 – 53.1 ng/mL) compared to the referent group (2.7 – 18.0 ng/mL PFOS maternal level).

A Monte Carlo PBPK simulation model was developed that incorporated significant points of pubertal development that included growth spurts and menarche (Wu et al. 2015). The model included compartments for plasma, gut, liver, fat, rest of body, kidney, filtrate, and storage. Tissue volumes and tissue blood flow rates were estimated based on body weight, body height, body surface area, and body mass index. Daily exposure to PFOS in plasma was from several sources. PFOS concentrations were simulated for a distribution of individuals 2 to 20 years of age with similar physiologic characteristics as those reported by Lopez-Espinosa et al. Models of growth were based on simulated population matches of the 5th, 50th, and 95th percentiles of the NHANES 2003-2004 data. Monte Carlo simulations showed the distribution of serum PFOS concentrations to be very similar between the PBPK
model and the Lopez-Espinosa et al. study population. The delay in menarche in days per
natural log of PFOS was approximately one-third that reported in the Lopez-Espinosa et al.
paper that was discussed above. See Table 2

In summary, the association between serum PFOS concentrations and delayed age at
menarche may be due, in part, to dilution (through growth of adolescents) and excretion (via
menstruation).

**Human Data Summary:** This review of three epidemiological associations with PFOS (time to
pregnancy, birth weight, and delayed menarche) demonstrates the confounding of the underlying
pharmacokinetics of PFOS as related to the pathophysiology of these outcomes studied. The above
three examples illustrate the challenges of interpreting the existing epidemiology literature. Several
other epidemiologic associations (e.g., decreased breast feeding duration, early onset menopause, and
endometriosis) that have been identified as statistically significant findings in the OEHHA
epidemiologic screen process are also confounded by the underlying pharmacokinetics of PFOS as
related to the pathophysiology of these outcomes. Whether confounding factors, bias, and effect
modifiers have been properly controlled in these epidemiologic associations, as well as others, is a
critical component of a proper evaluation of these studies for prioritization.

**Animal (mammalian) Data:**

A number of experimental animal (mammalian) toxicological studies on the reproductive and
developmental effects of PFOS have been published (Abbott et al. 2009; Butenhoff et al. 2009; Case
et al. 2001; Gortner 1980; Grasty et al. 2005; Lau et al. 2003; Luebker et al. 2005a; Thibodeaux et al.
2003, 2004). These studies included detailed information on the developmental toxicity with these
compounds as well as valuable insights on the role of maternal effects and its attribution to the
developmental outcomes in laboratory animals. Comprehensive review on the developmental
toxicity of the perfluoroalkyl acids was first reported in 2004 (Lau et al. 2004) and updated
subsequently (Abbott 2015; Andersen et al. 2008; Lau et al. 2004).

Overall, PFOS did not affect male or female reproductive functions in the laboratory animals.
These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive
organ morphology. The potential of PFOS to influence reproductive performance was evaluated in
2005a), and rabbits (Case et al. 2001). Gestational exposure to PFOS did not affect the number of
embryonic implantation sites in several strains of mice (CD-1, Sv129, or PPARα knockout) (Abbott
et al. 2009; Thibodeaux et al. 2003, 2004). Similarly, implantations were not affected in rabbits
either when exposed up to 3.75 mg/kg-d during GD 7 – 20 (period of organogenesis) albeit decreased
body-weight gain and food consumption were observed (Case et al. 2001). In rats, oral
administration of PFOS up to 10 mg/kg-d during GD 6 – 15 (period of organogenesis) also caused
reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of
the dams (Gortner 1980). In a two-generation reproduction/developmental study in rats (Luebker et
al. 2005a; Luebker et al. 2005b), potassium PFOS (given as potassium salt) doses as high as 3.2
mg/kg-d given to male and female rats for 6 weeks prior to mating, through mating and, for
females, through gestation and lactation. PFOS did not adversely affect mating or fertility
parameter in male or females, including fertility and pregnancy indices, estrous cycling, number of pregnancies per number of matings, number of days to inseminate, number of matings during the first week of cohabitation, epididymal sperm maturation, litter averages for corpora lutea, implantations, viable embryos, non-viable embryos, and reproductive organ histology. In particular, there were no statistically significant differences between control and potassium PFOS-treated females in the mean number of estrous cycles, rats with \( \geq 6 \) consecutive days of diestrus or estrus during the 28-day evaluation period. In a developmental neurotoxicity study with PFOS, pregnant female rats received PFOS doses up to 1 mg/kg/day from gestation to lactation. No PFOS treatment-related effects were noted on maternal health or reproductive outcomes (Butenhoff et al. 2009). Furthermore, the morphologic effects of PFOS on reproductive organs in non-human primate were evaluated from a six-month oral study and results indicated no abnormalities (Seacat et al. 2002).

