TECHNICAL SUPPORT DOCUMENT: INTERIM SPECIFIC GROUND WATER CRITERION FOR PERFLUOROOCTANE SULFONATE (PFOS)

(CAS #: 1763-23-1; Chemical Formula: $C_8HF_{17}O_3S$)

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Abbreviations

AFFF – aqueous fire fighting foam, also known as aqueous film forming foam
AIC — Akaike Information Criterion
ALP — alkaline phosphatase
ALT — alanine aminotransferase
APFO — ammonium perfluorooctanoate, the ammonium salt of PFOA
AST — aspartate aminotransferase
ATSDR – Agency for Toxic Substances and Disease Control
AUC — area under the curve
BMD — Benchmark Dose
BMDL — lower 95% confidence limit on the Benchmark Dose
BMDS — Benchmark Dose software
BMI — body mass index
BMR — Benchmark Response
BUN — blood urea nitrogen
C8 — a synonym for PFOA
C9 — a synonym for PFNA
CAR — constitutive androstane receptor
CDC — Centers for Disease Control
CL – clearance factor
DSR — NJDEP Division of Science and Research
DWQI — New Jersey Drinking Water Quality Institute
ER – estrogen receptor
FOSA — perfluorooctane sulfonamide
FOSE — perfluorooctane sulfonamidoethanol
FSH — follicle stimulating hormone
GAC — granular activated carbon
GD — gestational day
GFR — glomerular filtration rate
GGT — gamma-glutamyl transferase
HDL — high-density lipid cholesterol
HNF-4α — hepatocyte nuclear factor 4-α
HOMA-IR — Homeostatic model assessment-Insulin resistance
IARC — International Agency for Cancer Research
IRIS — USEPA Integrated Risk Information System
ISGWQC — Interim Specific Ground Water Quality Criterion
LDL — low-density lipid cholesterol
LH — luteinizing hormone
LOAEL — Lowest Observed Adverse Effect Level
MOA – mode of action

NHANES — National Health and Nutrition Examination Survey
NJDEP — New Jersey Department of Environmental Protection
NJDOH — New Jersey Department of Health
NOAEL — No Observed Adverse Effect Level
NTP — National Toxicology Program
OR — odds ratio
PFAA — perfluoroalkyl acid
PFAS — per- and polyfluoroalkyl substances
PFC — perfluorinated compound
PFHxS — perfluorohexane sulfonate
PFNA — perfluorononanoic acid
PFOA — perfluorooctanoic acid
PFOS — perfluorooctane sulfonate
PND — postnatal day
POD — Point of Departure
PPAR — peroxisome proliferator activated receptor
PTFE – polytetrafluoroethylene
PWS – public water supplies
PXR — pregnane X receptor
RfD — Reference Dose
RL — Reporting Level
RR — relative risk
RSC — Relative Source Contribution
SDWA — Safe Drinking Water Act
SHBG — sex hormone binding globulin
SMR — standardized mortality ratio
TSH — thyroid stimulating hormone
T3 — triiodothyronine
T4 — thyroxine
UCMR3 — Unregulated Contaminant Monitoring Rule 3
UF — uncertainty factor
V_d — volume of distribution
VLDL — very low-density lipid cholesterol
WT — wild type
USEPA — United States Environmental Protection Agency
WY — Wyeth 14,643; (4-Chloro-6-[2,3-xylidino]-2-pyrimidinylthio)acetic acid), a model PPAR-alpha activating compound

ABSTRACT

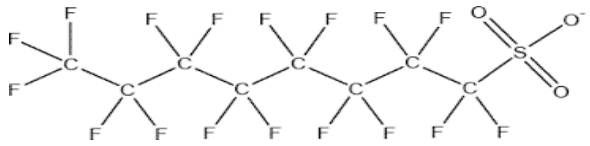
An Interim Specific Ground Water Quality Criterion (ISGWQC) for perfluorooctane sulfonate (PFOS) was developed using a risk assessment approach intended to protect for chronic (lifetime) drinking water exposure. A public health-protective approach in developing an ISGWQC based on animal toxicology data is supported by epidemiological associations of PFOS with health effects in the general population, as well as its biological persistence and bioaccumulation from drinking water in humans. Both non-carcinogenic and carcinogenic effects were evaluated for ISGWQC development. PFOS causes a number of different types of toxicological effects in animals including hepatic, endocrine, developmental, immune system toxicity, and hepatocellular and thyroid tumors. The most sensitive non-cancer effect with data needed for ISGWQC development was identified as immune suppression, specifically, a decrease in antibody response to an exogenous antigen challenge (i.e., plaque-forming cell response) following 60 days of PFOS exposure in adult male mice (Dong et al., 2009). Use of Dong et al. (2009) as the quantitative basis for the ISGWQC is supported by decreased plaque-forming cell response in mice in other studies and by the association of PFOS with decreased vaccine response in humans within the general population. A Target Human Serum Level (analogous to a Reference Dose but on a serum level basis) of 23 ng/ml was developed by applying a total uncertainty factor of 30 to the PFOS serum level, 674 ng/ml, at the No Observed Adverse Effect Level (NOAEL) in Dong et al. (2009). A clearance factor (8.1×10^{-5} L/kg/day), which relates serum PFOS concentrations to human external PFOS doses, was applied to the Target Human Serum Level to develop a Reference Dose of 1.8 ng/kg/day. Default values for drinking water exposure assumptions (2 L/day water consumption; 70 kg body weight) and Relative Source Contribution factor (20%) were used to develop a health-based water concentraton of 13 ng/L which was rounded to an ISGWQC of 10 ng/L. PFOS caused liver and thyroid tumors in a chronic rat study and was characterized as having "suggestive evidence of carcinogenic potential," consistent with the conclusion of USEPA Office of Water. Cancer risk was estimated based on dose-response modeling of liver tumors in female rats. It was concluded that the cancer risk assessment is too uncertain for use as the basis of the ISGWQC. However, the estimated cancer risk at the ISGWQC of 10 ng/L is close to the New Jersey cancer risk goal of one in one million. The ISGWQC of 10 ng/L based on immune system toxicity is therefore considered to be both scientifically appropriate and health protective.

EXECUTIVE SUMMARY

Introduction

Perfluorooctane sulfonate (PFOS) is a member of the group of substances called Perfluorinated compounds, chemicals that contain a totally fluorinated carbon chain which varies in length and a functional group such as carboxylic or sulfonic acid. Perfluorinated compounds are part of a larger group of chemicals called poly- and perfluoroalkyl substances (PFAS).

The chemical structure of PFOS is:



Development of an ISGWQC for perfluorooctane sulfonate (PFOS) was requested of the New Jersey Department of Environmental Protection (NJDEP) Division of Science, Research and Environmental Health by the NJDEP Site Remediation Program under N.J.A.C 7:9C. (http://www.state.nj.us/dep/wms/bears/gwqs.htm#/).

Interim specific ground water quality criteria are intended to be protective from lifetime cancer risk at the one in one million (10^{-6}) risk level and from any adverse non-cancer effects resulting from chronic (lifetime) exposure.

Human health risk assessment approaches used here to develop this ISGWQC generally follow USEPA risk assessment guidance.

Production and Use

Because carbon-fluorine bonds are among the strongest found in organic chemistry, PFOS and other PFCs are extremely stable and resistant to chemical reactions. Its structure gives PFOS both hydrophobic/lipophilic and hydrophilic properties that make it useful commercially and industrially. PFOS was produced in the U.S. for use in commercial products and industrial processes for over 50 years. The main worldwide producer of PFOS completed phasing out the manufacture of PFOS and its precursors in the U.S. and in other nations in 2002, although production continues in some Asian countries.

Many of the uses of PFOS stem from its surfactant properties and from its ability to repel both water and fats/oils. The following are some major uses of PFOS (continuing and discontinued):

- Stain/water repellants on clothing, bedding materials, upholstered furniture, carpets, and automobile interiors (e.g., ScotchGard™)
- Metal plating and finishing (continuing use)

- Aqueous film forming foams (AFFF, also known as aqueous fire fighting foams; continuing use; used for firefighting)
- Photograph development (continuing use)
- Aviation fluids (continuing use)
- Food containers and contact paper

The use of PFOS in AFFF is of particular importance as a source of environmental contamination. Whereas the U.S. no longer produces or imports PFOS-based AFFF, the use of existing stocks of these foams continues. This use results in release of PFOS to the environment, leading to contamination of soil, surface water, and groundwater. This is particularly the case at military bases, and military and civilian airports, where fire-fighting training and drills are carried out regularly.

Environmental Fate and Transport

Because of the extreme stability of their carbon−fluorine bonds, PFOS and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely. PFOS and other PFCs are found in many environmental media and in wildlife worldwide including in remote polar regions. PFOS is bioaccumulative in fish, and it is the PFC most commonly detected in fish monitoring studies. PFOS and other PFCs can be taken up into plants from contaminated soil or irrigation water. In general, PFOS and other longer chain PFCs are preferentially taken up into the root and shoot parts of the plant.

PFOS and some other PFCs are distinctive from other persistent and bioaccumulative organic compounds because of their importance as drinking water contaminants. PFOS migrates readily from soil to ground water and is highly water-soluble. These properties of PFOS differ from those of other well-known persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and polychlorinated biphenyls (PCBs) that have a high affinity for soil and sediments but low water solubility.

PFOS that is released into the environment can contaminate surface water and groundwater used as drinking water sources. Environmental sources include industrial discharge; release of AFFF; disposal in landfills; wastewater treatment plant discharge; and land application of biosolids. PFOS also enters the environment through the breakdown of precursor compounds. These precursor compounds are or were used industrially and are found in AFFF.

Although the production of PFOS and its precursors (e.g., perfluorooctanesulfonyl fluoride, POSF) were voluntarily phased out by the major global manufacturer of PFOS, environmental contamination and resulting human exposure to PFOS are anticipated to continue for the foreseeable future due to its environmental persistence, formation from precursor compounds, and continued production by other manufacturers.

Occurrence in Drinking Water

PFOS and other PFCs are not effectively removed from drinking water by standard treatment processes but can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis. Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOS detected in raw drinking water can be considered representative of concentrations in finished drinking water.

The occurrence of PFOS and other PFCs in public water supplies (PWS) has been evaluated more extensively in New Jersey than in most or all other states. More than 1,000 samples from 80 NJ PWS were analyzed with relatively low Reporting Levels (RLs; generally $\leq$ 5 ng/L) from 2006-2016. PFOS was a frequently detected PFC and was found in samples from approximately 42% of the 76 NJ PWS tested. In the 2013-2015 USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey of all large PWS (>10,000 users) and a subset of smaller PWS in the U.S., PFOS was detected more frequently in New Jersey PWS (3.4%) than nationally (1.9%). The RL in UCMR3 was 40 ng/L, much higher than the RLs for most other NJ PWS monitoring. PFOS has also been detected in NJ private wells near sites where contamination has occurred.

Human Biomonitoring

PFOS and other PFCs are found ubiquitously in the blood serum of the general population in the U.S. and worldwide. The most recent (2013-2014) National Health and Nutrition Examination Survey (NHANES), a representative sample survey of the U.S. general population conducted by the U.S. Centers for Disease Control and Prevention (CDC), determined the geometric mean and 95th percentile serum PFOS concentrations as 4.99 and 18.5 ng/ml, respectively. Serum PFOS levels in the U.S. general population have decreased over time, with an 84% decrease in the geometric mean in NHANES 2013-14 from the first NHANES monitoring in 1999-2000. In communities exposed through contaminated drinking water, serum PFOS levels are elevated compared to the general population. Exposures to industrially-exposed workers or others with occupational exposure are much higher than in the general population. Serum PFOS concentrations of greater than 10,000 ng/ml (10 ppm) have been reported in industrially exposed workers, although levels in most workers were lower.

Sources of Human Exposure

The human body burden of PFOS results from exposure to both PFOS itself and to precursor compounds that can be metabolized to PFOS. In the absence of the influence of specific sources of PFOS release to the environment, it appears that food and possibly house dust (reflecting consumer products use and breakdown) are the major sources of human exposure to PFOS. For high end consumers of fish and specifically for those who consume recreationally caught freshwater fish from contaminated waters, fish may be a particular source of PFOS in the diet.

The contribution of ingested drinking water to total exposure from all sources (e.g. diet, consumer products, etc.) is dependent on the concentration of PFOS in the drinking water, and relatively low concentrations in water substantially increase human body burden. Inhalation from showering, bathing, laundry, and dishwashing, and dermal absorption during showering, bathing, or swimming, are not expected to be significant sources of exposure from contaminated drinking water.

Exposures to PFOS may be higher in young children than in older individuals because of age- specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found.

Toxicokinetics

PFOS is well absorbed orally in animal studies, and it is reasonable to assume that PFOS is orally absorbed in humans with close to 100% efficiency. Unlike most other bioaccumulative organic compounds, it does not distribute to fat. Across species, liver accumulates the highest concentration of PFOS. However, with sufficiently long exposures and/or sufficiently sensitive analytical methods, PFOS is generally found in all tissues and organs. Although the brain is not a major site of PFOS accumulation, PFOS crosses the blood-brain barrier, and is found in the brain in humans and rodents. In the serum, PFOS is almost totally bound to albumin and other proteins. Since it is chemically non-reactive, it is not metabolized. Since it is chemically non-reactive, it is not metabolized. PFOS is slowly excreted in humans, and, with the exceptions of lactation and menstrual blood loss, urine is the most significant route of PFOS elimination in humans. The rate of excretion is likely dependent on the extent of secretion and reabsorption by organic anion transporters in the kidney. Although a significant fraction of PFOS is found in the bile in humans, PFOS is reabsorbed from the bile in the gastrointestinal tract, and, therefore, the feces is not a significant route of elimination. In rodents, however, the feces appears to be significant route of PFOS elimination.

The human half-life of PFOS is estimated at about five years. Because of its long half-life, it remains in the human body for many years after exposures ceases. The half-life of PFOS in laboratory animals is shorter than in humans, and varies widely among species. Because of the large variation in half-lives, the internal dose resulting from a given administered dose varies widely among species and, in some cases, genders of the same species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose.

Relationship between drinking water exposure and human serum levels

A human clearance factor for PFOS of 8.1×10^{-5} L/kg/day was developed by USEPA (2016a) to relate serum PFOS concentration to administered dose. Assuming an average U.S. daily water consumption rate, the clearance factor predicts a serum:drinking water ratio of 197:1.

Continued exposure to even low drinking water concentrations results in substantially increased serum PFOS levels. Based on the clearance factor, each 10 ng/L in drinking water is predicted to increase serum PFOS by 2.0 ng/ml with an average water consumption rate, and 3.6 ng/ml with an upper percentile water consumption rate. These increases in serum PFOS from drinking water can be compared to the most recent NHANES medians, 5.2 ng/ml, and 95th percentile, 18.5 ng/ml, serum PFOS concentrations. Increases in serum PFOS levels predicted from average and upper percentile drinking water consumption at various drinking water PFOS concentrations are shown in Figure E-1.

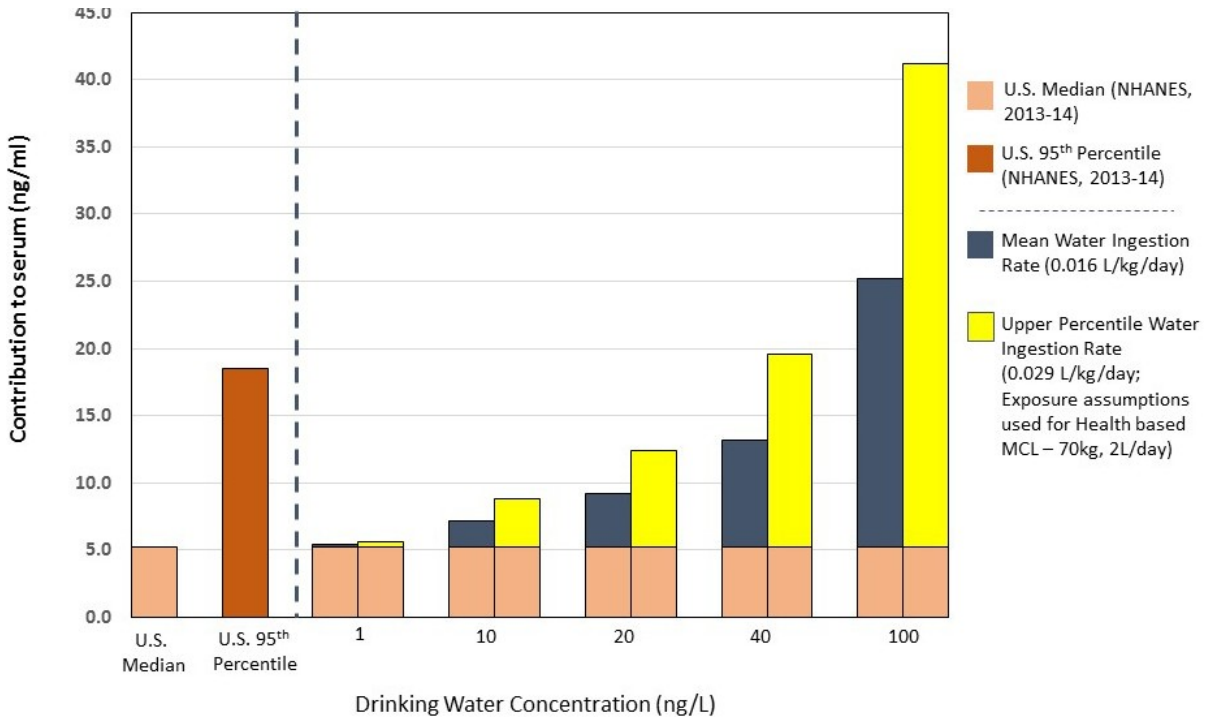


Figure E-1. Increases in serum PFOS concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOS, as compared to U.S median and 95th percentile serum PFOS levels (NHANES, 2013-14).

Exposures to infants

In humans, PFOS has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. Serum PFOS concentrations in infants at birth are lower than those in maternal serum. Both breast-fed infants whose mothers ingest contaminated drinking water and infants fed with formula prepared with contaminated drinking water receive much greater exposures to PFOS than older individuals who consume drinking water with the same PFOS concentration. PFOS exposure in breast-fed infants is greatest during the first few months of life because both PFOS concentrations in breast milk and the rate of fluid consumption are highest then. As a result, serum PFOS concentrations in breast-fed infants increase several-fold from levels at birth within the first few months of life. Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. While serum PFOS levels peak during the first year of life, they remain elevated for several years. These elevated exposures during infancy and early childhood are of particular concern because early life may be a sensitive time period for the toxicity of PFOS.

Health Effects

Literature Search and Screening

A comprehensive literature search was conducted for literature published through the end of 2014 using the PubMed and Toxline databases and was updated with relevant literature through 2016. Additional databases or websites of other state, federal, and international regulatory or authoritative health entities were searched for relevant references. This literature search aimed to identify all references relevant to health effects of PFOS in animals or humans.

Based on screening of the approximately 2860 references identified in the literature search, approximately 700 references were ultimately considered as potentially useful for the assessment of the health effects of PFOS.

Hazard Identification

Animal toxicology studies identified from the literature search and screening were categorized into different levels of review for use in risk assessment. Approximately 75 studies that fulfilled a set of criteria (for example, but not limited to, subchronic or greater exposure duration or *in utero* exposure, multiple dose groups, assessment of appropriate observable endpoints) were reviewed in detail and summarized in evidence tables. These studies were used to identify potential health hazards (i.e., hazard identification) and were evaluated for potential use for dose-response modeling. The remaining approximately 40 animal studies that did not meet the criteria mentioned above, but were nonetheless potentially useful as supporting studies underwent a less intensive review and were summarized in tabular form. These studies were used to further inform the weight of evidence for identified health hazards.

All human (epidemiology) studies that were identified (approximately 120) were reviewed in detail and summarized in evidence tables for use in identifying potential health hazards.

The mode of action evaluation of PFOS was based on relevant studies identified through the literature search, as well as other sources (e.g., previous evaluations by NJDEP and New Jersey Drinking Water Quality Institute (DWQI), review articles, other regulatory or health effects documents).

Non-cancer endpoints

The toxicological effects of oral PFOS exposure were assessed in studies of varying duration in several species including mice, monkeys, rabbits, and rats. In adult animals, endocrine/metabolic (e.g., thyroid hormone), hepatic (e.g., liver enlargement, histopathological lesions, and changes in serum chemistry), immune, and neurological effects were determined to be toxicologically important endpoints based on consistency across studies and appropriate for consideration of dose-response analysis. Following gestational exposure to PFOS, increased mortality, body weight, developmental (e.g., delays in eye opening, neurotoxicity, structural defects), endocrine/metabolic (e.g., changes in thyroid hormone levels, insulin resistance, increased fasting serum glucose), hepatic, and immune effects were observed in perinatal or adult offspring and were determined to be toxicologically important endpoints appropriate for consideration of dose-response analysis.

A number of human populations have been investigated for potential health effects from PFOS exposure in epidemiology studies. Such investigations have included the general population, occupationally exposed individuals, and people living within communities contaminated with high levels of PFOA but with general population level exposures to PFOS. Notably, epidemiological studies have not been conducted in communities with drinking water contaminated by PFOS. In most studies, serum PFOS levels are used as the exposure metric. Epidemiologic studies of PFOS have investigated associations with developmental, endocrine/metabolic, hepatic, immune, lipid metabolism, renal, and reproductive effects. However, some of

these studies have yielded inconsistent results, lacked proper controlling for confounding, or could only provide weak suggestions of causality. Among the epidemiologic studies, the studies of immune effects, and most particularly those investigating effects on vaccine response, were generally consistent in showing adverse responses to PFOS. There was also a consistency of findings among studies of PFOS exposure and increased serum uric acid/hyperuricemia as well as increased total cholesterol.

The epidemiologic data for PFOS are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings from experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population. These features of the epidemiologic data distinguish PFOS from most other organic drinking water contaminants and justify concerns about exposures to PFOS through drinking water. Notwithstanding, the human data have limitations and therefore are not used as the quantitative basis for the ISGWQC. Instead, the ISGWQC is based on a sensitive and well-established animal toxicology endpoint, decreased plaque forming cell respose which is an indicator of decreased immune response. This effect is considered relevant to humans based on epidemiological and mode of action data.

Cancer endpoints

In animals, only one study was identified that assessed tumor formation following PFOS exposure. Following chronic PFOS exposure, hepatocellular tumors in male and female rats, and thyroid tumors in male rats, were observed.

In humans, a limited number of epidemiological studies assessed cancer risk from PFOS exposure in occupationally exposed populations or in the general population. Although individual studies have shown borderline or weak (albeit statistically significant) associations between PFOS exposure and specific cancer types (e.g., bladder, breast, prostate) or cancer- related mortality (e.g., liver), there is no consistent indication of an association between PFOS exposure and cancer in general, or any specific form of cancer. Nonetheless, the database cannot be considered strong. Exposure characterization and case ascertainment was problematic in the occupational studies with high levels of exposure, and the non-occupational studies generally had small sample sizes.

Based on the tumors observed in rats, it is concluded that the designation of "Suggestive Evidence of Carcinogenic Potential" as described the 2005 USEPA Guidelines for Carcinogen Risk Assessment is appropriate for PFOS.

Mode of Action

At a minimum, strong evidence exists from animal and/or epidemiological studies for effects on the liver, the immune system, birth weight, and neonatal survival. In addition, PFOS causes liver tumors and possibly thyroid tumors in rats. The breadth of these effects suggests that PFOS may cause toxicity through multiple modes of action (MOAs). However, the mode(s) of action of PFOS have not been fully characterized. Based on the information reviewed here, the toxicological effects of PFOS are considered relevant to humans for the purposes of risk assessment.

PFOS is not chemically reactive. Thus, it is not metabolized to reactive intermediates and does not covalently bind to nucleic acids and proteins. Consistent with these properties, available data indicate that it is not genotoxic.

Hepatic effects

Much attention has been focused on the potential human relevance of hepatic effects of xenobiotics that occur through activation of the nuclear receptor, peroxisome proliferator-activated receptor-alpha (PPARα). Since many PPARα activating compounds cause rodent liver tumors; the human relevance of these tumors is subject to debate due to lower levels and/or differences in intrinsic activity of PPARα in human liver. While MOA data are most abundant for PFOS effects on the liver, most of the evidence relates to ruling out PPARα-dependent MOAs. Based on some hepatic effects (e.g., increased liver weight) in rodents that are similar to those caused by potent PPARα activators, cancer and non-cancer liver effects of PFOS have sometimes been assumed to be PPARα-dependent. However, several lines of evidence do not support a conclusion that liver effects due to PFOS exposure are PPARα-dependent. For some PPARα activators, non-cancer and cancer liver effects are clearly linked to PPARα activation. In contrast, PFOS effects on the rodent liver do not appear to primarily operate through a PPARα- dependent MOA, including at doses resulting in liver tumors. PPARα may make only a minor contribution, if any, to PFOS liver effects in rodents. Thus, there does not appear to be clear evidence to discount the human relevance of PFOS to cause hepatic effects in rodents. Other receptors including PPARβ/δ, PPARγ, constitutive activated receptor (CAR), pregnane X receptor (PXR), hepatocyte nuclear factor 4-α (HNF-4α), and possibly estrogen receptorα (ERα), may also be activated by PFOS, suggesting alternative, non-PPARα-dependent MOAs.

Immune effects

Following PFOS exposure in animals, immunosuppression as well as effects on immune organs, cell populations, and mediators have been observed. In humans, an association with suppression of vaccine response has been reported. Despite research efforts, the mode(s) of action by which PFOS exposure results in immune effects is unclear.

It appears that PPARα may play a role in some immune effects caused by PFOS in rodents. Unlike the case for liver effects, there are no data to suggest that immune effects mediated by PPARα are not relevant to humans. Therefore, these effects are assumed relevant to humans for the purposes of risk assessment. In addition to the possible role of PPARα, other mechanistic considerations may inform the MOA for PFOS-mediated immunotoxicity. Some evidence suggests a possible involvement of an alteration of cell signaling response. Stress is known to influence immune effects following chemical exposure. However, as reviewed in this assessment, an increase in serum corticosterone, a marker of stress, was a high dose phenomenon, whereas immune effects (i.e., decrease in plaque forming cell response) occurred at lower PFOS doses. The possibility has also been suggested that changes in lipid balance resulting from PFOS activity in the liver could affect the immune response. However, there does not appear to be specific evidence to support this hypothesis.

Developmental/fetal effects

Gestational exposure to PFOS is associated with several different endpoints, including decreased birth weight, malformations, and most notably, neonatal mortality. The MOAs for these effects are not known. However, it appears that the observed developmental effects do not necessarily share similar MOAs.

Research in WT and PPARα null mice suggests that developmental effects following gestational PFOS exposure are PPARα-independent. Neonatal mortality following gestational PFOS exposure has been noted in several rodent studies and is a striking and salient endpoint. The underlying toxicity for this effect occurs with maternal exposure during late gestation. Due to the observation of labored breathing associated with this mortality and the late developmental nature of the toxicity, immature lung development, possibly related to PFOS interference with lung surfactant has been suggested as a possible MOA. Oxidative stress and apoptosis have also been implicated in offspring lung injury that may be responsible for neonatal mortality.

Additionally, defects in cardiopulmonary function observed following gestational PFOS exposure have also been postulated as possible contributors to neonatal mortality. Nonetheless, there is no clear MOA responsible for PFOS-mediated newborn mortality.

Carcinogenicity

Hepatocellular

PFOS does not appear to be genotoxic or mutagenic. There is limited evidence that the formation of hepatocellular tumors from PFOS exposure may operate through a MOA involving sustained cell proliferation and inhibited apoptosis. However, given the lack of additional PFOS-specific data, it is not clear that this hypothesized MOA is either necessary or relevant. In rats, in addition to hepatic tumors, many PPARα activators produce Leydig cell and pancreatic acinar cell tumors. These tumor types are commonly referred to as the tumor triad. Although hepatic tumors were observed in the single chronic exposure study in rats there was no increased incidence of either Leydig cell or pancreatic acinar cell tumors. Along with other data discussed above, this provides further evidence for a PPARα-independent hepatic cancer MOA. In addition, similar to the discussion of the potential role of PPARα in non-cancer liver toxicity, PFOS does not demonstrate key molecular markers of PPARα activity/peroxisome proliferation. Further, PFOS and WY-14,643, a strong PPARα agonist and peroxisome proliferator that is often used as a model for PPARα-related liver effects cause grossly different effects on gene expression in mice. In summary, there is little evidence that PFOS operates through a PPARα- dependent MOA, at least at the doses that have been observed to cause liver tumors. As with non-cancer liver effects, other nuclear receptors, such as PXR and CAR, may play a role. In all, there does not appear to be evidence to suggest that the (unknown) MOA that is operative in rat liver tumors is not relevant to human cancer risk.

Thyroid follicular cell

In the only chronic PFOS exposure study, thyroid follicular cell tumors were observed in male rats only at the highest dose following recovery from dosing. The human relevance of these PFOS-mediated tumors is not clear and there is no evidence to inform a possible MOA.

Identification of Most Sensitive Endpoints

Dose-response analysis focused on health endpoints from animal studies with exposure durations greater than 30 days, as well as on shorter-term reproductive and developmental endpoints from animal studies involving exposures during gestation and/or the immediate post-natal period (i.e., reproductive/developmental studies). Endpoints were selected for dose-response analysis based on their reporting of serum PFOS concentrations at relevant timepoints. Only those endpoints in the animal studies associated with LOAELs in the lower end of the range of serum PFOS concentrations were considered for dose-response modeling, and potentially for RfD derivation. These most sensitive endpoints were identified by stratifying the endpoints from animal studies into quartiles of serum PFOS concentrations. In the lowest quartile, the maximum LOAEL serum PFOS concentration was approximately 24,000 ng/mL. Within that quartile, there was a general clustering of animal endpoints with a LOAEL serum PFOS concentration ≤ 10,000 ng/mL. Endpoints occurring at or below this serum PFOS concentration were considered to be within the group of most sensitive animal endpoints (n = 21). Not all of these endpoints were considered for dose-response modeling due to study-specific concerns and/or lack of biological significance. Ultimately, four endpoints were carried forward to non-cancer dose-response analysis:

- increased relative liver weight, adult mice (Dong et al., 2009)
- decreased plaque forming cell response, adult mice (Dong et al., 2009)
- increased hepatocellular hypertrophy, adult rats (Butenhoff et al., 2012)

- increased relative liver weight, adult mice (Dong et al., 2012a)

For the cancer endpoints, dose-response analysis was performed on the incidence of hepatocellular tumors in male and female rats in Butenhoff et al. (2012). The thyroid follicular cell tumors in rats were excluded from dose-response assessment due to questionable biological significance and inconsistencies in dose-response.

Dose-Response Analysis for non-cancer endpoints

For PFOS and other contaminants for which animal-to-human comparisons are based on serum concentrations (internal dose), dose-response analysis is based on serum PFOS concentrations (internal dose) rather than administered doses. The dose-response for the non-cancer and cancer endpoints was investigated using USEPA benchmark dose modeling (BMD) software (ver. 2.6.0.1). Fitting and assessing the benchmark dose model fit was carried out using USEPA benchmark dose modeling guidance.

For the non-cancer increased hepatocellular hypertrophy endpoint and the hepatocellular tumors, from Butenhoff et al. (2012), serum PFOS concentrations measured over the course of this 105- week study rose and then declined. The serum PFOS concentration at each dose was summarized across the study duration based on area under the curve (AUC) of serum concentration and time. For quantal data, the recommended benchmark response (BMR) value of 10% was used. For continuous data, except for liver weight endpoints, the recommended BMR of 1 SD was used. For liver weight endpoints, a BMR of 10% was used to accommodate relatively small increases in liver weight that could be considered adaptive. All available models in the USEPA software were evaluated.

Non-cancer

Data for two of the four endpoints provided acceptable fits to one or more of the available dose- response models included in the BMD software. The following BMDLs (as serum PFOS concentrations) were derived and were considered as points of departure (PODs) for potential Reference Dose (RfD) development:

- Relative liver weight increase – 5,585.5 ng/ml (Dong et al., 2009)
- Hepatocellular hypertrophy - 4,560.8 ng/ml (Butenhoff et al., 2012)

For two other endpoints, BMD modeling did not yield a valid POD. The PODs for these studies were based on the NOAELs:

- Relative liver weight increase – 4,350 ng/ml - NOAEL (Dong et al., 2012a)
- Decreased plaque-forming cell response – 674 ng/ml - NOAEL (Dong et al., 2009)

There were PODs for relative liver weight from two studies, both from the same laboratory (Dong et al., 2009; Dong et al., 2012a). The POD from Dong et al. (2012a) was lower than the POD from Dong et al. (2009) and was therefore carried forward for RfD development.

Dose-response analysis for hepatocellular tumors is presented in the section on Estimation of Cancer Risk from PFOS in Drinking Water below.

ISGWQC Derivation

The following graphic describes the process followed in criterion derivation.

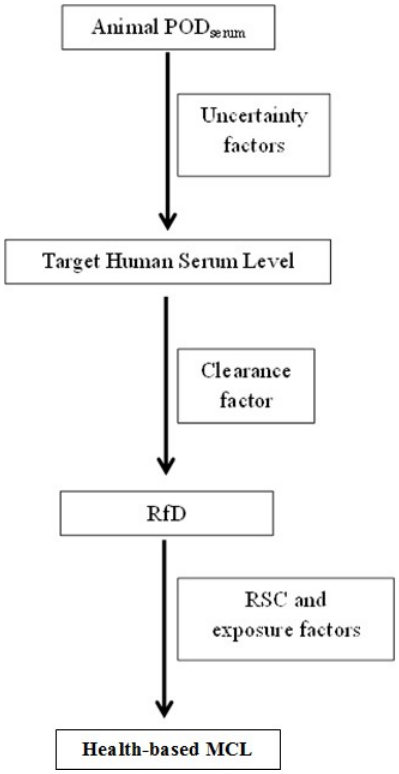


Figure E-2. Graphical representation of representation of the approach used to derive the ISGWQC

Non-Cancer Endpoints

Development of Target Human Serum Levels and Reference Doses

Target Human Serum Levels are analogous to Reference Doses (RfDs) but in terms of internal dose rather than administered dose. While Reference Doses (RfDs) are developed by applying uncertainty factors (UFs) to PODs (NOAELs, LOAELs, or BMDLs) based on administered dose (mg/kg/day), Target Serum Levels are developed by applying UFs are applied to POD serum concentrations.

For each of the three candidate non-cancer PODs, a UF of 3 was applied to account for interspecies differences in toxicodynamics. The typical UF of 3 for toxicokinetic variability between species was not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose. In addition, for each of the candidate studies the default UF of 10 was applied to account for potential differences in sensitivity to PFOS among humans including sensitive sub-populations. These two UFs result in a total UF of 30.

For the POD for increased liver weight, a UF of 3 was also applied. This POD was derived from a study that was of less than chronic duration, and longer duration exposures could potentially result in the same or additional effects at lower doses. Since two UFs of 3 are considered to be equivalent to a UF of 10, the additional UF of 3 applied to this endpoint yielded a total UF of 100.

Although the POD for decreased plaque forming cell response is from a subchronic study, a UF for the less than chronic duration of the endpoint was not applied because the dose-response for this effect was similar in several studies of shorter duration. This suggests that this effect does not become more severe or occur

at lower internal doses with longer durations of exposure.

The following table shows the POD, total UF and Target Human Serum Level for each of these endpoints.

Table E-1. Calculation of Target Human Serum Levels

Study	**Animal POD_{serum} (ng /ml)**	**UF_{TOTAL}**	**Target Human Serum Level (ng/ml)**
Butenhoff et al. (2012) (Hepatocellular hypertrophy)	4,561	30	152
Dong et al. (2012a) (Increased relative liver weight)	4,350	100	43.5
Dong et al. (2009) (Decreased plaque forming cell response)	674	30	22.5

Deriving an RfD as a human intake dose that corresponds to the Target Human Serum Level at steady state requires a constant that relates the two parameters. This constant is referred to as the Clearance Factor (CL). USEPA derived a CL for PFOS of 8.1 x 10^{-5} L/kg/day based on empirical data. This value was used to derive the RfD for each of the candidate studies. The following table shows the Target Human Serum Level and corresponding RfD for each of the candidate studies after application of the CL.

Table E-2. RfDs derived from Target Human Serum Levels

Study	**Target Human Serum Level (ng/ml)**	**RfD (ng/kg/day)**	**RfD (mg/kg/day)**
Butenhoff et al. (2012) (Hepatocellular hypertrophy)	152	12.3	1.23 x 10^{-5}
Dong et al. (2012a) (Increased relative liver weight)	43.5	3.5	3.5 x 10^{-6}
Dong et al. (2009) (Decreased plaque forming cell response)	22.5	1.8	1.8 x 10^{-6}

Relative Source Contribution Factor (RSC)

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used by USEPA and NJDEP in the development of health-based drinking water concentrations based on non-carcinogenic effects. The default value for the RSC is 20%, meaning that 20% of total exposure is assumed to come from drinking water and 80% from non-drinking water sources. If supported by available data, a higher chemical-specific value (up to 80%) can be used. It was concluded that there are insufficient data to develop a chemical-specific RSC for PFOS. USEPA UCMR3 monitoring shows that PFOS occurs (at concentrations greater than 40 ng/L) more frequently in PWS located throughout New Jersey (3.4%) than nationwide (1.9%), and PFOS has also been found in additional NJ PWS in NJDEP occurrence studies and other data reported to NJDEP.

There are no New Jersey-specific biomonitoring data for PFOS, and the more frequent occurrence in NJ PWS suggests that New Jersey residents, particularly in communities with contaminated drinking water, may also have higher exposures from non-drinking sources, such as contaminated soils, house dust, or other environmental media, than the U.S. general population. Importantly, residents may be exposed through consumption of recreationally caught fish from contaminated waters.

Additionally, the default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the greater exposures to infants who are breast-fed or consume formula prepared with contaminated drinking water, as compared to older individuals. These higher exposures during infancy must be considered because short term exposures to infants are relevant to the most sensitive effect (decreased immune response). Therefore, the default RSC of 20% was used to develop the ISGWQC.

ISGWQC (Interim Specific Ground Water Quality Criterion)

The ISGWQC is calculated based on the following equation, using default exposure assumptions of 2 L/day drinking water consumption, 70 kg adult body weight, and 20% (0.2) Relative Source Contribution (RSC).

$$MCL\ (ng/L)\ = \left(\frac{RfD\ \ (ng/kg/day)\ \times Body\ weight\ (kg)}{Daily\ drinking\ water\ intake\ (L/day)}\right) \times RSC$$

For each of the three candidate endpoints, the following table gives the RfD and corresponding potential health-based water concentration and ISGWQC.

Table E-3. Calculation of Potential ISGWQCs

Study	**Endpoint**	**RfD (ng/kg/day)**	**Health-based Water Conc. (ng/L = ppt)**	**ISGWQC * (ng/L=ppt)**
Butenhoff et al. (2012)	Hepatocellular hypertrophy	12.0	84	80
Dong et al. (2012a)	Increased relative liver weight	3.5	25	30
Dong et al. (2009)	Decreased plaque forming cell response	1.8	13	10

*ISGWQC are rounded to one significant figure.

ISGWQC

The ISGWQC of 10 ng/L value based on decreased plaque forming cell response from Dong et al. (2009) is the lowest of the potential ISGWQCs for non-carcinogenic effects. This endpoint is an appropriate basis for the ISGWQC because of the clear toxicological relevance of decreased immune response to foreign antigens and the substantial epidemiological evidence for the association of decreased vaccine response with general population level exposure to PFOS. Due to the uncertainties associated with the cancer risk assessment of PFOS (discussed below), the non-cancer endpoint (immune system toxicity) was judged to be the most appropriate basis for the ISGWQC.

Estimation of cancer risk from PFOS in drinking water

We conclude that PFOS is most appropriately described as having "Suggestive Evidence of Carcinogenic Potential," and that estimated cancer risks for PFOS are too uncertain for use as the basis of a ISGWQC.

The only chronic study of PFOS reported an increased incidence of liver and thyroid tumors in rats (Butenhoff et al., 2012). The hepatocellular tumor data is appropriate for dose-response analysis to develop a cancer slope factor, while the thyroid tumor data could not be used for cancer slope factor development. The cancer risk estimates were based on data from female rats, since the cancer slope factor for male rats is highly uncertain because liver tumors occurred only in the high dose group, while they occurred in all dosed groups in females. The cancer potency factor for hepatocellular tumors in female rats was 9.0×10^{-6} $(ng/kg/day)^{-1}$. Among the uncertainties associated with the cancer slope factor for liver tumors in females are uncertainties regarding inclusion of the recovery group data in dose-response analysis and uncertainties about the dose metric based on AUC serum levels.

The lifetime cancer risk at the ISGWQC of 10 ng/L, based on default assumptions for body weight (70 kg) and drinking water consumption (2 L/day), was estimated as 3×10^{-6} (3 in one million).

The estimated cancer risk of 3 in one million is slightly above the cancer risk goal for New Jersey ISGWQCs of one in one million. The NJDEP has a policy of applying an additional uncertainty factor of 10 to an RfD for a non-cancer endpoint to account for potential cancer risk when a cancer potency factor (slope factor) is not available or is considered uninformative. However, since the estimated cancer risk at the ISGWQC based on a sensitive non-carcinogenic effect is close to the New Jersey cancer risk goal of one in one million, application of this uncertainty factor is not necessary.

Potential for additive toxicity with other PFCs

We note that available information indicates that the target organs and modes of action may be generally similar for PFOS and some other PFCs. Therefore, the toxicity of PFOS and other PFCs may be additive. Although PFOS and other PFCs are known to co-occur in some NJ public water supplies, the potential for additive toxicity of PFOS and other PFCs was not considered in development of the ISGWQC.

The ISGWQC is 10 ng/L (0.01 µg/L).

INTRODUCTION

Development of ISGWQC by NJDEP

Development of an Interim Specific Ground Water Quality Criterion (ISGWQC) for perfluorooctane sulfonate (PFOS) was requested of the New Jersey Department of Environmental Protection (NJDEP) Division of Science, Research and Environmental Health by the NJDEP Site Remediation Program under N.J.A.C 7:9C (http://www.state.nj.us/dep/wms/bears/gwqs.htm#/).

ISGWQC are intended to be protective from lifetime cancer risk at the one in one million (10^{-6}) risk level and from any adverse non-cancer effects resulting from chronic (lifetime) exposure.

Human health risk assessment approaches used herein to develop this ISGWQC generally follow USEPA risk assessment guidance.

Document Development Process

The ISGWQC is based primarily on an evaluation of PFOS by the Health Effects Subcommittee of the New Jersey Drinking Water Quality Institute (DWQI). The information in this document is very similar to that in the DWQI Health-Based Maximum Contaminant Level Support Document: Perfluorooctane Sulfonate (DWQI, 2018). The text has been revised by the New Jersey Department of Environmental Protection to describe the development of the ISGWQC.

Literature Search and Screening

A comprehensive literature search was conducted for literature published through the end of 2014 using the PubMed and Toxline databases and was updated with relevant literature through 2016. Additional databases or websites of other state, federal, and international regulatory or authoritative health entities were searched for relevant references. This literature search aimed to identify all references relevant to health effects of PFOS in animals or humans. Detailed documentation of the database and website literature searches can be found in Appendix 1 (Tables A-1 and A-2).

Approximately 2860 references were identified from the literature search. These references were manually screened (i.e., by title, abstract and/or full text) for relevance to the areas of hazard identification, toxicity value derivation, or human exposure to determine whether they provided information on at least one of the following: effects in animals or humans; toxicokinetics; exposure to humans; or mode of action. References considered relevant to informing these areas were selected for further consideration during the preparation of this document. Table A-3 in Appendix 1 describes the criteria used to decide whether each reference will be further considered or excluded.

Backward searches (i.e., searches of citations to identified previously unidentified references) of selected key references (i.e., review articles or health assessments published from 2012 onwards) identified from the literature screening were employed to augment the database and website searches (Appendix 1, Table A-4).

Based on this screening, approximately 700 references were ultimately considered as potentially useful for the assessment of the health effects of PFOS. Some references that were excluded as not being relevant to hazard identification, toxicity values derivation, or human exposure were used to inform supporting sections of this assessment, such as the "Background Information" and "Environmental Sources, Fate, and Occurrence" sections. Additional references, including general background references (e.g., review

articles) not specific to PFOS but germane to relevant scientific issues, guidance documents, and other health assessments not identified from the above literature search, were identified based on previous knowledge or *ad hoc* literature or website searches.

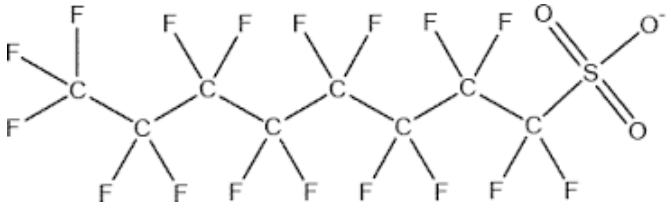
Figure A-1 in Appendix 1 summarizes the results of the literature search and screening.

BACKGROUND INFORMATION

PFOS is a member of a class of anthropogenic chemicals called perfluorinated chemicals (PFCs) or perfluoroalkyl acids (PFAAs). These chemicals have structures consisting of a totally fluorinated carbon chain of varying length and a charged functional group, such as carboxylate or sulfonate (Lindstrom et al., 2011). PFCs are members of a larger class of compounds, poly- and perfluoroalkyl substances (PFAS) which also includes fluorinated compounds with structures that differ from PFCs (Buck et al., 2011). The eight- carbon PFCs, PFOA and PFOS, were the most extensively investigated compounds in earlier studies, while current research focuses on a wider range of PFAS.

Physical and Chemical Properties

ATSDR (2018) and USEPA (2016a) have summarized the physical and chemical properties of PFOS. The backbone of the PFOS molecule is an eight-carbon chain that is fully fluorinated except for a terminal carbon, two of whose available bonds are fluorinated and the remaining bond of which forms a sulfonate. PFOS has a molecular weight of 500.03 Da, and its molecular structure of PFOS:



The fluorocarbon portion of the molecule is hydrophobic and lipophilic. However, the sulfonate end of the molecule is hydrophilic. The combination of these properties allows PFOS to bridge lipid/water interfaces and to act as a surfactant. PFOS is a fully fluorinated sulfonic acid. Because carbon-fluorine bonds are among the strongest found in organic chemistry due to fluorine's electronegativity, PFOS and other PFCs are extremely stable and resistant to chemical reactions. Therefore, PFOS is extremely stable in the environment, and it is resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis. Its melting temperature is ≥ 400°C. The potassium salt of PFOS is relatively soluble in water (570 mg/L) (ATSDR, 2018); 680 mg/L (USEPA, 2016a). Its vapor pressure is very low and has been reported variously as 2.48×10^{-6} mm Hg at 20°C (ATSDR, 2018) and 2.0×10^{-3} mm Hg at 25°C (USEPA, 2016a). The octanol-water partition coefficient (log K_{ow}) for PFOS is not measurable (USEPA, 2016b). Its pK_a is reported as <1 (PubChem, 2017).

Production and Use

The main worldwide producer of PFOS began production of "PFOS equivalents" (PFOS and/or starting materials such as perfluorooctane sulfonyl fluoride [POSF] that are used to produce to PFOS) in 1949 and completed phasing out the manufacture of these compounds in 2002 (Lindstrom et al., 2011). In 1994 and in 2002, the U.S. production of PFOS as reported in the USEPA Inventory Update Rule was 10,000-500,000 lbs (ATSDR, 2018). USEPA has also taken several actions (Significant New Use Rules; SNURs)

to require EPA notification and review of the manufacture or import of a number of chemicals that related to PFOS or can degrade to PFOS, with exceptions for "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." (USEPA, 2017). As of the 2018 ATSDR review, while the U.S. and most industrialized nations have stopped producing PFOS, China is still major producer and user, and hias increased its production while other countries have decreased their production.

Many of the uses of PFOS stem from its surfactant properties and from its ability to repel both water and fats/oils. The USEPA (2016a) reports the following as among the significant uses of PFOS:

Stain/water repellants on clothing, bedding materials, upholstered furniture, carpets, and automobile interiors (e.g., ScotchGard™); these materials can be a particularly important exposure route for infants and children because of their hand-to-mouth behaviors.

- Metal plating and finishing (continuing use)
- AFFF (continuing use; used for firefighting)
- Photograph development (continuing use)
- Aviation fluids (continuing use)
- Semiconductor industry
- Flame repellants
- Food containers and contact paper
- Oil and mining
- Cleaning products
- Paints, varnishes, sealants
- Textiles and leather

Of particular note on this list, is the use of PFOS in AFFF. Whereas the U.S. no longer produces or imports PFOS-based AFFF, the use of existing stocks of these foams continues (Seow, 2013). As discussed in the section on Environmental Fate and Transport, discharge of AFFF to the environment is a major source of PFOS drinking water contamination.

GUIDANCE AND STANDARDS DEVELOPED BY USEPA, ATSDR, AND OTHER STATES

USEPA (2016) Drinking Water Health Advisory

In May 2016, the USEPA Office of Water finalized a drinking water Health Advisory for PFOS of 70 ng/L (USEPA, 2016a). This Health Advisory is intended to apply to both lifetime exposure and short-term exposure. It replaces the earlier 2009 USEPA Office of Water (USEPA, 2009) Provisional Health Advisory for PFOS of 200 ng/L which was intended to protect for "short-term exposure" (defined by the USEPA Integrated Risk Information System [IRIS] as up to 30 days; USEPA, 2011a).

USEPA (2016c) also finalized a Health Advisory for PFOA of 70 ng/L, and USEPA (2016d) states that the total combined concentration of PFOS and PFOA in drinking water should not exceed 70 ng/L.

A detailed discussion of the basis for the USEPA (2016a) Health Advisory for PFOS and a comparison

with this ISGWQC are provided in Appendix 2. In summary, the USEPA Health Advisory is based on a Reference Dose (RfD) of 20 ng/kg/day based on decreased neonatal body weight in the F_2 generation (Luebker et al., 2005a). The default Relative Source Contribution factor of 20% was used to account for non-drinking water exposures. The USEPA Health Advisory uses a drinking water consumption rate of 0.054 L/kg/day, based on the 90th percentile for lactating women, which is higher than the default consumption rate based on adult exposure factors.

Figure 1 shows the predicted increases in serum PFOS levels from ongoing exposure in drinking water at the USEPA Health Advisory (70 ng/L) and the ISGWQC (10 ng/L) developed in this document. Predictions based on both average (0.016 L/kg/day) and upper percentile (0.029 L/kg/day) drinking water ingestion rates are shown. A clearance factor (1.4 x 10^{-4} L/kg/day) developed by USEPA (2016d) to relate human PFOS exposures to human serum PFOS levels was used to predict the increases in serum PFOS from exposures to these levels in drinking water. With average water consumption, ongoing exposure to 70 ng/L (the USEPA Health Advisory) is predicted to increase serum PFOS by 13.8 ng/ml, a 3.7-fold increase from the U.S. general population (NHANES) median of 5.2 ng/ml (CDC, 2017). With upper percentile water consumption, the increase in serum PFOS level from 70 ng/L is predicted as 25.1 ng/ml, resulting in a 5.8-fold increase from the general population (NHANES) median.

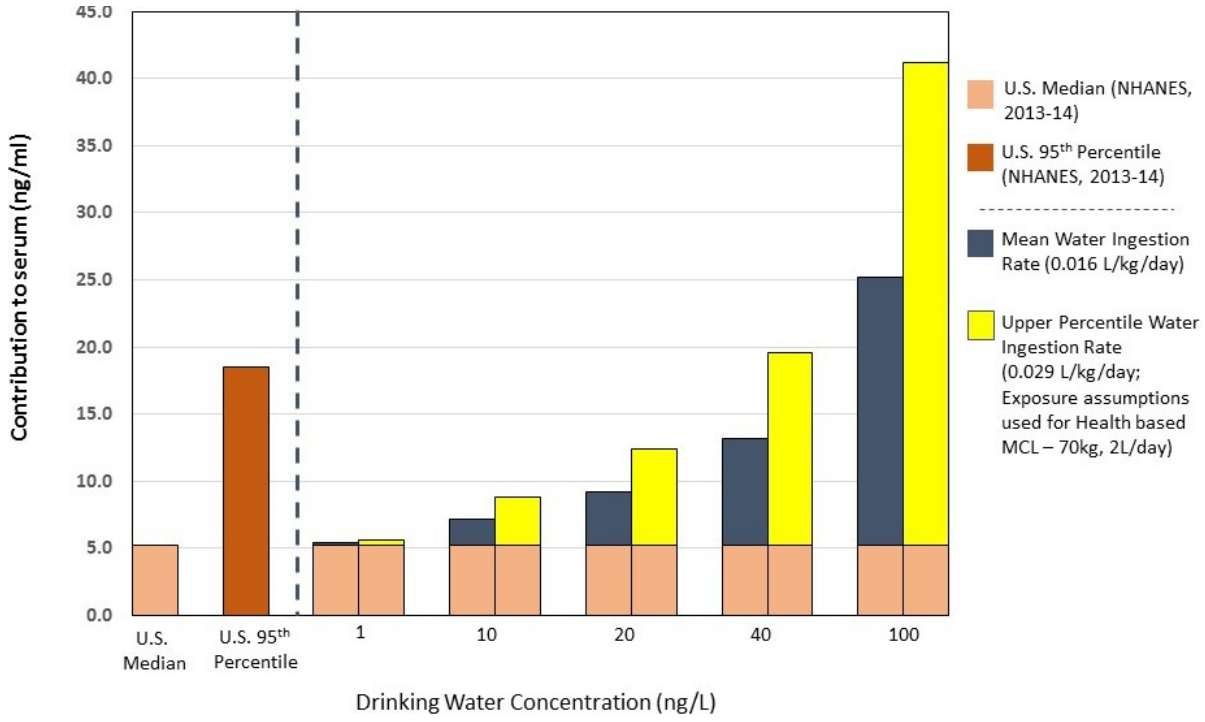


Figure 1. Increases in the median U.S. serum PFOS concentration (right of dotted line) predicted from mean and upper percentile consumption of drinking water for PFOS concentrations in drinking water at the ISGWQC (10 ng/L) and the USEPA Health Advisory (70 ng/L) levels, as compared to U.S median and 95th percentile serum PFOS levels (NHANES, 2013-14). Mean and upper percentile water ingestion rates are based on consumers of community water (USEPA, 2011b). The upper percentile consumption rate is between the 75th and 90th percentile.

Agency for Toxic Substances and Disease Registry (ATSDR) Draft Minimum Risk Level (MRL)

ATSDR (2018) has recently released a draft Toxicological Profile for Perfluoroalkyls that includes Intermediate MRLs for several PFCs including PFOS. ATSDR (2018) states that "an MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure." MRLs are therefore similar in concept to RfDs developed by USEPA, NJDEP, and DWQI, except that RfDs are intended to protect for chronic (lifetime) exposure, while MRLs are developed for several different exposure durations (Acute – up to 14 days; Intermediate – 15 to 364 days; Chronic – 365 days or longer). ATSDR (2018) concluded that decreased immune response in mice is the most sensitive toxicological endpoint for PFOS, and that it is a valid and relevant basis for risk assessment. However, it was not used as the critical effect for the MRL because ATSDR only considered endpoints for which time weighted average serum PFOS concentrations were available for the strains of animals used in the relevant toxicity studies, and the data needed to estimate the time weighted average serum PFOS concentrations in the mouse strains used in the PFOS immunotoxicity studies were not available. Therefore, the ATSDR Intermediate MRL is based on the same endpoint used as the basis for the USEPA PFOS Health Advisory, decreased offspring body weight in rats (Luebker et al., 2005), but it incorporates an additional uncertainty factor of 10 for more sensitive immunotoxicity observed in several mouse studies. It is notable that the ATSDR (2018) MRL of 2 ng/kg/day, based on offspring body weight in rats and on the application of a modifying factor for more sensitive immunotoxic effects, is essentially identical to the RfD presented herein of 1.8 ng/kg/day, based on immunotoxicity as the critical effect.

Guidance and standards of other states

The Interstate Technology and Regulatory Council (ITRC, 2018) has developed tables that provide the PFOS drinking water standards and guidance developed by USEPA, states, and other nations (Table 4 of ITRC, 2018), and the basis for the USEPA and state PFOS values (Table 5-2 of ITRC, 2018).

California (California State Water Resources Control Board, 2018; California EPA, 2018) has recently adopted the NJ DWQI Health-based MCL of 13 ng/L, which has the same technical basis as the basis for the ISGWQC proposed herein, as an interim Notification Level for detections of PFOS in California public water systems.

Vermont has adopted drinking water and ground water standards (Vermont DEC, 2017) for PFOS, PFOA, and the total of the two compounds of 20 ng/L. These Vermont values are based on the Reference Dose (RfD) of 2×10^{-5} mg/kg/day from the USEPA (2016a) PFOS Health Advisory (which is the same as the RfD in the final USEPA [2016a] PFOS Health Advisory), drinking water exposure assumptions for a child less than 1 year of age (instead of default adult exposure assumptions), and the default Relative Source Contribution (RSC) factor of 20%. Vermont (2018) drinking water guidance applies the total of 20 ng/L to PFOA, PFOS, and three additional PFCs (perfluoroheptanoic acid [PFHpA], perfluorononanoic acid [PFNA], and perfluorohexane sulfonate [PFHxS]).

Minnesota Department of Health (2017) has updated its earlier Health Risk Limit (HRL) for PFOS in drinking water to 27 ng/L. This value is based on a Reference Dose of 5.1 ng/kg/day and exposure modeling for breast-fed and formula-fed infants. The Reference Dose was derived by incorporation of an additional database uncertainty factor of 3, for potentially more sensitive immunotoxic effects, into the USEPA PFO Reference Dose (USEPA, 2016a) which is based on decreased offspring weight as described above. Several

other states use the USEPA (2016a) Health Advisory of 70 ng/L for PFOS, PFOA, or the total of both compounds as drinking water guidance or have adopted it as an enforceable standard. Connecticut (2016) and Massachusetts (2018) use 70 ng/L as guidance for the total of PFOS, PFOA, PFHpA, PFNA, and PFHxS.

ENVIRONMENTAL FATE, TRANSPORT, AND OCCURRENCE

Environmental Fate and Transport

PFOS and other perfluorinated compounds are found in many environmental media (e.g. drinking water, surface water, groundwater, air, sludge, soils, sediments, outdoor and indoor dust, and ice caps) in locations around the world including remote polar regions (Lau et al., 2007). PFOS in these environmental media arises from discharges of both PFOS and precursors that can convert to PFOS in the environment (Paul et al., 2017). Because of the extreme stability of their carbon−fluorine bonds, PFOS and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely (Buck et al., 2011; Lindstrom et al., 2011). Although the production of PFOS and its starting materials (e.g., perfluorooctanesulfonyl fluoride, POSF) were voluntarily phased-out by the major global manufacturer of PFOS (USEPA 2000a), environmental contamination and resulting human exposure to PFOS are anticipated to continue for the foreseeable future due to its environmental persistence, formation from precursor compounds, and continued production by other manufacturers.

PFOS has been found in soil, surface water, and groundwater near fluorochemical manufacturing facilities and disposal sites (USEPA, 2016a). Similarly, PFOS contamination has been observed in soil, surface water, and groundwater near sites where AFFF was used, such as civilian and military airports, industrial sites, and firefighting training facilities (Health Canada, 2016; USEPA, 2016a). Wastewater treatment plants are another source of PFOS to the environment as PFOS has been detected in treatment plant effluent and receiving waters (Health Canada, 2016; USEPA, 2016a). Additionally, the land application of PFOS-containing biosolids from wastewater treatment plants has resulted in the contamination of agricultural fields and nearby surface and well water (USEPA, 2016a).

Two major pathways have been proposed for long-range transport of PFOS and other perfluorinated compounds to remote locations worldwide, including the Arctic (Figure 2; Lau et al., 2007, 2012; Butt et al., 2010). The relative contributions of each of these pathways are not known. The first pathway involves the atmospheric transport of volatile precursors such as perfluorinated sulfonamide alcohols, followed by oxidation of the precursors to PFOS and other perfluorinated compounds which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of emitted perfluorinated sulfonates such as PFOS in their anionic forms to remote locations by currents on the ocean's surface.

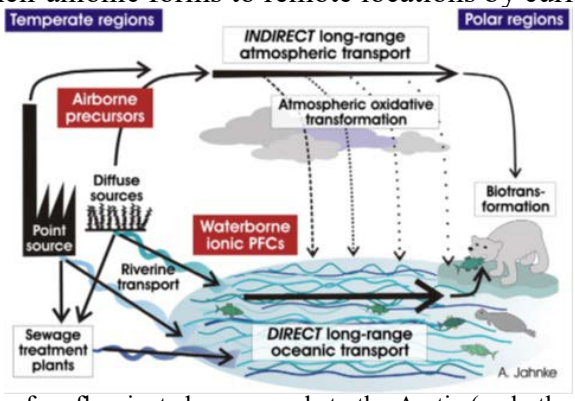


Figure 2. Major transport pathways of perfluorinated compounds to the Arctic (and other remote locations), by Annika Jahnke

(Butt et al., 2010)

Perfluorinated compounds are also found in wildlife (fish, birds, mammals) in studies from many locations throughout the world including in remote polar regions. PFOS and long chain perfluorocarboxylates (e.g., PFNA; perfluoroundecanoic acid, C11; perfluorotridecanoic acid, C13) generally predominate in wildlife in remote locations (Butt et al., 2010). PFOS and other PFCs with eight or more fluorinated carbons (e.g. PFNA) are considered to be bioaccumulative in fish, while those with seven or fewer fluorinated carbons (e.g. PFOA; perfluorohexane sulfonate, PFHxS) do not bioaccumulate signficantly (Martin et al., 2003; Conder et al., 2008). Additionally, PFOS is more bioaccumulative than the perfluorocarboxylate of the same fluorinated carbon chain length (i.e., PFNA) (Conder et al., 2008). In fish, PFOS is the PFC found most frequently and at the highest concentrations (Houde et al., 2011), although long chain perfluorocarboxylates are frequently reported. USEPA conducted a national study of PFCs in fish from 164 urban rivers in 38 states in 2008-09 (Stahl et al., 2014). PFOS was detected (>5.35 ppb) in 70% of 162 composite samples of 682 fish (skin-on fish fillets; 25 species represented with the majority smallmouth bass, largemouth bass, and channel catfish). The highest level detected was 127 ppb. PFOS levels in fish can be extremely high (i.e. > 9000 ppb; 9 ppm) in locations impacted by major contamination (e.g. Wurtsmith AFB, MI - MDHHS, 2015; Barksdale AFB, LA - Lanza et al., 2017).

Occurrence in drinking water

PFOS and other PFCs occur in raw and finished drinking water from both groundwater and surface water sources in New Jersey, other parts of the United States, and nations around the world (reviewed by Mak et al., 2009; Post et al., 2013; Hu et al., 2016). As discussed above, sources of PFOS in drinking water can include discharges from industrial facilities, release of AFFF, wastewater treatment plant effluent, and contaminated biosolids applied to agricultural land. PFOS and other PFCs are not effectively removed from drinking water by standard treatment processes such as coagulation/flocculation, sand filtration, sedimentation, medium-pressure ozonation, chloramination, and chlorination. However, PFOS and other PFCs can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis (Rumsby et al., 2009, Tagaki et al., 2011; Eschauzier et al., 2012; Appleman et al., 2014; DWQI, 2015b). Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOS and other PFCs detected in raw drinking water are representative of concentrations in finished drinking water (Post et al., 2013)

Occurrence in New Jersey drinking water

Considerable information is available on the occurrence of PFOS and other PFCs in New Jersey public water systems (PWS). This includes data from 53 PWS included in two NJDEP occurrence studies of PFCs, substantial additional data submitted to NJDEP by PWS and other parties, and data from the nationwide USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey. For the two NJDEP occurrence studies and most of the additional data submitted to NJDEP, analysis of samples was performed by certified laboratories with Reporting Levels (RLs) that were generally 4-5 ng/L or lower. To our knowledge, statewide drinking water studies of PFOS with sensitive RLs such as these have not yet been completed in states other than New Jersey. In contrast, the RL for PFOS in USEPA UCMR3 is much higher (40 ng/L).

NJDEP studies of occurrence in New Jersey public water systems

Following detection of PFOA in a New Jersey PWS at up to 190 ng/L in a groundwater source and up to 64 ng/L in tap water, two statewide studies of the occurrence of PFOA, PFOS, and other PFCs in drinking water were conducted by NJDEP in 2006 and 2009-10. The 2006 study tested 23 PWS for PFOA and PFOS, and the 2009-10 study tested 33 additional PWS for PFOA, PFOS, and eight other

PFCs (NJDEP, 2007b; NJDEP, 2014; Post et al., 2009a; Post et al., 2013).
The 2006 NJDEP study included 29 samples of raw and/or finished water from 23 NJ PWS including 14 with groundwater sources, 8 with surface water sources, and one using both groundwater and surface water. Of the PWS in this study, PFOS was detected in both surface water and ground water sources, with the highest detected concentration of 19 ng/L. It was found in 7 of 23 systems (30%) at or above the RL (4 ng/L), and in 6 of 23 systems (27%) below the RL. In this study, PFOA was detected (>4 ng/L) more frequently (65% of PWS) than PFOS (NJDEP, 2007; Post et al., 2009a).

The 2009-2010 NJDEP study tested raw water from 30 PWS for PFOA, PFOS, and 8 other PFCs. The sites for this study were chosen for geographic diversity, representing 19 of NJ's 21 counties. The study included 18 PWS with groundwater sources (17 unconfined, one confined) and 12 PWS with surface water sources. One or more PFC was detected (>5 ng/L) at 21 sites (70%), with the number of individual compounds detected varying from one (in 8 samples) to a maximum of 8 in one sample. PFOS was found in 8 of 29 PWS sampled (28%), including in 5 of 18 ground water sources (28%) at up to 12 ng/L and 3 of 11 surface water sources (27%) at up to 43 ng/L. As in the 2006 study, PFOA was the most commonly detected PFC (55% of the PWS tested).

NJDEP database of PFCs in New Jersey public water systems

The NJDEP Division of Science, Research, and Environmental Health maintains an internal database of PFC results from NJ PWS including the two NJDEP occurrence studies, additional raw and finished water data submitted to NJDEP by PWS and other parties, and detections from UCMR3 data. As of January 2016, the database included 1035 samples (423 raw water, 549 finished water, and 63 distribution system) from 282 sampling locations in 80 PWS (including 72 PWS with data from NJDEP studies and/or submitted to NJDEP, and 8 additional PWS with PFC detections in UCMR3). Of these samples, 374 were analyzed for only PFOA and PFOS, and 661 were analyzed for a broader suite of PFCs.

Table 1. PFOS concentration in raw or finished water from PWS included in NJDEP database*

PFOS Concentration (ng/L)	Number of PWS	% of PWS
ND**	44	57.89%
RL-<10**	14	18.42%
10-<20**	8	10.53%
20-<40**	3	3.95%
>40	7	9.21%

*Data shown are highest concentration found in raw or finished water from the PWS. Levels in finished water from some water supplies included may be lower because several raw water sources are blended in the treatment plant.

**Reporting levels (RLs) vary among samples and range from 1-40 ng/L. Therefore, the percentage of PWS with RL-<10, 10-<20, 20-<40 may actually be higher than shown.

Comparison of NJ occurrence to nationwide UCMR3 data and studies from other nations

Data on PFOS in PWS in New Jersey and nationwide is available through the USEPA UCMR3. Under UCMR3, nationwide monitoring of finished water for 30 unregulated contaminants, including PFOS and five other PFCs, was conducted in 2013−2015 by all large PWS (serving more than 10,000 people) and 800 representative smaller PWS (serving less than 10,000 people) (USEPA, 2012b). UCMR3 data therefore provide useful information on occurrence of PFCs in NJ in comparison to the rest of the United States. However, comparison of the UCMR3 PFC data with other New Jersey PFC occurrence data is complicated

by the fact that the UCMR3 RLs for PFOS (40 ng/L) and other PFCs (generally 10-90 ng/L) are much higher than the RLs for other PFC data in the NJDEP database (generally ≤5 ng/L).

UCMR3 monitoring in New Jersey includes all 165 large community PWS and a small number of small community PWS. A comparison of national versus New Jersey PFC data from UCMR3 is shown in Table 2 (data obtained from USEPA, 2016e). PFOS was detected (≥ 40 ng/L) in 6 of 175 PWS tested at locations throughout the state, including PWS using ground water and surface water sources. The occurrence frequency of PFOS in NJ PWS was 3.4%, which is slightly higher than the national frequency of 1.9%. In contrast, PFOA and PFNA were found much more frequently (5-10 fold) in NJ than nationally.

Table 2. New Jersey versus national UCMR3 PFC occurrence data as of January 2016

*Compound**	*Reporting Level (RL) (ng/L)*	*New Jersey*			*United States (other than NJ)*		
		Number of PWS	*Number above RL*	*Percent above RL*	*Number of PWS*	*Number above RL*	*Percent above RL*
PFOA	20	175	18	10.2 %	4734	90	1.9 %
PFNA	20	175	4	2.3 %	4734	10	0.2 %
PFHpA	10	175	6	3.4 %	4734	79	1.7 %
PFOS	***40***	***175***	***6***	***3.4 %***	***4734***	***89***	***1.9 %***
PFHxS	30	175	2	1.1 %	4734	53	1.1 %
PFBS	90	175	0	0 %	4734	8	0.2 %

*PFHpA – perfluoroheptanoic acid (C7); PFBS – perfluorobutane sulfonate

Occurrence in NJ private wells

A statewide study of PFOS or other PFCs in New Jersey private wells has not been conducted. Information from the NJDEP Site Remediation Program shows that PFOS has been found at levels above the USEPA Health Advisory (total of PFOA and PFOS of 70 ppt), and above the ISGWQC (10 ng/L), in several private wells near New Jersey sites where groundwater has been contaminated by PFOS through discharge of AFFF.

HUMAN BIOMONITORING

Human biomonitoring studies show that exposure to PFOS and/or its precursors is ubiquitous in the U.S. and throughout the world. PFOS has a human half-life of several years and remains in the body for many years after exposure ends. Data on blood serum concentrations from the general population, communities with contaminated drinking water, and workers with occupational exposure are summarized below. PFOS is detected in human breast milk, amniotic fluid, and umbilical cord blood, demonstrating that exposure occurs during prenatal and postnatal development, and it has also been detected in human seminal fluid.

Blood serum

General population

PFOS and other long chain perfluorinated chemicals are persistent in the human body and are found

ubiquitously in various world-wide populations. This topic was recently comprehensively reviewed by Kato et al. (2015). Through 2007-2008, PFOS was found in over 99% of a representative sample of the general U.S. population ages ≥ 12 years old (Kato et al., 2011). PFOS was also detected in essentially 100% of blood samples from individuals living in Asia, Europe, and or South America (Kannan et al., 2004).

The U.S. Centers for Disease Control and Prevention (CDC) conducts an ongoing assessment of health and nutrition of adults and children in the U.S., the National Health and Nutrition Examination Survey (NHANES). NHANES generates data on demographic, socioeconomic, dietary, and health-related parameters as well as medical, dental, and physiological measurements, and laboratory tests. The data collected from NHANES is intended to provide a cross-sectional view of selected health and nutrition data for the entire U.S. population. This is accomplished by a complex sampling scheme that begins with 15 nationwide counties identified on the basis of a series of characteristics and proceeds through selected areas in each county to individual selected households (CDC, 2016). Because the 15 counties are selected to be representative of pre-selected population and geographic characteristics rather than individual states, the aggregate data generated provide an estimate that is intended to be generalizable to the U.S. population, but is not necessarily specific to any given state (including New Jersey).

One component of NHANES has consisted of measurement of human exposure to selected environmental chemicals (CDC, 2017). Measurement of exogenous substances in human media is referred to as biomonitoring. This component analyzes blood and urine samples collected as part of the larger NHANES effort to determine the concentration of these chemicals using state of the art analytical methods and quality control procedures. Serum PFOS concentration data have been included since 1999. The most currently available NHANES serum PFOS data are from 2013-2014 (CDC, 2017). The 2013-2014 NHANES serum PFOS data are provided for total PFOS, linear (n-PFOS), and branched PFOS isomers. Unless otherwise indicated, PFOS serum concentrations discussed in this document refer to total PFOS. Because the population selected for NHANES is selected without reference to specific sources of PFOS exposure, it is assumed that serum PFOS concentrations reported by NHANES reflect general population level exposures. That is, they represent exposure to essentially ubiquitous levels of PFOS in the environment (e.g., from consumer products, food, soil, air, and water) and do not represent PFOS exposure from specific sources of release (e.g. industrial facilities that made or used PFOS; discharge of AFFF at airports, military bases, or fire training facilities). Table 3 presents a summary of the 2011-2012 and 2013-2014 data taken from the NHANES Fourth Annual Report on Human Exposure to Environmental Chemicals (CDC, 2017). In 2013-14, the median and 95th percentile serum PFOS concentrations were 5.2 ng/L and 18.5 ng/L, respectively.

Table 3. Total serum PFOS concentrations reported by NHANES for 2011-2012 and 2013-2014 (CDC, 2017)

	Survey years	Geometric mean (95% conf. interval)	50th Percentile (95% conf. interval)	75th Percentile (95% conf. interval)	90th Percentile (95% conf. interval)	95th Percentile (95% conf. interval)	Sample size
Total	11-12	**6.31** (5.84-6.82)	**6.53** (5.99-7.13)	**10.5** (9.78-11.1)	**15.7** (14.7-17.5)	**21.7** (19.3-23.9)	1904
	13-14‡	**4.99** (4.50-5.52)	**5.20** (4.80-5.70)	**8.70** (7.90-9.40)	**13.9** (11.9-15.5)	**18.5** (15.4-22.0)	2165
Age group							
12-19 years	11-12	**4.16** (3.70-4.68)	**4.11** (3.48-4.65)	**5.90** (5.14-7.25)	**9.05** (6.49-10.8)	**10.8** (8.52-14.2)	344
	13-14‡	**3.54** (3.17-3.96)	**3.60** (3.10-4.20)	**5.20** (4.60-6.20)	**7.80** (7.00-8.90)	**9.30** (7.90-11.7)	401
20 years and older	11-12	**6.71** (6.24-7.20)	**7.07** (6.65-7.52)	**11.0** (10.4-11.9)	**17.0** (15.3-18.5)	**22.7** (20.4-24.8)	1560
	13-14‡	**5.22** (4.70-5.81)	**5.60** (5.10-6.00)	**9.10** (8.20-10.2)	**14.5** (12.9-16.1)	**19.5** (15.8-23.0)	1764
Gender							
Males	11-12	**7.91** (7.19-8.70)	**8.31** (7.35-9.15)	**12.5** (11.4-13.5)	**19.3** (15.7-21.4)	**24.1** (22.2-28.5)	966
	13-14‡	**6.36** (5.62-7.20)	**6.40** (5.70-7.30)	**10.2** (8.70-11.5)	**15.5** (13.2-19.8)	**22.1** (16.7-26.9)	1031
Females	11-12	**5.10** (4.70-5.53)	**5.27** (4.67-5.64)	**8.57** (7.87-9.30)	**12.5** (11.0-14.9)	**17.5** (14.9-20.5)	938
	13-14‡	**3.96** (3.60-4.35)	**4.00** (3.60-4.60)	**7.20** (6.40-7.70)	**11.8** (9.70-13.6)	**15.1** (13.9-17.3)	1134
Race/ethnicity							
Mexican Americans	11-12	**4.79** (4.07-5.64)	**5.18** (3.92-6.33)	**7.91** (6.18-9.48)	**10.5** (8.50-12.6)	**12.1** (10.0-14.4)	211
	13-14‡	**3.47** (2.90-4.16)	**3.70** (3.00-4.40)	**5.20** (4.60-6.40)	**8.80** (6.40-10.3)	**10.8** (9.20-11.8)	332
Non-Hispanic blacks	11-12	**6.35** (5.41-7.46)	**6.57** (5.71-7.65)	**11.3** (9.74-13.9)	**21.8** (13.9-31.3)	**30.7** (21.6-45.1)	485
	13-14‡	**5.32** (4.12-6.88)	**5.30** (4.30-6.80)	**10.2** (7.60-13.7)	**17.4** (12.4-24.5)	**24.5** (16.3-39.7)	455
Non-Hispanic whites	11-12	**6.71** (6.15-7.32)	**6.83** (6.07-7.73)	**10.7** (9.89-12.2)	**15.7** (14.8-18.1)	**21.3** (18.7-23.5)	666
	13-14‡	**5.31** (4.72-5.98)	**5.70** (5.10-6.40)	**8.90** (8.20-9.90)	**14.1** (12.2-15.6)	**18.0** (15.5-20.4)	861
All Hispanics	11-12	**4.63** (3.86-5.55)	**5.18** (4.41-6.19)	**8.10** (6.64-9.78)	**11.0** (9.96-12.6)	**13.4** (11.5-16.1)	406
	13-14‡	**3.51** (3.09-3.98)	**3.70** (3.20-4.20)	**5.50** (4.90-6.40)	**8.80** (8.00-9.70)	**10.8** (9.70-12.1)	537
Asians	11-12	**7.10** (5.80-8.68)	**7.53** (5.96-9.25)	**12.6** (10.8-17.0)	**24.6** (19.1-33.3)	**35.1** (26.4-42.3)	291
	13-14‡	**6.18** (5.08-7.52)	**6.30** (5.00-7.90)	**13.2** (9.40-15.4)	**23.8** (15.2-33.9)	**33.6** (20.1-69.0)	234

Limit of detection (LOD, see Data Analysis section) for Survey year 11-12 is 0.2.
‡.See Calculation of PFOS and PFOA as the Sum of Isomers for additional information about Survey years 2013-2014.

Figure 3 below presents the geometric mean serum PFOS concentration for the total NHANES (CDC, 2017) biomonitoring population from the NHANES biomonitoring data from 1999-2000; 2003-2004; 2005-2006; 2007-2008; 2009-2010; 2011-2012; and 2013-2014.

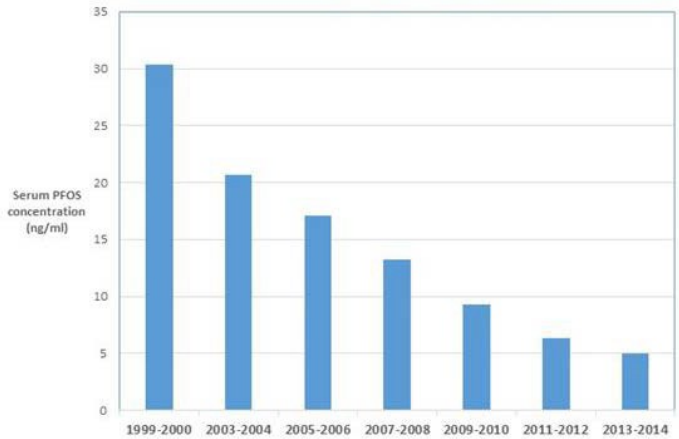


Figure 3. Geometric mean serum PFOS concentraton as reported by NHANES by reporting cycle, 1999-2014.

Starting from the first PFOS serum data collected under NHANES in 1999, the geometric mean PFOS concentration for the total sample population has decreased continuously. The 2013-2014 value represents an approximately 84% decrease from 1999. A similar pattern of decreasing serum PFOS concentrations over time was seen in three studies of American Red Cross blood donors in 2000-2001, 2006, 2010, and 2015 (Olsen et al., 2017). Each study included samples from 600-645 subjects from six locations throughout the U.S., with an approximately equal number in each of five 10-year age categories (20-29

through 60-69 years of age) from each location. Age and sex-adjusted geometric means were 35.1 ng/ml in 2000-01, 14.5 ng/ml in 2006, 8.4 ng/ml in 2010, and 4.3 ng/ml in 2015. This represents an approximately 88% decrease between 2000-01 and 2015.

For perspective, a phase-out of PFOS production was completed in 2002 by the principal worldwide manufacturer of PFOS (ATSDR, 2018). However, manufacture of PFOS has continued in some locations, primarily in China (ATSDR, 2018). As discussed above, NHANES data are an estimate of the PFOS exposure in the U.S. as a whole and likely reflect relatively ubiquitous and non-specific sources of exposure. It is not clear to what extent they can be applied to any particular region or sub-population, including New Jersey. At present, PFOS biomonitoring studies have not been conducted in the New Jersey population.

Communities with drinking water exposure

As shown in Figure 1, continued exposure to even relatively low concentrations of PFOS in drinking water concentrations results in substantial increases in serum levels. The quantitative relationship between drinking water exposure and human serum PFOS levels is discussed in the Toxicokinetics section.

Mean and/or median PFOS serum levels were higher than in the general population in several communities with drinking water contaminated by PFOS from industrial discharge and waste disposal (MDH, 2013), contaminated biosolids applied to agricultural land (ATSDR, 2013), and use of AFFF (NH DHHS, 2015).

A recent study (Hurley et al., 2016) found substantially increased serum PFOS levels in individuals served by PWSs reporting detection of PFOS in UCMR3 monitoring. PFOS detections were relatively low, ranging from 41 ng/L (the UCMR3 RL=40 ng/L) to 156 ng/L, with a mean of 58 ng/L. The study group consisted of middle aged and older California women (n=1,333; 70% between 60 and 79 years of age). Of this group, 5.9% resided in a zipcode where a PWS reporting detection of PFOS in UCMR3 monitoring is located. The distribution of serum concentrations differed significantly ($p = 0.0007$) in those served by a PWS where PFOS was detected ("exposed") as compared to those served by a PWS without a detection ("unexposed"). The median serum PFOS concentrations in the "exposed" group was 29% higher (9.11 ng/ml) than in the "unexposed" group (7.08 ng/ml). The authors note that the contribution of drinking water to serum PFOS is actually likely to be greater than the increase reflected in the study results. Some subjects who were classified as "exposed" because their PWS reported detection of PFOS may have received their drinking water from a point of entry (e.g. treatment plant) within the PWS that is not contaminated with PFOS. Additionally, the serum PFOS levels of some participants classified as "not exposed" may have been increased by PFOS in drinking water at concentrations below the UCMR3 RL of 40 ng/L.

Occupationally exposed workers

Serum PFOS levels in workers at facilities where PFOS or its starting material POSF were made or used were much higher than in the general population. Biomonitoring data from workers at such facilities were reviewed by Olsen (2015). Mean or median serum concentrations of several hundred ng/ml were reported for some job categories at some facilities, with maximum serum concentrations of over 10,000 ng/ml (10 ppm).

Other human biological matrices

Seminal plasma

PFOS and other PFCs were found in human seminal plasma in a study of Sri Lankans. The mean and median PFOS concentrations were 0.118 and 0.103 ng/ml, respectively, and PFOS serminal plasma concentrations were significantly correlated with serum PFOS concentrations (Guruge et al., 2005).

Amniotic fluid

PFOS was detected in amniotic fluid in a study in the United States (Stein et al., 2012). The median blood serum:amniotic fluid concentration ratio was about 20:1.

Umbilical cord blood serum and breast milk

PFOS and other PFCs were detected in numerous studies of umbilical cord blood from the general population worldwide, as reviewed by Kato et al. (2015) and MDH (2017). The ratio of serum PFOS levels in cord blood:maternal blood in these studies was reported by Kato et al. (2015) as about 0.5:1, and MDH (2017) reported that the average ratio in studies reviewed was 0.42:1. These lower levels in cord blood than maternal blood for PFOS, are in contrast to PFOA, for which serum levels in cord blood and maternal blood were similar.

Breast milk

PFOS has been detected in human breast milk in studies from locations worldwide. ATSDR (2018) summarized data from studies from Massachusetts, Sweden, Germany/Hungary, and China published between 2006 and 2008. Concentrations in breast milk were generally similar in these studies from different parts of the world. PFOS was detected in almost all samples, with minimum concentrations in the four studies ranging from <32 - 60 ng/L, and maximums ranging from 360-639 ng/L.

SOURCES OF HUMAN EXPOSURE

The human body burden of PFOS results from exposure to both PFOS itself and to precursor compounds such as perfluorooctane sulfonamidoethanols (FOSEs) and perfluorooctane sulfonamides (FOSAs) used in consumer products that can be metabolized to PFOS. Sources of exposure to PFOS and/or its precursors include food, drinking water, treated fabrics (carpets, upholstery, and clothing), food packaging, house dust, and indoor air (USEPA, 2016a). Gebbink et al (2015) assessed the daily exposure to PFOS arising from PFOS and PFOS precursors and estimated that between 11 and 33% of daily PFOS exposure results from precursors that are metabolized into PFOS.

Food

Egeghy and Lorber (2011), as reviewed by USEPA (2016a), suggest that food may be the primary route of exposure to PFOS in the general U.S. population, and Gebbink et al. (2015) also concluded that diet is the major pathway of exposure to PFOS. It appears that, in part, this is due to the historic use of PFOS in food packaging. D'Hollander et al. (2010), in a review of sources of human exposure to perfluorinated compounds note that among food items, the highest PFOS concentration was found in microwave popcorn (3.6 ng/g). They also note that in a Canadian study, a concentration of 2.7 ng/g was found in beef steak.

As mentioned above, PFOS is bioaccumulative in fish. It bioaccumulates in both freshwater and marine food chains, and is the PFC found most frequently in studies from worldwide locations. PFOS levels in fish can be extremely high (i.e. > 9000 ppb; 9 ppm) in locations impacted by major contamination (e.g. Wurtsmith AFB, MI. MDHHS, 2015; Barksdale AFB, LA. Lanza et al., 2017). Consumption of fish from

such impacted waters can result in high exposures, and fish consumption advisories for PFOS have been issued by several states (ADPH, undated; MDH, 2008; MDHHS (2015); WDNR, 2011).

As reviewed by the USEPA (2016a), PFOS has been found in plants grown in contaminated soil. Available information suggests that PFOS levels in roots and shoots of plants are higher than in other compartments. Consumption of plants grown in soil contaminated with PFOS may serve as a source of exposure to PFOS.

House dust

Exposure to PFOS in house dust is believed to occur through the ingestion route (Egeghy and Lorber, 2011; Gebbink et al., 2015; Trudel et al., 2008). D'Hollander et al. (2010) discuss the occurrence of PFOS in house dust. Dust samples were generally collected from vacuum cleaner bags. The median PFOS levels from North Carolina and Ohio homes and day care facilities was 201 ng/g and the maximum level was 12,100 ng/g. Median levels of PFOS in house dust from Canada and western Europe cited by D'Hollander et al. (2010) ranged from 16-85 ng/g. Thus, house dust can also constitute an ongoing source of exposure. D'Hollander et al. (2010) suggest that PFOS in house dust in locations without specific sources of contamination can arise from perfluorinated compound-treated materials in the home such as stain resistant coatings on carpets and furniture. However, as shown by Su et al., (2016), in homes impacted by specific significant sources of perfluorinated compound release to soil and/or air, such as industrial releases, house dust concentrations and exposures from house dust can be much greater.

Air

PFOS has low volatility, and inhalation exposure is primarily to PFOS bound to aerosol particles (Trudel et al., 2010). Data on PFOS concentration in ambient air are very limited. EPA (2016a) cites data from summertime air sampling in Albany, New York showing a concentration of 1.7 pg/m^3 in the vapor phase and 0.6 pg/m^3 in the particulate phase.

Exposures from drinking water

As discussed in the Biomonitoring section (above), serum levels higher than those prevalent in the general population have been observed in communities with highly contaminated drinking water resulting from environmental discharges, as well as in communities with relatively low levels of PFOS in drinking water identified through UCMR3. As discussed in Toxicokinetics (below), continued exposure to even relatively lower drinking water concentrations can substantially increase total human exposure, as indicated by serum PFOS levels.

PFOS exists in drinking water in its non-volatile anionic form, and the formation of inhalable water droplets during showering or bathing is minimal. Therefore, inhalation exposure is not expected to be significant from non-ingestion uses of drinking water such as showering, bathing, laundry, and dishwashing (USEPA, 2016f). In contrast, these are important exposure routes for volatile drinking water contaminants. Although dermal absorption of PFOS has not been evaluated, dermal absorption of the related compound PFOA during showering, bathing, or swimming is not expected to be significant compared to exposure through ingestion, based on analysis by NJDOH (2014) using skin permeability data from Franko et al. (2012).

Summary of sources of human exposure to PFOS

In the absence of the influence of specific sources of PFOS release to the environment, it appears that food and possibly house dust (reflecting consumer products use and breakdown) are the primary sources of human exposure to PFOS. For high end consumers of fish and specifically consumers of freshwater fish from contaminated waters, fish may be a particular source of PFOS in the diet. In communities with drinking water contaminated by PFOS, drinking water can be an important exposure source even if PFOS

concentrations are relatively low. In locations near release of PFOS to the environment (e.g. from manufacturing facilities), house dust may be a source of significant PFOS exposure.

TOXICOKINETICS

Absorption

Data on PFOS oral absorption are limited. Chang et al. (2012) reports that in rats, a single oral dose of 4.2 mg/kg of radiolabeled PFOS was 99% absorbed based on whole body recovery. This dose is at least five orders of magnitude greater than the Reference doses derived for the candidate critical effects in this assessment. Thus, at these much smaller doses, oral absorption of at least 99% can reasonably be assumed. Consistent with this estimate, ATSDR (2018) cites an estimate of >95% absorption of radiolabeled PFOS in rats at the same gavage dose as in Chang et al. (2012) from unpublished data submitted to the USEPA. Despite the absence of additional data, it is reasonable to assume that PFOS is systemically absorbed in rodents and humans with close to 100% efficiency.

No pharmacokinetic data for inhalation of PFOS were located. However, USEPA (2016b) reports that an acute inhalation study conducted by Rusch et al. (1979) identified an LC_{50} (concentration lethal to 50% of animals), indicating that PFOS is absorbed through inhalation. Additionally, ATSDR (2018) reports that "higher serum levels in [fluoropolymer production] workers compared to the general population probably reflects a predominant contribution from inhaled perfluoroalkyls."

ATSDR (2018) summarizes a dermal absorption study in which Johnson (1995a, 1995b) applied single doses up to 0.3 mg/kg of potassium PFOS and up to 20 µg/kg of the diethanolamine salt of PFOS to clipped, intact skin of rabbits. Total organic fluoride in the liver was not increased in treated animals compared to controls 28 days after dosing, indicating that dermal absorption was not substantial.

Distribution

Transport and binding

PFOS binds strongly, but non-covalently to plasma (serum) proteins, including albumin, gamma-globulin and alpha globulin. USEPA (2016b) has summarized the information on the initial binding sites of PFOS to these plasma proteins. Chen and Gao (2009) report a binding constant of PFOS to human albumin of $2.2 \times 10^4\ M^{-1}$ and a PFOS/human albumin molar ratio of 14. USEPA (2016b) cites an unpublished study by Kerstner-Wood, et al. (2003) indicating that, similar to the case with human serum, PFOS also binds strongly to serum proteins in rats and monkeys.

Organ distribution

Unlike many other biopersistent and bioaccumulative compounds, PFOS does not accumulate in adipose tissue. In humans and rodents, the highest concentrations of PFOS were found in liver. Pérez et al. (2013) analyzed PFOS concentrations in tissue samples from human autopsies of organ donors (n =20 subjects) in Catalonia, Spain. PFOS concentrations by tissue (in mean ng/g wet weight) were liver (102 ng/g) > kidney (75.6 ng/g) > lung (29.1 ng/g) > brain (4.9 ng/g).

In rats (Cui et al., 2008), following a 28-day exposure to 5 mg/kg/day, PFOS concentration was highest in liver > kidney > blood > lung > testis, spleen > brain. In male mice (Bogdanska et al. (2011), following 5 days of exposure to 23 mg/kg/day PFOS through feed, the highest concentration was observed in the liver > lung > blood > whole bone.

Although the fraction of the absorbed dose that deposits in the brain is relatively low, the presence of PFOS in the brains of humans and rodents provides clear evidence that PFOS crosses the blood-brain barrier.

Sex differences

In human liver and serum samples from organ donors, there do not appear to be significant differences in tissue distribution between men and women, or by age (5-74 years old) (Olsen et al., 2003a). Based on 2013-2014 NHANES data (see Table 3), the geometric mean serum PFOS concentration in men (n = 1031) is 6.36 ng/ml compared to 3.96 ng/ml in women (n = 1134). It is not clear whether this reflects a sex dependent difference in toxicokinetics and/or a difference in exposure.

In cynomolgus monkeys (Seacat et al., 2002), following 183 days of exposure, serum PFOS concentrations were equivalent in males and females for exposure to 0.03 mg/kg/day. With higher levels of exposure (0.15 and 0.75 mg/kg/day), serum PFOS concentrations in males became somewhat higher than in females as the exposure time increased. However, even for the high dose, the difference at 26 weeks of exposure was only on the order of 10%.

In contrast to the monkey data discussed above, serum levels were much higher in female rats than male rats at the end of a study in which males and females were given the same doses of PFOS for 105 weeks. In this study, the serum and liver concentrations had decreased by 2-fold or more at 105 weeks from the levels at the latest previous time point sampled (14 weeks or 53 weeks, depending on the dose). In contrast, this striking increase in serum levels at 105 weeks was not observed in females. This decrease in males, but not females, is consistent with the age dependent chronic progressive loss of kidney function known to occur in male rats (Goldstein et al., 1988; Hard et al., 2013) and is not necessarily associated with the PFOS exposure of the rats in this study.

Metabolism

Because of its carbon-fluorine bonds, PFOS is chemically stable and does not undergo chemical reactions even under severe conditions. Therefore, PFOS is not metabolized, as reviewed by USEPA (2016b).