The developmental effects reported in the laboratory animals for PFOS were primarily mediated by maternal effects. Experimental evidences demonstrate that developmental effects associated with PFOS exposures in offspring are observed when maternal animals were effected such as body weights. Evidence involving maternal effects in the outcome of the developmental toxicity include the following examples.

PFOS developmental toxicity has been evaluated in several laboratory species. In rabbits, oral PFOS administration ranging from 0.1 – 3.75 mg/kg/day was given from GD 6 – 20 and decreased maternal body-weight gain was observed at 1 mg/kg dose group or higher. No abnormal fetal effects were noted except decreased fetal body weight, which was observed with 2.5 and 3.75 mg/kg/day dose groups only. Study authors concluded that “The fetal effects occurred at maternally toxic dose levels and no fetal changes were present at nontoxic maternal doses” (Case et al. 2001). In mice, there was a statistically significant \((p < 0.05)\), dose-related increase in maternal liver weight when pregant dams were treated during gestation at a dose as low as 1 mg/kg potassium PFOS (Thibodeaux et al. 2003). Various developmental effects were reported (e.g., decreased postnatal survival and growth deficits) but primarily for litters from dams receiving 10 mg/kg/day potassium PFOS or higher (Lau et al. 2003). In addition to mice, the developmental toxicity of PFOS has also been evaluated in rats. Oral administration of PFOS during gestation to pregnant rats caused reduced maternal body-weight gain and fetal body-weight gain at 2 mg/kg-d maternal dose group or higher (Lau et al. 2003). In a two-generation reproduction/developmental study in rats by Luebker et al. (2005), described in details above, reported reduced body weight and body weight-gain at parental generation at 0.4 mg/kg or higher. Developmental hallmarks similar to previously reported by others \((i.e., \text{decreased fetal body weight, decreased postnatal survival, and developmental delays})\) were observed in pups from 1.6 mg/kg/day maternal dose groups or higher.

In the recent years, there have been toxicological studies reporting on the endocrine disturbance potential with PFOS exposures. Most of these studies were done either under \textit{in vitro} conditions (to which high concentrations of PFOS were employed) or \textit{in vivo} but only with a limited set of endpoints evaluated such as selected gene expressions (Feng et al. 2015; Lopez-Doval et al. 2015; Lopez-Doval et al. 2014; Pereiro et al. 2014; Wang et al. 2011). Endocrine is a very complicated system and evaluation of endocrine functions is a very highly specialized field (this is especially true in human clinical medicine). Given that PFOS is a strong surfactant, the toxicity
effects reported from the typical mono-layered in vitro tissue culture system offered very little insight and scientific value because the data were often comprised by the surfactant-induced toxicity. Similarly, gene expressions do not represent functionality and endocrine function is an intricate network.

Based on data from the large scale 2-generation reproductive and developmental studies (which are considered as the most comprehensive test by various agencies for evaluating endocrine functions), PFOS clearly did not alter the endocrine functions as the reproductive functions and performances in both males and females were normal (vide supra). If PFOS is indeed an endocrine disrupting compound, then one would expect it to directly activate endocrine receptors such as estrogen receptors or thyroid receptors. Ishibashi et al. (2007) reported that PFOS did not activate human estrogen receptor α or β. Due to a known methodological bias, Chang et al. demonstrated that PFOS did not disrupt hypothalamus-pituitary-thyroid axis and the thyroid hormone homeostasis in rats were maintained in toxicological studies, including rats that were exposed during gestation and lactation (Chang et al. 2009; Chang et al. 2008; Chang et al. 2007). There was no increase in thyroid follicular cell proliferation in rats after 28 days of dietary exposure with PFOS (Elcombe et al. 2012b). In a developmental neurotoxicity study with PFOS in rats, there were no changes in serum TSH levels in either dams or pups and thyroid tissue morphology were normal (Chang et al. 2009). Furthermore, while triiodothyronine (T3, the active form of thyroid hormone) elicits a dose-response activation of human thyroid receptor α from 0.000001 – 0.01 uM, under the same study condition, there was no activation of human thyroid receptor α when exposed to ammonium PFOS up to 100 uM (3M Company, unpublished data).

The listing process also requires OEHHA staff to consider “sufficient evidence in experimental animals (mammals), such that extrapolation to humans is appropriate.” In toxicology studies, liver is the primary target organ when the laboratory animals were exposed to PFOS and mechanistic research has shown that many intermediary metabolic effects can be explained by the activation of xenosensor nuclear receptors such as PPARα, constitutive androstane receptor (CAR), and pregnane X receptor (PXR) in the liver (Elcombe et al. 2012a; Elcombe et al. 2012b). Even though study with PFOS using PPARα knockout mice did not completely attenuate the developmental effects compared to the wildtype, the % neonatal survival did improve (Abbott et al. 2009). The roles of other nuclear receptors such as CAR and PXR and developmental toxicity in rodents is not known at present time. Because humans are considerably less sensitive to the pleiotrophic effects of PPARα or CAR/PXR activation compared to rodents (Gonzalez and Shah 2008; Klaunig et al. 2003; Lake 2009; Ross et al. 2010), the qualitative differences brings into question the relevance of rodent developmental effects and biological significance to humans.

**Animal (mammalian) Data Summary:** The developmental effects reported in the laboratory animals for PFOS were primarily mediated by maternal effects and based on the recent mode of action data, rodents may not be the most appropriate species for the hazard assessment of PFOS on developmental toxicity in humans.

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4. Even If PFOS Was A Strong Candidate for Listing (which is not supported by the data), the Margins of Safety Are Large Enough That It Should Not Be Assigned A High Priority for Review By OEHHA.

For all of the reasons articulated above, we believe that PFOS should not be considered for high prioritization for review as a reproductive toxicant by OEHHA. Moreover, the steady decline of PFOS serum concentrations in the United States general population (independently documented by CDC and 3M) is a reflection of effective risk management steps taken by 3M and EPA to eliminate production and restrict almost all use of PFOS, thereby greatly reducing exposures.

Information on margins of exposure (margins of safety) may also be informative in setting priorities. We can identify the margin of exposure between the residual serum PFOS concentrations in people and the serum concentration in rats associated with the no effect level for developmental effects. Because the comparison is based on measured serum concentrations, these margins already account for species differences in toxicokinetics.

The levels of PFOS causing a potential in reproductive / developmental toxicity in rats are two to three orders of magnitude higher than the levels experienced by the general population, demonstrating an ample margin of safety. From the two-generation reproductive study (Luebker et al. 2005b), the maternal doses associated with overall NOAEL for parental effects and offspring effects were 0.1 mg/kg-d and 0.4 mg/kg-d, respectively. The corresponding serum PFOS concentration at the end of gestation were 4,520 ng/mL and 26,200 ng/mL, respectively. Based on the 2012 NHANES data, compared to the geometric mean and 95% percentile serum PFOS concentration in the United States (6.3 ng/mL and 21.7 ng/mL, respectively).

<table>
<thead>
<tr>
<th>Human Exposure Level U.S. General Population 2012 Data (NHANES)</th>
<th>NOAEL in Rats</th>
<th>Margin of Exposure (Human Exposure Compared to NOAEL in Rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Serum PFOS Concentration (6.3 ng/mL)</td>
<td>4,520 – 26,200 ng/mL</td>
<td>717 - 4158</td>
</tr>
<tr>
<td>95th Percentile Serum PFOS Concentration (21.7 ng/mL)</td>
<td>4,520 – 26,200 ng/mL</td>
<td>208 - 1207</td>
</tr>
</tbody>
</table>

For this reason, as well as the others above, PFOS should not be assigned a high priority for review by OEHHA.
References:


Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., 2012a. Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. Toxicology 293, 16-29.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Noker, P.E., Butenhoff, J.L., 2012b. Evaluation of hepatic and thyroid responses in male Sprague Dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. Toxicology 293, 30-40.


Table 1. Association between PFOS Plasma Concentrations (ng/mL) and Subfecundity among 910 Subjects (416 Cases, 494 Controls) Subjects from the Norwegian Mother and Child Cohort Study, Norway, 2003-2004 (Whitworth et al. 2004)

<table>
<thead>
<tr>
<th>PFOS (ng/mL)</th>
<th>Fei et al. 2009;</th>
<th>Fei et al. 2012</th>
<th>Adjusted OR (95% CI)</th>
<th>Fei et al. 2009;</th>
<th>Fei et al. 2012</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects Nulliparous Parous</td>
<td>All Subjects Nulliparous Parous</td>
<td>Adjusted OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.4 – 26.0</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>26.1 – 33.3</td>
<td>2.07 (0.62)</td>
<td>0.70 (0.36 – 0.87)</td>
<td>0.79 (0.61 – 3.08)</td>
<td>0.62 (0.46 – 0.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.4 – 43.2</td>
<td>2.52 (1.24 – 5.13)</td>
<td>0.67 (0.53 – 0.84)</td>
<td>0.63 (0.43 – 0.91)</td>
<td>0.64 (0.47 – 0.87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 43.3</td>
<td>1.59 (0.75 – 3.37)</td>
<td>0.74 (0.58 – 0.93)</td>
<td>0.60 (0.41 – 0.87)</td>
<td>0.83 (0.61 – 1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = 0.025</td>
<td>P = 0.036</td>
<td>P = 0.26</td>
<td>P = 0.002</td>
<td>P = 0.006</td>
<td>P = 0.32</td>
<td></td>
</tr>
</tbody>
</table>