Elimination

Routes of elimination

Humans

Data on the mechanism of PFOS elimination are sparse and PFOS-specific mechanisms have not yet been established (USEPA, 2016b). It appears reasonable that the organic anion transporter (OAT) family of proteins that function in the renal tubular reabsorption processes for PFOA also function in the reabsorption of PFOS. ATSDR (2018) has summarized the human data on the routes of clearance and elimination of PFOS. With the exceptions of lactation and menstrual blood loss, PFOS is cleared primarily through urine. However, in humans, the PFOS bound to serum proteins is not filtered by the kidneys, and only about 1% of the serum PFOS is unbound and available for glomerular filtration. Of this, less than 0.1% of the glomerular filtered PFOS is excreted in the urine per day. This indicates substantial renal tubular reabsorption. A significant fraction of the PFOS in the body is contained in the bile. However, the bile clearance rate greatly exceeds the total body clearance rate. This occurs because bile PFOS is reabsorbed in the gastrointestinal tract with an estimated efficiency of 97%. This suggests that biliary excretion in the feces may also play a minor role in PFOS elimination.

Loss of serum through menstruation can be a significant route of elimination of PFOS in younger (as opposed

to post-menopausal) women. This is suggested both by the simple calculation of fractional serum loss, and pharmacokinetic modeling, (USEPA, 2016b). Although NHANES data indicate that the PFOS serum concentration is higher in men compared to women in the U.S. (see Table 3), it is unclear to what extent this reflects differences in exposure versus sex differences in half-life of elimination.

As reviewed by ATSDR (2018), transfer from serum to breast milk is a substantial route of elimination for perfluorinated compounds in general. Specifically, lactation reduces the maternal serum concentration of PFOS by 2-3% per month of breastfeeding.

Rats

Chang et al. (2012) compared the fraction of the total radiolabeled single IV dose (4.2 mg/kg) of PFOS administered to male Sprague-Dawley rats that was recovered in urine and feces during 89 days post-dose. Although urine was the predominant route of elimination (30.2% of the dose), feces (12.6% of the dose) was a significant route of elimination. In contrast, 48 hours after a single oral PFOS dose of 4.2 mg/kg, a larger fraction of the total dose (3.24%) was recovered in the feces compared to urine (2.52%). Given the very high rate of absorption of PFOS from the rat GI tract (see above), PFOS recovered in the feces presumably reflects absorbed PFOS eliminated via the bile.

Mice

Chang et al. (2012) similarly compared the fraction recovered in urine and feces after a single oral dose (1 or 20 mg/kg) of radiolabeled PFOS was given to male and female CD-1 mice. Although the authors did not report the cumulative recovery, the graphs of percent recovery over time indicate a similar distribution to that observed in the rats in this study.

Thus, in rodents, in contrast to humans, feces, via bile, appears to be a significant route of elimination and may contribute to the shorter half-life of PFOS in rodents compared to humans.

Half-life of elimination

USEPA (2016b) has summarized the available data for the half-life of elimination of PFOS by species. This is presented in Table 4.

Table 4. Summary of data for PFOS elimination half-life (USEPA, 2016b –Table 2-20)

Source	Human	Monkey	Rat	Mouse	Strain
Spliethoff et al. 2008	4.1 years	ND	ND	ND	Infants
3M Company 2000	4–8.67 years	ND	ND	ND	Occupational
Olsen et al. 2007	5.4 years	ND	ND	ND	Occupational
Butenhoff and Chang 2007	ND	ND	48.2 days (M) 46.9 days (F)	ND	SD; 28 days oral
Chang et al. 2012	ND	ND	39.8 days (M) 66.7 days (F)	ND	SD; single oral dose
	ND	ND	ND	39.6 days (M) 34.2 days (F)	CD-1; single oral dose
	ND	132 days (M) 110 days (F)	ND	ND	Cynomolgus; single IV dose
Seacat et al. 2002	ND	200 days (M/F)	ND	ND	Cynomolgus; oral, 182 days

Note: ND = No Data
M = male: F = female

Regarding the human data in Table 4, it should be noted that the Spliethoff et al (2008) data are based on changes in population levels in infant PFOS blood concentration over time and do not directly reflect longitudinal measurements in individuals. Additionally, the estimates of human half-life in adults shown in the table are derived from occupational cohorts that are mostly composed of retired workers and contain few women.

A more recent study by Li et al. (2018) provides estimates of the half-life of PFOS elimination in a community from Ronneby, Sweden, with drinking water contaminated by AFFF. The PFOS half-life was estimated based on decline of serum PFOS levels after exposure to the contaminated drinking water ended. It should be noted that the authors state that future reanalysis of all samples from the same individual in the same analytical batch will provide more definitive results. The study included 106 subjects, ranging from 4 to 83 years old at baseline, of which 20 were men and 30 were women 15-50 years old. The median serum PFOS concentration at the initial collection was 345 ng/ml (55% of the median in the retired worker study by Olsen et al., 2007). The estimates of half-life for all subjects, as well as for men and women 15-50 years old, are presented separately. The mean half-life estimates were 3.4 years for the entire study population, 3.1 years for women age 15-50 (95% CI = 2.7-3.7 years), and 4.6 years for men age 15-50 (95% CI = 3.7-6.1 years). Some subjects had very long half-lives of 8 - >10 years. Although the men in Olsen et al. (2007) were all older than 50 years of age, the mean half-life of 4.6 years for men age 15-50 years from Li et al. (2018) is in reasonable agreement with the mean half-life of 5.4 years from Olsen et al. (2007). Additionally, the 95% CI of 3.9-6.9 years from Olsen et al. (2007) overlaps with the 95% CI of 3.7-6.1 years for men age 15-50 from Li et al. (2018).

Because of its long half-life of several years, PFOS remains in the human body for many years after exposures cease. Because of the large variation in half-lives, the internal dose resulting from a given administered dose varies widely among species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose. Because PFOA is very rapidly eliminated in female rats with a half-life of 2-4 hours, the rat is not an ideal model for evaluation of

developmental effects of PFOA (DWQI, 2017). In contrast, PFOS is slowly excreted in female rats, and both rats and mice are suitable models for evaluation of developmental effects of PFOS.

Toxicokinetics relevant to developmental exposure

Summary

It is important to consider toxicokinetics relevant to developmental exposures of PFOS since PFOS causes developmental toxicity in experimental animals (see Health Effects section below).

Offspring of rodent dams dosed with PFOS during gestation are exposed *in utero* and postnatally through breast milk. In humans, PFOS has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. PFOS concentrations are lower in umbilical cord blood serum, reflective of serum levels in the newborn, then in maternal serum. PFOS exposure in breast-fed infants is greatest during the first few months of life because both PFOS concentrations in breast milk and the rate of fluid consumption are highest during this time period. As a result, serum PFOS concentrations in breast-fed infants increase several-fold from levels at birth within the first few months of life. Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. These greatly elevated exposures during the first months of life are of special concern because the neonatal period may be a sensitive time period for the toxicological effects of PFOS.

Trans-placental transfer

Trans-placental transfer of PFOS occurs in humans, as demonstrated by the presence of PFOS in cord blood and by studies comparing maternal and cord blood PFOS concentrations. The PFOS concentration in the cord blood, on average, is lower than in maternal blood, although the ratio between levels in cord blood and maternal blood varies among individuals. A recent review of the current literature (Kato et al., 2015) concluded that, overall the serum PFOS levels in cord blood were about 50% of the concentration in maternal blood in these studies. Zhang et al. (2013) found that in paired maternal blood and cord blood samples, the cord blood concentration of PFOS was, on average, 21% of the maternal blood concentration at delivery, and the correlation coefficient was 0.9. Fei et al. (2007) found a correlation coefficient of 0.72 comparing cord blood and second trimester maternal blood PFOS concentrations. On average, the cord blood PFOS concentration was 29% of the first trimester maternal blood concentration and 34% of the second trimester maternal concentration.

Trans-placental transfer of PFOS also occurs in rodents. In contrast to humans, it appears that fetal serum concentrations of PFOS in rats and mice are equal to or greater than maternal serum concentrations. Luebker et al. (2005a) found a variable ratio on GD 20 between rat maternal and fetal serum PFOS concentrations for maternal gestational doses between 0.1 and 3.2 mg/kg/day. For three of the four doses, the fetal/maternal ratio was 2.0-1.1. However, for an intermediate maternal dose of 1.6 mg/kg/day, the ratio was 0.74. Chang et al. (2009) found fetal maternal ratios on GD 20 of 2.3, 1.7 and 1.2 for maternal gestational PFOS doses of 0.1, 0.3 and 1.0 mg/kg/day, respectively. In mice, Borg et al. (2010) comparing maternal and fetal blood PFOS concentrations following a single maternal dose of 12.5 mg/kg on GD 16, found a mean fetal/maternal ratio of 2.3 on GD 18 and 1.1 on GD 20. For both rats and mice, it is not clear how, or to what extent the maternal/fetal serum (blood) ratio varies by maternal dose and/or length of gestation. Maternal-to-fetal transfer of PFOS results in a reduced maternal body burden during gestation under conditions of constant exposure.

Exposure to infants through breast milk and infant formula

As mentioned in the Biomonitoring section above, PFOS is detected in human breast milk worldwide. Factors which may potentially affect the concentration of PFOS in breast milk include whether the mother has previously nursed other infants and how soon after birth the sample is taken (Tao et al., 2008a; Haug et al., 2011; Thomsen et al., 2010). Thomsen et al. (2010) found that average PFOS breast milk concentrations were highest initially and decreased by about 3.1% per month, or about 37% during the first year of breast feeding, presumably due to decreased maternal body burden resulting from excretion into breast milk.

PFOS is also transferred to offspring through breast milk in rodents, as shown by Luebker et al. (2005a). This study used a cross-fostering design in which litters from treated and untreated dams were fostered after birth, resulting in four treatment groups: untreated dam with unexposed pup, treated dam with unexposed pup, untreated dam with pup exposed during gestation, and treated dam with pups exposed during gestation. For treated dams with a serum PFOS concentration at the end of lactation of 83 μg/ml, and pups born to unexposed dams (litter average), the pup:maternal PFOS serum ratio was 0.27. Minnesota Department of Health (MDH, 2017) reviewed the current literature on the relationship between PFOS concentrations in maternal serum and breast milk. They found that the mean breast milk:serum ratios reported in these studies ranged from 0.018 to 0.026, with an average among studies of 0.013 (i.e. 1.3:100 or 2.6:200). Based on a breast milk:maternal serum ratio and a serum:drinking water ratio of 200:1 or greater (discussed below), the initial PFOS concentration in breast milk is expected to be greater the concentration in the maternal drinking water source (See similar analysis for PFOA in Post et al., 2012 and ATSDR, 2018).

Exposures to infants to PFOS from breast milk or formula are higher than in older individuals exposed to the same concentration of PFOS in drinking water. Mean breast milk consumption is 150 ml/kg/day during the first post-partum month when PFOS levels in breast milk are highest (Thomsen et al., 2010), and it is 83 ml/kg/day from 6-12 months of age (USEPA, 2008). Similarly, the mean drinking water intakes in infants who consume drinking water (e.g. in formula prepared with water) are 137 ml/kg/day from birth to 1 month of age, and 53 ml/kg/day at 6-12 months of age (USEPA, 2011b). These fluid intakes are much higher than the mean drinking water consumption rates in lactating women, 26 ml/kg/day (USEPA, 2011b), and the general population (11 years of age or older), 13 ml/kg/day (USEPA, 2008). Although breast milk or formula consumption on a body weight basis decreases as the infant gets older, it remains much higher than adult water consumption throughout infancy.

As noted above, serum PFOS levels are generally lower in newborns than in their mothers. Several studies, summarized below, have consistently demonstrated that serum PFOS concentrations in breast-fed infants increase by several fold during the first few months of life, presumably because both breast milk PFOS concentrations and intake of breast milk on a body weight basis are highest during this time period. Infants fed with formula prepared with contaminated drinking water also receive the greatest exposures during the first few months of life because the rate of fluid intake is highest then.

Serum PFOS levels were measured in umbilical cord blood at delivery and at 6 month and 19 months of age in infants from the German general population (Fromme et al., 2010). Average body burdens, as indicated by serum levels, increased by several-fold from birth to 6 months in most infants, as a result of exposure through breast milk. Levels generally declined between 6 months and 19 months, a time point at which breast feeding had stopped or was decreased, but generally remained higher at 19 months than at birth (Figure 4).

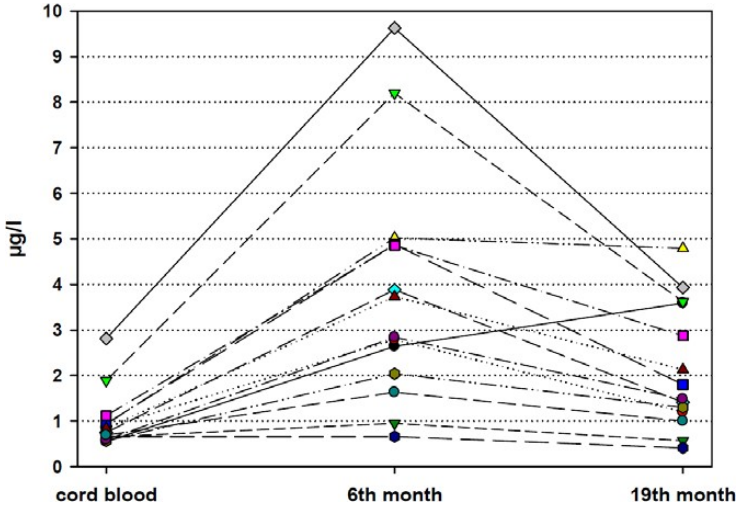


Figure 4. PFOS concentration in cord blood and blood collected in infants around six and nineteen months after birth (Fromme et al., 2010)

Similarly, a study of Faroese infants (n= 80) with serum PFOS data at birth and 11, 18, and 60 months estimated an increase in serum PFOS concentrations of about 29% per month during the period of exclusive breast feeding (median of 4.5 months in the study group) and about 4% per month during the period of partial breast feeding (median of 4 additional months) (Mogensen et al., 2015). Serum PFOS concentration increased little or not at all during periods when the infants being studied were not breast fed (e.g. were formula-fed); presumably, the drinking water in this location was not contaminated with PFOS. Data for 12 infants from the study are shown in Figure 5.

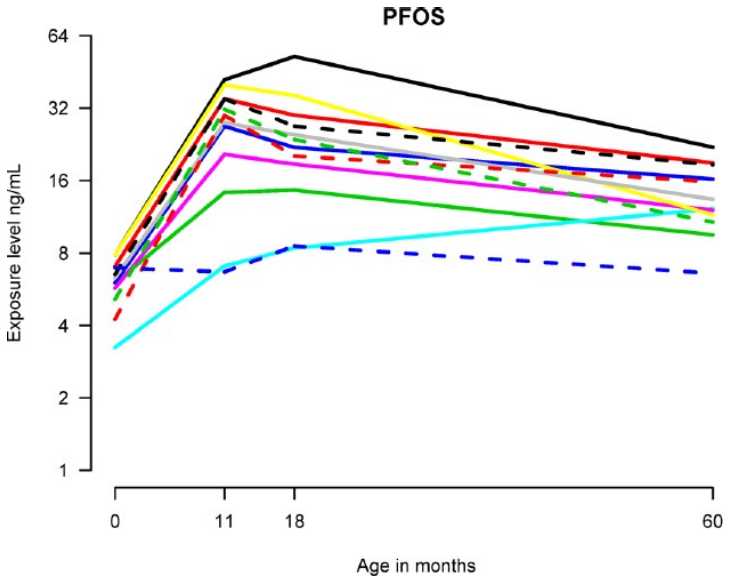


Figure 5. Serum PFOS concentrations over time in 12 infants from Mogensen et al. (2015). Data shown by dotted blue line are from an infant who was not breastfed.

Finally, Verner et al. (2016a,b) developed a pharmacokinetic model that predicts PFOS doses and plasma levels in breastfed infants and children, and their mothers. Monte Carlo simulations were used to predict the distribution of child:mother ratios for doses and plasma levels starting at birth (Figure 7). Predicted doses (ng/kg/day) to infants were highest right after birth and remained higher than in their mothers during the first year of life (Figure 6, right side). Theinfant:mother plasma level ratio, as discussed above, was less than 1 at birth, but this ratio increased to greater than 1 during the first year of life, with predicted ratios of about1.5-fold (median), 3-fold (95th percentile), and 7-fold (maximum) higher plasma PFOS concentrations in infants than in their mothers during the period of greatest infant exposure (Figure 7, left side).

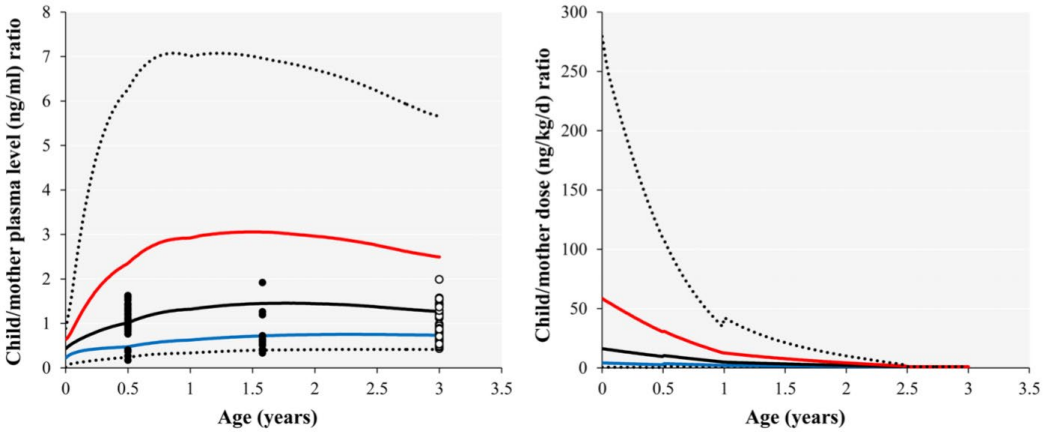


Figure 6. Monte Carlo simulations (n = 10 000) of child/mother ratios of plasma PFOS levels (ng/ml; right side of figure) and doses (ng/kg/day; left side of figure) for a breastfeeding period of 30 months. The black line represents the 50th percentile, the blue line represents the 5th percentile, the red line represents the 95th percentile, and the dotted lines represent minimum and maximum values (Verner et al., 2016b).

While peak serum PFOS concentrations occur during the first year of life, levels remain elevated for at least several additional years. In the study of Faroese children (Mogensen et al., 2015), serum PFOS levels declined after their peak in infancy but remained elevated above initial levels at birth until at least age 5 years, the last time point assessed. Similarly, the model developed by Verner et al. (2016a) predicts that plasma PFOS concentrations will remain several fold higher than at birth until at least age 3 years, the last time point modeled.

In summary, both breast-fed and formula-fed infants receive greater exposures to PFOS from contaminated drinking water (directly or indirectly) than older individuals. Serum PFOS levels peak during the first year of life and remain elevated for several years. These elevated exposures during early life are of concern because effects from neonatal exposure may be sensitive endpoints for the toxicity of PFOS.

Relationship between dose and serum concentration

A chemical-specific clearance factor (CL) of 8.1 x 10^{-5} L/kg/day (8.1 x 10^{-2} ml/kg/day) that relates PFOS serum levels to dose in humans at steady-state was developed by USEPA (2016b).

Dose (ng/kg/day) = Serum Level (ng/ml) x CL (ml/kg/day)

The clearance factor was based on the human half-life ($t_{1/2}$) from a study of retired workers (Olsen et al., 2007) and the volume of distribution (V_d) from Thompson et al. (2010a, b) using the equation below:

$CL = V_d \times (\ln 2 / t_{1/2})$

Where:

V_d = 0.23 L/kg
ln 2 = 0.693
$t_{1/2}$ = 5.4 years = 1,971 days

Thompson et al. (2010a,b) based the PFOS V_d value on a previously developed V_d for PFOA of 0.17 L/kg that had been calibrated with human data. The PFOA V_d was adusted by 35%, based on the observation of Andersen et al. (2006) that the V_d for PFOS can be 20 to 50% greater than for PFOA in monkeys. Thompson et al. (2010a) used the PFOS V_d of 0.23 L/kg in a steady-state toxicokinetic model to predict PFOS intake in a study of Australian drinking water consumers with mean serum PFOS concentration of 21.3 ng/ml (Thompson et al., 2010b), which is comparable to 95th percentile adult serum PFOS concentration reported from NHANES for 2013-2014 of 19 ng/ml (CDC, 2017).

The V_d of 0.23 L/kg for PFOS is supported by the observations of Egeghy and Lorber (2011). Using high (3 L/kg) and low (0.2 L/kg) bounding estimates of the V_d, Egeghy and Lorber (2011) compared predicted modeled PFOS intake with estimates of intakes based on the analyses of exposure pathways. The lower estimate (0.2 L/kg) provided modeled intake predictions similar to modeled intake based on exposure assessment. The derivation of this relationship involves several parameters whose values were estimated based on data for related chemicals or related species. See also Appendix 3 for an alternate derivation of the CL that does not require the estimation of V_d. This alternate derivation produces an estimate of CL that is in close agreement with the value derived by the USEPA (2016b).

Estimated increases in serum levels associated with PFOS in drinking water

The serum:drinking water ratio from ongoing exposure to a given concentration of PFOS in drinking water can be estimated as follows:

Human Dose (µg/kg/day) = Drinking Water Concentration (µg/L) x 0.016 L/kg/day

Where: 0.016 L/kg/day is the **mean** U.S. daily water ingestion rate (USEPA, 2011b).

Therefore:

Drinking Water Conc. (µg/L) x 0.016 L/kg/day = Serum Conc. (µg/L) x Clearance (8.1×10^{-5} L/kg/day)

And:

$$\frac{\text{Serum Concentration (µg/L)}}{\text{Drinking Water Concentration (µg/L)}} = \frac{0.016 \text{ L/kg/day}}{8.1 \times 10^{-5} \text{ L/kg/day}} = \mathbf{197:1}$$

The daily water ingestion rate based on the upper percentile factors (2 L/day water consumption; 70 kg body weight) used to derive ISGWQCs is 0.029 L/kg/day. Using the same equation shown above, the serum:drinking water ratio from **upper percentile** consumption is estimated as **358:1**.

For each 10 ng/L in drinking water, on average, ongoing exposure at the mean ingestion and upper percentile ingestion rates are predicted to increase serum PFOS by 2.0 ng/ml and 3.6 ng/ml, respectively. Increases in serum levels from various concentrations of PFOS in drinking water, and the percent increases from the most recent median serum level, 5.2 ng/ml, from NHANES (2013-14; CDC, 2015) are shown in Table 5 and Figure 7.

Table 5. Increase in serum PFOS concentrations predicted from various concentrations of PFOS in drinking water						
Drinking Water Conc. (ng/L)	Mean Water Ingestion Rate (0.016 L/kg/day)			Upper Percentile Water Ingestion Rate (0.029 L/kg/day)		
	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*
1	0.2	5.4	4%	0.4	5.6	8%
10	2.0	7.2	38%	3.6	8.8	69%
20	3.9	6.1	75%	7.2	12.4	138%
40	7.9	13.1	152%	14.3	19.5	275%
70	13.8	19.0	265%	25.1	30.3	483%
200	39.4	44.6	758%	71.6	76.8	1377%

*Total serum concentrations and % increases from drinking water are based on assumption of 5.2 ng/ml in serum (U.S. median value from NHANES, 2013-14; CDC, 2017) from non-drinking water exposures.

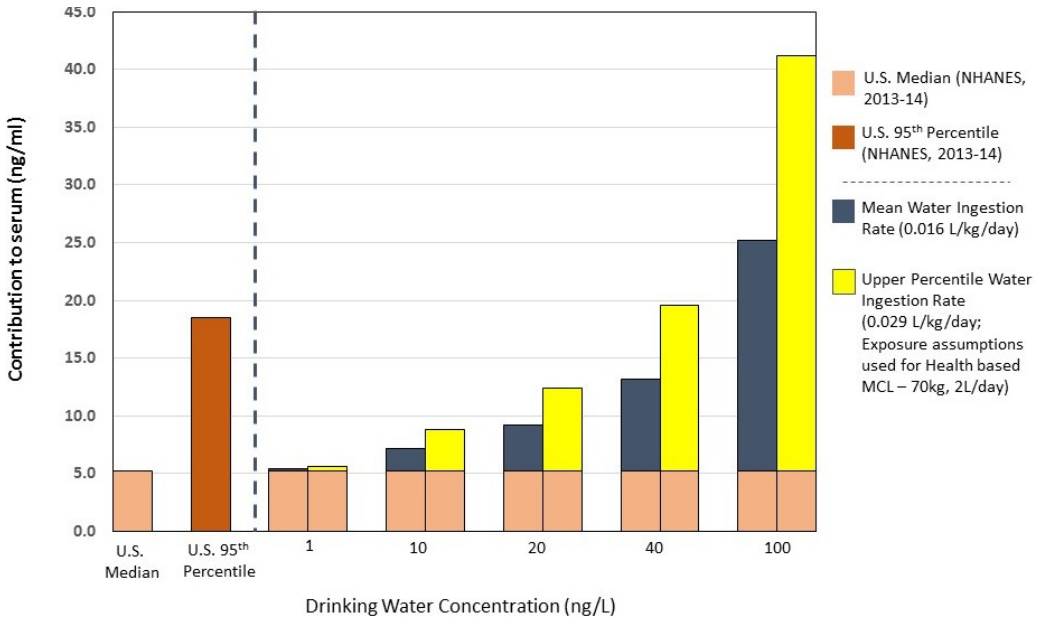


Figure 7. Increases in serum PFOS concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOS, as compared to U.S median and 95th percentile serum PFOS levels (NHANES, 2013-14).

It is evident from Table 5 and Figure 7 that relatively low concentrations of PFOS in drinking water are associated with substantial increases in serum PFOS concentrations; this has recently been observed in a study of serum PFOS levels in individuals served by PWS with PFOS detections in UCMR3 (mean UCMR3 detection – 58 ng/L; Hurley et al., 2016). For example, ongoing exposure to 40 ng/L (the UMCR3 Reporting Level) at the upper percentile ingestion rate is predicted to result in a serum concentration of 19.5 ng/ml, which is above the 95th percentile in the U.S population of 18.5 ng/ml (NHANES, 2013-14; CDC, 2017). With an average (mean) water ingestion rate, exposure to 70 ng/L (the USEPA Health Advisory) is expected to result in an elevation in serum level to 19.0 ng/ml, also above the 95th percentile from NHANES. Additionally, it should be kept in mind that (as discussed above), the increases in serum levels in infants who consume formula

prepared with contaminated water are expected to be substantially higher than those shown in Table 5 and Figure 7.

HAZARD IDENTIFICATION

Review of animal toxicology studies

As described in Literature Search and Screening, approximately 700 studies were identified as potentially useful for assessment of health effects of PFOS, including studies of effects in humans and animals, toxicokinetics, human exposure, and mode of action. Of these studies, 76 animal studies were considered further for use in hazard identification based on their use of typical laboratory species (e.g., rodents, non-human primates, and rabbits). Due to the relatively robust database for animal studies, studies were categorized for different levels of review for use in identifying possible health hazards and potentially dose-response analyses.

Of the 76 studies, 34 studies were reviewed and summarized in evidence tables. An evidence table was developed for studies that met all of the following criteria:

- Assessed an apical endpoint (i.e. an observable outcome in a whole organism, such as a clinical sign of pathologic state that is indicative of a disease state that can result from exposure to a toxicant (Krewski et al., 2010). These can include, but are not limited to: effects on body or organ weight, hematological, blood chemistry, or urinary markers, histopathology, pre-neoplastic or neoplastics lesions, reproductive indices, immunologic competence, results of neurobehavioral tests, or teratogenic outcomes);
- Was peer-reviewed (technical reports were considered if a corresponding peer-review
- publication was available);
- Contained primary data (i.e., not a review article or re-publication of data);
- Employed oral route of exposure (e.g., by drinking water, food, gavage, pill);
- Utilized a relevant duration of exposure (i.e., subchronic or greater [>30 days] exposure
- regimen or reproductive/developmental study);
- Contained >1 dose groups (i.e., a control group and at least 2 additional dose groups);
- Used a relevant animal model (i.e., mice, rats, non-human primates, rabbits).

Evidence tables for animal studies are found in Appendix 4. These tables briefly summarize important methodological information and salient results for each appropriate study. In addition, comments that might influence the interpretation and usefulness of data for health endpoints are noted for each study.

Studies that were reviewed and summarized in evidence tables were the primary sources for identifying potential hazards resulting from PFOS exposure. Additionally, the studies that were considered for dose-response analyses and potentially, criterion development, were chosen from this set of studies. For some studies, multiple evidence tables were prepared because that study reported the results from multiple species (e.g., both rats and mice were exposed) and/or multiple study designs (e.g., a study reporting the results following a multi-generation exposure in one cohort of animals and the results from a cross-fostering exposure in a different cohort of animals)

Of the 76 animal studies that were identified, 41 studies did not fulfill all of the above criteria and underwent a less detailed review. While these studies were not used for quantitative aspects of this assessment, they were

used to further inform the weight of evidence for identified health hazards. These studies are summarized in tabular review tables; one study (Zeng et al., 2011) was not included in either type of table because, based on in-depth review, it only reported mechanistic information.

While tabular review tables provided less methodological detail and study commentary than evidence tables, they include NOAEL/LOAELs for relevant endpoints reported in the study. Tabular review tables for animal studies can be found in Appendix 5.

A synthesis of the information from the evidence tables and the tabular review tables was then prepared in order to identify health effects following PFOS exposure. In considering the health hazards of PFOS, endpoints were categorized into general groupings.

For animal, the following effect groups were utilized:

- Body weight effects
- Endocrine/metabolic effects
- Hepatic effects
- Immune effects
- Neurological effects
- Renal effects
- Other systemic effects (e.g., clinical chemistry, hematology)

For reproductive/developmental studies in which offspring were assessed following gestational exposure, the same categories of effects listed above were utilized, as well as reproductive competency, offspring survival, and markers of development (e.g., eye opening). Also considered within the reproductive/developmental section are studies in which adult animals were exposed with subsequent assessment of reproductive organs.

Following the text describing the results from animal studies of PFOS, study summary tables provide salient information extracted from the evidence tables in Appendix 4, including endpoint, NOAEL/LOAELs, and serum PFOS concentrations at the LOAEL. While information from tabular review tables is not included in the summary, information from these tables is discussed as appropriate in the narrative synthesis for each category of endpoint. Multiple endpoints investigated in a single study are included in a single evidence table, but they may be summarized in multiple summary tables and discussed in narrative syntheses for multiple endpoints as appropriate.

Reporting of exposure levels in animal studies

For animal studies reported in the Hazard Identification section, the goal is to identify adverse endpoints of potential human relevance. For that purpose, exposure metrics are reported as given by the study authors (e.g., mg/L-water, mg/kg/day, mg/kg-feed). In contrast, in the Dose-Response section, studies are compared on the basis of the common metric of serum PFOS concentration.

Review of human epidemiology studies

Following literature screening, 124 studies were identified which assessed associations between human health effects and PFOS and were included in epidemiology evidence tables (Appendix 6). An individual evidence table for each study summarizes the design, location, study population characteristics, outcome and exposure assessment, study population exposure, statistical methods, results, and comments that might influence the interpretation and usefulness of data for health endpoints. Summaries of the studies evaluating each endpoint are provided below in tables following the relevant section.

The studies were conducted on populations in the U.S., Canada, and several European and Asian countries. The epidemiological studies come from populations with exposure levels prevalent in the general population and from workers with higher occupational exposures. In contrast to PFOA (DWQI, 2017), epidemiological data are not available from communities with elevated exposures to PFOS from drinking water or other environmental media. However, studies of people living within communities whose drinking water is contaminated with PFOA, but with general population level exposures to PFOS, have contributed to the epidemiological database for PFOS.

Epidemiologic studies of PFOS have investigated associations with developmental, endocrine/metabolic, hepatic, immune, lipid metabolism, renal, and reproductive effects. Among the epidemiologic studies, the studies of immune effects, and most particularly those investigating effects on vaccine response, were generally consistent in showing adverse responses to PFOS. There was also a consistency in findings between PFOS exposure and increased serum uric acid/hyperuricemia as well as increased total cholesterol.

The epidemiologic data for PFOS are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings from experimental animals for immune effects, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population. These features of the epidemiologic data distinguish PFOS from most other organic drinking water contaminants and justify concerns about exposures to PFOS through drinking water. Notwithstanding, the human data have limitations and therefore are not used as the quantitative basis for the ISGWQC. Therefore, the ISGWQC is based on a sensitive and well-established animal toxicology endpoint that is considered relevant to humans based on epidemiological and mode of action data.

In human environmental health effect studies in general, confounding by co-exposure to contaminants other than the one being evaluated may be particularly important since it may bias results. In some instances, PFOS has been shown to be strongly correlated with other co-occurring PFCs which may not have been controlled for, and the same may be true for co-occurrence with other environmental contaminants.

As is the case for epidemiologic studies of environmental contaminants in general, the nature of these observational epidemiology studies, in contrast to experimental studies, limits our ability to definitively conclude that PFOS causes health effects. However, the findings from observational epidemiology studies are useful in assessing consistency, strength of association, exposure response, temporality, specificity, and biologic plausibility - criteria which are useful in assessing causation.

Studies of exposure levels found in the general population

The majority of studies evaluated the general population and/or study populations with general population-level exposures to PFOS. The serum PFOS concentrations (based on a measure of central tendency, which was presented as median, mean, or geometric mean) in these studies range from 1.6-51.9 ng/L.

A number of studies involved the C8 Health Project which is a community health study of approximately 70,000 Ohio and West Virginia residents of all ages (infants to very elderly) with at least one year of exposure to drinking water contaminated with PFOA at >50 ng/L to over 3000 ng/L (Frisbee et al, 2009; C8 Science Panel, 2014). The C8 Health Project was conducted by the C8 Science Panel, which consisted of three epidemiologists chosen jointly by the parties involved in the legal settlement. This study, primarily interested in evaluating effects of PFOA exposure, is notable because of its large size, the wide range of exposure levels, and the large number of parameters evaluated. Data collected included serum levels of PFOA and other PFCs

(including PFOS), clinical laboratory values, and health histories. The median serum PFOA concentration in this population was 28 ng/ml (ppb), yet serum concentrations of PFOS were reflective of general population level exposure (median 5.2 ppb).

A strength of the general population studies is their use of serum PFOS levels as the basis for exposure assessment. Because of the long human half-life of PFOS, serum levels do not rapidly fluctuate with short term variations in exposure, and serum levels taken at a single time therefore reflect long-term exposures. Serum levels thus provide an accurate measure of internal exposure for each study participant, an advantage over studies based on external exposure metrics such as drinking water concentrations.

Among these studies, the large majority are cross-sectional. A general limitation of cross- sectional studies is that they evaluate information on both exposure and outcome at the same point in time, limiting their ability to establish temporality.

Occupational studies

Occupational studies are often considered useful for evaluating effects of environmental contaminants because exposure levels are generally higher than in general population or in communities exposed through site-specific environmental contamination. Mean or median serum PFOS levels in occupational studies reviewed in this report were generally over 1,000 ng/ml (ppb), several orders of magnitude higher than the median concentrations in the general population.

Occupational studies may also have a selection bias from a "healthy worker effect" whereby workers usually have lower overall mortality and morbidity than individuals of the same age as a whole, since severely ill and disabled persons are typically not included in the workforce, especially in industrial settings (Shah, 2009). Longer duration of employment may also increase the effects of this bias, since sick people will be more likely to leave or change to safer work. Therefore, data based on duration of employment may not accurately reflect higher prevalence or larger magnitude of effects that are associated with longer exposures to the contaminant being evaluated.

Another issue with occupational studies of PFOS is the small number of exposed female employees which limits the ability of the occupational epidemiology to adequately address specific effects among women. An additional issue is the possibility of effect modification due to exposure to other chemicals. Exposure to other PFCs, including PFOS at the 3M Decatur plant, may have played a role in the observed associations. Differences in exposures to other chemicals among manufacturing facilities may result in differences in degree of association with various effects.

Some occupational studies are also noted to have used alternative estimates of PFOS exposure (e.g., air concentrations, exposure to relative concentrations based on job title), instead of serum concentrations which provide a more accurate exposure assessment.

Hazard Identification for Specific Endpoints

Body weight

Animal studies

A summary of body weight effects in animals can be found in the study summary tables at the end of the following review (Table 6). Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

In general, terminal body weight and body weight changes were assessed in rats and mice following dietary and oral gavage exposures. For some studies, data on food consumption were available, which may inform whether changes in animal body weight were due to poor palatability of PFOS (e.g., in dietary studies) or a potentially toxic effect of PFOS. Not discussed in this section are body weight data of female animals exposed to PFOS during pregnancy.

Rats

Following exposures of >30 days to PFOS, decreases in body weight were observed in rats exposed via diet (Kawamoto et al. 2011; LOAEL = 2.1 mg/kg/day) and gavage (Luebker et al. 2005a; LOAEL = 0.4 mg/kg/day in F_0 prior to mating). In both studies, decreases in food consumption were reported at the coresponding LOAEL for decreased body weight. No decrease in body weight was reported following dietary exposures ≤ 1.6 mg/kg/day, even when decreases in food consumption were reported (Seacat et al. 2003; Butenhoff et al. 2012). Additionally, no change in body weight was observed in rats exposed to PFOS via drinking water for 91 days (Yu et al. 2009a; NOAEL = 15.0 mg/L). Food consumption data were not reported for this study.

With shorter durations of dietary exposure (≤ 28 days), decreases in body weight were reported with > 3 mg/kg/day (Curran et al., 2008; Lefebvre et al., 2008), and Elcombe et al. (2012a) reported decreased body weight with exposure to 5.6 mg/kg/day. Concurrent decreases in food consumption were also observed in these studies (Curran et al., 2008; Elcombe et al., 2012a; Lefebvre et al., 2008). Elcombe et al. (2012b) reported decreased body weight following 7 days of dietary exposure to 1.9 mg/kg/day but no change in food consumption (NOAEL = 9.7 mg/kg/day).

Following gavage exposure, decreases in body weight and food consumption were reported following 28 days of exposure ≤ 20 mg/kg/day (Cui et al., 2009; Kim et al., 2011). Following a single exposure to 250 mg/kg, decreased body weight was observed 14 days after exposure; however, information on food consumption was not reported (Sato et al., 2009). No decrease in body weight was observed in male rats exposed to PFOS for 28 (Kim et al., 2011; NOAEL = 10 mg/kg/day) or 5 days (Martin et al., 2007; NOAEL = 10 mg/kg/day). A decrease in body weight and food consumption was observed in rats exposed to 10 mg/kg/day via intraperitoneal injection for 14 days (Austin et al., 2003).

In total, some studies, but not all, report a decrease in adult rat body weight following PFOS exposure via diet, gavage, or intraperitoneal injection. In addition, there is evidence that a decrease in body weight following dietary PFOS is accompanied with decreased food consumption. This evidence suggests that rats may have avoided their food (i.e., ate less) due to the presence of PFOS in their chow, which could have caused the decreased body weight. However, concurrent decreases in rat body weight and food consumption following non-dietary PFOS exposures (i.e., gavage and intraperitoneal) suggest that PFOS may have affected appetite, which may have led to the decreased body weight.

Mice

With dietary exposure, decreased body weight in mice was observed following either 10 days (Qazi et al., 2009a, 2009b; 2012; LOAEL = ~40 mg/kg/day) or 28 days (Qazi et al., 2010a; LOAEL = 0.25 mg/kg/day) of exposure to PFOS, with a decrease in food consumption only occurring with the 10-day exposures. In contrast, no effect on body weight and food consumption was observed in mice exposed to PFOS in the diet for up to 6 weeks (Bijland et al., 2011; NOAEL = 3 mg/kg/day) or in mice exposed to 6 mg/kg/day for 10 days (Qazi et al., 2013).

Following gavage exposure to PFOS, decreased body weight in mice was observed following 60 days of exposure to ≥ 0.42 mg/kg/day PFOS (Dong et al., 2009, 2011, 2012a, 2012b). In these studies, a decrease in

food consumption was also observed. With shorter durations (≤ 28 days) of gavage exposure to PFOS, decreased body weight was observed with doses ≥ 10 mg/kg/day (Zheng et al., 2009; Mollenhauer et al., 2011; Wang et al., 2011a; Zheng et al., 2011; Wan et al., 2012; Wang et al., 2014a). When data were available, a decrease in food consumption was also observed (Zheng et al., 2009; Wang et al., 2011a; Zheng et al., 2011; Wang et al., 2014a). Following a single exposure to 250 mg/kg, decreased body weight was observed 14 days after exposure; however, information on food consumption was not reported (Sato et al., 2009).

In contrast, no significant change in body weight was observed in mice exposed up to 0.17 mg/kg/day PFOS for between 21 to 28 days (Peden-Adams et al., 2008; Guruge et al., 2009; Fair et al., 2011). Additionally, no change in body weight was observed in 4-week old mice exposed once to 11.3 mg/kg at age 10 days (Johansson et al., 2008). No information on food consumption was provided in these studies. In total, some studies, but not all, report a decrease in adult mouse body weight following PFOS exposure via diet or gavage. As with rats, a concurrent decrease in mouse body weight and food consumption following non-dietary (i.e., gavage) PFOS exposures suggests that PFOS may affect appetite and/or metabolism and ultimately body weight.

Monkeys

In monkeys, a decrease in body weight gain (LOAEL = 0.75 mg/kg/day) was observed in males and females exposed to PFOS for 182 days via intragastric intubation of a capsule (Seacat et al., 2002). Data on food consumption were not reported.

Overall Summary of body weight effects in animals

In summary, data are mixed regarding the ability of PFOS to affect the body weights of rats and mice. In monkeys, a decrease in body weight gain was observed. Studies that report decreased animal body weight and decreased food consumption following non-dietary exposures suggest that PFOS may have an effect on appetite and/or metabolism that may then lead to a decrease in body weight.

Table 6. Study summary table for body weight effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	≤104 weeks	Body weight (final) for males and females (overall mean daily food intake reported to increase linearly with PFOS dose)	Males: 1.0 Females: 1.3	--------	Serum and liver PFOS concentrations determined	--------
Dong et al. (2009)	Mice, C57BL/6	0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day (reported as mg/kg/day when representing a NOAEL and/or LOAEL) Oral gavage	60 days	↓ final body weight and body weight change (↓ food intake reported for ≥833.33 ug/kg/day) (determined at day 61)	0.083	0.417	Serum PFOS concentrations determined Only males used	21,640 (serum collected on day 61)

Table 6. Study summary table for body weight effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Dong et al. (2011)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day Oral gavage	60 days	↓ final body weight change (↓ reported for day 60 to day 61 [day of sacrifice] for 0.8333 ug/kg/day) (determined at day 61)	0.4167	0.8333	Serum PFOS concentrations determined Only males used Small sample size (n=6)	51,710 (serum collected on day 61)
Dong et al. (2012b)	Mice, C57BL/6	0, 0.0167, 0.0833, 0.833 mg/kg/day Oral gavage	60 days	↓ change in body weight (over 60 days of exposure) (↓ food intake on day 60 with 0.833 mg/kg/day) (determined at day 60)	0.0833	0.833	Serum PFOS concentrations determined Only males used	59,740 (serum collected on day 61)
Dong et al. (2012a)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333, 2.0833 mg/kg/day Oral gavage	60 days	↓ change in body weight (over 60 days of exposure) (↓ food intake on day 60 with≥0.4167 mg/kg/day) (determined at day 60)	0.0833	0.4167	Serum PFOS concentrations determined Only males used Small sample size (n=6)	24,530 (serum collected on day 61)

Table 6. Study summary table for body weight effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Kawamoto et al. (2011)	Rats, Wistar	0, 2, 8, 32, 128 ppm Dietary Daily PFOS dose (estimated as the mean of the daily PFOS doses reported weekly by study authors) 0, 0.1, 0.5, 2.1, 8.5 mg/kg/day	13 weeks	↓ body weight (↓ food consumption with ≥32 ppm) (determined after 13 weeks)	0.5	2.1	Serum, brain, liver, and kidney PFOS concentrations determined Only males used Internal PFOS concentrations not reported for controls	(serum samples collected after 13 weeks)

Table 6. Study summary table for body weight effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Luebker et al. (2005a)	Rats, Crl:CD® (SD)IGS BR VAF®	0, 0.1, 0.4, 1.6, 3.2 mg/kg/day Oral gavage	F0 males: pre-mating (42 days) and mating (≤14 days)	↓ overall body weight gain (day 0 to termination) (statistically significant reductions in body weight gain at various time points and terminal body weight observed at higher doses) (statistically significant reductions in absolute and relative feed consumption observed during exposure) (termination was 42 to 56 days of exposure)	0.1	0.4	Serum and liver PFOS concentrations determined Control values for internal PFOS measurements not reported Offspring effects summarized elsewhere in appropriate summary table	45,400 (determined after 42 to 56 days of exposure)
Seacat et al. (2002)	Monkeys, cynomolgus	0, 0.03, 0.15, 0.75 mg/kg/day capsule	26 weeks	↓ body weight change (from day 0 to sacrifice, males and females) (sacrifice was following 26 weeks of exposure)	0.15	0.75	Serum and liver PFOS concentrations determined Sample sizes generally 2 to 6 per group with multiple measurements during course of exposure	Males: 173,000 Females: 171,000 (determined after 183 days of exposure)
				Body weight (at sacrifice)	0.75	--------		--------

Table 6. Study summary table for body weight effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Seacat et al. (2003)	Rats, Crl:CD® (SD) IGS BR	0, 0.5, 2.0, 5.0, 20 ppm Dietary Estimated daily dose of PFOS (as reported by study authors) Males: 0, 0.03, 0.13, 0.34, 1.33 mg/kg/day Females: 0, 0.04, 0.15, 0.40, 1.56 mg/kg/day	14 weeks	Body weight (↓ food consumption with 20 ppm, no effect on food efficiency)	Males: 1.3 Females: 1.6	--------	Serum and liver PFOS concentrations determined Sample size ≤5 rats per endpoint	--------
Yu et al. (2009a)	Rats, Sprague-Dawley	0, 1.7, 5.0, 15.0 mg/L Drinking water	91 days	Body weight	15.0 mg/L	--------	Serum PFOS concentrations determined Only males used	--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased
-------- = not applicable

Human epidemiology studies

A summary of body weight effects in humans can be found in Table 7 (below). Detailed methodological information and additional study results can be found in the corresponding individual study tables in Appendix 6. Studies of PFOS exposure and associations with body weight and body mass index (BMI) are discussed here, while studies that reported on endpoints relevant to endocrine/metabolic effects (e.g., glucose homeostatis, metabolic syndrome) are discussed in the Endocrine/Metabolic section below.

Few epidemiology studies investigated body weight/BMI and other body weight related endpoints associations with PFOS. One study (Nelson et al., 2010) suggests an association with *increased* body weight in older adults only. Another study found no association of BMI, skinfold thickness, waist circumference or leptin with PFOS exposure in children (Timmermann et al., 2014).