Whitworth et al. 2012

<table>
<thead>
<tr>
<th>PFOS (ng/mL)</th>
<th>Whitworth et al. 2012</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects Nulliparous Parous</td>
<td>Adjusted OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>&lt; 10.34</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10.34 – 13.09</td>
<td>1.3 (0.9 – 1.9)</td>
<td>0.8 (0.4 – 1.6)</td>
<td>1.5 (0.9 – 2.5)</td>
</tr>
<tr>
<td>13.10 – 16.60</td>
<td>1.4 (1.0 – 2.0)</td>
<td>0.8 (0.4 – 1.4)</td>
<td>1.5 (0.9 – 2.6)</td>
</tr>
<tr>
<td>≥ 16.61</td>
<td>1.6 (1.6 – 2.3)</td>
<td>0.7 (0.4 – 1.3)</td>
<td>2.1 (1.2 – 3.8)</td>
</tr>
<tr>
<td>Test for trend</td>
<td>P = 0.02</td>
<td>P = 0.30</td>
<td>P = 0.01</td>
</tr>
</tbody>
</table>

Bach et al. 2015

<table>
<thead>
<tr>
<th>PFOS (ng/mL)</th>
<th>All Subjects</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects Nulliparous Parous</td>
<td>Adjusted OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>&lt; 21.0</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>21.0 – 27.9</td>
<td>1.08 (0.81 – 1.44)</td>
<td>1.16 (0.77 – 1.75)</td>
<td>1.04 (0.69 – 1.55)</td>
</tr>
<tr>
<td>&gt; 27.9 – 36.2</td>
<td>0.99 (0.73 – 1.34)</td>
<td>1.01 (0.65 – 1.57)</td>
<td>1.05 (0.69 – 1.60)</td>
</tr>
<tr>
<td>&gt; 36.2</td>
<td>0.99 (0.74 – 1.33)</td>
<td>0.97 (0.62 – 1.51)</td>
<td>1.04 (0.70 – 1.30)</td>
</tr>
<tr>
<td>P = 0.025</td>
<td>P = 0.30</td>
<td>P = 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Vestergaard et al. 2012

<table>
<thead>
<tr>
<th>PFOS (ng/mL)</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td>Adjusted OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
</tr>
<tr>
<td>&lt; 36.28</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥ 36.28</td>
<td>0.98 (0.54 – 1.77)</td>
<td>1.03 (0.72 – 1.47)</td>
</tr>
<tr>
<td>Log-transformed (continuous)</td>
<td>1.39 (0.92 – 2.09)</td>
<td></td>
</tr>
</tbody>
</table>

Buck Louis et al. 2013

<table>
<thead>
<tr>
<th>PFOS Log-transformed and rescaled by the SD</th>
<th>Buck Louis et al. 2013</th>
<th>All Subjects Adjusted FOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.99 (0.85 – 1.17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Velez et al. 2014

<table>
<thead>
<tr>
<th>PFOS Log-transformed and rescaled by the SD</th>
<th>Velez et al. 2014</th>
<th>All Subjects Adjusted FOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14 (0.98 – 1.34)</td>
<td>P = 0.17</td>
<td>0.96 (0.91 – 1.02)</td>
</tr>
</tbody>
</table>
Table 2. Comparison of association between plasma PFOS concentrations and age at menarche in simulated (Wu et al. 2014) and observed (Lopez-Espinosa et al. 2011) girls.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Simulated Wu et al. study</th>
<th>Lopez-Espinosa study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI Delay (days)</td>
<td>OR 95% CI Delay (days)</td>
</tr>
<tr>
<td>PFOS-Q2</td>
<td>0.94 0.87 – 1.02 15</td>
<td>0.72 0.47 – 1.10 79</td>
</tr>
<tr>
<td>PFOS-Q3</td>
<td>0.86 0.80 – 0.94 47</td>
<td>0.55 0.35 – 0.86 141</td>
</tr>
<tr>
<td>PFOS-Q4</td>
<td>0.75 0.70 – 0.82 73</td>
<td>0.55 0.35 – 0.87 138</td>
</tr>
<tr>
<td>LnPFOS</td>
<td>0.85 0.81 – 0.88 42</td>
<td>0.60 0.43 – 0.83 120</td>
</tr>
</tbody>
</table>