Table 7. Summary of Epidemiology Studies of Body weight/BMI

Endpoint	Effect and Direction	Serum PFOS concentration (ng/ml) (mean, median, etc.)	Study reference
Body weight	BMI ↑ (M 60-80 yrs old only, not younger M or F)	Med. 21.0	Nelson et al. (2010)
	BMI = (children)	Med. 41.5	Timmermann et al. (2014)
	Skinfold thickness = (children)	Med. 41.5	Timmermann et al. (2014)
	Waist circumference = (children)	Med. 41.5	Timmermann et al. (2014)
	Leptin = (children)	Med. 41.5	Timmermann et al. (2014)

↑ statistically significant positive association
↓ statistically significant negative association
= no statistically significant association/equivocal association
(Statistical significance reflects reporting by authors – generally $p < 0.05$)

Overall conclusions regarding the hazard identification for body weight effects

Both animal and human data provide little support for an effect of PFOS exposure on body weight. The overall weight of evidence does not appear to justify the identification of body weight effects as critical endpoints for consideration of dose-response.

Endocrine/metabolic effects

Animal studies

A summary of endocrine/metabolic effects in animals can be found in Table 8 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

Changes in the thyroid (e.g., histopathology, weight) and thyroid hormones were assessed in animals. Effects on other endocrine and metabolic organs and tissues (e.g., adipose tissue, adrenal glands, hypothalamus, and pituitary glands) and hormones (e.g., corticosterone, estradiol, and testosterone) were also investigated following PFOS exposure. These findings are briefly reviewed below. In addition, data regarding changes in glucose and urea levels are discussed as clinical chemistry parameters relevant to endocrine and metabolic effects.

Thyroid

Thyroid gland weight and histopathology

Effects of PFOS on weight and histopathology of the thyroid gland were assessed in rats. Following 52 weeks of exposure to 1.0 mg/kg/day PFOS, a decrease in relative (to brain) weight of the left thyroid gland was observed in male, but not female, rats (Butenhoff et al., 2012). In this study, no effect was observed in the right thyroid gland of either sex. Increased relative thyroid weight was observed in rats exposed to 100 mg/kg feed (> 6.3 mg/kg/day) of PFOS for 28 days (Curran et al., 2008). Yu et al. (2009a) observed no effect on relative thyroid weight in rats exposed for 91 days ≤ 15.0 mg/L PFOS in drinking water. Yu et al. (2009a) do not provide an estimate of the intake dose of rats in this study. No histopathological effects were observed in rat thyroid glands following chronic (NOAEL = 1.0 mg/kg/day; Butenhoff et al., 2012) or 7-day (NOAEL = 9.7 mg/kg/day; Elcombe et al., 2012b) exposures to PFOS. However, as reviewed in the cancer hazard identification section, an increase in the incidence of thyroid follicular cell tumors was observed in male rats exposed to 1.0 mg/kg/day (20 ppm) for 52 weeks followed by 52 weeks of recovery (Butenhoff et al., 2012).

Thyroid hormones

Levels of thyroid hormones were assessed in rats, mice, and monkeys following PFOS exposure.

Several studies in rats assessed the effect of PFOS on the levels of thyroid hormones. Following 91 days of drinking water exposure to PFOS, total thyroxine levels were decreased with doses ≥ 1.7 mg/L (Yu et al., 2009a). In contrast to this decrease, Yu et al. (2009a) observed no consistent effect on free T4, total triiodothyronine (T3), and thyroid stimulating hormone (TSH) across dose groups (NOAEL = 15.0 mg/L). With a shorter duration of exposure (28 days), decreases in total T4 were observed in male and female rats exposed ≥ 1.3 mg/kg/day PFOS (Curran et al., 2008). Decreases in total T3 were also observed in males and females but at doses ≥ 50 mg/kg feed; TSH was not assessed in these rats. Decreased total and free T4 and total T3 were observed in rats exposed to 10 mg/kg/day PFOS for 5 days (Martin et al., 2007). Following a single oral dose of 15 mg/kg, decreases in total T4 and total and reverse T3 were observed with no effect on free T4 (Chang et al., 2008).

In mice, PFOS was reported to have no effect on total T3 and T4 levels following 28 days of exposure to 0.17 mg/kg/day (Fair et al., 2011).

In monkeys, thyroid hormone levels were assessed after 182 days of exposure to PFOS (Seacat et al., 2002). While there were no effects on free and total T4 (NOAEL = 0.75 mg/kg/day), both free T3 and total T3 levels decreased at 0.75 and 0.15 mg/kg/day, respectively, in males and females. Additionally, TSH levels increased following exposure to 0.75 mg/kg/day. These thyroid hormone effects were observed in the absence of any change in thyroid gland histopathology.

Effects on other endocrine and metabolic organs and tissues

The effect of PFOS on adipose tissue, the adrenal glands, hypothalamus, and the pituitary glands were investigated in animals.

Studies in mice have assessed the effect of PFOS exposure on adipose tissue. Decreases in epididymal fat weight have been observed in mice exposed for 10 days to 0.02% PFOS in feed (~40 mg/kg/day; Qazi et al., 2009a, 2009b, 2012). This decrease was not observed in PPARα null mice (Qazi et al. (2009b) or in mice exposed to lower doses of PFOS for either 10 (6 mg/kg/day) or 28 days (0.14 mg/kg/day; Qazi et al., 2013). When fed a regular (i.e., non-high fat) diet, mice exposed to 20 mg/kg/day PFOS for 14 days had decreased relative fat weight compared to controls (Wang et al., 2011a, 2014a).

The effects of PFOS on the adrenal glands were assessed in rats and mice. Following 52 weeks of exposure, relative (to brain weight) adrenal gland weights were reduced in female rats exposed to 1.3 mg/kg/day PFOS, whereas such a decrease was not observed in male rats exposed to 1.0 mg/kg/day (Butenhoff et al., 2012). Decreased relative adrenal gland weight was observed in male rats exposed to 0.5 to 6.0 mg/kg/day PFOS for 28 days (Pereiro et al., 2014). However, decreased relative adrenal gland weight was not observed in male and female rats exposed ≤ 6.34 mg/kg/d for males or 7.58 mg/kg/d for females for 28 days, although there was a shallow, but statistically significant trend toward increased adrenal weight across doses from 0.14-7.58 mg/kg/day (Curran et al., 2008). In mice, exposure to PFOS of ≤ 0.17 mg/kg/day had no effect on adrenal gland histopathology (Fair et al., 2011).

Effects on the hypothalamus were assessed in rats and mice following PFOS exposure. No effect on relative hypothalamus weight was observed in rats exposed ≤ 6.0 mg/kg/day PFOS for 28 days (Lopez-Doval et al., 2014; Pereiro et al., 2014). To assess the effect of PFOS exposure on the hypothalamus, rats and mice were exposed to PFOS via intracerebroventricular injection (Asakawa et al., 2007). Exposed animals experienced a decrease in food intake (LOAEL = 0.1 mg/kg) as well as changes in gastro-duodenal motility and rate of gastric emptying (LOAEL = 0.3 mg/kg).

The effect of PFOS on the pituitary glands was investigated in rats. After 28 days of exposure, histopathological changes were observed in the pituitary glands of male rats exposed to 0.5 mg/kg/day (Lopez-Doval et al., 2014). However, no change in relative pituitary weight was observed after 28 days exposure to ≤ 6.0 mg/kg/day PFOS (Lopez-Doval et al., 2014; Pereiro et al., 2014).

Effects on other endocrine and metabolic hormones

In addition to thyroid hormone, the effect of PFOS on various other hormones were investigated in animals. Data are mixed for an effect of PFOS on corticosterone levels in mice, as both an increase (LOAEL = 0.83 mg/kg/day; Dong et al., 2009) and no change (NOAEL = 0.83 mg/kg/day; Dong et al., 2011) in this hormone was observed following 60 days of exposure.

A decrease in estradiol was observed in male monkeys but not females following 182 days of PFOS exposure at 0.75 mg/kg/day (Seacat et al., 2002). Decreased leptin was observed in rats following 2 weeks of exposure to 10 mg/kg/day (Austin et al., 2003).

Lopez-Doval et al. (2014) observed decreased luteinizing hormone and increased follicle stimulating hormone in rats following 28 days of exposure to 0.5 mg/kg/day.

A decrease in testosterone was observed in rats following 28 days of exposure to 0.5 mg/kg/day (Lopez-Doval et al., 2014), whereas no change in testosterone was reported for rats exposed ≤ 5 days to 10 mg/kg/day (Martin et al., 2007). No effect on testosterone levels was found in monkeys exposed to 0.75 mg/kg/day PFOS for 182 days (Seacat et al., 2002).

Glucose

In monkeys, no effect on serum glucose levels was observed following 182 days of exposure (Seacat et al., 2002; NOAEL = 0.75 mg/kg/day).

In rats, decreased serum glucose levels were observed in males (LOAEL = 1.0 mg/kg/day) and females (LOAEL = 0.1 mg/kg/day) following 53 weeks of exposure (Butenhoff et al., 2012). Curran et al. (2008) reported that 28 days of PFOS exposure caused a decrease in serum glucose in female (LOAEL = 7.6 mg/kg/day) but not male (NOAEL = 6.3 mg/kg/day) rats. Elcombe et al. (2012a) reported decreased glucose in male rats exposed to 5.6 mg/kg/day for 28 days.

In mice, no effect on serum glucose was observed in females exposed to PFOS for 28 days (Fair et al., 2011; NOAEL = 0.17 mg/kg/day). However, decreased serum glucose was observed in males exposed for 14 days (Wang et al., 2014a; LOAEL = 20 mg/kg/day).

In total, animal studies have reported either no effect or a decrease in serum glucose levels following PFOS exposure.

Urea/ Blood Urea Nitrogen

Effects on urea levels in blood/serum (often reported as blood urea nitrogen; BUN) can result from changes in liver metabolism or kidney function. For simplicity of presentation, changes in blood/serum urea in animals in response to PFOS exposure are addressed here. Following 182 days of PFOS exposure in monkeys, no effect on blood urea nitrogen (BUN) was observed (Seacat et al., 2002; NOAEL = 0.75 mg/kg/day). Increased BUN was observed in male (LOAEL= 0.1 mg/kg/day) and female (LOAEL = 0.3 mg/kg/day) rats following 53 weeks of exposure (Butenhoff et al., 2012). At an interim observation (14 weeks of exposure) in the Butenhoff et al. (2012) study, increased BUN was observed at ≥ 1.3 mg/kg/day in males and females (Seacat et al., 2003). Following 28 days of exposure, Curran et al. (2008) reported a statistically significant decrease in serum urea in female rats exposed to 3.7 mg/kg/day. At 7.6 mg/kg/day, a decrease was also observed in females, but was not statistically significant. In male rats, no effect on serum urea was observed (NOAEL = 6.3 mg/kg/day).

In total, data are mixed for the effect of PFOS on urea in animals. Available data suggest no effect in monkeys and mice; however, increased and decreased urea levels in serum have been observed in rats.

Summary of endocrine/metabolic effects in animals

In summary, studies in multiple species with differing durations of exposure have demonstrated that PFOS can cause endocrine and metabolic effects in animals. Data are mixed regarding an effect of PFOS on the thyroid gland with some studies, but not all, finding changes in thyroid weight. Although a lack of histopathological changes have been observed in the thyroid gland following PFOS exposure, an increased incidence of thyroid follicular cell tumors was noted following chronic exposure (Butenhoff et al., 2012). While not always consistent, PFOS has been reported to affect the level of thyroid hormones. In some studies, decreases in T3 and T4 were not accompanied by a compensatory increase in TSH, which is a classical indicator of hypothyroidism. Additionally, some thyroid hormone measurements need to be interpreted with caution, as analytical methods may influence free T4 measurements (Chang et al., 2007).

Aside from the thyroid gland, PFOS can have an effect on adipose tissue and may affect some functions associated with the hypothalamus. There are few data regarding an effect on the adrenal and pituitary glands although there is a suggestion of histopathological effects. For corticosterone and testosterone, the data are contradictory, and it is unclear whether PFOS has a substantive effect on these hormones. There is only one

study each for the effect of PFOS on levels of estradiol, leptin, luteinizing hormone, and follicle stimulating hormone. Thus, there is insufficient information to draw clear conclusions. Glucose levels in animals following PFOS exposure have either been decreased or unchanged. The effect of PFOS on serum levels of urea is unclear as no effect, increases, and decreases have all been observed in animals.

Table 8. Study summary table for endocrine/metabolic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	52 weeks	↓ adrenal gland absolute weight (left) and relative to brain weight (left and right), females only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure	Males: 1.0 Females: ----	Males: ---------- Females: 1.3	Serum and liver PFOS concentrations determined Only one dose reported for this endpoint	Males: ---- Females: 223,000 (week 14) 233,000 (week 105) (female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)
				↓ thyroid (left, with parathyroid) absolute weight and relative to brain weight, males only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	Males: ---------- Females: 1.3	Males: 1.0 Females: ----		Males: 146,000 Females: ---- (determined at week 53)

Table 8. Study summary table for endocrine/metabolic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	<53 weeks	↑ follicular cell adenoma (thyroid), males only following <53 weeks of exposure then exposure to control diet until terminal sacrifice between weeks 103 and 106	-------- (doses <20 ppm not part of recovery study)	Males: 1.0 Females: ----	Serum and liver PFOS concentrations determined Due to conflation of interim and term data in outcome reporting for thyroid adenomas, neither significance, nor dose-response for term outcomes are interpretable	Males: 2,420 Females: ---- (determined at week 106)
Dong et al. (2009)	Mice, C57BL/6	0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day (reported as mg/kg/day when representing a NOAEL and/or LOAEL) Oral gavage	60 days	↑ serum corticosterone (after 60 days of exposure)	0.417	0.833	Serum PFOS concentrations determined Only males used	65,430 (serum collected on day 61)
Dong et al. (2011)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day Oral gavage	60 days	Serum corticosterone	0.8333	--------	Serum PFOS concentrations determined Only males used Small sample size (n=6)	--------

Table 8. Study summary table for endocrine/metabolic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Seacat et al. (2002) 1-year recovery data not summarized herein	Monkeys, cynomolgus	0, 0.03, 0.15, 0.75 mg/kg/day Capsule	26 weeks	↑ adrenal gland weight (left, relative to body weight, males only) (limited sample size prevented determination of NOAEL and LOAEL)	--------	--------	Serum and liver PFOS concentrations determined Sample sizes generally 2 to 6 per group with increased frequency of endpoint measurements	--------
				↑ TSH (males and females) (determined on days 182 and 184)	0.15	0.75		Males: 173,000 Females: 171,000 (determined after 183 days of exposure)
				Total T4 (no consistent changes with dose or duration)	0.75	--------		--------
				↓ Total T3 (males and females) (on days 182 and 184)	0.03	0.15		Males: 82,600 Females: 66,800 (determined after 183 days of exposure)
				Free T4 (only measured on day 184)	0.75	--------		--------

Table 8. Study summary table for endocrine/metabolic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↓ free T3 (males and females) (only measured on day 184)	0.15	0.75		Males: 173,000 Females: 171,000 (determined after 183 days of exposure)
				↓ estradiol (males only) (on day 182)	Males: 0.15 Females: 0.75	Males: 0.75 Females: ----		Males: 173,000 Females: ---- (determined after 183 days of exposure)
				Testosterone (for entire duration of exposure)	0.75	--------		--------
Yu et al. (2009a)	Rats, Sprague-Dawley	0, 1.7, 5.0, 15.0 mg/L Drinking water	91 days	Thyroid weight (absolute and relative)	15.0 mg/L	--------	Serum PFOS concentrations determined Only males used Unclear whether thyroid hormone measurements were subject to negative bias due to analytical method used	--------
				Total T3 (statistically significant increase with 1.7 mg/L but no statistically significant effects at higher doses)	15.0 mg/L	--------		--------
				↓ Total T4 (determined after 91 days of exposure)	--------	1.7 mg/L		5,000 (determined after 91 days of exposure)

Table 8. Study summary table for endocrine/metabolic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Free T4 (statistically significant decrease at 5.0 mg/L but no statistically significant effects at other doses)	15.0 mg/L	--------		--------
				TSH	15.0 mg/L	--------		--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

↑ = increased; ↓ = decreased
-------- = not applicable

Human epidemiology studies

A summary of endocrine/metabolic effects in humans can be found in Tables 9 to 11 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Thyroid hormones/thyroid disease

Nine studies were identified that investigated a possible association between free T4 and PFOS exposure in adults. The central tendency serum PFOS concentration in these studies was mostly in the range of 8-20 ng/ml, consistent with general population exposure. However, one study of an occupational cohort (Olsen et al., 2003b) had mean serum PFOS concentrations of 800-1,320 ng/ml. With one exception, these studies did not find a statistically significant association between serum PFOS and serum free T4. Dallaire et al. (2009), found a significant positive association between serum PFOS and free T4 in an Inuit population in Nunavik, Quebec, Canada.

Six studies investigated the possible association between serum PFOS and total T4. An additional study, Kim et al. (2000) included PFOS and total T4 in cord blood serum as well as maternal serum. In general, the central tendency PFOS exposure in the populations in these studies were consistent with general population exposures. However, the C8 Study population in Knox et al. (2011) (median concentration 21-26 ng/ml) and the population in several northern New York State counties (Shrestha et al., 2015) (geom. mean 31.6 ng/ml) had serum PFOS levels that were somewhat higher. One of these studies (Lopez-Espinosa et al., 2012a) reported a statistically significant positive association of total T4 with serum PFOS. None of the other studies reported a statistically significant association. A study of children, de Cock et al. (2014b), also did not find a significant association.

Two studies (Dallaire et al., 2009); Kim et al., 2011) reported a significant negative association between total T3 and adult serum PFOS. The significant association of PFOS and T3 in the Kim et al. (2011) study was specific to T3 in maternal serum. Linked results for T3 in fetal cord serum did not yield a significant association with PFOS. A third study that examined T3 uptake (Knox et al., 2011) found a significant negative association with serum PFOS. Two additional studies, Jain et al (2013b), and the previously mentioned Shrestha et al. (2015) study with elevated PFOS serum concentrations did not find a significant association between serum PFOS and total T3.

Eleven studies evaluated the association between adult serum PFOS and thyroid stimulating hormone (TSH). In addition, the aforementioned Kim et al. (2011) study also investigated the association of TSH in fetal cord serum with fetal cord serum PFOS. Dallaire et al. (2009) found a significant negative association, while the study of Lopez-Espinosa et al. (2012a) found a significant positive association. The remaining studies found no significant associations between serum PFOS and TSH.

Two studies addressed the association between adult serum PFOS and thyroxine binding globulin (TBG). Dallaire et al. (2009) found a significant negative association, while Jain et al. (2013b) found no significant association.

Lopez-Espinosa et al. (2012a) investigated the association between serum PFOS and clinical hypothyroidism, sub-clinical hypothyroidism and sub-clinical hyperthyroidism. None of these conditions were significantly (positively or negatively) associated with serum PFOS. Melzer et al. (2010) found no significant associations between serum PFOS and self-reported ever or current thyroid disease.

Summary of thyroid hormones/thyroid disease studies

With the possible exception of T3, none of the thyroid hormones or measures of thyroid function showed

consistent evidence of an association with PFOS exposure. There is a suggestion that PFOS exposure is associated with decreased total T3 and/or T3 uptake. However, the significance of this observation is not clear.

Metabolic function

Glucose homeostasis

Several studies examined the association between PFOS exposure and insulin levels. Lin et al. (2009) found a significant positive association in adults, and Timmermann et al. (2014) found a significant positive association for overweight children, but not for normal weight children. In the Timmermann et al. study, the central tendency level of PFOS in serum (median 41.5 ng/ml) is higher than in other studies that reflect general population exposure. In contrast, Fisher et al. (2013) found no significant association of PFOS with insulin in adults.

No significant associations were observed between serum glucose (adults or children) in three studies (Fisher et al. (2013); Lin et al. (2009); Timmermann et al. (2014)), or in a single study of glucose homeostasis (Lin et al., 2011).

Several studies addressed PFOS and HOMA-IR (Homeostatic model assessment-Insulin resistance). This is essentially a measure of the efficiency of insulin utilization and β cell production of insulin, with higher insulin resistance values indicating less efficient insulin efficiency/glucose utilization. Lin et al. (2009) found a significant positive association of HOMA-IR and serum PFOS in adults. Timmermann et al. (2014) found a significant positive association for overweight (but not for normal weight) children. Two other studies in adults (Fisher et al., 2013; Nelson et al., 2010) found no significant associations. Lin et al. (2009) found that β cell function was significantly positively associated with adult serum PFOS. Since decreased β cell function is a component of an increased value for HOMA-IR, this appears to contradict the findings from the same study regarding HOMA-IR. Adolescent β cell function in this study, however, was negatively associated with serum PFOS with borderline statistical significance. Lind et al. (2014) did not observe a significant association between the pro-insulin/insulin ratio (a measure of insulin secretion) in a population of 70 year-olds.

Metabolic syndrome/body weight/obesity

Metabolic syndrome is a cluster of conditions — increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels — that are predictive of the risk of heart disease, stroke and diabetes. Two studies, Fisher et al. (2013) and Lin et al. (2009) examined the association of metabolic syndrome with serum PFOS in adults, defining metabolic syndrome as having at least three of the five contributing definitions. Neither study found a significant association with serum PFOS.

Nelson et al. (2010) found that serum PFOS was significantly positively associated with body weight for the portion of their NHANES sample 60-80 years-old, but not for other adult ages. Timmermann et al (2014) did not find a significant association between children's serum PFOS and either BMI, skinfold thickness, or waist circumference.

Adiponectin and leptin are both hormones that function (at least in part) in the regulation of fat stores. Adiponectin is also involved in glucose regulation. No significant association was found between serum PFOS and adiponectin (Lin et al. (2011), 12-30-year-olds); Timmermann et al. (2014), children) or leptin (Timmermann et al. (2014), children). Obesity is associated with low-grade chronic inflammation, which inhibits adiponectin. In the Lin et al. (2011) study, no association was found between inflammatory markers

and serum PFOS.

Uric acid

Uric acid is the final product of purine metabolism and may be associated with decreased kidney function or other underlying toxicity. For simplicity of presentation, epidemiology studies investigating associations between uric acid and/or hyperuricemia and PFOS exposure are addressed here. Geiger et al. (2013) (children) and Gleason et al. (2015) (adolescents and adults) found that uric acid concentration in blood was positively associated with serum PFOS. Steenland et al. (2010), also found a significant positive association of both serum uric acid and hyperuricemia with serum PFOS in a very large population of adults. Geiger et al. (2013) found that having hyperuricemia is positively associated with serum PFOS.

Summary of metabolic function studies

There is a suggestion that PFOS is associated with inhibition of insulin function and utilization. However, the evidence for this comes from only two studies (Lin et al., 2009, Timmermann et al., 2014). Other studies did not find these associations. There is also a suggestion that PFOS is associated with increased uric acid levels and an increased risk of hyperuricemia. The evidence for the association of elevated serum uric acid with PFOS exposure is supported by three studies (Geiger et al., 2013; Gleason et al., 2015; Steenland et al., 2010). The evidence for an association of PFOS exposure with hyperuricemia is supported by Geiger et al. (2013) and Steenland et al. (2010). There is a relatively strong consistency in findings among these studies, all of which are relatively large studies (particularly the Steenland et al. (2010) study, n = 53,454). Overall there is moderately strong evidence that PFOS exposure in humans is associated with elevated serum uric acid including the potential for progression to hyperuricemia.

Sex Hormones

A number of epidemiology studies have investigated the potential association between serum PFOS and sex hormones. These include, testosterone (5 studies), estradiol (5 studies), sex hormone binding globulin (SHBG) (5 studies), follicle stimulating hormone (FSH) (4 studies), luteinizing hormone (LH) (4 studies), inhibin-B (3 studies), free androgen index (4 studies), dehydroepiandrosterone, anti-Müllerian hormone, and gonadotrophin hormones (1 study each). One study which found statistically significant negative association with total and free testosterone and free androgen index (Joensen et al. 2013), while the other studies did not find a significant association between these sex hormones and serum PFOS (Table 11).

Table 9. Summary of Epidemiology Studies of Thyroid Function

Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
T4	transthyretin-bound T4 =	Geo. mean 10.92	Audet-Delage (2013)
	Free T4 =	Geo. mean 19.57	Bloom et al. (2010)
	Free T4 =	Geo. mean cases 7.08 controls 7.50	Chan et al. (2011)
	Free T4 ↑	Geo. mean 18.28	Dallaire et al. (2009)

	Free T4 =	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
	Free T4 =	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
	Free T4 =	Geo. mean 31.60	Shrestha et al. (2015)
	Free T4	Geo. mean 7.78	Lin et al. (2013a)
	Free T4 =	Mean 800-1,320	Olsen et al. (2003b)
	Total T4 =	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
	Total T4 =	Med. 7.16- 9.58	Ji et al. (2012)
	Total T4 = (maternal and fetal serum)	Mean 2.93 (maternal)	Kim et al. (2011)
	Total T4 =	Med. 20.97-26.15	Knox et al. (2011)
	Total T4 ↑	Med. 20	Lopez-Espinosa et al. (2012a)
	Total T4 =	Mean 800-1,320	Olsen et al. (2003b)
	Total T4 =	Geom. mean 31.60	Shrestha et al. (2015)
	T4 (apparently total) = (children)	Med. 1.6 (maternal)	de Cock et al. (2014b)
T3	T3 ↓	Geo. mean 18.28	Dallaire et al. (2009)
	Free T3 =	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
	T3 ↓ (maternal serum, not sig for fetal serum)	Mean 2.93	Kim et al. (2011)
	T3 uptake =	Med. 20.97-26.15	Knox et al. (2011)
	T3 ↑ (M only)	Mean 800-1,320	Olsen et al. (2003b)
	T3 =	Geo. mean 31.60	Shrestha et al. (2015)

TSH	=	Geo. mean 9.57	Bloom et al. (2010)
	=	Geo. mean cases 7.08 controls 7.50	Chan et al. (2011)
	↓	Geo. mean 18.28	Dallaire et al. (2009)
	=	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
	=	Med. 7.16- 9.58	Ji et al. (2012)
	=	Mean 2.93	Kim et al. (2011)
	=	Med. 20.97-26.15	Knox et al. (2011)
	=	Geo. mean 7.78	Lin et al. (2013a)
	↑	Med. 20	Lopez-Espinosa et al. (2012a)
	=	Mean 800-1,320	Olsen et al. (2003b)
	=	Geo. mean 31.60	Shrestha et al. (2015)
Thyroxine-binding globulin (TBG)	↓	Geo. mean 18.28	Dallaire et al. (2009)
	=	Not reported (NHANES 2007-8 pop)	Jain et al (2013b)
Thyroid disease	Clinical hypothyroidism =	Med. 20	Lopez-Espinosa et al. (2012a)
	Sub-clinical hypothyroidism =	Med. 20	Lopez-Espinosa et al. (2012a)
	Sub-clinical hyperthyroidism =	Med. 20	Lopez-Espinosa et al. (2012a)
	Thyroid disease ever/curren (self-reported) =	Geo. mean = 25.08 - 19.14	Melzer et al. (2010)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Table 10. Summary of Epidemiology Studies of Metabolic Function			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Glucose homeostastis	Insulin =	Geo. mean 8.40	Fisher et al. (2013)
	Insulin ↑ (for >20 yrs old)	Mean 22.42 - 24.29 (diff age ranges)	Lin et al. (2009)
	Insulin ↑ (for overweight)	Med. 41.5	Timmermann et al. (2014)
	Glucose =	Geo. mean 8.40	Fisher et al. (2013)
	Glucose (homeostasis) =	Med. 8.93	Lin et al. (2011)
	Glucose =	Mean 22.42 - 24.29 (diff age ranges)	Lin et al. (2009)
	Glucose =	Med. 41.5	Timmermann et al. (2014)
	HOMA-IR =	Geo. mean 8.40	Fisher et al. (2013)
	HOMA-IR =	Med. 21.0	Nelson et al. (2010)
	HOMA-IR ↑ (for >20 yrs old)	Mean 22.42 - 24.29 (diff age ranges)	Lin et al. (2009)
	HOMA-IR ↑ (for overweight)	Med. 41.5	Timmermann et al. (2014)
	Metabolic syndrome =	Geo. mean 8.40	Fisher et al. (2013)
	Metabolic syndrome =	Mean 22.42 - 24.29 (diff age ranges)	Lin et al. (2009)
	Adiponectin =	Med. 8.93	Lin et al. (2011)
	Adiponectin =	Med. 41.5	Timmermann et al. (2014)
	β cell function ↑ (for >20 yrs old)	Mean 22.42 - 24.29 (diff age ranges)	Lin et al. (2009)
	Diabetes =	Mean 13.2	Lind et al. (2014)
	Pro-insulin/insulin ratio =	Mean 13.2	Lind et al. (2014)
Uric acid	Serum uric acid ↑	Mean 18.4	Geiger et al. (2013)
	Serum uric acid ↑	Med. 11.3	Gleason et al. (2015)
	Hyperuricemia ↑	Mean 18.4	Geiger et al. (2013)
	Uric acid, hyperuricemia ↑	Med. 20.2	Steenland et al. (2010)
Inflammmation	Inflammatory markers =	Med. 8.93	Lin et al. (2011)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Table 11. Summary of Epidemiology Studies of Sex Hormones			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Sex hormones	Testosterone =	Med. 24.5	Joensen et al. (2009)
	Testosterone =	Med. 3.6	Kristensen et al. (2013)
	Testosterone =	Mean 8.1-51.9 (multiple pops.)	Specht et al. (2012)
	Testosterone =	Med. 21.2 (maternal)	Vested et al. (2013)
	Testosterone (total and free) ↓	Mean 8.46	Joensen et al. (2013)
	Estradiol =	Med. 24.5	Joensen et al. (2009)
	Estradiol =	Med. 3.6	Kristensen et al. (2013)
	Estradiol =	Mean 8.1-51.9 (multiple pops.)	Specht et al. (2012)
	Estradiol =	Med. 21.2 (maternal)	Vested et al. (2013)
	Estradiol =	Mean 8.46	Joensen et al. (2013)
	SHBG =	Med. 24.5	Joensen et al. (2009)
	SHBG =	Med. 3.6	Kristensen et al. (2013)
	SHBG =	Mean 8.1-51.9 (multiple pops.)	Specht et al. (2012)
	SHBG =	Med. 21.2 (maternal)	Vested et al. (2013)
	SHBG =	Mean 8.46	Joensen et al. (2013)
	FSH =	Med. 24.5	Joensen et al. (2009)
	FSH =	Med. 3.6	Kristensen et al. (2013)
	FSH =	Med. 21.2 (maternal)	Vested et al. (2013)
	FSH =	Mean 8.46	Joensen et al. (2013)
	LH =	Med. 24.5	Joensen et al. (2009)
	LH =	Med. 3.6	Kristensen et al. (2013)
	LH =	Med. 21.2 (maternal)	Vested et al. (2013)
	LH =	Mean 8.46	Joensen et al. (2013)
	Inhibin B =	Med. 24.5	Joensen et al. (2009)
	Inhibin B =	Med. 21.2 (maternal)	Vested et al. (2013)
	Inhibin B =	Mean 8.46	Joensen et al. (2013)
	Free androgen index =	Med. 24.5	Joensen et al. (2009)
	Free androgen index =	Med. 3.6	Kristensen et al. (2013)
	Free androgen index =	Med. 21.2 (maternal)	Vested et al. (2013)
	Free androgen index ↓	Mean 8.46	Joensen et al. (2013)
	Dehydroepiandrosterone=	Med. 3.6	Kristensen et al. (2013)
	Anti-mullerian hormone=	Med. 3.6 n	Kristensen et al. (2013)
	Gonadotrophin hormones =	Mean 8.1-51.9 (multiple pops.)	Specht et al. (2012)

↑ statistically significant positive association
↓ statistically significant negative association
= no significant association/equivocal association

Overall conclusions regarding the hazard identification of endocrine and metabolic effects

There is some evidence from animal studies for decreased levels of T4 and T3 due to PFOS exposure. The epidemiological literature provides some support for a role of PFOS in reducing total T3 and possibly T3 uptake. PFOS may affect thyroid weight, but the direction of the effect (decrease/increase) is not consistent. With the exception of thyroid follicular cell tumors, histopathological changes of the thyroid have not been noted in thyroid in response to PFOS exposure. The observation of thyroid follicular cell tumors in rats with chronic exposure contributes to the overall assessment of carcinogenic potential, but there is no suggestion of a mode of action for these tumors.

There is limited evidence for PFOS effects on the hypothalamus. There is limited evidence from the epidemiological literature for an association of PFOS with inhibition of insulin function and utilization.

There is moderately strong evidence for an association of PFOS with increased uric acid levels and the occurrence of hyperuricemia. It is unclear whether (or to what extent) the association of PFOS with uric acid reflects an underlying toxicity. Despite the suggestion of an association of PFOS and uric acid in humans, the lack of data on uric acid levels in animals exposed to PFOS makes the identification of an appropriate animal model uncertain.

Of the endocrine and metabolic endpoints for which there is some evidence for the potential for PFOS to cause adverse effects, the strongest evidence from animal studies relates to the thyroid. The strongest evidence from epidemiologic studies relates to uric acid. For both thyroid effects and uric acid effects, observations in animals are not strongly supported by observations in animals and vice-versa. The animal evidence for thyroid effects is sufficient to include this as an endpoint for consideration of dose-response. While the human evidence for uric acid effects, would suggest that such effects would be an appropriate endpoint for consideration of dose-response, the epidemiologic evidence does not support dose response modeling, and the animal evidence is insufficiently consistent to support dose-response modeling.

Hepatic effects

Animal studies

A summary of hepatic effects in animals can be found in Table 12 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

In general, the following endpoints were identified in animals: increases in liver weight (absolute and relative to body weight), changes in liver histopathology (hepatocellular hypertrophy and other microscopically observed changes), changes in liver carbohydrate and fat content, and increased of incidence tumors (e.g., adenomas and carcinomas). Of these endpoints, histopathological effects and liver weight, and tumor findings (although related to carcinogenicity) are briefly reviewed below. Cchanges in serum enzymes typically associated with liver damage as well as data on bilirubin are also discussed. Note that effects of PFOS on blood/serum levels of urea are discussed in the section on Endocrine and Metabolic Effects.

Liver weight

Increased liver weight (both absolute and relative to body weight) has been consistently observed in mice, monkeys, and rats following subchronic or greater exposure durations to PFOS (see Table 12). Similarly, numerous shorter duration (i.e, <30 days) studies have also reported that PFOS exposure can cause an increase in relative liver weight in mice (e.g., Qazi et al., 2009b; Zheng et al., 2009; Rosen et al., 2010) and rats (e.g.,

Martin et al., 2007; Elcombe et al., 2012a, 2012b). In these shorter duration studies, increased relative liver weight was reported to occur with 5 or 7 days of exposure in rats (Martin et al., 2007) and mice (Zheng et al., 2009; Rosen et al., 2010), respectively.

Following exposures ≥30 days, representative LOAELs for increased relative liver weight were reported to be 0.083, 0.75, and 1.0 mg/kg/day in mice, monkeys, and rats, respectively (Seacat et al., 2002; Dong et al., 2009; Butenhoff et al., 2012). At shorter durations of exposure (<30 days), representative LOAELs for increased relative liver weight were reported to be 5 mg/kg/day in mice (Zheng et al., 2011) and 1.3 mg/kg/day in rats (Elcombe et al., 2012a). However, some low-dose studies in mice did not observe an increase in relative liver weight with PFOS exposures of up to 28 days (e.g., Peden-Adams et al., 2008, NOAEL = 0.17 mg/kg/day; Guruge et al., 2009, NOAEL = 0.025 mg/kg/day).

In addition to studies using standard rat and mouse strains, WT (wild-type) and PPARα null mice have been compared with respect to their hepatic effects of PFOS. Rosen et al. (2010) reported increased relative liver weights in both WT and PPARα null mice following 7 days of exposure. Similarly, Qazi et al. (2009b) reported an increase in absolute liver weight in WT and PPARα null mice following 10 days of exposure; relative liver weight was not reported in this study.

Liver enzymes

While a number of enzyme parameters can be measured as part of clinical chemistry panels, data are reviewed below for alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST), which are indicative of liver effects, following PFOS exposure. Data on the effects of PFOS exposure on liver enzymes and bilirubin are discussed below and summarized in the table for Clinical Chemistry.

ALT

In male and female monkeys, no effect on ALT levels were reported following 182 days of PFOS exposure (Seacat et al., 2002; NOAEL = 0.75 mg/kg/day).

In rats, increased ALT levels were reported in males exposed to 1.0 mg/kg/day for 53 weeks (Butenhoff et al., 2012). This increase was also observed at an interim observation (14 weeks) in these male rats (Seacat et al., 2003). In contrast, there was no effect of PFOS exposure on ALT levels in female rats (Seacat et al., 2003; Butenhoff et al., 2012; NOAEL = 1.3 mg/kg/day). Elcombe et al. (2012a) reported no effect on ALT levels in male rats exposed for ≤ 28 days (NOAEL = 7.9 mg/kg/day). However, a decrease in ALT was observed in male rats exposed to 1.9 mg/kg/day for 7 days (Elcombe et al., 2012b).

In mice, no effect on ALT was observed following exposures up to 28 days or at doses ≤ 6 mg/kg/day (Qazi et al., 2010b, 2013).

ALP

Data are somewhat limited regarding the effect of PFOS exposure on levels of ALP in animals. Seacat et al. (2002) reported no effect of PFOS exposure on ALP in male and female monkeys exposed for 182 days (NOAEL = 0.75 mg/kg/day). Curran et al. (2008) observed no effect of PFOS exposure on ALP in male (NOAEL = 6.3 mg/kg/day) and female (NOAEL = 7.6 mg/kg/day) rats exposed for 28 days. Qazi et al. (2010b) found an increase in ALP in male mice (LOAEL = 0.005% in feed) exposed for 10 days.

AST

No effect on AST levels were observed in male and female monkeys exposed to PFOS for 182 days (Seacat et al., 2002; NOAEL = 0.7 mg/kg/day).

In rats, no effect on AST levels were observed in male (NOAEL = 1.0 mg/kg/day) and female (NOAEL = 1.3 mg/kg/day) rats exposed for 53 weeks (Butenhoff et al., 2012). However following shorter durations of PFOS exposure, data for AST are mixed in rats. Following 28 days of exposure, Curran et al. (2008) found decreased AST in female (LOAEL = 7.6 mg/kg/day) but not male (NOAEL =6.3 mg/kg/day) rats, whereas Kim et al. (2011) observed increased AST in male (LOAEL = 10 mg/kg/day) but not female (NOAEL = 10 mg/kg/day) rats. Additionally, no effect on AST was reported after 28 days (Elcombe et al., 2012a, NOAEL = 1.3 mg/kg/day) or 7 days (Elcombe et al., 2012b, NOAEL = 9.7 mg/kg/day) of PFOS exposure.

In mice, no effect on AST was observed following 28 days (Qazi et al., 2013; NOAEL = 0.14 mg/kg/day) or 10 days (Qazi et al., 2010b; 2013; NOAEL = 6 mg/kg/day) of exposure.

For the serum enzymes discussed above, effects following PFOS exposure vary. While there is some evidence that PFOS can affect ALT levels in animals, data generally suggest no effect on this serum enzyme following PFOS exposure. For ALP, the data, while limited, were negative in monkeys and rats but indicate an effect in mice. AST levels were generally not affected by PFOS exposure; however, some rat studies have reported increased or decreased levels of this enzyme.

Bilirubin

Various observations on bilirubin have been reported following PFOS exposure. Seacat et al. (2002) reported a decrease in total bilirubin in male monkeys following 182 days of exposure to 0.75 mg/kg/day, whereas no effect was observed in females (NOAEL = 0.75 mg/kg/day). No effect on total bilirubin was reported in male (NOAEL = 1.3 mg/kg/day) and female (NOAEL =1.6 mg/kg/day) rats following 14 weeks of exposure (Seacat et al., 2003). However, Curran et al. (2008) observed an increase in conjugated bilirubin in male (LOAEL = 6.3 mg/kg/day) and female (LOAEL = 3.7 mg/kg/day) rats following 28 days of exposure.

In total, data are mixed (i.e., increases, decreases, or no effect have been observed) regarding whether PFOS exposure affects bilirubin levels in animals.

Histopathological lesions

Following PFOS exposure, a number of different histopathological lesions have been reported in the liver including cystic hepatocellular degeneration (Butenhoff et al., 2012), hepatocellular hypertrophy/hepatomegaly (Seacat et al., 2002, 2003; Martin et al., 2007; Curran et al., 2008; Qazi et al., 2010b; Kim et al., 2011; Butenhoff et al., 2012; Elcombe et al., 2012a, 2012b), hepatocyte vacuolation (Seacat et al., 2002, 2003; Wang et al., 2014a), and hepatocyte necrosis (Butenhoff et al., 2012).

Of these lesions, hepatocellular hypertrophy and vacuolation have been assessed in multiple species. Hepatocellular hypertrophy following PFOS exposure has been observed in mice (Qazi et al., 2010b), monkeys (Seacat et al., 2002), and in multiple rat studies (e.g., Martin et al., 2007; Butenhoff et al., 2012; Elcombe et al., 2012a, 2012b). Similarly, hepatocellular vacuolation following PFOS exposure has been observed in mice (Wang et al., 2014a), monkeys (Seatcat et al., 2002) and rats (Seacat et al., 2003). Vacuole formation was observed in both wild-type (WT) and PPARα null mice (Rosen et al., 2010) following PFOS exposure.

While observed following subchronic (i.e., >30 days) and longer exposure durations (see Table 12), lesions such as hepatocellular hypertrophy have also been reported with PFOS exposures of 7 days or less in rats (Martin et al., 2007; Elcombe et al., 2012a, 2012b). In mice, vacuole formation was observed following 7 days of PFOS exposure (Rosen et al., 2010), whereas hypertrophy (Qazi et al., 2010b) and vacuolation (Wang et al., 2014a) were observed following 14 days of exposure.

With subchronic and greater exposure durations, hepatic lesions, specifically cystic hepatocellular degeneration, in rats have been observed at administered doses as low as 0.02 mg/kg/day (Butenhoff et al., 2012). At higher doses, hypertrophy (0.1 mg/kg/day) and necrosis (1.0 mg/kg/day) have been observed (Butenhoff et al., 2012). In monkeys, centrilobular vacuolation and hypertrophy were observed with 0.75 mg/kg/day exposure (Seacat et al., 2002). No chronic mouse studies assessed histopathological lesions. At shorter durations of PFOS exposure (i.e., <30 days), hepatic lesions occurred at higher doses. For example, 1.3 mg/kg/day of PFOS exposure caused hypertrophy in rats (Elcombe et al., 2012a), and vacuolation was observed in mice exposed to 5 mg PFOS/kg/day (Wang et al., 2014a).

While the presence of histopathological lesions in the liver has been a common observation following PFOS exposure, some studies assessing hepatic endpoints have reported no histopathological changes. For example, Fair et al. (2011) found no histopathological changes in the livers of mice exposed up to 0.17 mg/kg/day for 28 days. Additionally, some studies have reported histopathological lesions in males but not in female animals following PFOS exposure. Butenhoff et al. (2012) reported an increase in cystic hepatocellular degeneration in male rats but no increase in females at any dose. Other studies also report that male rats appear to be more sensitive than females to the formation of histopathological lesions in the liver following PFOS exposure (Seacat et al., 2003; Curran et al., 2008; Kim et al., 2011).

Hepatic tumors

Although they are related to carcinogenicity, tumors are discussed here because they may result from a progression that begins with earlier non-neoplastic hepatic damage.

The Butenhoff et al. (2012**)** study in male and female rats was the only identified study that assessed the formation of liver tumors. In both males and females exposed to PFOS for 104 weeks, a statistically significant increase in the incidence of hepatocellular adenomas was reported for the highest dose groups. No statistically significant increases in hepatocellular carcinomas were observed in males or females. However, when adenomas and carcinomas were combined, a statistically significant increase in hepatocellular adenomas/carcinomas was observed in females only.

In summary, studies with multiple species and durations have consistently demonstrated hepatic effects in laboratory animals following PFOS exposure. The apparent succession of some of these lesions occurs in a dose-related manner. For example, as reported in Butenhoff et al. (2012), cystic hepatocellular degeneration in male rats was observed in the lowest dose group (0.02 mg/kg/day). With increasing dose up to 1.0 mg/kg/day, additional effects were observed including hypertrophy, vacuolation, necrosis, and adenomas. This increase in the number of and severity of effects with dose suggests that these effects occur along a continuum starting with cystic degeneration towards more severe effects (e.g., necrosis and tumors).

Table 12. Study summary table for hepatic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	52 weeks	↑ liver absolute weight (males), relative to body weight (males and females), and relative to brain weight (males) (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	Males: ---------- Females: ----	Males: 1.0 Females: 1.3	Serum and liver PFOS concentrations determined Only one dose reported for this endpoint	Males: 146,000 Females: 223,000 (week 14) 233,000 (week 105) (male serum PFOS concentrations determined after 53 weeks of exposure, female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 12. Study summary table for hepatic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	≤104 weeks	↑ cystic degeneration (males only) (determined in rats from scheduled [week 14 and 53], unscheduled, and terminal sacrifices)	Males: ---------- Females: 1.3	Males: 0.02 Females: ---------- Due to conflation of interim and term data in outcome reporting both significance and dose-response for term outcomes are not interpretable	Serum and liver PFOS concentrations determined Other pathological effects reported by study authors but not summarized herein	Males: 910 (week 4) 4,040 (week 14) 1,310 (week 105) Females: ---- (male serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 12. Study summary table for hepatic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ hepatocellular hypertrophy (centrilobular), males and females (determined in rats from scheduled [week 14 and 53], unscheduled, and terminal sacrifices)	Males: 0.02 Females: 0.1	Males: 0.1 Females: 0.3		Males: 4,330 (week 4) 17,100 (week 14) 7,600 (week 105) Females: 12,600 (week 4) 64,400 (week 14) 75,000 (week 105) (male serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks, female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 12. Study summary table for hepatic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ individual hepatocyte necrosis, males and females (determined in rats from scheduled [week 14 and 53], unscheduled, and terminal sacrifices)	Males: 0.2 Females: 0.3	Males: 1.0 Females: 1.3		Males: 41,800 (week 4) 148,000 (week 14) 146,000 (week 53) 69,300 (week 105) Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (male serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks, female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 12. Study summary table for hepatic effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ hepatocellular adenoma, males and females (presumably determined in rats from scheduled [week 14 and 53], unscheduled, and terminal sacrifices)	Males: 0.2 Females: 0.3	Males: 1.0 Females: 1.3		Males: 41,800 (week 4) 148,000 (week 14) 146,000 (week 53) 69,300 (week 105) Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (male serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks, female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 12. Study summary table for hepatic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ hepatocellular adenoma plus carcinoma, combined only for females (presumably determined in rats from scheduled [week 14 and 53], unscheduled, and terminal sacrifices)	0.3	1.3		Males: ---- Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)
Dong et al. (2009)	Mice, C57BL/6	0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day (reported as mg/kg/day when representing a NOAEL and/or LOAEL) Oral gavage	60 days	↑ liver weight relative to body weight (determined after 60 days of exposure)	0.008	0.083	Serum PFOS concentrations determined Only males used	7130 (serum collected on day 61)
Dong et al. (2011)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day Oral gavage	60 days	↑ liver weight relative to body weight (determined after 60 days of exposure)	0.0833	0.4167	Serum PFOS concentrations determined Only males used Small sample size (n=6)	21,640 (serum collected on day 61)

Table 12. Study summary table for hepatic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Dong et al. (2012b)	Mice, C57BL/6	0, 0.0167, 0.0833, 0.833 mg/kg/day Oral gavage	60 days	↑ liver weight relative to body weight (determined after 60 days of exposure)	0.0167	0.0833	Serum PFOS concentrations determined Only males used	8,210 (serum collected on day 61)
Dong et al. (2012a)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333, 2.0833 mg/kg/day Oral gavage	60 days	↑ liver weight relative to body weight (determined after 60 days of exposure)	0.0167	0.0833	Serum PFOS concentrations determined Only males used Small sample size (n=6)	8,210 (serum collected on day 61)
Kawamoto et al. (2011)	Rats, Wistar	0, 2, 8, 32, 128 ppm Dietary Daily PFOS dose (estimated as the mean of the daily PFOS doses reported weekly by study authors) 0, 0.1, 0.5, 2.1, 8.5 mg/kg/day	13 weeks	↑ relative liver weight (↑ absolute liver weight at highest dose) (determined after 13 weeks)	0.5	2.1	Serum, brain, liver, and kidney PFOS concentrations determined Only males used Internal PFOS concentrations not reported for controls	(serum samples collected after 13 weeks)

Table 12. Study summary table for hepatic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Seacat et al. (2002) 1-year recovery data not summarized herein	Monkeys, cynomolgus	0, 0.03, 0.15, 0.75 mg/kg/day Capsule	26 weeks	↑ relative liver weight (i.e., relative to body weight) (↑ absolute and relative [to brain] liver weight in females only with 0.75 mg/kg/day) (determined after 183 days of exposure)	Males: 0.15 Females: 0.15 (based on relative to body weight)	Males: 0.75 Females: 0.75 (based on relative to body weight)	Serum and liver PFOS concentrations determined Sample sizes generally 2 to 6 per group with increased frequency of endpoint measurements	Males: 173,000 Females: 171,000 (determined after 183 days of exposure)
				Cetrilobular vacuolation, hypertrophy, mild bile stasis (sex, incidence, and severity not reported) (determined after 183 days of exposure)	0.15	0.75		172,000 (determined after 183 days of exposure)

Table 12. Study summary table for hepatic effects in animals

Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Seacat et al. (2003)	Rats, Crl:CD® (SD) IGS BR	0, 0.5, 2.0, 5.0, 20 ppm Dietary Estimated daily dose of PFOS (as reported by study authors) Males: 0, 0.03, 0.13, 0.34, 1.33 mg/kg/day Females: 0, 0.04, 0.15, 0.40, 1.56 mg/kg/day	14 weeks	↑ relative liver weight (to body weight, males and females) (↑ absolute liver weight males only with 20 ppm) (determined after 14 weeks of exposure)	Males: 0.3 Females: 0.4 (based on relative liver weight)	Males:1.3 Females: 1.6 (based on relative liver weight)	Serum and liver PFOS concentration determined Sample size ≤5 rats per endpoint	Males: 148,000 Females: 223,000 (determined after 14 weeks of exposure)
				Centrilobular hepatocyte hypertrophy, midzonal to centrilobular vacuolation (determined after 14 weeks of exposure)	Males: 0.1 Females: 0.4	Males: 0.3 Females: 1.6		Males: 43,900 Females: 223,000 (determined after 14 weeks of exposure)
Yu et al. (2009a)	Rats, Sprague-Dawley	0, 1.7, 5.0, 15.0 mg/L Drinking water	91 days	↑ liver weight (absolute and relative) (determined after 91 days of exposure)	1.7 mg/L	5.0 mg/L	Serum PFOS concentrations determined Only males used	33,600 (determined after 91 days of exposure)

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased

-------- = not applicable

Human epidemiology studies

A summary of hepatic effects in humans can be found in Table 13 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Liver enzymes

The increase of liver enzymes in serum is generally considered to be an indicator of liver toxicity. Several studies investigated the association between serum liver enzymes and PFOS exposure. No overall consistent pattern is apparent. While some studies, including Gallo et al. (2012) and Olsen et al. (2003b), found significant positive associations of serum ALT with serum PFOS at median and mean PFOS concentrations in the study population, other studies by Gleason et al. (2015), Olsen et al. (2012), and Jiang et al. (2014) failed to find a significant association. There is some suggestion that those studies that did find a significant positive association involved cohorts with higher PFOS exposure. Only one study (Olsen et al., 2003b) found a positive association of PFOS with gamma glutamyl transferase (GGT; in females only), while two other studies did not. The occupational cohort of Olsen et al. (2003b) had a much greater exposure than the non-occupational cohorts in the other studies. No significant positive associations were found between serum PFOS and AST. Of the three studies that measured ALP, only the Olsen et al. (2003b) occupational cohort found a significant positive association.

Bilirubin

Elevated serum bilirubin can be an indirect measure of liver toxicity and/or an indication of bile duct blockage (cholestastis). A component of total bilirubin is direct bilirubin, a product of hemoglobin metabolism for which increased serum concentrations reflect increases in liver and bile duct disease. Therefore, total bilirubin serves only as an inferential measure of liver function. The available studies of serum bilirubin in various cohorts showed both significant positive and negative associations with no clear pattern.

Table 13. Summary of Epidemiology Studies of Hepatic Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Liver enzymes			
	ALT ↑	Med. 20.3	Gallo et al. (2012)
	ALT =	Med. 11.3	Gleason et al. (2015)
	ALT =	Δ+4.2	Olsen et al. (2012)
	ALT ↑ (M only)	Mean. 800-1,320	Olsen et al. (2003b)
	ALT =	Mean 4.75	Jiang et al. (2014)
	GGT =	Med. 20.3	Gallo et al. (2012)
	GGT =	Med. 11.3	Gleason et al. (2015)
	GGT ↑ (F only)	Mean. 800-1,320	Olsen et al. (2003b)
	AST =	Med. 11.3	Gleason et al. (2015)
	AST =	Δ+4.2	Olsen et al. (2012)
	AST =	Mean. 800-1,320	Olsen et al. (2003b)
	AST =	Mean 4.75	Jiang et al. (2014)
	ALP =	Med. 11.3	Gleason et al. (2015)
	ALP =	Δ+4.2	Olsen et al. (2012)
	ALP ↑	Mean. 800-1,320	Olsen et al. (2003b)
Bilirubin	Direct ↑	Med. 20.3	Gallo et al. (2012)
	Total ↑	Med. 11.3	Gleason et al. (2015)
	Total ↓	Δ+4.2	Olsen et al. (2012)
	Total ↓, direct ↓	Med. 1,000-3,000	Olsen et al. (1999)
	Total ↓	Mean. 800-1,320	Olsen et al. (2003b)
	Total ↑ (for 2-branched PFOS only)	Mean 4.75	Jiang et al. (2014)
↑ statistically significant positive association ↓ statistically significant negative association =no significant association/equivocal association Δ+ positive change			

Overall conclusions regarding the hazard identification of hepatic effects

There is evidence from animal studies that the liver is a target organ for PFOS exposure. In animals, PFOS has produced a variety of hepatic effects including histopathological changes, increased liver weight, and tumors. In humans, studies of hepatic effects have focused on changes in serum enzymes that are typically associated with liver damage. Such studies have reported mixed results following PFOS exposure.

Based on the strength of the observations from animal studies, hepatic effects are identified as endpoints for consideration of dose-response.

Immune effects

Animal studies

A summary of immune effects in animals can be found in Table 14 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

In general, the following endpoints were identified in laboratory animals and are briefly reviewed below: immunosuppression (e.g., host resistance, natural killer cell activity, plaque forming cell response), as well as effects on immune organs (e.g., cellularity, histopathology, weight), cell populations, and immune mediators (e.g., cytokines, immunoglobulins).

Immunosuppression

Although no chronic studies assessed immunosuppression, subchronic (i.e., ≥30-90 days of exposure) and shorter duration studies of PFOS were found to cause such effects. Dong et al. (2009) observed decreased plaque forming cell response (i.e., a measurement of the ability of an organism to form reactive antibodies to an extrinsic antigen) in adult male mice (following sheep red blood cell [SRBC] challenge) after 60 days of PFOS exposure (LOAEL = 0.083 mg/kg/day). At shorter durations of exposure, decreased plaque forming cell response was observed in male mice following 7 (Zheng et al., 2009; LOAEL = 5 mg/kg/day) or 28 days of PFOS exposure (Peden-Adams et al., 2008; LOAEL = 0.002 and 0.02 mg/kg/day for males and females, respectively). In contrast, Qazi et al. (2010a) found no effect on plaque forming response in male mice following 28 days of exposure (NOAEL = 0.25 mg/kg/day). With *in utero* exposure (GD1 to GD17) to PFOS, decreased plaque forming cell response was observed in male (LOAEL = 5 mg/kg/day), but not female (NOAEL = 5 mg/kg/day), mouse offspring at 8 weeks of age (Keil et al., 2008). At these LOAELs, decreases in plaque forming cell response compared to controls were: 30% (Dong et al., 2009), 52 to 78% (for males, Peden-Adams et al., 2008), 63% (Zheng et al., 2009), and 53% (Keil et al., 2008).

In addition to effects on plaque forming cell response, other indicators of immunosuppression have been reported in mice. For example, following 60 days of PFOS exposure, decreased natural killer cell activity was observed at doses of > 0.83 mg/kg/d (although there was an increase in natural killer cell activity at a lower dose of 0.08 mg/kg/day) (Dong et al., 2009). At the same exposure duration, no effect on delayed-type hypersensitivity was observed in mice (Dong et al., 2011) at any dose (i.e., ≤ 0.83 mg/kg/day). Following 21 days of exposure, increased mortality in response to influenza A virus was reported in Guruge et al. (2009; LOAEL= 0.025 mg/kg/day).

Effects on immune organs

Following PFOS exposure, effects assessed in immune organs (spleen and thymus) included changes in cellularity, histopathology, and organ weight. Decreases in splenic and thymic cellularity have consistently been observed in mice following PFOS exposure. While these decreases have been observed following subchronic exposure (Dong et al., 2009, 2012a, 2012b) and in shorter 7 or 10 days studies (Zheng et al., 2009; Qazi et al., 2012).

Decreases in splenic and thymic cellularity have been observed in mice with relatively high doses (20 mg/kg/day) following 7 days of PFOS exposure (Zheng et al., 2009). However, longer durations of PFOS exposure (e.g., 60 days) caused decreases in splenic and thymic cellularity at 0.4 mg/kg/day (Dong et al., 2009, 2012a). No decrease in splenic and thymic cellularity was observed following 28 days of exposure to 0.17 mg/kg/day (Peden-Adams et al., 2008).

There is limited information regarding the histopathological effects of PFOS exposure on the spleen and thymus. Following 14 days of exposure, histopathological effects in mouse spleen (dilation of splenic sinus) and thymus (vasodilation, congestion) were observed with 5 mg/kg/day (Wang et al., 2011a). At lower doses in mice, no effects on spleen and thymus histopathology were observed with 0.17 mg/kg/day for 28 days (Fair et al., 2011). In rats, spleen histopathology (congestion, mild dilation of the splenic antrum) was observed with 28 days of exposure at 5 mg/kg/day (Cui et al., 2009).

In general, decreased relative spleen and thymus weights were observed in mice following PFOS exposure. Following subchronic exposure, these decreases occurred with PFOS doses >0.4 mg/kg/day (Dong et al., 2009, 2011, 2012a, 2012b). With shorter durations of exposure (i.e.,<14 days), decreased relative spleen and thymus weights were observed following higher PFOS doses, >20 mg/kg/day (Qazi et al., 2009b, 2012; Zheng et al., 2009, 2011; Wang et al., 2011a). In contrast, no changes in spleen and thymus weights were observed when PFOS doses were <0.25 mg/kg/day (Peden-Adams et al., 2008; Guruge et al., 2009; Qazi et al., 2010a). In addition to observations in standard strains of mice, 40 mg/kg/day of PFOS for 10 days decreased absolute spleen weights in wild-type (WT) and PPARα null mice (Qazi et al., 2009b). Absolute thymus weights were reduced, but with statistical significance only in WT mice.

In rats following 52 weeks of exposure, relative (to body weight) spleen weight decreased in males (LOAEL = 1.0 mg/kg/day) but increased in females (LOAEL = 1.3 mg/kg/day; Butenhoff et al., 2012). Following 28 days of exposure, relative spleen weight increased in female (LOAEL = 7.6 mg/kg/day), but not male rats (NOAEL = 6.3 mg/kg/day; Lefebvre et al., 2008). No effect on relative thymus weight was observed in these rats.

Effects on specific cell populations

Exposure to PFOS has been reported to affect immune cell populations in mice. For example, 60 days of PFOS exposure decreased splenic and thymic T cell CD4/CD8 subpopulations (LOAEL 35= 0.4 mg/kg/day) and splenic lymphocyte proliferation (LOAEL = 0.8 mg/kg/day; Dong et al., 2009). At lower doses, PFOS exposure caused an increase in the percentage of peritoneal cavity macrophages (LOAEL = 0.02 mg/kg/day; Dong et al., 2012a). At a shorter duration of exposure (i.e., 7 days), 5 mg/kg/day of PFOS caused a decrease in lymphocyte proliferation (Zheng et al., 2009).

Effects on immune mediators

PFOS has been reported to affect immune mediators (i.e., cytokines, immunoglobulins) in mice. Following 60 days of exposure, PFOS was reported to either increase (IL-1beta, IL-4, IL-6, IL- 10, TNFα) or decrease (IL-2) the *ex vivo* production of cytokines by isolated splenocytes or peritoneal cells (Dong et al., 2011, 2012a). Following inoculation with sheep red blood cells, decreases in serum IgM levels have been observed with 60 days of exposure to 0.83 mg/kg/day PFOS (Dong et al., 2011). At a shorter duration of exposure (i.e., 7 days), 5 mg/kg/day PFOS increased IgG and decreased IgM levels in serum (Zheng et al., 2011).

Summary of immune effects in animals

In summary, animal studies, primarily in mice, have demonstrated various immune effects following PFOS exposure. Immunosuppression has consistently been reported (in all but one study) in the form of decreased immune system function (e.g., plaque forming cell response to a foreign antigen) and decreased host resistance. Although the total number of studies examining immunosuppression in animals is relatively small (n = 5), the consistency of the effect provides strong support for identifying immunosuppression as an effect of PFOS exposure. At the organ level, decreases in spleen and thymus cellularity and relative weights have been observed. Additionally, there is evidence that PFOS can affect immune cells populations, serum immunoglobulin levels, and immune mediators. These effects at different levels of the immune system

provide evidence that supports a conclusion that PFOS is immunotoxic in laboratory animals.

Table 14. Study summary table for immune system effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	52 weeks	↓ spleen absolute weight, relative to body weight, and relative to brain weight, males only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	--------	Males: 1.0	Serum and liver PFOS concentrations determined Only one dose reported for this endpoint	146,000 (determined after 53 weeks of exposure)
				↑ spleen weight relative to body weight, females only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	--------	Females: 1.3		Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)

Table 14. Study summary table for immune system effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Dong et al. (2009)	Mice, C57BL/6	0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day (reported as mg/kg/day when representing a NOAEL and/or LOAEL) Oral gavage All animals appear to have been immunized with sheep red blood cells (SRBC) four days prior to sacrifice.	60 days	↓ spleen weight relative to body weight (determined at day 61)	0.083	0.417	Serum PFOS concentrations determined Only males used	21,640 (serum collected on day 61)
				↓ thymus weight relative to body weight (determined at day 61)	0.083	0.417		21,640 (serum collected on day 61)
				↓ splenic cellularity (determined at day 61)	0.083	0.417		21,640 (serum collected on day 61)
				↓ thymic cellularity (determined at day 61)	0.083	0.417		21,640 (serum collected on day 61)
				↓ splenic and thymic T cell CD4/CD8 subpopulations Effects on splenic B cells observed at higher doses (determined at day 61)	0.083	0.417		21,640 (serum collected on day 61)

Table 14. Study summary table for immune system effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↓ splenic NK cell activity (↑ activity reported at 83.33 ug/kg/day) (determined at day 61)	0.417 Based on decreased activity	0.833 Based on decreased activity		65,430 (serum collected on day 61)
				↓ splenic lymphocyte proliferation (determined at day 61)	0.417	0.833		65,430 (serum collected on day 61)
				↓ plaque forming cell response (determined at day 61)	0.008	0.083		7,130 (serum collected on day 61)
Dong et al. (2011)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day Oral gavage All animals appear to have been immunized, at least once (7 days prior to sacrifice) with SRBC. Animals used for the delayed-type	60 days	↓ spleen weight relative to body weight (determined at day 61)	0.4167	0.8333	Serum PFOS concentrations determined Only males used Small sample size (n=6)	51,710 (serum collected on day 61)
				↓ thymus weight relative to body weight (determined at day 61)	0.4167	0.8333		51,710 (serum collected on day 61)

Table 14. Study summary table for immune system effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
		hypersensitivity response assay also received a booster SRBC immunization one day prior to sacrifice.		↑ cytokine secretion (IL-4), splenocytes (↓ INF-gamma reported for 0.8333 ug/kg/day) (determined at day 61)	0.0167 (based on IL-4 data)	0.0833 (based on IL-4 data)		10,750 (serum collected on day 61)
				Number of T-cells (from splenocytes) secreting cytokines: ↓ for IL-2+ cells ↑ for IL-10+ cells (determined at day 61)	0.4167	0.8333		51,710 (serum collect on day 61)
				↓ serum IgM levels (↑ IgG, IgG1, and IgE with 0.8333 ug/kg/day) (determined at day 61)	0.0167 (based on IgM data)	0.0833 (based on IgM data)		10,750 (serum collected on day 61)
				Delayed-type hypersensitivity (footpad thickness)	0.8333	--------		--------

Table 14. Study summary table for immune system effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL*** ***(day assessed)***
Dong et al. (2012b)	Mice, C57BL/6	0, 0.0167, 0.0833, 0.833 mg/kg/day Oral gavage	60 days	↓ spleen weight relative to body weight (determined at day 61)	0.0833	0.833	Serum PFOS concentrations determined Only males used	59,740 (serum collected on day 61)
				↓ thymus weight relative to body weight (determined at day 61)	0.0833	0.833		59,740 (serum collected on day 61)
Dong et al. (2012a)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333, 2.0833 mg/kg/day Oral gavage A separate cohort of seven groups of animals were immunized with lipopolysaccharide on day 61 (i.e, one day after the final exposures) to assess innate immune response (e.g., cytokine levels).	60 days	↓ spleen weight relative to body weight (determined at day 61)	0.0833	0.4167	Serum PFOS concentrations determined Only males used Small sample size (n=6)	24,530 (serum collected on day 61)
				↓ thymus weight relative to body weight (determined at day 61)	0.0833	0.4167		24,530 (serum collected on day 61)
				↓ splenic cellularity (↑ percentage of splenic macrophages with ≥0.833 mg/kg/day) (determined at day 61)	0.0833 (based on cellularity data)	0.4167 (based on cellularity data)		24,530 (serum collected on day 61)

Table 14. Study summary table for immune system effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ percentage of peritoneal cavity macrophages (↓ peritoneal cavity cellularity with 2.0833 mg/kg/day) (determined at day 61)	0.0083	0.0167		4,530 (serum collected on day 61)
				↑cytokine production (TNF-alpha) by peritoneal cells (↑ production of IL-1beta and IL-6 at higher doses) (determined at day 61)	0.0833 (based on TNF-alpha data)	0.4167 (based on TNF-alpha data)		24,530 (serum collected on day 61)
				↑cytokine production (TNF-alpha and IL-1beta) by splenic cells (↑ production of IL-6 at higher dose) (determined at day 61)	0.4167 (based on TNF-alpha data)	0.8333 (based on TNF-alpha data)		59,740 (serum collected on day 61)

Table 14. Study summary table for immune system effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL*** ***(day assessed)***
				↑ serum cytokines (IL-1beta and IL-6), without LPS stimulation (↑ serum cytokine with LPS stimulation but at higher PFOS doses) (determined at day 61)	0.4167	0.8333		59,740 (serum collected on day 61)

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased

-------- = not applicable

Ig = immunoglobulin; IL = interleukin; INF = interferon; LPS = lipopolysaccharide; NK = natural killer; TNF = tumor necrosis factor

Note: For some endpoints animals were administered sheep red blood cells or other antigen to assess immune response. Such immunizations are noted in the "Administered Doses and Route" column.

Human epidemiology studies

A summary of immune effects in humans is found in Table 15 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Vaccine response/antibody titers

Five studies evaluated associations of serum PFOS concentrations and antibody concentrations following vaccination for measles, mumps, rubella, diphtheria, tetanus and/or influenza (Grandjean et al., 2012, Granum et al., 2013, Stein et al., 2016, Kielsen et al., 2016, and Looker et al., 2014). These epidemiology studies are discussed in detail because they provide support for the toxicological effect that was ultimately selected as the basis for the ISGWQC that is developed later in this document.

In a prospective study of a birth cohort from the Faroe Islands (n = 380-509) that was followed post vaccination and then pre-and post-booster vaccination (geometric mean maternal pregnancy serum PFOS = 27.0 ng/ml; 5-year old serum PFOS = 16.7 ng/ml), Grandjean et al. (2012) found a statistically significant negative association between serum PFOS concentration at age 5 (but not maternal PFOS concentration during pregnancy) and post-booster tetanus antibody concentration. For post-booster antibody concentration, there was a 29% decrease for each doubling of serum PFOS. There was a negative, but not statistically significant association with post-booster tetanus antibody concentration at 7 years. For pre-booster tetanus antibody levels at 5 years, there was a negative, but not significant association with the 5-year old PFOS serum concentration. It should be noted that in general, the various measurements of tetanus antibody concentrations were negatively (even if not significantly) associated with measures of PFOS concentration. The odds ratio (OR) for antibody levels being below the clinically protective level (0.1 IU/ml) was elevated (but not significantly) for both maternal and 5-year old serum PFOS levels. For diphtheria antibodies, maternal pregnancy PFOS concentrations were significantly negatively associated with 5-year old pre-booster antibody levels with a 39% decrease in diphtheria antibodies for each doubling of maternal serum PFOS. Pre- and post- booster antibody concentrations at 5 years old were negatively (but not significantly) associated with the 5-year old PFOS serum concentration. However, diphtheria antibody concentrations at 7 years old were significantly negatively associated with PFOS concentrations at 5 years old. All measures of diphtheria antibody concentrations were negatively associated with the measures of PFOS concentration even when not significantly associated. The ORs for diphtheria antibody levels being below the clinically protective level were significantly elevated for maternal and 5-year old PFOS serum concentrations. In this cohort, PFOS and PFOA exposures were highly correlated, and similar results were obtained when these analyses were conducted for PFOA.

In a cohort study nested in a birth cohort from Norway (mean maternal post-partum serum PFOS concentration = 5.6 ng/ml, n = 49-51), vaccine antibody levels were measured in the serum of 3 year olds (approximately 2-3 years post vaccination) (Granum et al. (2013). Maternal, post-partum serum PFOS concentration was significantly negatively associated with rubella antibody levels. There was also a negative (but not statistically significant) association with measles, *Haemophilus* influenza, and tetanus antibody levels. Similar associations were observed with other perfluorinated chemicals.

In a cross-sectional study of children 12-19 years old, nested in the U.S. NHANES study cohort (n = 1,188), (geometric mean serum PFOS concentration = 20.8 ng/ml) (Stein et al., 2016), mumps and rubella antibody levels were significantly negatively associated with concurrent serum PFOS concentrations (including when the analysis was limited to sero-positive individuals as an indication of a prior vaccination). The

decrease in antibody levels for mumps and rubella for a doubling of PFOS was 5.9 and 13.3%, respectively. PFOS concentration was also negatively (but not significantly) associated with measles antibodies. Although negative associations were also seen between other PFCs and these antibodies, the association with PFOS was the strongest.

In a prospective study of adult volunteers from among the staff of a hospital in Copenhagen, Denmark (n = 12), with a median age of 37.9 years and a median PFOS concentration of 9.52 ng/ml (Kielsen et al., 2016), the increase in diphtheria antibodies (but not tetanus antibodies) following a booster vaccination was significantly decreased as a function of serum PFOS (p =0.044). The decrease in diphtheria antibody production for each doubling of serum PFOS was 11.9%. Tetanus antibody production was also negatively associated with serum PFOS (3.6% decrease for each doubling of PFOS), but was not statistically significant. The sample size in this study was small (n = 12), but the subjects were followed closely post-vaccination (6 samples over 30 days) for antibody determination to monitor the time course of response. Eight perfluorinated chemicals were measured. The strongest negative effect on diphtheria antibody production was found for PFHxS, although the effect was borderline significant (p = 0.055). PFOS accounted for the second strongest effect.

The only study to report an overall lack of association between antibody levels and serum PFOS (Looker et al., (2014)), was conducted with adults > 18-years old (n = 403) nested in the C8 study panel cohort in Ohio/West Virginia (median PFOS serum concentration = 9.12 ng/ml). Serum levels of influenza vaccine were measured approximately 21 days post-vaccination. Neither the influenza-specific titer, nor the OR for sero-conversion were negatively associated with PFOS. It may be notable that influenza vaccine response was the only antibody response evaluated in this study.

Infection

In a longitudinal study in Denmark following a birth cohort through average 8.2-years old (Fei et al., 2010b), there was a significant association of hospitalization for infectious disease and maternal pregnancy serum PFOS (mean = 35.3 ng/ml) for girls only at the two highest quartiles of exposure and overall for trend. Dalsager et al. (2016), in a longitudinal prospective study nested in the Odense (Denmark) Child Cohort, obtained serum PFOS concentration from mothers during their first trimester of pregnancy. The median serum PFOS concentration was 8.1 ng/ml. When the children (n = 346) were between one and three years old, the mothers were prompted by text to report every two weeks, during the course of one year, on the number of days during each two-week period that the children had specific categories of health symptoms. Although cough, nasal discharge, diarrhea, and vomiting were not associated with PFOS, both the number and proportion of days with fever among the highest tertile exposed group were statistically significantly associated. Although the proportion of days with fever did not remain significant following Bonferroni adjustment, the rate ratio for fever remained positively statistically significantly associated. A prospective birth cohort of 1,558 mother-child pairs (mean maternal PFOS serum concentration at 28-32 weeks gestation = 5.5 ng/mL) found a significantly increasing trend (P for trend=0.0008) for total infectious disease collected from self-administered questionnaires up to 4 years of age (Goudarzi et al., 2017). Impinen et al., 2018 (mean PFOS cord blood=5.6 ng/ml) followed a nested cohort in Oslo, Norway of 641 children through age 10 years of age found a statistically significant association with the number of parentally reported lower respiratory tract infection infections by 10 years of age, but not with number of episodes of the common cold by age 2.

Two other studies (Okada et al., 2012, mean PFOS = 5.2 ng/ml; Granum et al., 2013, mean PFOS = 5.5

ng/ml) did not find a significant association between infectious disease in young children (under 3 years old, maternal serum PFOS). It should be noted that in these studies, the number of subjects were considerably smaller (Okada et al. (2010), n = 343; Granum et al. (2013), n = 65-93) than in Fei et al. (2010b; n = 1,400) and Goudarzi et al. (2017; n=1,558).

The Looker et al. (2014) study in adults also did not find a significant association between concurrent serum PFOS and episodes/diagnosis of infectious disease.

Asthma

The only study showing a clear association of serum PFOS with asthma was a case-control study of 10-15-year olds in Taiwan [mean serum PFOS = 33.4 (controls) and 45.5 ng/ml (cases)] (Dong et al., 2013). The OR and trend for ever having received a diagnosis of asthma was significant for PFOS (as well as for most other perfluorinated chemicals). The OR for the association of serum PFOS and serum IgE was significant for the highest quartile of PFOS as was the overall trend. This was also the case for other perfluorinated chemicals. No relationship was observed for absolute eosinophil count or eosinophil cationic protein.

Three other studies [Humblet et al. (2014), mean serum PFOS = 16.7-17.2 ng/ml; Stein et al. (2016), mean serum PFOS = 15.0 ng/ml; and Impinen et al. (2018), mean cord blood PFOS = 5.6 ng/ml)] did not find an association between serum PFOS and ever or current asthma or wheeze (Humblet et al., 2014, Impinen et al., 2018), reduced lung function (Impinen et al., 2018), rhinitis (Stein et al., 2016), or rhinoconjunctivis (Impinen et al., 2018).

A nested cohort study of 641 children through age 10 years of age found a statistically significant association with severity of obstructive airways among the moderately exposed group compared to the reference group, but this association was not observed in the highest exposed group (Impinen et al., 2018).

Allergy

Several studies examined the association of PFOS with blood/serum IgE. Wang et al. (2011b) found that cord blood PFOS (median = 5.5 ng/ml) was significantly positively associated with cord blood IgE, but not with 2-year old blood IgE. Okada et al. (2012) found no significant association between maternal blood PFOS (median 5.2 ng/ml) and cord blood IgE. Stein et al. (2016) found that serum IgE from 12-19-year olds was significantly positively associated with concurrent serum PFOS (geom. mean = 20.8 ng/ml) for mold-specific IgE only, but not for total IgE, or for six other common allergens. Impinen et al. (2018) found no association of rhinitis and IgE, or rhinoconjunctivitis, with cord blood PFOS (mean = 5.6 ng/ml) among 10 year olds.

No significant associations were found between cord blood PFOS (median = 5.5 ng/ml, Wang et al., 2011b; mean 5.6 ng/ml, Impinen et al., 2018) and atopic dermatitis at age 2 years (Wang et al., 2011b) or age 10 years (Impinen et al., 2018). Additionally, no significant associations were found between maternal PFOS (median 5.02 ng/ml) and overall allergic conditions at age12-24 months (Okada et al., 2014) or allergic sensitization in 10 year olds (Impinen et al., 2018).

Autoimmunity

Osuna et al. (2014) found no significant association between autoimmune antibodies in cord blood or at 7-years old and cord blood or 7-year old blood PFOS (3.1 and 27.0 ng/ml, respectively).

Summary of epidemiological studies of associations between immune effects and PFOS

The total number of epidemiology studies examining antibody response to vaccines is relatively small (n = 5), and not all vaccine types were evaluated in each study. Nonetheless, the study findings are consistent and support a potential for PFOS to reduce vaccine response, particularly for some vaccine types in children. The effects of PFOS on suppression of vaccine response appears to occur at or close to levels of PFOS exposure prevalent in the general population. However, there is not sufficient information to evaluate associations of PFOS and vaccine response in adults. The sole study that did not show a significant association between PFOS exposure and any antibody response (Looker et al., 2014) was conducted in adults and assessed influenza vaccine response only. Consistent with this finding, the only other study that evaluated influenza vaccine response (Granum et al., 2013) also did not find a statistically significant association between influenza vaccine response and PFOS exposure in children, although it did find a significant association of rubella vaccine response and PFOS exposure. It may be the case that PFOS affects antibody response differentially for different vaccine challenges.

Studies of associations of PFOS and infectious disease provide mixed results. The longitudinal study of Fei et al. (2010b) found a significant positive association between maternal PFOS and infectious disease in girls, but not for boys. Dalsager et al. (2016) found a positive association with symptoms of fever, Goudarzi et al. (2017) found a positive association with total infectious diseases up to 4 years of age and maternal serum PFOS, and Impinen et al. (2018) found a positive association with number of lower respiratory tract infections about 10 years olds but not with the common colds among two year olds. Three additional studies did not find significant associations.

There is a suggestion from a single study (Dong et al., 2013) of an association of PFOS and childhood asthma.

Table 15. Summary of Epidemiology Studies of Immune Effects

Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Asthma	Previous diagnosis ↑	Median 28.9 controls; 33.9 cases	Dong et al. (2013)
	Ever = Wheeze= Current =	Mean 16.7-17.2	Humblet et al. (2014)

Table 15 (continued). Summary of Epidemiology Studies of Immune Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
	- IgE titre in cases ↑ - Eosinophil count ↑ - Eosinophil cationic protein ↑	Median 28.9 controls; 33.9 cases	Dong et al. (2013)
	Ever = Wheeze = Rhinitis =	Geo mean 15.0	Stein et al. (2016)
	- Current = - Ever = - Wheeze = - Severity of obstructive airways ↑ - Reduced lung function =	Mean 5.6 (cord blood)	Impinen et al. (2018)
Infection	hospitalization, (children) – girls only ↑	Mean 35.3	Fei et al. (2010b)
	Infectious diseases –18 mos =	Med. 5.2	Okada et al. (2012)
	Episodes/diagnosis infectious disease (1-3 yrs old) =	Med. 5.5	Granum et al. (2013)
	Cold, influenza (> 18 yrs old) =	Med. 9.12	Looker et al. (2014)
	Total infectious diseases (up to 4 years of age) ↑	Mean 5.5 (maternal)	Goudarzi et al. (2017)
	Symptoms of infections: (fever) ↑ (cough, nasal discharge, diarrhea, vomiting) =	Med. 8.1 (maternal)	Dalsager et al. (2016)
	Number of episodes of common cold = Number of episodes of lower respiratory tracts infections ↑	Mean 5.6 (cord blood)	Impinen et al. (2018)

Antibody response following vaccination	<u>Tetanus antibody response</u> maternal PFOS = 5 yr old PFOS - 5 yr old (post- booster) response ↓ - 7 yr old response = - <u>Diphtheria antibody response</u> Maternal PFOS - 5 yr old response ↓ 5 yr old PFOS - 7 yr old response ↓	Maternal (geo. mean)– 27.0 5 yrs old (geo. mean) – 16.7	Grandjean et al. (2012)
	Rubella antibody levels ↓ Measles = Tetanus = Haemophilus influenza = (3 yr-olds)	Med. 5.5	Granum et al. (2013)
	Rubella antibody levels ↓ Mumps ↓ Measles = (12-19 yr-olds)	Geo mean 20.8	Stein et al. (2016)
	Diphtheria antibody levels ↓ Tetanus = (Adults (med 37.9 yrs old)	Med. 9.52	Kielsen et al. (2016)

	Influenza antibody levels = Sero-conversion = Sero-protection = (Adults > 18 yrs old)	Med. 9.12	Looker et al. (2014)
Allergy	IgE (18 mos) = Allergies (18 mos) =	Med. 5.2	Okada et al. (2012)
	Cord blood IgE ↑	Med. 5.5 (cord blood)	Wang et al. (2011b)
	IgE 2 yr old =	Med. 5.5 (cord blood)	Wang et al. (2011b)
	Allergic diseases (12-24 mos) = Eczema =	Med. 5.02	Okada et al. (2014)
	Atopic dermatitis (2 yr old)	Med. 5.5 (cord blood)	Wang et al. (2011b)
	Atopic dermatitis = Rhinitis & IgE = Allergic sensitization = Rhinoconjunctivitis =	Mean 5.6 (cord blood)	Impinen et al. (2018)
	Total IgE = Mold IgE ↑ Plant = Cockroach = Dust mites = Pets = Rodents = Food =	Geo. mean 15.0	Stein et al. (2016)
Auto antibodies	Pre-natal and 7 yr old =	Geo. mean cord blood = 3.1 7 yrs = 27	Osuna et al. (2014)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Overall conclusions regarding the hazard identification of immune effects
There is strong evidence from animal studies for various immune effects: immunosuppression; changes in spleen and thymus weight and cellularity; and effects on the levels of circulating populations of immunologically active cells, serum immunoglobulins and immune mediators. Epidemiologic evidence for immune effects of PFOS is strongest for suppression of vaccine response. Although the total number of animal studies and epidemiology studies for immunosuppression is relatively small, the consistency of the observations of this effect in both animal and human studies mutually reinforces the identification of immunosuppression as an effect of PFOS that is appropriate for consideration of dose-response.

Neurological effects

Animal studies
A summary of neurological effects in animals can be found in Table 16 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

In general, structural and behavioral effects were assessed in rats and mice following PFOS exposure. Structural effects included changes in organ (i.e., brain) weight and histopathology, Behavioral effects included, for example, changes in learning, locomotion, or reaction to stimulus. These findings are briefly reviewed below.

Structural effects
Following 52 weeks of exposure, statistically significant increased relative brain weights were observed in female rats exposed to 1.3 mg/kg/day (Butenhoff et al., 2012). In this study, there was no effect on the brain weights of male rats (NOAEL = 1.0 mg/kg/day). However, statistically significant increased relative brain weight was observed in male rats following 91 days of exposure to ≥ 2.1 mg/kg/day (Kawamoto et al., 2011). No histopathological changes (i.e., to the neuronal or glial cells of the cerebrum and cerebellum) were observed in these rats (NOAEL = 8.5 mg/kg/day).

With shorter duration (28 days) exposures to PFOS, statistically significant increased relative brain weight in males and females was reported (Curran et al., 2008; LOAEL = 3 mg/kg/day). In addition, changes in brain histopathology were observed, such as alterations to hypothalamic neuron structure (Lopez-Doval et al., 2014; LOAEL = 3 mg/kg/day) and gliocyte hyperplasia and focal hemorrhage (Cui et al., 2009; LOAEL = 20 mg/kg/day).

Overall, there is evidence in rats that exposure to PFOS can have effects on brain weight and brain histopathology.

Behavioral effects
During the course of a 91-day exposure in rats, Kawamoto et al. (2011) reported an increase in convulsions in rats following ultrasonic stimulus (at week 6, LOAEL = 8.5 mg/kg/day). However, these authors observed no other behavioral abnormalities in these rats (NOAEL = 8.5 mg/kg/day). Behavioral abnormalities (e.g., reduced activity; LOAEL = 5 mg/kg/day) were reported in rats following 28 days of exposure (Cui et al., 2009). After a single exposure to PFOS, Sato et al. (2009) observed increased locomotion in rats following ultrasonic stimulus (LOAEL = 250 mg/kg) but for the authors' summary category of "other signs of neurobehavioral effects" no other other signs of adverse neurobehavioral effects were seen (NOAEL for this category = 500 mg/kg).

In mice, impaired spatial learning and memory (LOAEL = 2.2 mg/kg/day) as assessed by water maze were observed following 3 months of exposure (Long et al., 2013). Following 28 days of exposure, effects on the open field test (e.g., decreased time in the center area, LOAEL = 3 mg/kg/day) but not on the functional observation battery (NOAEL = 6 mg/kg/day) were reported (Fuentes et al., 2007a).

After a single exposure to PFOS, Sato et al. (2009) observed increased locomotion in mice following ultrasonic stimulus (LOAEL = 125 mg/kg). For the authors summary category of "other signs of neurobehavioral effects" no other signs of adverse neurobehavioral effects were seen (NOAEL for this category = 500 mg/kg).

Following a single exposure in 10-day old mice, Johansson et al. (2008) reported changes in spontaneous behavior (locomotion, rearing, total activity), habituation, and activity in response to a nicotine challenge when assessed at either 2 or 4 months of age (LOAEL = 11.3 mg/kg). However, no effect was observed on performance in the elevated plus-maze. In summary, exposure to PFOS is reported to cause reduced activity in rats and effects on learning, behavior, and habituation in mice. Data in rats and mice also suggest that exposure to PFOS can cause behavioral changes (e.g., increased locomotion) following ultrasonic stimulus in the absence of other neurobehavioral effects. A study in mice indicates that a single exposure during the neonatal period can cause behavioral changes in adulthood.

Summary of neurological effects in animals

In summary, a limited number of rodent studies have assessed the neurotoxicity of PFOS. These studies have demonstrated some effects on the brain (e.g., increased relative weight and histopathological changes). In all studies in both rats and mice, behavioral effects were observed in response to PFOS exposure. The studies did not all examine the same effects and some studies observed some behavioral effects, but not others. Behavioral effects that were observed in response to PFOS exposure included changes in learning, memory, activity, and habituation.

Table 16. Study summary table for neurological effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	52 weeks	↑ brain weight relative to body weight, females only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	Males: 1.0 Females: -----	Males: ---------- Females: 1.3	Serum and liver PFOS concentrations determined Only one dose reported for this endpoint	Males: ---- Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)
Kawamoto et al. (2011)	Rats, Wistar	0, 2, 8, 32, 128 ppm Dietary Daily PFOS dose (estimated as the mean	13 weeks	↑ relative brain weight (determined after 13 weeks of exposure)	0.5	2.1	Serum, brain, liver, and kidney PFOS concentrations determined Only males used	(serum samples collected after 13 weeks)

Table 16. Study summary table for neurological effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
		of the daily PFOS doses reported weekly by study authors) 0, 0.1, 0.5, 2.1, 8.5 mg/kg/day		↑ convulsions following ultrasonic stimulus (observed only during week 6 and then ceased afterward due to death of 1 rat out of 6 in group) (determined at week 6)	2.1	8.5	Internal PFOS concentrations not reported for controls	-------- (serum samples collected after 13 weeks) Note: difference in time points for endpoint analysis and serum PFOS analysis
				Behavioral abnormalities: startle response, touch response, pain response, righting reflex, visual placing, abdominal tone, limb tone	8.5	--------		--------
				Brain histology (neuronal or glial cells of cerebrum and cerebellum) and ultrastructure (neurons in cortex, hippocampus, and cerebellum)	8.5	--------		--------

Table 16. Study summary table for neurological effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Long et al. (2013)	Mice, C57BL6	0, 0.43, 2.15, 10.75 mg/kg Oral (presumed gavage)	3 months	Impaired spatial learning (↑ escape latency) (data for 0.43 mg/kg/day group not reported)	--------	2.15	Internal PFOS concentrations not determined PFOS purity not reported Missing information (e.g., lowest dose data for escape latency on day 3, number of poor swimmers)	--------
				Impaired spatial memory (↓ time spent in target quadrant)	0.43	2.15		--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased

-------- = not applicable

Human epidemiology studies

A summary of neurological effects in humans can be found in Table 17 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Memory/function in older adults

No association of self-reported memory loss with PFOS was observed for a large sample of the C8 Study cohort ≥ 50 years old (Gallo et al., 2013). No association of self-reported difficulty in remembering/confusion or self-reported difficulties with daily life/senility were found for a sub- sample of the NHANES cohort 60-85 years old (Power et al., 2013).

Learning

In a test of differential reinforcement of low-rates of responding that reflected both learning and impulsivity in children 9-11 years old (Gump et al., 2011), there was some indication that PFOS was associated with decreased learning response (increased impulsivity). However, the effect was not consistently significant across learning periods.

There was a suggestion of a negative association between self-reported learning problems and PFOS exposure in a large sub-set of children 5-18 years old from the C8 Study cohort (Stein and Savitz, 2011).

In a Danish birth cohort with a 22-year follow-up (Storm et al., 2014), there was no association between maternal serum PFOS at 30 weeks of gestation and children's academic performance on a standardized 9th grade performance test.

Attention/Attention deficit hyperactivity disorder (ADHD)

Of five studies that investigated an association between PFOS exposure and ADHD, only one found a positive association between PFOS exposure and reported ADHD. In a subset of the NHANES population 12-15 years old (Hoffman et al., 2010), based on parental reporting of children's ADHD diagnosis, there was a small, but statistically significant increase in the OR for ADHD (OR = 1.03-1.05 depending on the stringency of the reporting definition) for each ng/ml increase in children's serum PFOS. There was a larger and significant OR (1.60) for an inter-quartile range increase in PFOS. . This study had comparable (and generally consistent with general population) maternal PFOS serum levels as the studies that found no significant association of PFOS and ADHD.

Autism

No significant association was observed between maternal gestational PFOS exposure and autism in a single case-control study (Liew et al., 2015).

Depression

No significant association was observed in a prospective pregnancy cohort between maternal gestational exposure and 22 years of follow-up of the offspring through a Danish national health registry (Storm et al., 2014).

Summary of epidemiological findings

There is little evidence from epidemiological studies for an association between PFOS exposure and neurological effects in either older adults or children. The PFOS exposures in the available studies were all in the range of the general population.

Table 17. Summary of Epidemiology Studies of Neurologic Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Memory	Memory loss =	Med. ~ 24	Gallo et al. (2013)
	Difficulty remembering/confusion =	Geom. mean 22.63	Power et al. (2013)
Senility	Difficulty with daily life/senility =	Geo. mean 22.63	Power et al. (2013)
Learning	Task learning (children) =	Med. 9.90	Gump et al. (2011)
	Learning problems =	Mean 22.9	Stein and Savitz (2011)
	Academic achievement =	Med. 21.4	Strom et al. (2014)
Attention	ADHD ↑	Med. 22.6	Hoffman et al. (2010)
	ADHD ↑	Med. 25-27	Liew et al. (2015)
	ADHD –	Med. Cases 6.92 Controls 6.77	Ode et al. (2014)
	ADHD =	Mean 22.9	Stein and Savitz (2011)
	ADHD =	Med. 21.4	Strom et al. (2014)
Autism	=	Med. 25-27	Liew et al. (2015)
Depression	=	Med. 21.4	Strom et al. (2014)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Overall conclusions regarding the hazard identification of neurotoxicity

The available animal studies do not provide strong support for the neurotoxicity of PFOS, although the neonatal period may be a sensitive lifestage for neurobehavioral effects based on animal studies. Similarly, the available human data do not show strong associations between PFOS exposure and neurological effects. Therefore, the available evidence does not appear to justify neurological effects as endpoints for dose-response.

Renal effects

Animal studies

A summary of renal effects (kidney weight and histopathology) in animals can be found in Table 18 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

Kidney weight

Following 52 weeks of exposure, Butenhoff et al. (2012) reported increased relative kidney weights (for right and left kidneys) for female rats exposed to 1.3 mg/kg/day but not for male rats (NOAEL = 1.0 mg/kg/day). No effect on relative kidney weight was reported in male rats exposed to PFOS for 91 days (Kawamoto et al., 2011; NOAEL = 8.5 mg/kg/day). Following 28 days of exposure, increased relative kidney weight was reported in male (LOAEL = 6.3 mg/kg/day) and female (LOAEL = 3.7 mg/kg/day) rats (Curran et al., 2008). Cui et al. (2009) reported increased relative kidney weights in male rats (LOAEL =5 mg/kg/day).

Following 60 days of PFOS exposure in mice, data suggest an effect on relative kidney weight. Statistically significant decreases in relative kidney weight were reported by Dong et al. (2009, 2012a) with a LOAEL of 0.83 mg/kg/day. In two additional studies, these authors also reported decreased (although not statistically significant) relative kidney weight following exposure to ≤ 0.83 mg/kg/day (Dong et al., 2011, 2012b). Following shorter durations (21 or 28 days) of PFOS exposure, no effect on relative kidney weight was observed in mice exposed up to 0.17 mg/kg/day PFOS (Peden-Adams et al., 2008; Guruge et al., 2009).

No effect on kidney weight was observed in cynomolgus monkeys from 26 weeks of oral exposure to PFOS doses of up to 0.75 mg/kg/day (Seacat et al. 2002; not shown in Table 15).

In total, data are mixed regarding increased kidney weight in rats following PFOS exposure. Data are also mixed in mice with some evidence suggesting decreased relative kidney weights following PFOS exposure. No effects were reported in monkeys.

Histopathology

Three studies evaluated kidney histopathology following PFOS exposure. Results from these studies are mixed. Cui et al. (2009) reported a change in kidney histopathology (e.g., turbidness/tumefaction in epithelium of proximal convoluted tubules) in rats exposed to PFOS for 28 days (LOAEL = 20 mg/kg/day). However, Fair et al. (2011) reported no effect on kidney histopathology in mice exposed to PFOS for 28 days (NOAEL = 0.17 mg/kg/day). No effect on kidney histopathology was observed in cynomolgus monkeys from 26 weeks of oral exposure to PFOS doses of up to 0.75 mg/kg/day (Seacat et al. 2002; not shown in Table 15).

Summary of renal effects in animals

A limited number of studies assessed renal effects in rodents. Data are mixed regarding the ability of PFOS to increase or decrease relative kidney weights in rats and mice, respectively. Further, histopathological effects were observed in rats but not mice. No effects on kidney weight or histopathology were found in monkeys.

Table 18. Study summary table for renal effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	52 weeks	↑ kidney weight relative to body weight (left and right), females only (only data from controls and 20 ppm group presented by authors) (determined after 52 weeks of exposure)	Males: 1.0 Females: ----	Males: --------- Females: 1.3	Serum and liver PFOS concentrations determined Only one dose reported for this endpoint	Males: ---- Females: 54,000 (week 4) 223,000 (week 14) 233,000 (week 105) (female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)
Dong et al. (2009)	Mice, C57BL/6	0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day (reported as mg/kg/day when representing a NOAEL and/or LOAEL) Oral gavage	60 days	↓ kidney weight relative to body weight (determined at day 61)	0.417	0.833	Serum PFOS concentrations determined Only males used	65,430 (serum collected on day 61)
Dong et al. (2011)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day Oral gavage	60 days	Kidney weight relative to body weight	0.8333	--------	Serum PFOS concentrations determined Only males used Small sample size (n=6)	--------

Table 18. Study summary table for renal effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Dong et al. (2012b)	Mice, C57BL/6	0, 0.0167, 0.0833, 0.833 mg/kg/day Oral gavage	60 days	Kidney weight relative to body weight	0.833	--------	Serum PFOS concentrations determined Only males used	--------
Dong et al. (2012a)	Mice, C57BL/6	0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333, 2.0833 mg/kg/day Oral gavage	60 days	↓ kidney weight relative to body weight (determined at day 61)	0.4167	0.8333	Serum PFOS concentrations determined Only males used Small sample size (n=6)	59,740 (serum collected on day 61)
Kawamoto et al. (2011)	Rats, Wistar	0, 2, 8, 32, 128 ppm Dietary Daily PFOS dose (estimated as the mean of the daily PFOS doses reported weekly by study authors) 0, 0.1, 0.5, 2.1, 8.5 mg/kg/day	13 weeks	Kidney weight	8.5	--------	Serum, brain, liver, and kidney PFOS concentrations determined Only males used Internal PFOS concentrations not reported for controls	--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased
-------- = not applicable

Human epidemiological studies

A summary of renal effects in humans can be found in Table 19 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Renal function

Two studies evaluated renal function. Shankar et al. (2011a) examined the association between serum PFOS concentration and the estimated glomerular filtration rate (eGFR) in adults (≥ 20 years old) in a cross-sectional study of the NHANES cohort (n = 4,587). The eGFR was significantly negatively associated with PFOS for the overall study population. The association was strongest for those < 60 years old (borderline significant for those ≥ 60 years old). This was not significantly influenced by sex or BMI. These findings are further supported by a large (n=9,660) cross-sectional study among children and adolescents (1 to <18 years of age) from the C8 study population (Watkins et al., 2013) which found a statistically significant negative association and a significant negative trend across quartiles of PFOS.

These two cross-sectional studies may have suffered from reserve causation such that decreased eGFR (e.g., poor kidney function) could plausibly lead to increased serum PFOS. Shankar et al. (2011a) stratified the study population by the presence of chronic kidney disease (defined on the basis of eGFR) and the association was strengthened for those without chronic kidney disease, possibly suggesting that the association between eGFR and PFOS exposure in the full cohort was not influenced by reverse causality. Conversely, Watkins et al. (2013) utilized predicted serum PFOA levels from modeled drinking water exposure in addition to measured serum PFOA to minimize susceptibility to reverse causation. Although associations were significant with measured serum PFOA levels and eGFR, in contrast, predicted serum PFOA was not associated. Although, predicted PFOS serum concentrations were not evaluated, atleast with PFOA, reverse causality is likely to explain association with eGFR.

Chronic kidney disease

The Shankar et al. (2011a) study discussed above, also investigated the relationship between serum PFOS concentration and the prevalence of chronic kidney disease (eGFR < 60 mL/min/1.73 m^2, n = 230). The OR for chronic kidney disease was significantly > 1.0 across the 2^{nd}-4^{th} quartiles of PFOS exposure (compared to the first quartile), and the association with PFOS exposure was significant for trend. The maximum OR (4^{th} quartile) was 1.82. These findings are suggestive of a dose-response relationship.

Summary of epidemiologic studies

The evidence for the association of PFOS exposure with renal effects in humans is based on two cross-sectional studies (Shankar et al., 2011a and Watkins et al., 2013) with large sample sizes and consistent evidence of a dose-response trend, However, reverse causation requires further investigation. The Shankar et al. (2011a) study provides limited evidence that general population levels of PFOS exposure are associated with chronic kidney disease.

Table 19. Summary of Epidemiology Studies of Renal Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Function	eGFR (est. glomerular filtration rate) ↓	Med. 18.7	Shankar et al. (2011a)
	eGFR ↓	Med. 20.0	Watkins et al. (2013)
Kidney disease	Chronic kidney disease ↑	Med. 18.7	Shankar et al. (2011a)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Overall summary of renal effects

Only a small number of animal and epidemiological studies have assessed renal effects following PFOS exposure. Therefore, the limited available evidence does not appear to justify renal effects as critical endpoints for dose-response.

Clinical chemistry

Animal studies

A summary of clinical chemistry parameters in animals can be found in Table 20 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

In general, clinical chemistry analyses following PFOS exposure have been conducted in monkeys, rats, and mice. The clinical chemistry parameters measured in blood or serum have included bilirubin, enzymes (e.g., alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase), glucose, lipids (e.g., cholesterol, lipoproteins, triglycerides), and urea. Because some of these parameters are traditionally considered indicative of effects on specific organs (e.g., liver or kidneys), the textual review of these endpoints are discussed in the relevant sections elsewhere in the hazard identification. For example, data regarding liver enzymes and bilirubin are reviewed in the hepatic section. Data regarding glucose and urea are reviewed in the endocrine/metabolic section. Effects on serum lipids are discussed in this section.

Lipids

A number of lipid parameters (e.g., cholesterol, lipoproteins, triglycerides) have been measured in animals following PFOS exposure. These data are reviewed below by species.

Monkeys

In monkeys, serum lipids were assessed following 182 days of exposure to PFOS (Seacat et al., 2002). Decreases were observed for high-density lipoprotein (HDL; LOAEL = 0.03 mg/kg/day in males) and total cholesterol (LOAEL = 0.75 mg/kg/day in males and females). However, PFOS exposure had no effect on very low-density lipoprotein (VLDL) and triglyceride levels (NOAEL = 0.75 mg/kg/day).

Rats

In a 104-week bioassay with rats, statistically significant decreases in total cholesterol were observed in males at week 53 (LOAEL = 1.0 mg/kg/day) and females at week 27 (LOAEL = 0.1 mg/kg/day) but not at sacrifice (Butenhoff et al., 2012). Seacat et al. (2003) reported interim observations of Butenhoff et al. (2012) and observed decreased total cholesterol in males at week 14 (LOAEL = 1.3 mg/kg/day) but no effect in females (NOAEL = 1.6 mg/kg/day).

Following 28 days of exposure to PFOS, decreased total cholesterol was observed in male and female rats exposed to ~3 mg/kg/day (Curran et al., 2008) and in male rats exposed to 1.3 mg/kg/day (Elcombe et al., 2012a). Decreased total cholesterol was also observed in male rats exposed for 7 days (Elcombe et al., 2012b; LOAEL = 1.9 mg/kg/day) and for < 5 days (Martin et al., 2007; LOAEL = 10 mg/kg/day).

In addition to decreased total cholesterol following PFOS exposure, decreases in serum triglycerides were also observed in rats. Kim et al. (2011) reported decreased serum triglycerides in male, but not female, rats exposed to 10 mg/kg/day for 28 days. Similarly, decreases in serum triglycerides were also observed in male rats following exposure for 28 (Elcombe et al., 2012a; LOAEL = 5.6 mg/kg/day) or 7 days (Elcombe et al., 2012b; LOAEL = 9.7 mg/kg/day).

Mice

Following up to 6 weeks of exposure, decreased total cholesterol was observed in male mice exposed to 3 mg/kg/day (Bijland et al., 2011). At shorter durations of exposure (≤ 14 days), decreased total cholesterol was also observed by Wang et al. (2014a; LOAEL = 20 mg/kg/day) and Qazi et al. (2010b; LOAEL = 0.005% in feed). In contrast, following 28 days of PFOS exposure, ≤ 0.17 mg/kg/day did not cause a statistically significant decrease in cholesterol in female mice (Fair et al., 2011).

Exposure to PFOS also caused a reduction in HDL in mice exposed ≤ 6 weeks (Bijland et al., 2011; LOAEL = 3 mg/kg/day) or 14 days (Wang et al., 2014a; LOAEL =5 mg/kg/day). Similarly, PFOS exposure caused a reduction in low-density lipoprotein (LDL) following ≤ 6 weeks (Bijland et al., 2011; LOAEL = 3 mg/kg/day) or 14 days (Wang et al., 2014a; LOAEL = 20 mg/kg/day).

Decreases in serum triglycerides were also reported following PFOS exposure. Bijland et al. (2011) reported decreased triglycerides following ≤ 6 weeks of exposure to 3 mg/kg/day. Wang et al. (2014a) also reported a decrease in triglycerides following 14 days of exposure to 20 mg/kg/day, whereas Qazi et al. (2010b) observed no change in triglycerides following 10 days of exposure (NOAEL = 0.005% in feed).

In total, the data suggest that PFOS exposure affects serum lipid levels in animals. Decreases in total cholesterol have typically been observed in monkeys, rats, and mice. Data also suggest that PFOS decreases other serum lipid parameters such as HDL, LDL, and triglycerides.

Summary of clinical chemistry findings in animals

In summary, several clinical chemistry parameters have been assessed in animals following PFOS exposure. Levels of total cholesterol, HDL, LDL, and triglycerides have consistently been reported to decrease with PFOS exposure. As reviewed in the hepatic section, data for bilirubin are mixed with respect to an effect of PFOS exposure. Data for serum enzymes (i.e., ALT, ALP, ASP), also reviewed in the hepatic section, typically show no effect. However, some studies have reported changes in these enzymes. As discussed in the endocrine/metabolic section, glucose levels in animals following PFOS exposure have either been decreased or unchanged. The effect of PFOS on serum levels of urea is unclear.

Table 20. Study summary table for clinical chemistry parameters in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	<53 weeks	↑ ALT (at weeks 14 and 53), males only (determined at weeks 4, 14, 27, and 53 but only statistically significant at weeks 14 and 53)	Males: 0.2 Females: 1.3	Males: 1.0 Females: ----	Serum and liver PFOS concentrations determined	Males: 41,800 (week 4) 148,000 (week 14) 146,000 (week 53) Females: ---- (male serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks)
				↓ AST (at week 4), females only (determined at weeks 4, 14, 27, and 53 but only statistically significant at week 4)	Males: 1.0 Females: 0.3	Males: ---------- Females:1.3		Males: ---- Females: 54,000 (week 4) (female serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks)

Table 20. Study summary table for clinical chemistry parameters in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↓ total CHOL (at weeks 14, 27, and 53 but not at term), males ↓ total CHOL (at week 27 only), females (determined at weeks 4, 14, 27, 53 and at termination, statistically significant results for each sex reported above)	Males: 0.2 Females: 0.03	Males: 1.0 Females: 0.1		Males: 148,000 ppm (week 14) 146,000 ppm (week 53) Females: Not reported (week 27) (male serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks; female serum PFOS concentrations reported for after exposure for 4, 14, and 102 weeks)

Table 20. Study summary table for clinical chemistry parameters in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↓ glucose (at weeks 4 and 53), males ↓ glucose (at weeks 14 and 53), females (determined at weeks 4, 14, 27, and 53, statistically significant results for each sex reported above)	Males: 0.2 Females: 0.03 (based on week 53)	Males: 1.0 Females: 0.1 (based on week 53)		Males: 146,000 ppm (week 53) Females: Not reported (week 53) (male serum PFOS concentrations reported for after exposure for 4, 14, 53, and 105 weeks; female serum PFOS concentrations reported for after exposure for 4, 14, and 102 weeks)

Table 20. Study summary table for clinical chemistry parameters in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
				↑ BUN (at weeks 14, 27, and 53), males and females (determined at weeks 4, 14, 27, and 53, statistically significant results for each sex reported above)	Males: 0.02 Females: 0.1 (both based on week 53)	Males: 0.1 Females: 0.3 (both based on week 53)		Males: Not reported (week 53) Females: Not reported (week 53) (male serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks; female serum PFOS concentrations reported for after exposure for 4, 14, and 105 weeks)
				↑ CREAT (at week 14 only), females only (determined at weeks 4, 14, 27, and 53, statistically significant results for each sex reported above)	Males: 1.0 Females: 0.03	Males: ---------- Females: 0.1 (higher doses produced no effect)		Males: ---- Females: 27,300 ppm (week 14) (females serum PFOS concentrations reported for after exposure for 4, 14, and 102 weeks)

Table 20. Study summary table for clinical chemistry parameters in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Seacat et al. (2002) 1-year recovery data not summarized herein	Monkeys, cynomolgus	0, 0.03, 0.15, 0.75 mg/kg/day Capsule	26 weeks	↓ total CHOL (on days 91 to 182)	Males: 0.15 Females: 0.15	Males: 0.75 Females: 0.75	Serum and liver PFOS concentrations determined Sample sizes generally 2 to 6 per group with increased frequency of endpoint measurements	Males: 173,000 Females: 171,000 (determined after 183 days of exposure)
				↓ HDL (on days 153 and 182) (for males, statistically significant reductions observed at 0.03 and 0.75 mg/kg/day, non-statistically significant reductions observed at 0.15 mg/kg/day)	Males: ---- Females: 0.03	Males: 0.03 Females: 0.15		Males: 15,800 Females: 66,800 (determined after 183 days of exposure)
				↓ total BILI (for males only, on days 91, 153, and 182)	Males: 0.15 Females: 0.75	Males: 0.75 Females: ----		Males: 173,000 Females: ---- (determined after 183 days of exposure)

Table 20. Study summary table for clinical chemistry parameters in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ SBA (for males only, on day 182)	Males: 0.15 Females: 0.75	Males: 0.75 Females: -----		Males: 173,000 Females: ---- (determined after 183 days of exposure)
				ALB, ALK, ALT, AST, BUN, CA, CL, CREAT, GLOB, GLUC, K, NA, PHOS, PROT, SDH, TRIG, VLDL (for males and females, any effects reported to be non-treatment related)	0.75	--------		--------
Seacat et al. (2003)	Rats, Crl:CD® (SD) IGS BR	0, 0.5, 2.0, 5.0, 20 ppm Dietary Estimated daily dose of PFOS (as reported by study authors) Males: 0, 0.03, 0.13, 0.34, 1.33 mg/kg/day Females: 0, 0.04, 0.15, 0.40, 1.56 mg/kg/day	14 weeks	↓ CHOL (males only) (determined after 14 weeks of exposure)	Males: 0.3 Females: 1.6	Males: 1.3 Females: -----	Serum and liver PFOS concentrations determined Sample size ≤5 rats per endpoint	Males: 148,000 Females: ---- (determined after 14 weeks of exposure)
				↑ ALT (males only) (determined after 14 weeks of exposure)	Males: 0.3 Females: 1.6	Males: 1.3 Females: -----		Males: 148,000 Females: ---- (determined after 14 weeks of exposure)

Table 20. Study summary table for clinical chemistry parameters in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ BUN (males and females) (determined after 14 weeks of exposure)	Males: 0.3 Females: 0.4	Males: 1.3 Females: 1.6		Males: 148,000 Females: 223,000 (determined after 14 weeks of exposure)
				ALB, AST, BILI (total), CA, CL, CREAT, GGT, GLOB, GLU, K, NA, PHOS, PROT	Males: 1.3 Females: 1.6	--------		--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased
-------- = not applicable

ALB = albumin; ALK = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BASO = basophils; BILI = bilirubin; BUN = blood urea nitrogen ; CA = calcium; CHOL = cholesterol; CL = chloride; CREAT = creatinine; GGT = gamma glutamyltransferase; GLOB = globulin; GLUC = glucose; EOSIN = eosinophil; HCT = hematocrit; HDL = high density lipoprotein cholesterol; HGB = hemoglobin; K = potassium; LYMPH = lymphocyte; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MONO = monocyte; N-SEG = segmented neutrophil; NA = sodium; PHOS = inorganic phosphate; PLT = platelet; PROT = total protein; RBC = red blood cell; RETIC = reticulocyte; SBA = serum bile acid; SDH = sorbitol dehydrogenase; TRIG = triglycerides; VLDL = very low-density lipoprotein; WBC = white blood cell

Human epidemiology studies

A summary of clinical chemistry parameters in humans can be found in Table 21 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Triglycerides

The results of twelve studies which evaluated PFOS and serum triglyceride data are conflicting. Only three studies showed a significant positive association of PFOS exposure with increased serum triglyceride levels (Timmermann et al. (2014) overweight children only; Olsen et al. (2003b); Steenland et al. (2009)). Olsen et al. (2003b) is an occupational cohort with a very high PFOS exposure (mean of 800-1,320 ng/ml). However, an earlier (but smaller) study by Olsen et al. (1999) at the same plant with an even higher level of exposure showed no significant association. Steeland et al. (2009) is a high-quality study with a very large study population (n =46,294), with a relatively low level of PFOS exposure (22.4 ng/ml) typical of the general population. In contrast, two studies showed a significant negative association of PFOS exposure and triglyceride levels: Frisbee et al. (2013; girls only); and Château-Degat et al. (2010; females only). Both of these studies had relatively large study populations with general population levels of PFOS exposure. Seven other studies showed no significant association of PFOS with triglycerides.

Overall, there may be a suggestion of a relatively weak association of PFOS with increased serum triglycerides that is observable with either very high levels of PFOS exposure or with very statistically powerful studies.

Total cholesterol

There is consistent evidence from nine studies for a positive association of PFOS exposure with serum total cholesterol: (Eriksen et al., 2013; females only); Frisbee et al. (2010; children); Geiger et al. (2014b); Jain (2013a); Nelson et al. (2010); Olsen et al. (1999, 2003b); Starling et al. (2014b); and Steenland et al. (2009). With the exception of the Olsen et al. occupational studies, all of these studies detected a significant positive association in populations within the exposure range prevalent in the general population. The Fu et al. (2014) study also showed an apparent, but not statistically significant trend of increasing total cholesterol with PFOS exposure. In addition, Steenland et al. (2009) showed a significant positive association between clinically defined hypercholesterolemia and PFOS exposure.

There is, therefore, strong evidence for a positive association of PFOS exposure and increased serum total cholesterol even at relatively low levels of PFOS exposure.

High density cholesterol (HDL)

The evidence for an association of PFOS exposure with HDL is weak. Three studies (Château- Degat et al. (2010), Frisbee et al. (2010) (boys only), Starling et al. (2014b) showed a significant positive association of PFOS exposure and HDL. However, eight studies showed no significant association. These included the two Olsen et al. (1999, 2003b) occupational studies with very high serum PFOS levels. With the exception of the Olsen et al. studies, all of the studies investigated populations with essentially general population levels of exposure.

Low density cholesterol (LDL)

There is a suggestion of an association between PFOS exposure and LDL. Four studies showed a clear significant positive association between PFOS exposure and serum LDL levels: Fitz- Simon et al. (2013); Frisbee et al. (2010; children); Geiger et al. (2014b); Olsen et al. (1999; for one of two consecutive years only); and Steenland et al. (2009). In addition, Olsen et al. (1999) showed a positive association in only one

of two non-consecutive years during which LDL levels were collected. In addition, two studies of non-HDL cholesterol (the majority of which is LDL) also showed a significant positive association with PFOS exposure (Nelson et al., 2010; Steenland et al., 2009). However, four studies showed no significant association between PFOS and LDL. Of these, however, Fu et al. (2014) showed an apparent, but non-significant trend. With the exception of the Olsen et al. (1999) occupational study, all of these studies were in populations with PFOS exposures prevalent in the general population. In addition, the Geiger et al. (2014b) study also showed a significant positive association between PFOS exposure and clinically defined LDL dyslipidemia.

Summary of epidemiologic studies

There is consistent evidence for an association between PFOS exposure and increased serum cholesterol levels, including at low levels of exposure prevalent in the general population (i.e. in populations with no known exposure to specific sources of PFOS contamination). However, the evidence is somewhat less clear for an association between PFOS exposure and increased levels of LDL, and weak, at best for an association between PFOS exposure and either HDL or triglyceride levels.

In contrast to studies of general population exposure levels, associations between PFOS and increased serum cholesterol were not observed in studies of occupationally exposed workers. As discussed in DWQI (2017), associations of PFOA with some clinical parameters, including cholesterol, liver enzymes, and uric acid, exhibit a steep dose-response curve in the lower exposure range found in the general population, with a much flatter slope (approaching a plateau) at higher exposures such as those found occupationally. For dose-response curves of this type, the associations found in populations with lower exposures may not be observed in workers because even the least exposed workers used as the comparison/reference group in occupational studies may have exposure levels that are high enough to fall on the much flatter upper portion of the dose-response curve. These conclusions may also be relevant to the discrepancy in results between occupational and general population studies of associations of PFOS and increased cholesterol described above.

Table 21. Summary of Epidemiology Studies of Serum Lipids			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Triglycerides	↑ (for overweight only)	Med. 41.5	Timmermann et al. (2014)
	↓(F only)	Mean 18.5	Château-Degat et al. (2010)
	=	Geo. mean 8.40	Fisher et al. (2013)
	= (Δ triglycerides as function of Δ PFOS)	Geo. mean baseline = 18.5 Follow-up = 8.2	Fitz-Simon et al. (2013)
	↓ (children -F only)	Mean 22.7	Frisbee et al. (2010)
	=	Mean 1.68	Fu et al. (2014)
	=	Mean 17.7	Geiger et al. (2014b)
	=	Med. Preg - 10.07 Non-preg – 12.11	Jain (2013a)
	=	Med. 1,000-3,000	Olsen et al. (1999)
	↑	Mean 800-1,320	Olsen et al. (2003b)
	=	Med. 13.03	Starling et al. (2014b)
	↑	Mean 22.4	Steenland et al. (2009)
HDL	↑	Mean 18.5	Château-Degat et al. (2010)
	=	Geom. mean 8.40	Fisher et al. (2013)
	= (Δ triglycerides as function of Δ PFOS)	Geom. mean baseline = 18.5 Follow-up = 8.2	Fitz-Simon et al. (2013)
	↑ (children – M only)	Mean 22.7	Frisbee et al. (2010)
	=	Mean 1.68	Fu et al. (2014)
	=	Mean 17.7	Geiger et al. (2014b)
	=	Med. 21.0	Nelson et al. (2010)
	=	Med. 1,000-3,000	Olsen et al. (1999)
	=(as Δ)	Mean Δ +4.2	Olsen et al. (2012)
	=	Mean 800-1,320	Olsen et al. (2003b)
	↑	Med. 13.03	Starling et al. (2014b)
	=	Mean 22.4	Steenland et al. (2009)
TC/HDL	↓	Mean 18.5	Château-Degat et al. (2010)
	=	Geo. mean 8.40	Fisher et al. (2013)
	=(as Δ)	Mean Δ +4.2	Olsen et al. (2012)
	=	Mean 22.4	Steenland et al. (2009)
HDL dyslipidemia	=	Mean 17.7	Geiger et al. (2014b)

Table 21. Summary of Epidemiology Studies of Serum Lipids			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study reference**
Total cholesterol	↑(F only)	Mean 36.1	Eriksen et al. (2013)
	↑	Geo. mean baseline=18.5 Follow-up = 8.2	Fitz-Simon et al. (2013)
	=	Geom. mean 8.40	Fisher et al. (2013)
	↑ (children)	Mean 22.7	Frisbee et al. (2010)
	=	Mean 1.68	Fu et al. (2014)
	↑	Mean 17.7	Geiger et al. (2014b)
	↑ (F)	Med. 10.07– 12.11	Jain (2013a)
	↑	Med. 21.0	Nelson et al. (2010)
	=(as Δ)	Mean Δ +4.2	Olsen et al. (2012)
	↑ (for 1 of 2 non-consecutive yrs)	Med. 1,000-3,000	Olsen et al. (1999)
	↑	Mean 800-1,320	Olsen et al. (2003b)
	↑	Med. 13.03	Starling et al. (2014b)
	↑	Mean 22.4	Steenland et al. (2009)
Hypercholestero l- emia	↑	Mean 22.4	Steenland et al. (2009)
Non-HDL cholesterol	↑	Mean 22.4	Steenland et al. (2009)
	↑	Median 21.0	Nelson et al. (2010)
LDL	=	Geo. mean 8.40	Fisher et al. (2013)
	↑(↓ in LDL w ↓ in PFOS)	Geo. mean baseline = 18.5 Follow-up = 8.2	Fitz-Simon et al. (2013)
	↑ (children)	Mean 22.7	Frisbee et al. (2010)
	=	Mean 1.68	Fu et al. (2014)
	↑	Mean 17.7	Geiger et al. (2014b)
	=	Med. 21.0	Nelson et al. (2010)
	↑ (for 1 of 2 non-consecutive yrs)	Med. 1,000-3,000	Olsen et al. (1999)
	=	Med. 13.03	Starling et al. (2014b)
	↑	Mean 22.4	Steenland et al. (2009)
LDL dyslipidemia	↑	Mean 17.7	Geiger et al. (2014b)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association Δ change			

Overall summary of lipid effects

The observations from animal studies and epidemiology studies are in apparent conflict. While, in general, the animal studies show a consistent decrease in total cholesterol, HDL, LDL, and triglycerides as a result of PFOS exposure (including monkeys), epidemiology studies provide consistent evidence for an association between PFOS exposure and increased total cholesterol. There is also suggestion for an association between PFOS exposure and increased LDL in humans. Although the evidence from epidemiology studies is less consistent for an association between PFOS exposure and increases in triglycerides or HDL, there is no evidence from epidemiology studies to suggest that these parameters decrease with increasing PFOS exposure in humans.

Of possible relevance to this discrepancy, PFOA also caused decreased serum lipids in rodents, while increased serum lipids were associated with PFOA exposure in humans. Recent studies reviewed in DWQI (2017) suggest that these differences may be related to the low fat diet generally used in laboratory rodent studies versus the higher fat content of a typical Westernized human diet, rather than solely to interspecies differences. However, such studies have not been conducted for PFOS.

The lack of an animal model for the observed relationships between PFOS exposure and serum lipids precludes consideration of lipid parameters as endpoints for dose-response consideration.

Hematological effects

Animal studies

A summary of hematological effects of PFOS in animals can be found in Table 22 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

Following PFOS exposure, some animal studies assessed hematological parameters associated with erythrocytes (e.g., red blood cell number, hemoglobin, and hematocrit), leukocytes, (e.g., white blood cell numbers), and thrombocytes (i.e., platelets). These findings are briefly reviewed below by species.

Monkeys

Following 182 days of PFOS exposure, decreased hemoglobin levels were observed in male monkeys exposed to 0.75 mg/kg/day (Seacat et al., 2002). No effect on hemoglobin was observed in female monkeys (NOAEL = 0.75 mg/kg/day). Additionally, no effect was observed in males and females for a number of other hematological parameters including erythrocytes, leukocytes, and thrombocytes (NOAEL = 0.75 mg/kg/day).

Rats

Following 104 weeks of exposure, Butenhoff et al. (2012) reported an increase in segmented neutrophils in males exposed to 1.0 mg/kg/day, but with no similar effect in females (NOAEL = 1.3 mg/kg/day). This increase in the male rats was first observed at an interim observation at 14 weeks of exposure (Seacat et al., 2002). No other effects on erythrocytes, leukocytes, and thrombocytes were observed in these rats either at 14 or 104 weeks of exposure (Seacat et al., 2002; Butenhoff et al., 2012).

Following a shorter duration of exposure (28 days), Curran et al. (2008) reported a decreased in red blood cells, hemoglobin, and hematocrit in females (LOAEL = 7.6 mg/kg/day) but not males (NOAEL = 6.3 mg/kg/day). In these rats, no effect on white blood cell numbers was observed. Also following 28 days of

exposure, Kim et al. (2011) observed no effects on various parameters assessing erythrocytes, leukocytes, and thrombocytes in male and female rats (NOAEL = 10 mg/kg/day).

Mice

In male mice, 10 days of exposure to PFOS (0.02% in feed) was reported to decrease total white blood cell numbers (Qazi et al., 2009a) and bone marrow cell content (Qazi et al., 2012). In contrast, 10 days of exposure to 0.005% PFOS in feed had no effect on hematocrit or hemoglobin levels in male mice (Qazi et al., 2010b).

Summary of hematological effects in animals

Although assessed in multiple species, data are somewhat limited regarding the hematological effects of PFOS in animals. Although some studies do report changes in certain parameters, the impact of PFOS on hematological parameters is unclear.

Table 22. Study summary table for hematological effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Butenhoff et al. (2012)	Rats, Sprague-Dawley	0, 0.5, 2, 5, 20 ppm Dietary Mean daily intake of PFOS (as reported by study authors) Males: 0, 0.024, 0.098, 0.242, 0.984 mg/kg/day Females: 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day	<53 weeks	↑ N-SEG (at week 14 only), males only (determined at 14 weeks of exposure)	Males: 0.2 Females: 1.3	Males: 1.0 Females: ----	Serum and liver PFOS concentrations determined	Males: 148,000 Females: ---- (determined at 14 weeks of exposure)
Seacat et al. (2002)	Monkeys, cynomolgus	0, 0.03, 0.15, 0.75 mg/kg/day Capsule	26 weeks	↓ HGB (at day 91, 153, and 182, males only) (values reported by authors to be within normal range)	Males: 0.15 Females: 0.75	Males: 0.75 Females: ----	Serum and liver PFOS concentrations determined Sample sizes generally 2 to 6 per group with increased frequency of endpoint measurements	Males: 173,000 Females: ---- (determined after 183 days of exposure)
				Counts for: BASO, EOSIN, HCT, HGB (females only), LYMPH, MCH, MCHC, MCV, MONO, PLT, RBC, RETIC, N-SEG and WBC and blood cell morphology (any statistically significant changes were not consistently observed over the	0.75	--------		--------

Table 22. Study summary table for hematological effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
				duration of exposure)				
Seacat et al. (2003)	Rats, Crl:CD® (SD) IGS BR	0, 0.5, 2.0, 5.0, 20 ppm Dietary Estimated daily dose of PFOS (as reported by study authors) Males: 0, 0.03, 0.13, 0.34, 1.33 mg/kg/day Females: 0, 0.04, 0.15, 0.40, 1.56 mg/kg/day	14 weeks	↑ N-SEG (males only) (determined after 14 weeks of exposure)	Males: 0.3 Females: 1.6	Males: 1.3 Females: ----	Serum and liver PFOS concentrations determined Sample size ≤5 rats per endpoint	Males: 148,000 Females: ---- (determined after 14 weeks of exposure)
				HCT, HGB, MCH, MCHC, MCV, PLT, RBC, WBC	Males: 1.3 Females: 1.6	--------		--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased
-------- = not applicable

ALB = albumin; ALK = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BASO = basophils; BILI = bilirubin; BUN = blood urea nitrogen; CA = calcium; CHOL = cholesterol; CL = chloride; CREAT = creatinine; GGT = gamma glutamyltransferase; GLOB = globulin; GLUC = glucose; EOSIN = eosinophil; HCT = hematocrit; HDL = high density lipoprotein cholesterol; HGB = hemoglobin; K = potassium; LYMPH = lymphocyte; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MONO = monocyte; N-SEG = segmented neutrophil; NA = sodium; PHOS = inorganic phosphate; PLT = platelet; PROT = total protein; RBC = red blood cell; RETIC = reticulocyte; SBA = serum bile acid; SDH = sorbitol dehydrogenase; TRIG = triglycerides; VLDL = very low-density lipoprotein; WBC = white blood cell

Human epidemiologic studies

A summary of hematological effects in humans can be found in Table 23 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Only one study (Jiang et al., 2014) reported on hematologic parameters. This was a study of pregnant women in Tianjin, China. There are a number of significant limitations to this study, including a relatively small sample size (n = 141), incomplete information on recruitment and demographics, and statistical investigation of associations by means of correlation analyses rather than regression analysis with controlling for confounders and/or co-variates. This study stratified the analyses on the basis of linear and branched forms of PFOS.

No significant correlation was observed between serum PFOS and RBC, WBC, hemoglobin, total blood protein, or albumin. Platelet count was significantly positively correlated with branched chain PFOS only.

Summary of hematological studies

The quality of the Jiang et al. (2014) study is not adequate to support conclusions about the effectof PFOS exposure on hematological parameters.

Table 23. Summary of Epidemiology Studies of Blood Chemistry (non-lipid)

Endpoint	Effect and Direction	Serum PFOS concentration (ng/ml) (mean, median, etc.)	Study reference
WBC	=	Mean 4.75	Jiang et al. (2014)
RBC	=	Mean 4.75	Jiang et al. (2014)
Hb	=	Mean 4.75	Jiang et al. (2014)
Platelet count	↑ (branched PFOS forms only)	Mean 4.75	Jiang et al. (2014)
Total protein	=	Mean 4.75	Jiang et al. (2014)
Albumin	=	Mean 4.75	Jiang et al. (2014)

↑ statistically significant positive association
↓ statistically significant negative association
= no significant association/equivocal association

Overall summary of hematological effects

The animal data do not present a clear picture of possible effects of PFOS on hematological parameters. The single epidemiological study is not of adequate quality to draw conclusions about human hematological effects. Based on these observations, the available evidence does not justify hematological effects as critical endpoints for dose-response.

Reproductive/developmental effects

Animal studies

A summary of reproductive/developmental effects in animals can be found in Table 24 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendices 3 or 4.

The first section of the review of the animal data focuses on PFOS exposure in adult animals and any resulting effects on reproductive organs.

The second part of the review of the animal data focuses on gestational (i.e., maternal) exposures and resulting effects in fetal, neonatal, and adult offspring. This review of endpoints resulting from maternal exposure during gestation, including neonatal exposure through lactation, proceeds according to the following general order:

1. Reproductive and developmental endpoints, including pregnancy outcomes, offspring survival, and structural defects in offspring.

2. All other endpoints, including body weight effects, endocrine/metabolic effects, hepatic effects, immune effects, neurological effects (i.e., developmental neurotoxicity), renal effects, and other effects (e.g., cardiovascular effects).

Studies in adult animals focusing on reproductive organ weight and histopathology

The effects of PFOS exposure on the reproductive organs following adult exposures have been assessed in monkeys, rats, and mice. Typically, these assessments have focused on male (e.g., epididymis, testes) and female (e.g., ovaries, uterus) reproductive organ weights and histopathology, including mammary glands.

Monkeys

Following 182 days of exposure to ≤ 0.75 mg/kg/day PFOS in monkeys, Seacat et al. (2002) reported no effect on reproductive organ weights in males (epididymis, testes) and females (ovaries). Additionally, no histopathological changes were observed in these males (i.e., prostate, seminal vesicle) and females (i.e., mammary glands, uterus, vagina).

Rats

In rats following 52 weeks of PFOS exposure, Butenhoff et al. (2012) reported no effect on reproductive organ weights in males (testes; NOAEL = 1.0 mg/kg/day) and females (ovaries, uterus; NOAEL = 1.3 mg/kg/day). No histopathological changes were observed in these males (epididymides, prostate, seminal vesicles, testes) and females (cervix, ovaries, uterus, vagina). While no histopathological changes were observed in the aforementioned female reproductive organs, Butenhoff et al. (2012) also examined the mammary glands of these PFOS-exposed females. No non-neoplastic effects were observed in mammary glands. However, as discussed in the Carcinogenicity section (below), a statistically significant increased incidence of mammary gland fibroadenomas and combined

fibroadenomas/adenomas was observed only in the low dose group, while there was a significantly lower incidence in the high dose group and a significantly decreased trend for these tumors overall.

For shorter durations of PFOS exposure (28 days) in rats, data are mixed for an effect of PFOS on male reproductive organ weights. Cui et al. (2009) reported an increase in relative gonadal weight in males exposed to 5 mg/kg/day. However, no effects on testes weights were reported following exposures of ~ 6 mg/kg/day (Curran et al., 2008; Lopez-Doval et al., 2014). Data for histopathological changes in male reproductive organs are also mixed. Lopez-Doval et al. (2014) reported changes in testes histopathology (interstitial edema, degeneration of sperm heads; LOAEL = 1.0 mg/kg/day) following PFOS exposure; however, Curran et al. (2008) observed no histopathological changes in the epididymis and testes (NOAEL = 6.3 mg/kg/day). In females, no histopathological changes were observed in mammary glands, ovaries, uterus, and vagina (Curran et al., 2008; NOAEL = 7.6 mg/kg/day).

Mice

In mice, data are relatively limited for the effects of PFOS on reproductive organs. Following 28 days of exposure to 0.17 mg/kg/day, Fair et al. (2011) reported decreased relative uterine weight but no change in uterine histopathology. Following 28 days of exposure in adult male mice, Qiu et al. (2013) observed a decrease in sperm count and changes in testicular histopathology (LOAEL = 2.5 mg/kg/day).

Summary of effects on reproductive organ weight and histopathology

In total, data are relatively limited for the effect of PFOS on male and female reproductive organs following adult exposures in monkeys, rats, and mice. Some data suggest that PFOS can affect reproductive organ weight or histopathology.

Studies assessing reproductive/developmental endpoints following gestational exposure

Reproductive and developmental effects following gestational exposure to PFOS have been assessed in rats, mice, and rabbits. In some studies, pre-mating and/or lactational exposures were combined with gestational exposures to determine the effects of PFOS on offspring.

Effects of gestational exposure were evaluated for reproductive indices such as implantation sites, length of gestation, fetal survival, as well as litter effects and neonatal survival. In addition, reports also included assessment of gestational exposure to PFOS on structural and morphological effects in perinatal offspring as well as other developmental effects such as developmental milestones.

Rats

Pregnancy and neonatal outcomes

Data suggest that gestational PFOS exposure may have a limited impact on pregnancy outcomes in rats. For example, following gestational exposures, Butenhoff et al. (2009) and Thibodeaux et al. (2003) found no effect on the number of implantation sites in dams exposed to ≤ 10 mg/kg/day from GD2-20. Maternal exposure to PFOS did not affect the length of gestation (Butenhoff et al., 2009; NOAEL = 1.0 mg/kg/day) during the entire length of gestation or the number of live fetuses at term (Thibodeaux et al., 2003; NOAEL = 10 mg/kg/day) with exposure during GD2-20.

Some studies in rats assessed the reproductive and developmental effects of PFOS following exposure from pre-mating through gestation (Luebker et al. 2005a, 2005b). For example, Luebker et al. (2005b) reported no effects on corpora lutea, implantations, viable fetuses, and dead fetuses at GD21 (NOAEL = 2.0 mg/kg/day). When assessed at GD21, the authors also observed decreases in the percentage of dead or resorbed concepti per litter and early resorptions per litter at a maternal dose of 2.0 mg/kg/day. Similarly, Luebker et al. (2005a) also observed at GD10 no effect on corpora lutea, implantations, and

viable embryos (NOAEL = 3.2 mg/kg/day). However, at the end of pregnancy, these authors observed decreases in the duration of gestation and the number of implantation sites per delivered litter, as well as an increase in the number of dams with stillborn pups (LOAEL = 3.2 mg/kg/day). A decrease in the number of liveborn pups and an increase in stillborn pups per litter were also observed (LOAEL = 3.2 mg/kg/day). Using the F_1 generation for subsequent mating, Luebker et al. (2005a) observed no effect on the duration of gestation, number of implantations, and number of live pups (NOAEL = 0.4 mg/kg/day).

Following birth, there is evidence for an effect of PFOS on litter size and offspring survival. Lau et al. (2003) observed a significant reduction in postnatal rat pup survival (LOAEL = 2 mg/kg/day) following maternal exposure from GD2 to GD21. While all offspring appeared normal at parturition, all neonates in the 10 mg/kg/day maternal dose group became pale and inactive and died around an hour after birth. Over 95% of offspring in the 5 mg/kg/day maternal dose group did not survive past PND1. Grasty et al. (2003, 2005) reported decreased litter sizes following exposure on GD19 to GD20 (LOAEL = 25 mg/kg/day). In contrast, Butenhoff et al. (2009) reported no effect on number of litters and live litter size following PFOS exposure from GD0 to term (NOAEL = 1.0 mg/kg/day).

Pup mortality was reported to increase following gestational PFOS exposure. When assessed at PND3, Wan et al. (2010) observed a decrease in the number of delivered pups and an increase in pup mortality following maternal exposure on GD2 to GD21 (LOAEL = 2.0 mg/kg/day).

Similarly, Chen et al. (2012a) observed increased postnatal mortality at PND3 following maternal exposure from GD1 to GD21 (LOAEL = 2.0 mg/kg/day). In contrast, Butenhoff et al. (2009) reported that following maternal exposure on GD0 to PND20, there was no effect on offspring survival when assessed on PND0 to PND4 and on PND4 to PND21 (NOAEL = 1.0 mg/kg/day).

Additional studies assessed neonatal survival following maternal exposures prior to and during gestation. When assessed at PND5, Luebker et al. (2005b) reported increased offspring mortality (LOAEL = 1.6 mg/kg/day). In a two-generation study, Luebker et al. (2005a) reported an increase in the number of dams with all F_1 pups dying between PND1 and PND4 (LOAEL = 3.2 mg/kg/day). In the 3.2 mg/kg/day maternal dose group, 100% of the F_1 pups died by PND2. Additionally, the F_1 offspring in the 1.6 mg/kg/day maternal dose group were in such poor condition at PND21 as not to be further assessed in the study. Following mating of the F_1 generation, no effect on F_2 mortality was observed through PND21 (NOAEL = 0.4 mg/kg/day).

Structural and morphological effects in perinatal offspring

Following gestational exposure, data suggest that PFOS can cause skeletal and visceral defects in rat offspring. Thibodeaux et al. (2003) reported that various defects were observed in at-term offspring of dams exposed to 10 mg/kg/day from GD2 to GD20. These abnormalities included cleft palate, sternal defects, anasarca, enlarged right atrium, and ventricular septal defects. Maternal toxicity was observed in terms of decreases in T3 and T4 (LOAEL = 1 mg/kg/day), weight gain (LOAEL = 2 mg/kg/day), and hepatic effects in the high dose group.

Studies in rats also found effects of PFOS on the lungs of offspring. Following maternal exposure on GD19 and GD20, Grasty et al. (2003, 2005) observed histological and morphometric changes in offspring lungs at GD21 and PND0 suggestive of a delay in lung maturation (LOAEL = 25 mg/kg/day). In the 25 mg/kg/day maternal dose group, dams experienced decreased weight gain. Similarly, Chen et al. (2012a) observed changes (e.g., alveolar hemorrhage, thickened inter-alveolar septa) in lung morphology of 21-day old offspring following maternal exposure to 2.0 mg/kg/day on GD1 to GD21.

Chen et al. (2012a) did not report on maternal toxicity. In contrast, no effect on fetal lung histology at GD18.5 was observed with maternal exposure from GD12 to GD18 (Ye et al., 2012; NOAEL = 20 mg/kg/day). No maternal deaths were observed during PFOS exposure; however, no other maternal endpoints of toxicity were examined.

Other developmental effects
Data are mixed for whether PFOS can affect developmental milestones in offspring. In terms of sexual maturation, Butenhoff et al. (2009) reported no effect of gestational and lactational PFOS exposure (GD0 to PND20; NOAEL = 1.0 mg/kg/day) on the ages at which female and male offspring reached vaginal patency or balanopreputial separation, respectively. Similarly, Luebker et al. (2005a) observed no effect of pre-mating, gestational, and lactational PFOS exposure on sexual maturation in F_1 males and females (NOAEL = 0.4 mg/kg/day). This study did, however, observe a delay in pinna unfolding in the F_1 offspring (LOAEL = 1.6 mg/kg/day). Lau et al. (2003) observed a delay in eye opening of rat offspring born to mothers exposed on GD2 to GD21 (LOAEL = 2 mg/kg/day).

<u>*Mice*</u>

Pregnancy and neonatal outcomes
Thibodeaux et al. (2003) reported a decrease in the percentage of live fetuses at term following maternal exposure from GD1 to GD17 (LOAEL = 20 mg/kg/day); no effect on the number of implantation sites was observed (NOAEL = 20 mg/kg/day). Similarly, Yahia et al. (2008) observed a decrease percentage of live fetuses along with increased percentages of resorbed fetuses and dead fetuses following maternal exposure from GD0 to GD17 (LOAEL = 20 mg/kg/day). At lower maternal doses on GD11 to GD16, Lee et al. (2015) reported decreases in placental capacity (i.e., the ratio of fetal weight to placental weight; LOAEL = 0.5 mg/kg/day) and the number of live fetuses (LOAEL = 2.0 mg/kg/day) as well as an increase in the number of resorptions and dead fetuses (LOAEL = 0.5 mg/kg/day). However, Lee et al. (2015) observed no effect on the number of implantations.

Fuentes et al. (2006) observed no effect on pregnancy outcome following maternal exposure on GD6 to GD18 (NOAEL = 6 mg/kg/day). These authors assessed the numbers of (per litter) implants, live fetuses, dead fetuses, early resorptions, and late resorptions. Additionally, no effect was observed on the numbers of litters with dead fetuses and post-implantation loss as well as the fetal sex ratio. Similarly, no effect on length of gestation and the number of litters and pups per litter were observed following gestational exposure on GD12 to GD18 (Fuentes et al., 2007b; NOAEL = 6 mg/kg/day). Additional studies reported no effects on the number of live pups, litter size, and sex ratio following maternal exposures ≤ 10 mg/kg/day (Fuentes et al., 2007b; Rosen et al., 2009; Onishchenko et al., 2011).

In addition to studies using standard mouse strains, wild-type (WT) and PPARα null mice have been compared with respect to the reproductive/developmental effect of PFOS. Following maternal exposure on GD15 to GD18, Rosen et al. (2010) reported no effect on the number of implantation sites, total number of pups at birth (alive and dead), and percentage litter loss from implantation to birth in either WT or null mice (NOAEL = 10.5 mg/kg/day).

Following birth, gestational PFOS exposure was reported to affect offspring survival. Lau et al. (2003) observed a significant reduction in postnatal mouse pup survival (LOAEL = 10 mg/kg/day) following maternal exposure from GD1 to GD18. Most offspring in the ≥ 15 mg/kg/day maternal dose group did not survive within 24 hours of birth. Yahia et al. (2008) reported a decrease in offspring survival at PND4 following maternal exposure (GD0 to GD18) to 10 mg/kg/day. Decreased postnatal survival at PND15 was also observed in WT (LOAEL = 4.5 mg/kg/day) and PPARα null (LOAEL = 8.5

mg/kg/day) mice (Abbott et al., 2009a).

Structural and morphological effects in perinatal offspring

Following gestational exposure, data suggest that PFOS can lead to skeletal, visceral, and external defects in mouse offspring. Thibodeaux et al. (2003) reported that various defects were observed in term offspring of dams exposed to 15 mg/kg/day from GD1 to GD17. These abnormalities included cleft palate, sternal defects, enlarged right atrium, and ventricular septal defects. Maternal toxicity was limited to increased relative liver weight and decreased serum triglycerides (LOAEL for both endpoints = 5 mg/kg/day) and decreased body weight gain (LOAEL = 20 mg/kg/day). Similarly, an increase in fetal cleft palate at GD17 was observed following gestational exposure from GD1 to GD17 (Era et al., 2009; LOAEL = 13 mg/kg/day); maternal effects were not determined. Following gestational exposure on GD0 to GD17, an increase in the percentage of fetuses with sternal defects (LOAEL = 1 mg/kg/day) was observed by Yahia et al. (2008). These authors also observed bilateral swelling in the back of the necks of fetal and neonatal offspring in the 20 mg/kg/day maternal dose group. Increased liver weight and decreased weight gain were observed in dams in the 10 and 20 mg/kg/day groups, respectively.

In contrast, Fuentes et al. (2006) observed no effect of gestational PFOS exposure (GD6 to GD18) on a number of developmental parameters including assymetrical sternebrae, diminished ossification of caudal vertebrae, supernumerary ribs, and total number of litters with skeletal defects (NOAEL = 6 mg/kg/day). Maternal effects were limited to increased absolute liver weight (LOAEL = 3 mg/kg/day) and increased relative liver weight (LOAEL = 6 mg/kg/day). Additionally, no effect on offspring lung histology was observed following maternal exposure from GD1 to GD17 (Rosen et al., 2009; NOAEL = 10 mg/kg/day). Although limited to the assessment of body weight and general appearance, no maternal toxicity was observed.

Other developmental effects

Data are mixed regarding the ability of PFOS to affect developmental milestones in mouse offspring. Lau et al. (2003) observed a delay in eye opening of mouse offspring born to mothers exposed on GD1 to GD17 (LOAEL = 1 mg/kg/day). Similarly, a delay in eye opening was observed in WT (LOAEL = 8.5 mg/kg/day) and PPARα null (LOAEL =10.5 mg/kg/day) mice following gestational exposure from GD15 to GD18 (Abbott et al., 2009a). Fuentes et al. (2007b) observed an increase in the time to testes descent in males (LOAEL = 6 mg/kg/day), while no effect was observed for other male maturation milestones or for any milestone in females (NOAEL = 6 mg/kg/day).

<u>*Rabbits*</u>

Pregnancy outcomes

Data indicate that PFOS does not affect pregnancy outcomes in rabbits. Following maternal exposure on GD7 to GD29, Case et al. (2001) observed no effects on corpora lutea, implantations, resorptions, and the number of live and dead fetuses (NOAEL = 3.8 mg/kg/day).

Structural and morphological effects in perinatal offspring

Gestational PFOS from GD7 to GD29 did not results in any external, soft tissue, or skeletal abnormalities in offspring (Case et al., 2001; NOAEL = 3.8 mg/kg/day).

<u>*Summary of effects on reproductive and developmental parameters in offspring*</u>

In total, there is evidence that gestational exposure to PFOS can have effects on some reproductive and developmental parameters. In rats, pregnancy outcomes (e.g., number of implantation sites, length of

gestation) did not appear to be affected by gestational PFOS exposure. However following birth, gestational PFOS exposure resulted in decreased pup survival. In mice, data are mixed regarding the impact of gestational PFOS exposure on pregnancy outcomes. However, gestational PFOS exposure caused increased mortality in mouse offspring. Data in rabbits suggest no effects from PFOS exposure on pregnancy outcomes. In rats and mice, skeletal and visceral defects were observed in offspring following gestational PFOS exposure. Additionally, lung defects were observed in rat, but not mouse, offspring. No structural or morphological effects were observed in rabbit offspring. The available data for rats and mice appear to be mixed regarding the ability of gestational PFOS exposure to impact developmental milestones (e.g., sexual maturation).

Body weight effects from developmental exposure

Body weight effects have been assessed in rats, mice, and rabbits following gestational exposure to PFOS. Decreases in body weight have been reported in fetal, neonatal, and adult offspring of pregnant animals exposed to PFOS. These findings are briefly reviewed below.

Rats

Gestational PFOS exposure of pregnant rat dams has led to body weight changes in fetal, neonatal, and weaned offspring. Following maternal PFOS exposure on GD2 to GD20, Thibodeaux et al. (2003) reported decreased fetal body weight on GD21 in the 10 mg/kg/day group, whereas the corresponding dams experienced decreased weight gain at doses ≥ 2 mg/kg/day. In studies with observations immediately following parturition (e.g., PND0 and PND1), there is a consistent finding of decreased offspring body weight following gestational exposure to PFOS at maternal doses ≥ 0.4 mg/kg/day (Grasty et al., 2003, 2005; Lau et al., 2003; Luebker et al., 2005a, 2005b; Wan et al., 2010; Wang et al., 2011c; Chen et al., 2012a; Lv et al., 2013; Rogers et al., 2014). For many of the studies that reported decreased pup body weight, maternal toxicity (e.g., decreased maternal weight gain), when available, was also reported at LOAELs similar to the offspring effect. In such cases, it is unclear whether maternal toxicity contributed to the decreased pup body weights or whether the pup body weights were independently sensitive to gestational PFOS exposure. Decreases in rat pup body weight have been reported to persist beyond the neonatal period to weaning (e.g., typically PND21; Lau et al., 2003; Luebker et al., 2005a; Wan et al., 2010; Chen et al., 2012a; Lv et al., 2013).

In a two-generation study, Luebker et al. (2005a) reported that maternal PFOS exposure prior to and during mating and then during gestation and lactation caused a decrease in pup (i.e., the F_1 generation) body weight in the 1.6 mg/kg/day group from PND1 through PND21. Using the F_1 generation males and females for breeding and following a similar exposure regimen, a decrease in pup (i.e., the F_2 generation) body weight was observed in the 0.4 mg/kg/day maternal dose group from PND1 through PND21, although this effect only reached statistical significance at PNDs 7 and 14.

In contrast, Butenhoff et al. (2009) observed no decreased pup body weight at PND1 through PND72 for all maternal exposure groups (NOAEL = 1.0 mg/kg/day, exposure from GD0 to PND20). Additionally, Butenhoff et al. (2009) reported *increased* offspring body weight at sexual maturation, an effect that was only statistically significant in the 0.1 mg/kg/day maternal dose group. Yu et al. (2009b) also observed no effect on pup body weight (on PNDs 0, 14, 21, and 35) following maternal exposure to 3.2 mg/kg feed throughout gestation.

Mice

Gestational PFOS exposure of pregnant mouse dams has led to body weight changes in fetal, neonatal, and adult offspring. Following maternal PFOS exposure on GD1 to GD17, Thibodeaux et al. (2003) reported decreased fetal body weight on GD18 in the 10 mg/kg/day group, whereas the corresponding dams experienced increase relative liver weights at 5 mg/kg/day. Similarly, Lee et al. (2015) reported

decreased fetal body weight on GD17 in the 2.0 mg/kg/day maternal dose group following exposure on GD11 to GD16. In this study decreased placental weight and increased placental necrosis were observed in the 0.5 mg/kg/day group. It is possible that the placental effects in this study influenced the observed decrease in fetal body weight. In neonates, decreased pup body weight was observed following maternal doses ≥ 10 mg/kg/day (Yahia et al., 2008). At these dose levels, dams were reported to have increased liver weight. In contrast to decreased offspring body weight, Ryu et al. (2014) reported that PFOS exposure (4 mg/kg feed) during gestation, lactation, and into adulthood caused an increase in body weight gain in offspring at 12 weeks of age.

In several studies where mouse dams were exposed to PFOS during pregnancy, no effect on offspring body weight was observed. At birth (i.e., PND0), no decrease in neonatal body weight was observed even at a maternal dose as high as 10 mg/kg/day (Lau et al., 2003; Ribes et al., 2010; Onishchenko et al., 2011). When assessed later in life, gestational PFOS exposure did not cause a decrease in offspring body weight. For example, no effect on body weight was observed in offspring at ages 3 weeks (Wan et al., 2014; NOAEL = 3.0 mg/kg/day), 8 weeks (Keil et al., 2008; NOAEL = 5 mg/kg/day), and 20 weeks (Ngo et al., 2014; NOAEL = 3.0 mg/kg/day). In addition to studies using standard mouse strains, WT (wild-type) and PPARα null mice have been compared with respect to the developmental/reproductive effects of PFOS. Abbott et al. (2009a) reported no effect on offspring body weight at PND1 and PND15 in either WT or PPARα null mice following maternal exposure to 10.5 mg/kg/day during GD15 to GD18.

Rabbits

PFOS exposure of pregnant does during GD7 to GD20 led to a decrease in fetal body weight at GD29 with maternal PFOS doses ≥ 2.5 mg/kg/day (Case et al., 2001). In this study, a decrease in maternal weight gain was reported to occur (LOAEL = 1.0 mg/kg/day).

Summary and conclusions for offspring body weight effects in animals

In total, animal studies have consistently shown a decrease in fetal or neonatal weight with gestational PFOS exposure. Decreased fetal/neonatal body weight has been reported to occur in multiple species (i.e., rats, mice, and rabbits). Post-natal effects on body weight are less consistent with some studies showing post-natal decreases in body weight and other studies showing no post-natal effects. Some studies have reported that decreased offspring body weight can persist to weaning and beyond. Although maternal toxicity has been observed at doses similar to those causing the decreased offspring body weight, this effect in the offspring may represent developmental toxicity from gestational PFOS exposure.

In summary, there is strong evidence from several animal species that exposure to PFOS during gestation causes decreased birthweight.

Endocrine/metabolic effects from developmental exposure

Endocrine and metabolic effects following gestational exposure to PFOS have been assessed in rats and mice. Findings for effects on the thyroid gland and hormones as well as on additional endocrine and metabolic endpoints (e.g., glucose metabolism, insulin resistance) are briefly reviewed below.

Rats

Thyroid gland

Following gestational and lactational exposure to PFOS, no effect on thyroid histology (e.g., number of follicles and distribution of follicle sizes) was observed in male and female offspring when assessed at GD20, PND4, and PND21 (Chang et al., 2009; NOAEL = 1.0 mg/kg/day). While morphometric analyses on PNDs 4 and 21 of offspring thyroid follicular colloid area revealed no effect from PFOS

exposure, increased follicular epithelial cell height in males were observed on PND21. Similarly, no effect on offspring thyroid histopathology at PND5 was observed in the highest maternal dose group (2.0 mg/kg/day) following pre-mating and gestational PFOS exposure (Luebker et al., 2005b).

Thyroid hormones
Following gestational exposure, thyroxine (T4), triiodothyronine (T3), and thyroid stimulating hormone (TSH) have been assessed in rat offspring.

Decreases in T4 levels have generally been observed in neonatal and post-weaning rats. Following gestational exposure (GD2 to GD21), Lau et al. (2003) reported decreased serum levels of total and free T4 (LOAEL = 2 mg/kg/day) in offspring when assessed between PNDs 1 and 35. Luebker et al. (2005b) reported a decrease in total T4 (LOAEL = 0.4 mg/kg/day) but not free T4 at PND5 in offspring following pre-mating, gestational, and lactational exposures. With gestational and lactational exposure until PND14, decreased total T4 was also observed in offspring at PNDs 7 and 14 (Wang et al., 2011c; LOAEL = 3.2 mg/kg feed). Similarly, decreased total T4 was observed at PNDs 21 and 35 in rat offspring following gestational exposure as well as in offspring further exposed to PFOS via lactation (Yu et al., 2009b; LOAEL= 3.2 mg/kg feed).

Data generally show no effect on offspring T3 levels. No change in serum T3 levels between PNDs 1 and 35 were observed in offspring following gestational exposure (Lau et al., 2003; NOAEL = 3 mg/kg/day). Yu et al. (2009b) reported no change through PND35 in total and reverse T3 in rat offspring following gestational exposure as well as in offspring further exposed to PFOS via lactation (NOAEL = 3.2 mg/k feed). Following maternal PFOS exposure prior to and during gestation, no effect on total and free T3 levels were observed in offspring at PND5 (Luebker et al., 2005b). In contrast, with a higher dose range (0, 3.2, and 32 mg/kg feed), Wang et al. (2011c) reported decreased total T3 in offspring at 2 weeks of age following gestational and lactational exposure until PND14 (LOAEL = 32 mg/kg feed).

Following gestational exposure, PFOS did not affect serum TSH levels in offspring assessed between PND1 and PND35 (Lau et al., 2003; NOAEL = 3 mg/kg/day). Similarly, no effect on offspring TSH was observed in rats exposed to PFOS via gestation and lactation (Chang et al., 2009; NOAEL = 1.0 mg/kg/day). However, an increase in offspring TSH at PND5 was observed in the 1.6 mg/kg/day maternal dose group following pre-mating and gestational exposure (Luebker et al., 2005b).

Other endocrine and metabolic effects
In addition to thyroid gland and hormone effects, additional endocrine and metabolic effects, such as those on other hormones and glucose metabolism, have been assessed in rats following gestational PFOS exposure. Lv et al. (2013) reported decreased serum adiponectin (LOAEL = 0.5 mg/kg/day) and increased serum leptin (NOAEL = 1.5 mg/kg/day) in adult offspring (age 21 weeks) following gestational and lactational exposure to PFOS.

Lv et al. (2013) also assessed the effects of gestational and lactational PFOS exposure on parameters associated with glucose metabolism. Following maternal exposure from GD0 to PND21, adult offspring had increased levels of fasting serum insulin at 21 weeks of age (LOAEL= 1.5 mg/kg/day). In addition, increased insulin resistance index (LOAEL = 1.5 mg/kg/day) and increased glucose intolerance (at 18 weeks of age; LOAEL = 0.5 mg/kg/day) were observed in these adult offspring. However, Lv et al. (2013) observed no effect on fasting serum glucose and fasting glycosylated serum protein levels in adult offspring at ages 13 and 18 weeks (NOAEL =1.5 mg/kg/day).

Mice

Thyroid hormone

Studies investigating thyroid effects of gestational PFOS exposure in mouse offspring are relatively limited. Following maternal exposure from GD1 to GD17, Lau et al. (2003) observed no effect on serum T4 levels in offspring when assessed between PNDs 3 and 35 (NOAEL = 20 mg/kg/day).

Other endocrine and metabolic effects

In addition to thyroid hormone effects, additional endocrine and metabolic effects, such as those on glucose metabolism, have been assessed in mice following gestational PFOS exposure.

Ngo et al. (2014) observed no effect on blood glucose levels in offspring (age 20 weeks) following maternal exposure from GD1 to GD17 (NOAEL = 3.0 mg/kg/day). Following gestational and lactational exposure, Wan et al. (2014) observed increased fasting serum insulin in adult offspring (age 9 weeks; LOAEL = 3 mg/kg/day). Additionally, in these offspring, increased fasting serum glucose (LOAEL = 0.3 mg/kg/day) and increased homeostatic model assessment for insulin resistance (HOMA-IR; LOAEL = 3 mg/kg/day) were reported. However, no effect was observed for the oral glucose tolerance test (NOAEL = 3 mg/kg/day).

Summary of thyroid, endocrine and metabolic effects

In total, there is evidence that gestational exposure to PFOS can affect several endocrine or metabolic endpoints. In rats, data suggest that maternal PFOS exposure can decrease levels of T4 in offspring. However, data suggest no effect on other thyroid endpoints (e.g., histology, T3 and TSH) in rat offspring. The relatively limited reported data show no effect on T4 levels in mouse offspring. Gestational and lactational PFOS exposure may lead to other endocrine and metabolic effects into adulthood, as changes in some glucose metabolism parameters (e.g., fasting insulin, insulin resistance index) have been observed in adult offspring of rats and mice.

Hepatic effects from developmental exposure

Hepatic effects have been assessed in rat and mouse offspring following gestational exposure to PFOS. Findings for histopathology, liver weight, and liver fat content are briefly reviewed below.

Rats

Histopathology

While data are limited, the liver histopathology observed with exposure of adult rats (e.g., hepatocyte hypertrophy, cytoplasmic vacuolation) was not observed in rats at weaning (age 21 days) following gestational (GD2 to GD21) PFOS exposure (Wan et al., 2010; NOAEL = 2.0 mg/kg/day).

Liver weight

In several studies where rat dams were exposed to PFOS during pregnancy, data are mixed regarding increases in offspring liver weight. Following PFOS exposures of ≤ 10 mg/kg/day from GD2 to GD20, no effects on relative liver weight were observed in offspring just prior to term (Thibodeaux et al., 2003; Bjork et al., 2008). Although transient increases in offspring relative liver weight were observed prior to and at PND5 in the 3 mg/kg/day maternal dose group, these increases in the offspring did not persist when assessed at PND35 (Lau et al., 2003). Increased relative liver weight was observed in weaned rats following maternal exposure (GD2 to GD21) to 2.0 mg/kg/day (Wan et al., 2010). Similarly, increased relative liver weight was observed in offspring at PND 21 and 35 with maternal exposure to 3.2 mg/kg

feed during gestation and lactation (Yu et al., 2009b). However, no increase in relative liver weight was observed in this study when rats were only exposed during gestation.

Liver fat content

Following gestational and lactational PFOS exposure, adult offspring were reported to have an accumulation of liver fat and liver triglycerides when assessed at ~22 weeks of age (Lv et al., 2013, LOAEL = 1.5 mg/kg/day). Luebker et al. (2005b) reported that maternal exposure during pre-mating through gestation resulted in no effect on fetal liver cholesterol or triglycerides at GD21 (NOAEL = 2.0 mg/kg/day). For 5-day old neonates in this study, liver triglycerides were decreased (LOAEL = 1.0 mg/kg/day) and no effect on liver cholesterol (NOAEL = 2.0 mg/kg/day) was observed.

Mice

Liver histopathology

Following gestational PFOS exposure from GD1 to GD17 to either 5 or 10 mg/kg/day, analyses of fetal livers revealed eosinophilic granules in the absence of an affect on maternal body weight and appearance (Rosen et al., 2009).

Liver weight

Following gestational exposure in mice and assessment of effects near term at or close to parturition, Thibodeaux et al. (2003) observed increased relative liver weight in offspring at GD18 (LOAEL = 20 mg/kg/day), whereas Onishchenko et al. (2011) observed no increase in offspring liver weight at birth (NOAEL = 0.3 mg/kg/day). In maturing or adult offspring, data for liver weight are also mixed following gestational exposures to PFOS. Lau et al. (2003) observed increased relative liver weight in offspring from PND1 to PND21 following maternal exposure (on GD1 to GD17) to 5 mg/kg/day. While not statistically significant, this increase persisted until the final reported observation at PND35. Following the same exposure scenario as Lau et al. (2003), Keil et al. (2008) observed an increase in relative liver weight in male but not female offspring at 4 weeks of age. At 8 weeks of age, there were no statistically significant increases in relative liver weight in either sex compared to controls. No increase in relative liver weight was observed in adult offspring (20 weeks of age) following gestational exposure (Ngo et al., 2014; NOAEL = 3.0 mg/kg/day).

Following gestational and post-gestational exposures, data suggest that PFOS can increase the liver weight in exposed offspring. Wan et al. (2014) reported increased relative liver weight in male but not female offspring at PND63 following maternal exposure to 3 mg/kg/day from GD3 to weaning at PND21. Increased relative liver weight was also observed in offspring at 12 weeks of age following gestational and lactational PFOS exposure with additional dietary exposure until 12 weeks of age (Ryu et al., 2014; LOAEL = 4 mg/kg feed).

In addition to studies using standard mouse strains, wild-type (WT) and PPARα null mice have been compared with respect to the reproductive/developmental effects of PFOS. Abbott et al. (2009a) reported increased relative weights at PND15 in both WT and null mice following maternal exposures on GD15 to GD18 (LOAEL = 10.5 mg/kg/day).

Summary of hepatic effects

Data in rats suggest a hepatic effect in offspring following gestational PFOS exposure. While the effects from PFOS were not observed in the only study that evaluated histopathology, liver weight data provide some evidence that PFOS can have an impact on offspring livers. Other indicators of hepatic effects, such as increases in hepatic lipid content, suggest an effect from gestational exposure. In mice, the

effect of gestational PFOS exposure on offspring livers is unclear. While there is evidence for a histopathological effect (i.e., eosinophilic granules), data are mixed as to whether gestational PFOS exposure affects offspring liver weight. In both species, continued PFOS exposure after gestation results in increased offspring liver weight.

Immune effects from developmental exposure

Immune effects have been assessed in mouse offspring following gestational exposure to PFOS. Findings for immune function, immune organs, specific cell populations, and hypersensitivity are briefly reviewed below.

Immunosuppression

Decreased immune function has been observed in offspring following gestational PFOS exposure. Keil et al. (2008) reported a decrease in natural killer cell activity in male (LOAEL =1.0 mg/kg/day) and female (LOAEL = 5.0 mg/kg/day) mouse offspring at 8 weeks of age, but not at 4 weeks of age, following maternal exposure during GD1 to GD17. Plaque forming cell response, while not assessed at 4 weeks in Keil et al. (2008), was decreased in 8-week old males (LOAEL = 5.0 mg/kg/day) but not females (NOAEL = 5.0 mg/kg/day).

Effects on immune organs

No effect on immune organs weight or histopathology has been consistently observed in offspring following gestational exposures to PFOS. Following maternal exposure on GD1 to GD17, no effect was observed for spleen and thymus endpoints (i.e., relative organ weight and cellularity) for male and female offspring assessed at 4 and 8 weeks of age (Keil et al., 2008; NOAEL = 5.0 mg/kg/day). Similarly, Ngo et al. (2014) observed no effect on relative spleen weight in 20-week old offspring (NOAEL = 3.0 mg/kg/day).

Effects on specific cell populations

Data suggest that gestational PFOS exposure may have some effect on specific immune cell populations in offspring. Following maternal exposure from GD1 to GD17, Keil et al. (2008) observed a decrease in splenic lymphocytes (B220) in 4-week old female offspring (LOAEL =5.0 mg/kg/day). This effect was not observed in 4-week old male offspring or either sex at 8 weeks of age (NOAEL = 5.0 mg/kg/day). Keil et al. (2008) observed no effect on thymic lymphocytes of offspring at 4 weeks of age (NOAEL = 5.0 mg/kg/day); however, decreased thymic lymphocytes (CD3+ and CD4+) were observed in 8-week old males but not females in the 5.0 mg/kg/day maternal dose group.

Hypersensitivity

Data are not consistent for an effect of PFOS exposure on airway hypersensitivity. Ryu et al. (2014) observed in 12-week old offspring, an effect on airway sensitivity following a methacholine challenge but no effects on airway hyperresponsiveness and allergen (ovalbumin)- induced airway hyperresponsiveness. In this study, the offspring had been exposed to PFOS during gestation and lactation (4 mg/kg feed maternal dose) followed by dietary PFOS exposure (4 mg/kg feed) until 12 weeks of age.

Summary of immunologic effects

PFOS may affect certain immune endpoints in mouse offspring following gestational PFOS exposure. Data suggest that PFOS can decrease immune function (e.g., natural killer cell activity, plaque forming cell response) and certain immune cell populations in offspring. However, data also suggest that PFOS

has no effect on histopathology and weight of immune organs (e.g., spleen and thymus) as well as airway hypersensitivity in offspring.

Neurological effects

In general, structural and behavioral effects were assessed in rats and mice following gestational PFOS exposure. Structural effects assessed include brain weight. Behavioral effects assessed include changes in learning, locomotion, or reaction to stimulus. These findings are briefly reviewed below.

Rats

Structural effects

No effects on brain measurements (weight, length, width) were observed in rat offspring when assessed at PNDs 21 and 72 following maternal PFOS exposure from GD0 to PND21 (Butenhoff et al., 2009; NOAEL = 1.0 mg/kg/day).

Behavioral effects

A reduction in learning ability was observed in offspring following gestational exposure (GD1 to parturition; LOAEL = 5 mg/L – no intake dose reported), as assessed by escape latency and escape distance in the Morris water maze. Using similar tests, a reduction in learning ability was also observed in offspring following gestational and lactational exposures (GD1 to weaning, LOAEL = 15 mg/L – no intake dose reported) (Wang et al., 2015). In contrast, no effect on learning behavior (T-maze) was observed following gestational exposure (GD2 to GD21) in weaned offspring (Lau et al., 2003; NOAEL = 3 mg/kg/day). Butenhoff et al. (2009) also reported no effect on learning and memory (Biel maze) in weaned offspring following gestational and lactational exposures (GD0 to PND20; NOAEL = 1.0 mg/kg/day). Luebker et al. (2005a) reported no indications of neurotoxicity, as assessed by passive avoidance and water maze performance, in weaned F_1 offspring born to dams exposed prior to (i.e., for ≤ 56 days before GD0) and during gestation and lactation (GD0 to PND20; NOAEL = 0.4 mg/kg/day). Increased locomotor activity was observed in male (at PND17; LOAEL = 0.3 mg/kg/day) and female (at PND21; LOAEL = 1.0 mg/kg/day) offspring exposed to PFOS during gestation and lactation (i.e., GD0 to PND20) (Butenhoff et al., 2009). Following maternal exposures (i.e., pre-mating through PND22), delays in surface righting and air righting in lactating offspring were observed (Luebker et al., 2005a; LOAEL = 1.6 mg/kg/day). In contrast, no effect on motor function and vision were observed in offspring exposed during gestation (GD1 to parturition) as well as in offspring exposed during gestation and lactation (GD1 to weaning) (Wang et al., 2015; NOAEL = 15 mg/L).

No effect on acoustic startle response was observed in offspring at PNDs 20 and 60 following gestational and lactational exposure (Butenhoff et al., 2009; NOAEL = 1.0 mg/kg/day).

A decrease in hind limb grip strength was observed in offspring at weaning following gestational and lactational PFOS exposure (Butenhoff et al., 2009; LOAEL = 1.0 mg/kg/day).

Mice

Structural effects

No effect on brain weight at birth was observed in offspring following gestational PFOS exposure (Onishchenko et al., 2011; NOAEL = 0.3 mg/kg/day).

Behavioral effects

Delayed learning, as assessed by a water maze test, was observed in female (LOAEL = 6 mg/kg/day),

but not male (NOAEL = 6 mg/kg/day), offspring (age 3 months) following maternal exposures on GD12 to GD18 (Fuentes et al., 2007c).

No effects on offspring locomotor activity have been typically observed following gestational PFOS exposure. Following maternal exposure (6 mg/kg/day) on GD12 to GD18, no effects were observed in open field test activity or coordination/balance in 3-month old offspring (Fuentes et al., 2007b, 2007c; Ribes et al., 2010). Onishchenko et al. (2011) also reported no effect on locomotor activity in 5- to 8-month old female offspring following gestational exposure (NOAEL = 0.3 mg/kg/day). However, a decrease in motor activity was observed in male offspring (LOAEL = 0.3 mg/kg/day). No effect on habituation as assessed in the open field test was observed in offspring following maternal PFOS exposure (Fuentes et al., 2007b; NOAEL =6 mg/kg/day).

Additional neurological measures suggest an effect in offspring following gestational exposure to PFOS. For example, Fuentes et al. (2007b) observed alterations in tail pull resistance, vertical climb, and forelimb grip of offspring (LOAEL = 6 mg/kg/day).

Some behavioral effects of gestational PFOS exposure may differ based on sex. Following maternal PFOS exposure (0.3 mg/kg/day) from GD1 to birth, weaned male but not female offspring were reported to have alterations in muscle strength, circadian activity, and emotion- related behavior (Onishchenko et al., 2011). However, both sexes of offspring showed altered motor coordination.

Summary of developmental neurological effects

Data do not provide conclusive evidence for developmental neurological effects following gestational PFOS exposure. No structural effects were observed in rat and mouse offspring. Data are mixed from studies in rats and mice regarding the ability of PFOS exposure to alter offspring learning ability and motor function.

Renal effects

Data are limited for the renal effects in offspring following gestational PFOS exposure. Rogers et al. (2014) reported a decrease in nephron endowment in 22-day old males rats born to dams exposed to 18.75 mg/kg/day from GD2 to GD6. This decrease was not accompanied by any statistically significant changes in offspring body weight or kidney weight. In mice, a decrease in offspring relative kidney weight was observed in females at 4 weeks of age following maternal exposure from GD1 to GD17 (Keil et al., 2008; LOAEL = 5 mg/kg/day). No such effect was observed in females at 8 weeks or in males at either time point (NOAEL = 5 mg/kg/day).

Other effects

Data are limited for the cardiovascular effects in offspring following gestational PFOS exposure. Rogers et al. (2014) reported an increase in systolic blood pressure of male (52 weeks of age) and female (65 weeks of age) offspring born to dams exposed to 18.75 mg/kg/day from GD2 to GD6. No effect on offspring heart histopathology at PND5 was observed in the 2.0 mg/kg/day maternal group following pre-mating and gestational exposure (Luebker et al., 2005b).

Overall Summary of reproductive and developmental effects in animals

In total, data are relatively limited for the effects of PFOS on male and female reproductive organs following adult exposures, but these data do not suggest an impact on reproductive organ weight or histopathology. This is discussed in more detail in the Carcinogenicity section.

Following gestational exposure, PFOS caused increased neonatal offspring mortality, structural deformities, and decreased offspring body weights at birth and beyond. Although not entirely consistent, data suggest that gestational PFOS exposure may have limited effects on pregnancy outcomes or developmental milestones in animals.

Endocrine and metabolic effects in offspring appear to include decreases in T4 levels as well as effects on glucose metabolism. Evidence of hepatic effects in offspring includes increased liver weight and increases in hepatic lipid content. Certain immune endpoints, such as natural killer cell activity and plaque forming cell response, in offspring appear to be affected by gestational PFOS exposure.

Data in offspring do not provide conclusive support for developmental neurobehavioral effects following gestational PFOS exposure; however, effects on offspring learning ability and motor function have been reported. For other effects in offspring, such as renal and cardiovascular effects, data are too limited to reach a definitive conclusion.

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Abbott et al. (2009a)	Mice, 129S1/ SvImJ wild type (WT) Mice, 129S1/ SvImJ knockout (KO)	WT: 0, 4.5, 6.5, 8.5, 10.5 mg/kg/day KO: 0, 8.5, 10.5 mg/kg/day Oral gavage	GD15–GD18	Maternal (WT and KO) body weight at GD18 and body weight gain (GD15–GD18) Maternal (WT and KO) body weight, liver weight (absolute and relative) at PND15	10.5	--------	Serum PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				For both WT and KO: number of implantation sites, total number of pups at birth (alive and dead), percent litter loss from implantation to birth	10.5	--------	Serum PFOS concentrations determined for pups Duration of exposure may not identify effects that might arise from exposures occurring earlier in gestation	--------
				For both WT and KO pups: birth weight, body weight on PND15, and weight gain from PND1–PND15	10.5	--------		--------
				Absolute liver weight on PND15 in WT and KO pups (compared to controls)	10.5	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ absolute liver weight on PND15 in WT pups (trend across doses); no trend across doses in KO pups (determined at PND15)	WT: 8.5 KO: 10.5	WT: 10.5 KO: --------		WT: 41,200 KO: ---- (determined at PND15)
				For WT and KO pups: ↑ relative liver weight on PND15 (compared to controls and trend across doses) (determined at PND15)	8.5	10.5		WT: 41,200 KO: 52,400 (determined at PND15)
				↓ postnatal survival on PND15 (determined at PND15)	WT: -------- KO: --------	WT: 4.5 (no statistically effect at next dose level but at higher dose levels) KO: 8.5		WT: 24,100 KO: 42,800 (determined at PND15)

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Delayed eye opening in WT (on PND13) and KO (on PND14) pups (determined around PND15)	WT: 6.5 KO: 8.5	WT: 8.5 KO: 10.5		WT: 40,700 KO: 52,400 (determined at PND15)
Butenhoff et al. (2009)	Rats, Crl:CD (SD)	0, 0.1, 0.3, 1.0 mg/kg/day Oral gavage	GD0–PND20	Maternal body weight (on GD0, GD20, and PND1) and change in body weight (from GD0–GD20 and PND1–PND21)	1.0	--------	Internal PFOS concentrations not determined Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days	--------
				↓ maternal body weight from PND4–PND21	0.3	1.0		--------
				Maternal food consumption (relative consumption GD0–GD20 and PND1–PND21; absolute PND1–PND21)	1.0	--------		--------
				Maternal absolute food consumption GD0–GD20	0.3	1.0		--------
				Internal macroscopic examination of dams that failed to deliver or necropsied on PND21	1.0	--------		--------
				Number of litters, length of gestation, implantation sites, unaccounted sites (potential resorption)	1.0	--------	Internal PFOS concentrations not determined Lack of histology	--------

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				↑ offspring body weight at vaginal patency and at balanopreputial separation	--------	0.1		--------
				Delivered litters, pups born/litter, live litter size PND0, % males/litter at birth,% survival PND0–4, % survival PND4–21, pup weight (male and female separately at PND 1, 21, 72), age at vaginal patency or balanopreputial separation	1.0	--------		--------
				↓ offspring hind limb grip strength on PND21 (males only, mean value reported to be in historical control range) Note: multiple time points also assessed but no effects observed	0.3	1.0		--------
				↑ offspring locomotor activity in males (PND17) and females (PND21)	Males: 0.1 Females: 0.3	Males: 0.3 Females: 1.0		--------

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Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Acoustic startle response in offspring	1.0	--------		--------
				Biel maze swimming in offspring	1.0	--------		--------
				Offspring brain measures (weight, length, width) at PND21 and 72	1.0	--------		--------
Case et al. (2001)	Rabbits, New Zealand white	0, 0.1, 1.0, 2.5, 3.75 mg/kg/day Oral gavage	GD7–GD29	↓ maternal body weight gain (during exposure period; no effect on body weight when exposure ended) Reduction in maternal body weight gains generally correlated with a reduction in feed consumption	0.1	1.0	Internal PFOS concentration not determined Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				↓ fetal weight	1.0	2.5	Internal PFOS concentration not determined	--------
				Corpora lutea, implantations, resorptions (early and late), and number of fetuses (alive and dead)	3.75	--------		--------
				External, soft tissue, or skeletal abnormalities	3.75	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Chang et al. (2009)	Rats, Sprague-Dawley	0, 0.1, 0.3, 1.0 mg/kg/day Oral gavage	GD0–PND20	Maternal TSH (at GD20, PND4, and PND21)	1.0	--------	Serum, brain, and liver PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects See also Butenhoff et al. (2009) for additional maternal effects (e.g., body weight)	--------
				Offspring TSH (at GD20, PND4, and PND21)	1.0	--------	Serum, brain, and liver PFOS concentrations determined for offspring Sample size varied for thyroid endpoints, sample size unclear	--------
				Offspring thyroid histology (at GD20, PND4, and PND21) Thyroids from 0.1 and 0.3 mg/kg/day groups not analyzed	1.0	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring thyroid morphometry: ↑ thyroid follicular epithelial cell height (at PND21 only), males only Study authors report low values in concurrent male controls Thyroids from 0.1 and 0.3 mg/kg/day groups not analyzed	Males: ---------- Females: 1.0	Males: 1.0 Females: ----	for TSH measurement	Males: 18,610 Females: ---- (determined at PND21)
				Offspring thyroid follicular colloid area (at PND4 and PND21), males and females Thyroids from 0.1 and 0.3 mg/kg/day groups not analyzed	1.0	--------		--------

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Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring thyroid cell proliferation: ↑ for females only Study author report wide range of control values Thyroids from 0.1 and 0.3 mg/kg/day groups not analyzed (determined at GD20)	Males: 1.0 Females: -----	Males: ---------- Females: 1.0		31,460 (determined at GD20 and pooled by litter)
Chen et al. (2012a)	Rats, Sprague-Dawley	0, 0.1, 2.0 mg/kg/day Oral gavage	GD1–GD21	↓ decrease in offspring body weight (from PND0–PND21) (determined at PND21)	0.1	2.0	Serum and lung PFOS concentrations determined for pups Sample size not explicit Only qualitative histology data	47,520 (determined at PND0) 4,460 (determined at PND21)
				↑ post-natal mortality (determined at PND3)	0.1	2.0		47,520 (determined at PND0)
				Offspring lung morphology including alveolar hemorrhage and thickened inter-alveolar septa (determined at PND0 and PND21)	0.1	2.0		47,520 (determined at PND0) 4,460 (determined at PND21)

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Era et al. (2009) (results from single dose regimens not summarized herein)	Mice, ICR	0, 9, 13, 20, 30 mg/kg/day Oral gavage	GD1–GD17	↑ cleft palate (see comments, LOAEL based on 7.3% incidence at 13 mg/kg/day versus ~0% in controls) (determined at GD17)	9	13	Serum and amniotic fluid PFOS concentrations determined Maternal effects not reported for this dosing regimen Statistical significance not reported	110,000 (as estimated from graphical representation of data) (determined at GD17)
Fuentes et al. (2006)	Mice, Charles River CD1	0, 1.5, 3, 6 mg/kg/day Oral gavage	GD6–GD18	Maternal effects: Body weight (GD18) and body weight gain; food consumption, gravid uterine weight, kidney weight (absolute and relative), maternal thyroid hormones or corticosterone	6	--------	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				Maternal effects: ↑ absolute liver weight (↑ relative liver weight at higher dose)	1.5 (based on absolute liver weight)	3 (based on absolute liver weight)		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Fetal effects (reproductive performance): implants/litter, live fetuses/litter, dead fetuses/litter, early resorptions/litter, late resorptions/litter, litters with dead fetuses post-implantation loss mean fetal weight fetal sex ratio	6	--------	Internal PFOS concentrations not determined for offspring PFOS purity not reported	--------
				Fetal effects (developmental): number of litters examined skeletally, assymetrical sternebrae, diminished ossification of caudal vertebrae, supernumerary ribs, total of litters with skeletal defects (↓ number of fetuses with diminished ossification [calcaneous] with 3 mg/kg/day but not at other doses)	6	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Grasty et al. (2003) (results from single dose regimen not summarized herein)	Rats, Sprague-Dawley	0, 25, 50 mg/kg/day Oral gavage	GD19–GD20	Maternal effects ↓ weight gain	--------	25	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				↓ live litter size	--------	25	Internal PFOS concentrations not determined for offspring PFOS purity not reported Qualitative reporting of lung histology	--------
				↓ percent survival	25	50		--------
				↓ offspring weight	--------	25		--------
				Difference in lung histology (i.e., thinning of epithelial walls) between exposed and control offspring	--------	25		--------
Grasty et al. (2005) (results from rescue studies not summarized herein)	Rats, Sprague-Dawley	0, 25, 50 mg/kg/day Oral gavage	GD19–GD20	Maternal effects ↓weight gain (Study authors did not assessment maternal toxicity in this study; however, the authors refer to Grasty et al. [2003], which used the same exposure regimen, for potential maternal effect)	--------	25	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------

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Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: ↓ live litter size	--------	25	Internal PFOS concentrations not determined for offspring Qualitative data reported for some endpoints	--------
				Offspring effects: ↓ pup birth weight	--------	25		--------
				Offspring effects: ↑ neonatal mortality	--------	25		--------
				Offspring effects: Lung histology at GD21 (alveolar wall thickness)	50	---------		--------
				Offspring effects, morphometric analysis of lung tissue: ↓ small airway proportion ↓ solid tissue:small airway ratio (↑ solid tissue proportion at the high dose)	--------	25		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Keil et al. (2008)	Mice, B6C3F1	0, 0.1, 1.0, 5.0 mg/kg/day Oral gavage	GD1–GD17	Maternal effects Body weight loss (quantitative data not reported by study authors)	5.0	--------	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				Offspring effects: Body weight (at 4 and 8 weeks of age)	5.0	--------	Internal PFOS concentrations not determined for offspring Adversity of immunotoxicity effects not clear	--------
				Offspring effects (at 4 weeks of age): ↑ relative liver weight in males ↓ relative liver weight in female with 0.1 mg/kg/day only	Males: 1.0 Females: 5.0 (based on no effect at higher doses)	Males: 5.0 Females: ----		--------
				Offspring effects (at 4 weeks of age): ↓ relative kidney weight, females only	Males: 5.0 Females: 1.0	Males: ---- Females: 5.0		--------
				Offspring effects (at 4 weeks of age): Relative spleen weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------

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Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (at 4 weeks of age): Relative thymus weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (at 8 weeks of age): Relative liver weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (at 8 weeks of age): Relative kidney weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (at 8 weeks of age): Relative spleen weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (at 8 weeks of age): Relative thymus weight	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (4 and 8 weeks of age): Spleen cellularity, for both males and females	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (4 and 8 weeks of age): Thymus cellularity, for both males and females	Males: 5.0 Females: 5.0	Males: ---- Females: ----		--------
				Offspring effects (at 4 weeks of age): NK cell function (genders analyzed together)	5.0	--------		--------
				Offspring effects (at 8 weeks of age): ↓ NK cell function (genders analyzed separately)	Males: 0.1 Females: 1.0	Males: 1.0 Females: 5.0		--------
				Offspring effects (at 8 weeks only): ↓ IgM response (to SRBC immunization), males only	Males: 1.0 Females: 5.0	Males: 5.0 Females: ----		--------
				Offspring effects (at 4 weeks of age): ↓ splenic lymphocytes (B220 cells only), females only	Males: 5.0 Females: 1.0	Males: ---- Females: 5.0		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (at 4 weeks of age): Thymic lymphocytes	Males: 5.0 Females: 5.0	Males: ---- Females: ---		--------
				Offspring effects (at 8 weeks of age): Splenic lymphocytes	Males: 5.0 Females: 5.0	Males: ---- Females: ---		--------
				Offspring effects (at 8 weeks of age): ↓ thymic lymphocytes (CD3+ and CD4+ cells only), males only	Males: 1.0 Females: 5.0	Males: 5.0 Females: ---		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Lau et al. (2003)	Rats, Sprague-Dawley	0, 1, 2, 3, 5 mg/kg/day Oral gavage	GD2–GD21 Endpoints measured through PND35	Offspring effects: ↓ body weight (generally observed within PND10 but then no statistically significant difference from controls afterwards, except for 5 mg/kg/day where effect was reported even at PND22) (body weight determinations made various days between PND0 and PND35, LOAEL based on PND5 determination)	3	5	Serum and liver PFOS concentrations determined for offspring Limited number of time points assessed for internal PFOS concentrations Serum PFOS concentrations determined for dams but reported in Thibodeaux et al. (2003) Maternal effects reported in Thibodeaux et al. (2003) Maternal exposure <30 days Thyroid hormone measurements may	110,000 (determined at PND0, as estimated from graphical representation of data) (offspring serum PFOS reported for PND0, 2, 5, except for 5 mg/kg group where reported only for PND0)
				Offspring effects: Absolute liver weight (only time point for 5 mg/kg/day was PND0)	3	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
				Offspring effects: ↑ relative liver weight (effect not consistent across doses and time points, only time point for 5 mg/kg/day was PND0)	3	--------	be subject to negative bias	--------
				Offspring effects: ↓ serum total and free T4 (only the decrease in serum free T4 persisted until PND35) (serum thyroid determinations made various days between PND0 and PND35, LOAEL based on PND2 for total T4)	1	2		70,000 (determined at PND2, as estimated from graphical representation of data) (offspring serum PFOS reported for PND0, 2, 5, expect for 5 mg/kg group where reported only for PND0)
				Offspring effects: Serum T3 and TSH	3	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: Learning behavior (T-maze) (only 3 mg/kg/day group tested)	3	--------		--------
		0, 1, 2, 3, 5, 10 mg/kg/day Oral gavage	GD2–GD21 Cross-fostering experiment (3 days) also conducted with pups from 5 mg/kg/day group	Offspring effects: ↓ survival (100% of pups in 10 mg/kg/day group died within 60 minutes of birth)	1	2	Internal PFOS concentrations not determined for offspring assessed for developmental milestones and those in the cross-fostering experiment Serum PFOS concentrations determined for dams but reported in Thibodeaux et al. (2003) Maternal effects reported in Thibodeaux et al. (2003)	--------
				Offspring effects: Delayed eye opening	1	2		--------
				Offspring effects: Vaginal opening, onset and profiles of estrous cycle, preputial separation (10 mg/kg/day group not assessed due to 100% mortality)	5	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects, cross-fostering experiment: ↓ survival (prenatally exposed pups with control dams) (all control pups cross-fostered with exposed dams survived)	--------	5		--------
	Mice, CD-1	0, 1, 5, 10, 15, 20 mg/kg/day Oral gavage	GD1–GD17	Offspring effects: ↓ survival (most pups in 15 and 20 mg/kg/day groups did not survive past 24 hour after birth)	5	10	Internal PFOS concentrations not determined for offspring Serum PFOS concentrations determined for dams but reported in Thibodeaux et al. (2003) Maternal effects reported in	--------
				Offspring effects: Body weight (only time point for 15 and 20 mg/kg/day was PND0)	10	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: Absolute liver weight (effect not consistent across doses and time points, only time point for 15 and 20 mg/kg/day was PND0)	10	--------	Thibodeaux et al. (2003) Thyroid hormone measurements may be subject to negative bias	--------
				Offspring effects: ↑ relative liver weight (effect generally statistically significant through PND21, only time point for 15 and 20 mg/kg/day was PND0)	1	5		--------
				Offspring effects: Serum T4 (only T4 measured in mice)	20	--------		--------
				Offspring effects: Delayed eye opening (data not available for 15 and 20 mg/kg/day groups)	--------	1		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Lee et al. (2015)	Mice, CD-1	0, 0.5, 2.0, 8.0 mg/kg/day Oral gavage	GD11–GD16	Maternal effects: ↓ change in body weight (statistically significant from GD14 through GD17)	2.0	8.0	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				Maternal effects: ↓ placental weight	--------	0.5		--------
				Maternal effects: ↑ placental necrosis (area of injury)	--------	0.5		--------
				Offspring effects: ↓ fetal weight	0.5	2.0	Internal PFOS concentrations not determined for offspring PFOS purity not reported	--------
				Offspring effects: ↓ placental capacity	--------	0.5		--------
				Offspring effects: ↑ number of resorptions and dead fetuses	--------	0.5		--------
				Offspring effects: ↓ number of live fetuses	0.5	2.0		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
Luebker et al. (2005a) (results from single-dose cross-foster experiment not summarized herein)	Rats, Crl:CD® (SD)IGS BR VAF®	0, 0.1, 0.4, 1.6, 3.2 mg/kg/day Oral gavage	F0 males: pre-mating (42 days) and mating (≤14 days) F0 females: pre-mating (42 days), mating, and then either until GD9 (caesarean group) or LD20 (natural delivery group)	Maternal effects: Mortality	3.2	--------	Serum and liver PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days Paternal effects summarized elsewhere in appropriate summary table(s)	--------
				Maternal effects: ↓ body weight gain (during periods with gestation and lactation) (statistically significant reductions in absolute and/or relative feed consumption observed during different periods of exposure) (determined at study day 42)	0.4	1.6		82,000 (determined at LD21)
				Maternal effects, general reproductive endpoints: Estrous cycle, number of pregnancies/matings, number of days to inseminate, number of matings during first week of cohabitation	3.2	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Maternal effects, general reproductive endpoints at GD10 (caesarean-section group): Corpora lutea, implantations, viable embryos	3.2	--------		--------
				Maternal effects, general reproductive endpoints following natural birth: ↓ duration of gestation ↓ implantation sites per delivered sites ↑ dams with stillborn pups ↑dams with all pups dying between PND1–PND4 (determined at or near PND0)	1.6	3.2		-------- (determined at LD21, serum PFOS not reported for 3.2 mg/kg group)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
		0, 0.1, 0.4, 1.6, 3.2 mg/kg/day Oral gavage post weaning (i.e., starting on LD22)	See description above for details regarding F0 exposure duration (i.e., pre-conception, gestation, and lactation exposures of F1) F1 started gavage exposure on LD22 at same dose level as parents, exposure continued through PND90 (i.e., the start of mating) and	Offspring effects (F1): ↓ number of liveborn pups ↑ stillborn pups/litter (100% mortality of pup in 3.2 mg/kg/day group after LD2)	1.6	3.2	Liver PFOS concentrations determined for F1 Internal PFOS concentrations determined after some effect were initially observed Control values for internal PFOS measurements not reported	--------
				Offspring effects (F1), prior to weaning: ↓ pup weight per litter (from LD1 to LD21) ↓pup weight gain per litter (from LD4 to LD21)	0.4	1.6		--------
				Offspring effects (F1), prior to weaning: Delays in pinna unfolding, eye opening, surface righting, and air righting	0.4	1.6		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
			continued ≤14 days	Offspring effects (F1), prior to weaning: Delays in eye opening	0.1	0.4		--------
				Offspring effects (F1), post weaning: Mortality (F1 pups in 1.6 mg/kg/day group observed to be in poor clinical condition and not evaluated past LD21)	0.4	--------		--------
				Offspring effects (F1), post weaning: Body weight and body weight gains (absolute and relative feed consumption similar between exposed and control groups)	0.4	--------		--------
				Offspring effects (F1), post weaning: Sexual maturation (male and females)	0.4	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (F1), post weaning: Neurotoxicity (passive avoidance, water maze performance)	0.4	--------		--------
				Offspring effects (F1), post weaning: Reproductive effects (duration of gestation, number of implantations, number of live pups)	0.4	--------		--------
		0, 0.1, 0.4 mg/kg/day	See description above for details regarding F1 exposure duration (i.e., pre-conception, gestation, and lactation exposures of F2), F2 lactation exposure ended on LD21	Offspring effects (F2): Mortality (throughout lactation period)	0.4	--------	Internal PFOS concentration not determined for F2	--------
				Offspring effects (F2): Body weight and body weight gain (any reductions were not statistically significant, or were statistically significant but transient)	0.4	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Luebker et al. (2005b) Authors conducted dose-response and pharmaco-kinetic studies. Only results from dose-response study are summarized herein	Rats, Crl:CD® (SD)IGS VAF/Plus®	0, 0.4, 0.8, 1.0, 1.2, 1.6, 2.0 mg/kg/day (natural delivery group) Oral gavage	F0 males: no exposure F0 females: pre-mating (42 days), mating (≤14 days), and then until LD4	Maternal (F0) effects: Mortality	2.0	--------	Serum and liver PFOS concentrations determined for dams Quantitative data for internal PFOS measurements not reported for controls Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days	--------
				Maternal (F0) effects: ↓ body weight gain (effect primarily observed during lactation with some reductions during pre-mating, no apparent differences between exposed and controls during gestation) (↓ relative feed consumption during lactation with ≥0.8 mg/kg/day, decreases during pre-mating and gestation with 2.0 mg/kg/day) (determined on LD5)	0.4	0.8		42,600 (determined on LD5)
				Maternal (F0) effects: ↑ relative liver weight (determined on LD5)	0.4	0.8		42,600 (determined on LD5)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Maternal (F0) effects, reproductive endpoints: Fertility index, number of implantation sites, gestation index, number of still liveborn pups	2.0	--------		--------
				Maternal (F0) effects, reproductive endpoints: ↓ gestation length (effects including dams with all pups dying by PND5 and viability index observed at higher doses; increases and decreases in dams with stillborn pups observed) (determined presumably at PND0/LD0)	0.4	0.8		42,600 (determined on LD5)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Maternal (F0) effects, serum biochemical parameters: ↓ total CHOL (determined on LD5)	--------	0.4		27,200 (determined on LD5)
				Maternal (F0) effects, serum biochemical parameters: ↓ TRIG (determined on LD5)	1.2	1.6		169,000 (determined on LD5)
				Maternal (F0) effects, serum biochemical parameters: ↑ GLUC (determined on LD5)	1.6	2.0		134,000 (determined on LD5)
				Maternal (F0) effects, serum biochemical parameters: HDL, LDL, MAL	2.0	--------		--------
				Maternal (F0) effects, milk biochemical parameters: CHOL	2.0	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	*Comment(s)*	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL*** ***(day assessed)***
				Maternal (F0) effects, liver biochemical parameters: ↑ TRIG (determined on LD5)	1.2	1.6		169,000 (determined on LD5)
				Maternal (F0) effects, liver biochemical parameters: CHOL Malic enzyme activity	2.0	--------		--------
				Maternal (F0) effects, thyroid hormones: ↓ total T4 (measured by analog RIA method) (↓ total T3 with ≥1.2 mg/kg/day and no effect on TSH when measured by analog RIA method) (determined on LD5)	--------	0.4		27,200 (determined on LD5)
				Maternal (F0) effects, thyroid hormones: Free T4 (measured by equilibrium dialysis RIA method)	2.0	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring (F1) effects: ↓ pup body weight (at birth and LD5) ↓ pup body weight gain (from birth to LD5) (determined on LD5)	--------	0.4	Serum and liver PFOS concentrations determined for offspring Quantitative data for internal PFOS measurements for control animals not reported Limited sample size for some endpoints (e.g., thyroid hormone measurements)	36,200 (determined on LD5)
				Offspring (F1) effects: ↑ pup mortality (through LD5) (determined on LD5)	1.2	1.6		-------- (determined on LD5, offspring serum PFOS concentration not reported for 1.6 mg/kg group)
				Offspring (F1) effects, serum biochemical parameters: CHOL, GLUC, HDL, LDL, TRIG	2.0	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL*** ***(day assessed)***
				Offspring (F1) effects, liver biochemical parameters: ↓ TRIG (statistically significant effect in females limited to 1.0, 1.2, and 1.6 mg/kg/day but not 2.0 mg/kg/day) (determined on LD5)	Males: 0.8 Females: 0.8	Males: 1.0 Females: 1.0		84,400 (determined on LD5, offspring serum PFOS concentration reported for litter not individual sexes)
				Offspring (F1) effects, liver biochemical parameters: CHOL, glycogen content, malic enzyme activity	2.0	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring (F1) effects, thyroid hormones: Total T3 (measured by analog RIA method) (reductions observed but were not statistically significant; reductions also observed when using an analog CL method but limited sample availability)	2.0	--------		--------
				Offspring (F1) effects, thyroid hormones: ↓ total T4 (measured by analog RIA method) (non-statistically significant reductions observed when using an analog CL method) (determined on LD5)	--------	0.4		36,200 (determined on LD5)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring (F1) effects, thyroid hormones: Free T3 and free T4 (measured by equilibrium dialysis RIA method) (limited sample size prevented determination of NOAEL and LOAEL)	--------	--------		--------
				Offspring (F1) effects, thyroid hormones: TSH (measured by analog RIA method) (limited sample size prevented determination of NOAEL and LOAEL)	--------	--------		--------
				Offspring (F1) effects, histopathology: Microscopic changes to heart and thyroid (limited sample size prevented determination of NOAEL and LOAEL)	--------	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
		0, 1.6, 2.0 mg/kg/day (caesarean group) Oral gavage	F0 males: no exposure F0 females: pre-mating (42 days), mating (≤14 days), and then until GD20	Maternal (F0) effects: ↓ dams with any resorptions	1.6	2.0	Internal PFOS concentration not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days	--------
				Maternal (F0) effects, serum biochemical parameters: CHOL, GLUC, HDL, LDL, MAL, TRIG	2.0	--------		--------
				Maternal (F0) effects, liver biochemical parameters: ↓ liver CHOL	------	1.6		--------
				Maternal (F0) effects, liver biochemical parameters: TRIG	2.0	--------		--------
				Offspring (F1) effects: Litter averages for corpora lutea, implantations, viable fetuses, and dead fetuses; percent live male fetuses, pooled fetal body weight	2.0	--------	Internal PFOS concentration not determined for offspring Only two doses used in the caesarean group	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring (F1) effects: ↓ percent dead or resorbed concepti/litter ↓ early resorptions/litter	1.6	2.0		--------
				Offspring (F1) effects, serum biochemical parameters: ↑ CHOL, LDL	--------	1.6		--------
				Offspring (F1) effects, serum biochemical parameters: GLUC, HDL, MAL, TRIG	2.0	--------		--------
				Offspring (F1) effects, liver biochemical parameters: CHOL, TRIG	2.0	--------		--------
Lv et al. (2013)	Rats, SPF Wistar	0, 0.5, 1.5 mg/kg/day Oral gavage	GD0–PND21	Neonatal deaths, Survival rates through PND21	1.5	--------	Serum and liver concentrations	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
			(i.e., weaning)	↓ body weight (at PND21) (effect also observed at PND0 with 1.5 mg/kg/day) (determined on PND21)	-------- (based on PND21 data)	0.5	determined for offspring Maternal effects not reported Only two dose levels used Maternal exposure >30 days	11,000 (determined on PND21, also determined on PND0 but not reported herein)
				↑ glucose intolerance (at 15 weeks after weaning, only statistically significant for 0.5 mg/kg/day group) (effect also observed at 10 weeks after weaning but only statistically significant for 1.5 mg/kg/day group) (determined 10 to 15 weeks after weaning on PND21)	--------	0.5		11,000 (determined on PND21, prior to endpoint assessment)
				Fasting serum glucose, fasting glycosylated serum protein levels (at 10 and 15 weeks after weaning)	1.5	--------		--------

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Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				↑ fasting serum insulin ↑insulin resistance index ↑ serum leptin (all 18 weeks after weaning on PND21)	0.5	1.5		71,350 (determined on PND21, prior to endpoint assessment)
				↓ serum adiponectin (determined 18 weeks after weaning on PND21)	--------	0.5		11,000 (determined on PND21, prior to endpoint assessment)
				↑ liver fat accumulation ↑ liver TRIG (determined 19 weeks after weaning on PND21)	0.5	1.5		71,350 (determined on PND21, prior to endpoint assessment)
				Serum CHOL and TRIG	1.5	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Ngo et al. (2014) Only maternal and WT data are summarized herein	Mice, C57BL/6J	0, 0.01, 0.1, 3.0 mg/kg/day (combined from two separate experimental blocks) Oral gavage	GD1–GD17	Maternal effects: Overt toxicity, Incidence of pregnancy, Body weight development	3.0	--------	Serum PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days PFOS degradation observed Potential PFOA contamination in some exposure groups	--------
				Offspring effects: Body weight development (for between weeks 3 to 11 and weeks 12 to 20) Terminal BMI (no statistically significant differences in feed intake between groups at week 20)	3.0	--------	Serum concentrations determined for offspring Data reporting sometimes combine WT and Min/+ data, which did not allow for determining how genotype affected the endpoint observation PFOS degradation observed	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: Blood glucose levels	3.0	--------	Potential PFOA contamination in some exposure groups	--------
				Offspring effects, organ weights: Liver (absolute and relative) Spleen (absolute and relative)	3.0	--------		--------
Rosen et al. (2009)	Mice, CD1	0, 5, 10 mg/kg/day	GD1–GD17	Maternal effects: Body weight General appearance	10	--------	Internal PFOS concentration not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				Offspring effects: Litter size	10	--------	Internal PFOS concentrations not	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects, histology: Liver (presence of eosoinphilic granules with ≥5 mg/kg/day) Lung (no apparent effects) (limited sample size prevented determination of NOAEL and LOAEL)	--------	--------	determined for offspring Small sample size for some observations Only qualitative data reported	--------
Thibodeaux et al. (2003)	Mice, CD-1	0, 1, 5, 10, 15, 20 mg/kg/day Oral gavage	GD1–GD17	Maternal effects: ↓ weight gain (no effect on food consumption)	15	20	Serum PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days Thyroid hormone measurements may be subject to negative bias based on analytical method used	--------
				Maternal effects, hepatic endpoints: ↑ liver weight (absolute and relative)	1	5		--------
				Maternal effects, clinical chemistry: ↓ TRIG	1	5		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Maternal effects, clinical chemistry: Total BILI, CHOL, GLUC, SBA, SDH	20	--------		--------
				Maternal effects, endocrine endpoints: Total T4 (transient reduction by GD6 but return to normal levels by end of pregnancy)	20	--------		--------
				Fetal effects: Implantation sites	20	--------	Serum PFOS concentrations not determined for fetal tissue	--------
				Fetal effects: ↓ percentage of live fetuses	15	20		--------
				Fetal effects, teratology: ↑ cleft palate, sternal defects, enlarged right atrium, ventricular septal defects	10	15		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Fetal effects, body weight: ↓ body weight (statistically significant reductions with 10 and 15 mg/kg but not 20 mg/kg)	5	10		--------
				Fetal effects, hepatic endpoints: ↑ liver weight (absolute and relative)	15	20		--------
	Rats, Sprague-Dawley	0, 1, 2, 3, 5, 10 mg/kg/day Oral gavage	GD2–GD20	Maternal effects, body weight: ↓ weight gain (reduction in food and water consumption with ≥5 mg/kg/day)	1	2	Serum and liver PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days Thyroid hormone measurements may be subject to negative bias based on	--------
				Maternal effects, hepatic endpoints: ↑ relative liver weight (no effect on absolute liver weight)	5	10		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Maternal effects, clinical chemistry: ↓ CHOL, TRIG	5	10	analytical method used	--------
				Maternal effects, clinical chemistry: Total BILI, GLUC, SBA, SDH	10	--------		--------
				Maternal effects, endocrine endpoints: Corticosterone, prolactin	10	--------		--------
				Maternal effects, endocrine endpoints: ↓ T3, T4 (no effect on TSH)	--------	1		--------
				Fetal effects: Number of implantation sites, percentage of live fetuses	10	--------	Serum PFOS concentrations not determined for fetal tissue Liver PFOS concentrations determined for fetal tissue	--------
				Fetal effects, body weight: ↓ body weight	5	10		--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
				Fetal effects, teratology: ↑ cleft palate, sternal defects, anasarca, enlarged right atrium, ventricular septal defects	5	10		--------
				Fetal effects, hepatic endpoints: Liver weight (absolute and relative)	10	--------		--------
Wan et al. (2010)	Rats, Sprague-Dawley	0, 0.1, 0.6, 2.0 mg/kg/day Oral gavage	GD2–GD21	Offspring effects: ↓ number of delivered pups per litter (at PND3) (determined on PND3)	0.6	2.0	Serum and liver PFOS concentrations determined for offspring Internal PFOS concentrations not determined for dams Maternal effects not reported Internal PFOS concentrations only	4,260 (determined on PND21, after endpoint assessment)
				Offspring effects: ↑ mortality (at PND3) (determined on PND3)	0.6	2.0		4,260 (determined on PND21, after endpoint assessment)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects, body weight: ↓ body weight (at PND21) (determined at PND21)	0.6	2.0	reported for PND21 and not PND3	4,260 (determined on PND21)
				Offspring effects, hepatic effects: ↑ relative liver weight (at PND21) (no effect on absolute liver weight) (determined on PND21)	0.6	2.0		4,260 (determined on PND21)
				Offspring effects, hepatic effects: Histopathology (e.g., hepatocyte hypertrophy, cytoplasmic vacuolation, at PND21)	2.0	--------		--------
Wan et al. (2014)	Mice, CD-1	0, 0.3, 3 mg/kg Oral gavage	GD3–PND21 (weaning)	Maternal effects, body weight: Body weight	3	--------	Serum and liver PFOS concentrations determined for dams	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Only results for standard diet summarized herein for PND63				Maternal effects, hepatic endpoints: ↑ relative liver weight (no effect on absolute liver weight) (determined on PND21)	0.3	3	Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days	131,720 (determined on PND21)
				Maternal effects (endocrine): ↑ HOMA-IR (non-statistically significant increases in fasting glucose and fasting insulin with ≥0.3 mg/kg) (determined on PND21)	--------	0.3		15,330 (determined at PND21)
				Offspring effects, body weight: Body weight (at PND21 and between PND21 to PND63)	3	--------	Serum and liver PFOS concentrations determined for offspring	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects, hepatic endpoints: ↑ relative liver weight (males and females at PND21, males only at PND63) (↑ absolute liver weight statistically significant in males only at PND21 and PND63 with 3 mg/kg) (determined at PND63)	Males: ---- Females: 3 (based on PND63 data for relative liver weight)	Males: 0.3 Females: ---- (based on PND63 data for relative liver weight)	Only two dose levels used	Males: 300 Females: ---- (determined at PND63)
				Offspring effects: ↑ fasting serum glucose (males and females at PND63) (no effects at PND21) (determined at PND63)	-------- (based on PND63 data)	0.3 (based on PND63 data)		Males: 300 Females: 510 (determined at PND63)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: ↑ fasting serum insulin (males and females at PND63) (↑ males only at PND21 with ≥0.3 mg/kg) (determined at PND63)	0.3 (based on PND63 data)	3 (based on PND63 data)		Males: 3,360 Females: 3,400 (determined at PND63)
				Offspring effects: ↑ HOMA-IR (males and females at PND63) (no effects at PND21) (determined at PND63)	0.3 (based on PND63 data)	3 (based on PND63 data)		Males: 3,360 Females: 3,400 (determined at PND63)
				Offspring effects: OGTT (males and females at PND63) (data not reported for PND21)	3 (based on PND63 data)	-------- (based on PND63 data)		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Wang et al. (2011c)	Rats, Wistar	0, 3.2, 32 mg/kg Dietary	GD1–PND14 (sacrifices on PNDs1, 7, and 14)	Maternal effects: General toxicity, food intake	32	--------	Serum and brain PFOS concentrations determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure >30 days	--------
				Maternal effects, endocrine endpoints: ↓ total T3 (at PND1) (data not complete for PNDs7 and 14) (determined at PND1)	3.2	32		16,900 (determined at PND1)
				Maternal effects, endocrine endpoints: ↓ total T4 (at PND1) (↓ at PND7 but high dose data not reported, data not complete at PND14) (determined at PND1)	-------- (based on PND1 data)	3.2 (based on PND1 data)		2,290 (determined at PND1)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects: ↓ pup body weight (at PNDs1, 7, and 14) (determined at PNDs1, 7, and 14)	3.2	32	Serum and brain PFOS concentrations determined for offspring Sample size not reported for every endpoint Only two doses used	32,900 (determined at PND1) 21,300 (determined at PND7) 25,200 (determined at PND14)
				Offspring effects, endocrine endpoints: ↓ total T3 (at PND14) (no effect at PNDs1 and 7) (determined at PND14)	3.2	32		25,200 (determined at PND14)
				Offspring effects, endocrine endpoints: ↓ total T4 (at PND 7 and 14) (↓ at PND1 with 32 mg/kg) (determined at PNDs7 and 14)	-------- (based on PNDs7 and 14 data)	3.2 (based on PNDs7 and 14 data)		3,650 (determined at PND7) 4,890 (determined at PND14)

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
Wang et al. (2015)	Rats, Wistar	0, 5, 15 mg/L Drinking water	Dams: GD1–weaning Offspring: weaning–PND35 Cross-fostering initiated on PND1[a]	Offspring effects, reproductive/ developmental endpoints: ↓ survival (from birth to PND1, percentage of pups per litter) (no effect on number of pups born per litter)	5 mg/L	15 mg/L	Hippocampus PFOS concentrations determined for offspring Internal PFOS concentrations in offspring only determined for PND35 Internal PFOS concentrations not determined for dams Maternal toxicity not reported Only two doses used	--------
				Offspring effects, neurotoxicity: Visual and motor functions (swimming speed and time to reach visible platform)	15 mg/L	--------		--------
				Offspring effects, neurotoxicity: ↑ escape latency (learning ability) (statistically significant effects observed for both doses in TC and CT groups and only in TT15 group)	-------- (based on TC and CT groups)	5 mg/L (based on TC and CT groups)		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects, neurotoxicity: ↑ escape distance (learning ability, at training day 7 for TC group) (statistically significant effects observed at various training days for other groups)	-------- (based on TC group)	5 mg/L (based on TC group)		--------
				Offspring effects, neurotoxicity: ↓ time spent in target quadrant and number of platform crossings (spatial memory, only observed for TT15)	5 mg/L	15 mg/L		--------
Yahia et al. (2008)	Mice, ICR	0, 1, 10, 20 mg/kg/day Oral gavage	Prenatal study: GD0–	Maternal effects: Deaths	20	--------	Internal PFOS concentrations not determined for dams	--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
			GD17, sacrifice on GD18 Postnatal study: GD0–GD18, sacrifice following natural birth	Maternal effects, body weight: ↓ weight gain (GD11 until end of gestation) (↓ daily feed consumption GD14 onward and ↑ daily water consumption GD11 onward with 20 mg/kg)	10	20	Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------
				Maternal effects, hepatic endpoints: ↑ liver weight (hypertrophy with 20 mg/kg)	1	10		--------
				Maternal effects, organ weights: Kidneys, lungs, brains	20	--------		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (prenatal study): ↓ percentage of live fetuses (non-statistically significant increases in percentage of resorbed fetuses and percentage of dead fetuses)	10	20	Internal PFOS concentrations not determined for offspring Strain of mouse not very common and appropriateness for endpoints unclear	--------
				Offspring effects (prenatal study): ↓ fetal body weight	1	10		--------
				Offspring effects (prenatal study): Bilateral swelling in back of neck (100% incidence)	10	20		--------
				Offspring effects (prenatal study): ↑ sternal defects (percentage of fetuses) (statistically significant increases in other structural defects observed with ≥10 mg/kg)	--------	1		--------

Table 24. Study summary table for reproductive/developmental effects in animals								
Reference	***Species/ Strain***	***Administered Doses and Route***	***Duration***	***Endpoint(s)***	***NOAEL**** (mg/kg/d unless noted)	***LOAEL**** (mg/kg/d unless noted)	***Comment(s)***	***Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)***
				Offspring effects (postnatal study): ↓ survival (percentage of pups at PND4)	1	10		--------
				Offspring effects (postnatal study): ↓ body weight	1	10		--------
				Offspring effects (postnatal study): Bilateral swelling in back of neck (100% incidence)	10	20		--------
Ye et al. (2012)	Rats, Sprague-Dawley	0, 5, 20 mg/kg	GD12–GD18	Maternal effects: Deaths	20	--------	Internal PFOS concentrations not determined for dams Maternal effects included to inform fetal/neonatal effects Maternal exposure <30 days	--------

Table 24. Study summary table for reproductive/developmental effects in animals

Reference	*Species/ Strain*	*Administered Doses and Route*	*Duration*	*Endpoint(s)*	*NOAEL** (mg/kg/d unless noted)	*LOAEL** (mg/kg/d unless noted)	*Comment(s)*	*Serum PFOS concentration (in ng/mL) corresponding to the LOAEL (day assessed)*
				Offspring effects: Lung histology	20	--------	Internal PFOS concentrations not determined for offspring Qualitative data reported Dam and fetal weights recorded by not reported PFOS purity not reported Only two doses used	--------

* NOAELs are defined herein as the highest dose that did not produce a statistically significant (e.g., $p<0.05$) effect and LOAELs are defined herein as the lowest dose with statistically significant (e.g., $p<0.05$) effects. For some endpoints, there were dose-related trends that included non-statistically significant changes at lower doses than the LOAEL.

↑ = increased; ↓ = decreased
-------- = not applicable

a = cross-fostering groups from Wang et al. (2015) defined as: CC = no prenatal and no postnatal exposure; TT5 or TT15 = prenatal and postnatal exposure to 5 or 15 mg/L, respectively; CT5 or CT15 = only postnatal exposure to 5 or 15 mg/L, respectively; TC5 or TC15 = only prenatal exposure to 5 or 15 mg/L, respectively

BILI = bilirubin; BMI = body mass index; CHOL = cholesterol; CL = chemiluminometric; GLUC = glucose; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment for insulin resistance; Ig = immunoglobulin; LD = lactation day; LDL = low density lipoprotein; MAL = mevalonic acid lactone; NK = natural killer; OGTT = oral glucose tolerance test; RIA = radioimmunoassay; SBA = serum bile acid; SDH = sorbitol dehydrogenase; SRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TRIG = triglycerides; TSH = thyroid stimulating hormone

Human epidemiological studies

A summary of reproductive/developmental effects in humans can be found in Tables 25 and 26 at the end of the following review. Detailed methodological information and additional study results can be found in the corresponding tables in Appendix 6.

Reproductive effects

Fertility

Studies evaluated the association between serum PFOS and several closely related measures of reproductive ability in populations with PFOS serum concentration levels prevalent in the general population: infertility (Caserta et al., (2013); Fei et al, (2009); Jørgensen et al. (2014)); La Rocca et al. (2014)); time to pregnancy (Fei et al., (2009, 2012); Jørgensen et al. (2014)); fecundity (the probability of conceiving within a fixed time period, generally one month or one menstrual cycle) (Fei et al (2009, 2012); Jørgensen et al. (2014); Vestergaard et al. (2012)); and sub-fecundity (time to pregnancy > 6 cycles) (Vestergaard et al. (2012)). Only the linked studies of Fei et al (2009, 2012) found significant associations between PFOS and measures of relative difficulty in conceiving (increased infertility, increased time to pregnancy, decreased fecundity).

Fei et al. (2012) was also the only one of these studies that stratified on the basis of parous/nulliparous (i.e., previous pregnancy/no previous pregnancy). In that study, the clearest indication of a significant association between PFOS exposure and time to pregnancy or fecundity was for nulliparous women. This may be relevant since pregnancy and lactation are known to reduce maternal PFOS body burden, and it has, therefore, been argued that the apparent association of PFOS and time to pregnancy could be the result of reverse causation (i.e., those with previous successful pregnancies have lower levels of serum PFOS as a result of the pregnancies). The positive association for nulliparous women, however, is not compatible with an explanation based on reverse causation.

Despite the consistent findings of the Fei et al. (2009, 2012) studies across related indicators of fertility and the evidence from Fei et al. (2012) that reverse causation was not responsible for those findings, there is no consistent evidence for an association of PFOS and reduced fertility.

Birth weight and related reproductive endpoints

Individual epidemiology studies addressing to birth weight and related reproductive endpoints are presented in Table 25. Endpoints from developmental studies are summarized in Table 26. Epidemiology studies have not shown a consistent decrease in birthweight with reference to maternal serum concentration of PFOS. In a birth sub-sample of a larger cohort from the UK with a median maternal serum PFOS concentration of 19.6 ng/ml (Maisonet et al., 2012), there was a significant negative association between maternal, gestational period, serum PFOS concentration and birthweight. The analyses adjusted for various maternal factors, including previous pregnancies. This is an important consideration since maternal PFOS body burden decreases during pregnancy. In this study, maternal serum PFOS concentration was also significantly negatively associated with birth length, but not with Ponderal Index [a measure of body leanness calculated as: body mass (kg)/height^3 (m^3)], or gestational age. In a study nested within the C8 Health Study cohort (Darrow et al., 2013) with a geometric mean maternal serum PFOS concentration of 13.1 ng/ml, maternal serum PFOS concentration was significantly negatively associated with continuous birthweight (for first pregnancies with prospective maternal serum PFOS measurements only). However, maternal PFOS was not associated with the category of low birthweight. In contrast, other studies (Fei et al. (2007, 2008); Hamm et al., (2010); Robledo et al. (2015)) with comparable exposures did not show a significant negative association between maternal PFOS exposure and birthweight, or

categorical low birth weight (Darrow et al. (2013), or Ponderal Index [Apelberg et al. (2007) for cord blood; Maisonet et al. (2012); Robledo et al. (2015)].

Summary of epidemiologic studies on birthweight effects

Although there is a suggestion of a relationship between maternal PFOS exposure and decreased birthweight from epidemiological studies, the evidence is not consistent. This lack of consistency among studies does not appear to be a direct function of differences in the range of exposures among the populations studied. However, these studies have addressed populations with a relatively narrow range of exposures (central tendency estimates of maternal serum PFOS concentrations in the range of 5-35 ng/ml) that are generally consistent with general population level exposures to PFOS. These observations therefore do not rule out an association at higher levels of PFOS exposure or more subtle effects in pregnancies at increased risk for low birthweight.

Puberty

Three studies were identified that investigated an association between PFOS and the onset of female puberty. Female puberty was determined based on the self-reported age at onset of menarche. In the case of the Lopez-Espinosa et al. (2011) study determination of puberty was based either on self-reported menarche or serum estradiol levels. In two of these studies [Christensen et al. (2011), Kristensen et al. (2013)], the PFOS concentration was based on a maternal pregnancy sample. In the Lopez-Espinosa (2011) study (C8 cohort, n = 2,931), the PFOS concentration was based on the girls' serum PFOS at the time of recruitment (8-18 years old). For the studies based on maternal PFOS, there was no association with onset of female puberty. In the Lopez-Espinosa et al. (2011) study there was a significant association between delayed onset of puberty and girls' serum PFOS concentration based on estradiol levels and age at menarche. There is a possibility of confounding of this result through reverse causality since earlier onset of menarche would result in a decreased body burden and serum concentration of PFOS, whereas delayed onset of menarche (independent of PFOS causation) would allow for retention of a larger body burden of PFOS.

Male puberty was only addressed in the same Lopez-Espinosa et al. (2011) C8 cohort study (n =3,076). Male puberty was determined on the basis of testosterone levels. PFOS was significantly associated with delayed onset of male puberty. Unlike the case for females, there is no obvious confounding of this association due to reverse causality.

While the Lopez-Espinosa et al. (2011) study found a significant association between childhood PFOS exposure and delayed onset of puberty for both females and males in a large-scale study, it is the only study to examine such an association. Similarly, there were only two available studies that showed a lack of association between maternal PFOS exposure and the onset of female puberty. Thus, there are insufficient data upon which to draw conclusions about associations between PFOS exposure (either maternal or childhood) and the onset of puberty.

Preterm birth

Five studies were identified that investigated a possible association between maternal serum PFOS and outcomes related to preterm birth or related outcomes (premature birth, length of gestation, gestational age). Of these, only one study (Stein et al., 2009) showed a significant association with maternal PFOS (for premature birth at < 37 wks). This was a study nested in the C8 cohort (n = 4,512; median PFOS concentration = 13.6 ng/ml). The OR for premature birth for each inter-quartile increase in PFOS concentration was 1.3, and the OR for the fourth quartile compared with the first quartile of PFOS exposure was 1.8. Fei et al. (2007) (n = 50), Darrow et al. (2013) (n = 1,630) and Hamm et al. (2010) (n = 252) found no significant assocation. Olsen et al. (2004) (n = 122) also found no association between high versus low occupational PFOS exposure and pre-term labor compiled as episodes of

care under the workers' health coverage. Exposure assessment in this study was based on air concentration rather than in serum, and even the low exposure group had an elevated level of exposure.

The positive finding in the large-sized Stein et al. (2009) study provides some support for an association between maternal PFOS exposure and preterm birth. However, the finding from this one study is not sufficient to draw overall conclusions.

Miscarriage

The possibility of an association between maternal PFOS exposure and miscarriage was only addressed by two studies, both of which investigated the C8 cohort. Stein et al. (2009) was a retrospective study based on self-reported outcomes up to five years prior to enrollment in the cohort. Darrow et al. (2013) was a prospective study that tracked women post-enrollment. Although neither found a significant association for the study cohorts as a whole, Darrow et al. (2013) found a significant OR (1.34) for miscarriage during first pregnancy.

Preeclampsia

Both of the C8 cohort studies referenced above in the discussion of miscarriage (Stein et al (2009) ($n \approx 5{,}000$, mean = 15.0 ng/ml) and Darrow et al. (2013) ($n = 1{,}630$, geo. mean = 13.1 ng/ml) found significant positive associations between maternal PFOS exposure and preeclampsia (pregnancy-induced hypertension combined with increased urinary protein). The much smaller, Starling et al. (2014a) study of the Norwegian Mother and Child Study cohort (cases = 466, controls = 510; median = 12.87 ng/ml) did not find such an association. The finding of a positive association in the large C8 cohort in both retrospective and prospective studies suggests the possibility of true association.

Placental weight

Fei et al. (2008) found no association of placental weight with maternal PFOS exposure in the large Danish National Birth Cohort ($n = 91{,}827$).

Duration of breast feeding

Only one study was identified that addressed a possible association between maternal PFOS exposure and the duration of breast feeding. Fei et al. (2010a), investigating the large Danish National Birth Cohort ($n = 91{,}827$), found a positive association between PFOS exposure and cessation of breast feeding at < 6 months, but not at < 3 months. The relationship for cessation at < 6 months was significant for both primaparous and multiparous women. For overall duration of breast feeding as a continuous variable, the association with PFOS was significant for multiparous women only.

Sperm/semen characteristics

In two studies examining sperm morphology (Joensen et al., 2009; Toft et al., 2012), no effect on sperm morphology was significantly associated with PFOS exposure. The only significant association of sperm morphology with men's serum PFOS was a negative association with the occurrence of coiled tail (Louis et al., 2015). As coiled tail is considered to be an adverse indicator of sperm viability, the significance of this observation is unclear. No association between men's serum PFOS concentration and semen volume was observed in four general population studies with moderate to high levels of exposure [Joensen et al. (2009), Raymer et al. (2012), Toft et al. (2012), Vested et al. (2013)]. Sperm count was not significantly associated with PFOS serum concentration in three studies [Joensen et al. (2009), Toft et al. (2012), Vested et al. (2013)]. Sperm concentration

was also not significantly associated with serum PFOS in four studies [Joensen et al. (2009), Raymer et al. (2012), Toft et al. (2012), Vested et al. (2013)]. Neither semen, pH, viscosity, nor liquification were found to be significantly associated with serum PFOS in a single study (Raymer et al., 2012). In four studies of various measures of sperm motility [Joensen et al. (2009), Raymer et al. (2012), Toft et al. (2012), and Vested et al. (2013)]. PFOS was not significantly associated with motility. The only significant association was for increased distance migrated as a function of PFOS exposure (Louis et al., 2015). As increased distance migrated is considered an indication of sperm viability, the interpretation of this outcome is unclear.

In a single study (Kvist et al., 2012) of multiple populations (Greenland, Poland, Ukraine) the Y:X chromosome ratio in sperm was significantly positively associated with serum PFOS for the pooled study population, but no significant relationship was observed when examining each population separately. However, in a MANOVA analysis, the Greenland population, with the highest serum PFOS concentration (mean = 51.65 ng/ml) was significantly negatively correlated with the Y:X ratio. This relationship was driven by the difference between the third and fourth quartiles of serum PFOS. It is difficult to draw conclusions from these data.

Overall, there is little to no evidence from epidemiologic studies linking adverse effects in either sperm or semen with PFOS exposure.

Testicular volume

In a single study (Vested et al., 2013), testicular volume was not associated with serum PFOS concentration.

Female reproductive organs/menstruation

No association was observed between serum PFOS and the incidence of endometriosis (either all cases, or stages 3-4) (Louis et al., 2012). No association was observed between the length of the menstrual cycle and serum PFOS in either a study in which serum PFOS and cycle length were determined in the same adult women (Lyngsø et al., 2014), or in a study in which maternal serum PFOS was measured during the second trimester of pregnancy and data on cycle length was determined in the daughters (Kristensen et al., 2013).

In a case-control study of individuals recruited from specialty clinics and advertisements, serum PFOS concentration was significantly higher in polycystic ovary syndrome cases (n = 52) compared to controls (n = 50) (OR = 5.76) (Vagi et al. 2014). However, there are some significant weaknesses in this study including small sample size and the potential for reverse causation. In a nested-cohort of the Danish National Birth Cohort (Kristensen et al., 2013), there was no significant association between maternal, second trimester PFOS exposure and the number of follicles per ovary in daughters either with (n = 171), or without (n = 75) hormonal contraception.

In a nested case-control (107 cases and 108 controls) study of cryptorchidism, there was no significant difference in cord blood PFOS concentration (Versterholm-Jensen et al., 2014).

Sex hormones

In analyses of possible associations of sex hormones (testosterone, estradiol, SHBG, FSH, LH, inhibin B, free androgen index, dehydroepiandrosterone, anti-mullerian hormone, and gonadotropin hormones) and PFOS exposure (adult and gestational) among four different studies (Joensen et al. (2009), Kristensen et al. (2013), Specht et al. (2012), Vested et al. (2013)) in males and females (not all parameters measured in each study), no significant associations were observed.

Menopause

No association was observed between the age-adjusted probability of having achieved menopause and serum PFOS (Taylor et al. (2014).

Summary of reproductive effects

Overall, there are no clear consistent observations of associations between reproductive effects and PFOS exposure. However, it is interesting to note that those studies that did observe significant associations of reproductive effects with PFOS exposure [decreased birthweight (Darrow et al., 2013); delayed onset of male and female puberty (Lopez-Espinosa et al., 2011); premature birth (Stein et al., 2009); miscarriage in first pregnancy (Darrow et al., 2014); and preeclampsia (Darrow et al.,2013; Stein et al., 2009)] tended to be studies of the C8 cohort. These studies had large sample sizes and, therefore, greater power to observe relatively low-probability outcomes.

Developmental effects

Neurobehavior

Neurobehavioral performance in neonates (Donauer et al., 2015) was not associated with maternal pregnancy serum PFOS concentration. Behavioral difficulties at seven years of age in the Danish National Birth Cohort (Fei and Olsen, 2011) were also not significantly associated with maternal pre-pregnancy serum PFOS exposure.

Neuromotor

Cord blood PFOS was significantly associated with decreased gross motor skills in 2-year olds in a Taiwanese cohort (Chen et al., 2013). PFOS exposure in this cohort was relatively low (mean= 7.0 ng/ml). Relatively elevated maternal pre-pregnancy PFOS exposure (median = 34.4 ng/ml) was significantly associated with negative (adverse) assessment of coordination disorders in the Danish National Birth Cohort (Fei and Olsen, 2011).

Cerebral palsy

In a case-control study nested within the Danish National Birth Cohort (Liew et al., 2014), the maternal pregnancy (1^{st} or 2^{nd} trimester) PFOS serum level was significantly higher in cerebral palsy cases (n = 156, 28.9 ng/ml) than in controls (n = 550, 27.6 ng/ml) for boys only (risk ratio= 1.7-2.1).

Morphogenic parameters

Only one study (Halldorsson et al., 2012) evaluated morphogenic parameters (BMI, waist circumference, overweight) at 20 years old as a function of maternal pregnancy PFOS exposure. None of these parameters were significantly associated with maternal PFOS exposure.

Summary of developmental effects

There is some suggestion of an association between gestational PFOS exposure and neuromotor effects including gross motor, coordination and cerebral palsy. However, since cerebral palsy can be related to delivery difficulties, it is not clear to what extent an association of gestational PFOS exposure with cerebral palsy is consistent with other measures of neuromotor performance.

Table 25. Summary of Epidemiology Studies of Reproductive Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study references**
Fetal or postnatal growth	Birthweight =	Mean 35 (maternal)	Fei (2007)
	Birthweight =	Mean 35.3	Fei et al. (2008)
	Birthweight =	Mean 9.0 (maternal)	Hamm et al. (2010)
	Birthweight ↓	Med. 19.6 (maternal)	Maisonet et al. (2012)
	Birthweight =	Med. 12.44 (maternal)	Robledo et al. (2015)
	Birthweight ↓	Geo. mean 13.1 (maternal)	Darrow et al. (2013)
	Low birthweight =	Geo. mean 13.1 (maternal)	Darrow et al. (2013)
	Child weight (1-11 mos) =	Mean 1.6 (cord)	de Cock et al. (2014a)
	Head circum. ↓	Med. 5 (cord)	Apelberg et al.(2007)
	Head circum. = (1-11 mos.)	Mean 1.6 (cord)	de Cock et al. (2014a)
	Head circum. =	Mean 35.3	Fei et al. (2008)
	Ponderal index = (equivocal)	Med. 5 (cord)	Apelberg et al.(2007)
	Ponderal index =	Med. 19.6 (maternal)	Maisonet et al. (2012)
	Ponderal index =	Med. 12.44 (maternal)	Robledo et al. (2015)

Table 25. Summary of Epidemiology Studies of Reproductive Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study references**
Fertility	Infertility =	18-32% > LOD	Caserta et al. (2013)
	Infertility ↑	Med. 33.7	Fei et al (2009, 2012)
	Infertility =	Med. 10.6	Jørgensen et al. (2014)
	Infertility =	Med. < 0.4	La Rocca et al. (2014)
	Time to pregnancy ↑	Med. 33.7	Fei et al (2009, 2012)
	Time to pregnancy =	Med. 10.6	Jørgensen et al. (2014)
	Fecundity ↓	Med. 33.7	Fei et al (2009, 2012)
	Fecundity =	Med. 10.6	Jørgensen et al. (2014)
	Sub-fecundity/fecundity ratio	Med. Non-preg 35.75, preg -Preg 36.29	Vestergaard et al. (2012)
Puberty	Menarche Decreased age =	Med. 19.8 (maternal)	Christensen et al. (2011)
	Menarche =	Med. 3.6 (maternal)	Kristensen et al. (2013)
	Menarche/puberty ↓	Med. 18	Lopez-Espinosa et al. (2011)
	Male (testosterone cutoff) ↓	Med. 20	Lopez-Espinosa et al. (2011)
Gestation	Preterm birth =	Mean 13.1	Darrow et al. (2013)
	Preterm birth =	Mean 9.0	Hamm et al. (2010)
	Premature birth ↑	Med. 13.6	Stein et al. (2009)
	Length of gestation =	Mean 35	Fei (2007)
	Length of gestation =	Mean 9.0	Hamm et al. (2010)
	Gestational age =	Med. 19.6	Maisonet et al. (2012)
	Miscarriage =	Geo. mean 14.3	Darrow et al. (2014)
	Miscarriage (1st preg) ↑	Geo. mean 14.3	Darrow et al. (2014)
	Miscarriage =	Med. 13.6	Stein et al. (2009)
	Pre-term labor =	Air conc. H = 0.6-2.0 ppm L = 0.4 ppm Minimal = 0.1-0.2 ppm	Olsen et al. (2004)
	Preeclampsia (preg induced hypertension) ↑	Mean 13.1	Darrow et al. (2013)
	Preeclampsia =	Med. 12.87	Starling et al. (2014a)
	Preeclampsia ↑	Med. 13.6 ng/ml	Stein et al. (2009)
	Placental weight =	Mean 35.3	Fei et al. (2008)
Breast feeding	Weaning < 3 mos (first child) =	Med. 32.3 -37.0	Fei et al. (2010a)
	Weaning < 6 mos (first child) ↑	Med. 32.3 -37.0	Fei et al. (2010a)
	Duration First child = (sig only for multiparous)	Med. 32.3 -37.0	Fei et al. (2010a)

Table 25. Summary of Epidemiology Studies of Reproductive Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study references**
Sperm/semen	Morphology =	Med. 24.5	Joensen et al. (2009)
	Morphology (coiled tail) ↓	Med. 19.5-21.6	Louis et al. (2015)
	Morphology (% normal)	Med. 18.4	Toft et al. (2012)
	Volume =	Med. 24.5	Joensen et al. (2009)
	Volume =	Med. 32.3	Raymer et al. (2012)
	Volume =	Med. 18.4	Toft et al. (2012)
	Volume =	Med. 21.2 (maternal – long. Study)	Vested et al. (2013)
	Count =	Med. 24.5	Joensen et al. (2009)
	Count =	Med. 18.4	Toft et al. (2012)
	Count =	Med. 21.2 (maternal – long. Study)	Vested et al. (2013)
	Concentration =	Med. 24.5	Joensen et al. (2009)
	Concentration =	Med. 32.3	Raymer et al. (2012)
	Concentration =	Med. 18.4	Toft et al. (2012)
	Concentration =	Med. 21.2 (maternal – long. Study)	Vested et al. (2013)
	Motility =	Med. 24.5	Joensen et al. (2009)
	Motility (dist migrated) ↑	Med. 19.5-21.6 ng/ml	Louis et al. (2015)
	Motility =	Med. 32.3	Raymer et al. (2012)
	Motility =	Med. 18.4	Toft et al. (2012)
	Motility (% progressive) =	Med. 21.2 ng/ml (maternal – long. Study)	Vested et al. (2013)
	pH =	Med. 32.3	Raymer et al. (2012)
	Liquification =	Med. 32.3	Raymer et al. (2012)
	Viscosity =	Med. 32.3	Raymer et al. (2012)
	Testicular volume =	Med. 21.2 (maternal – long. Study)	Vested et al. (2013)
Sex ratio	X:Y chromosome ratio (pooled) ↑ (for pop. w highest conc ↓)	8.2-51.65 (multiple populations)	Kvist et al. (2012)
Endometriosis	All and stage 3-4 =	Geo. mean 6.11-7.41	Louis et al. (2012)
Menstrual cycle	Length =	Med. 5.0 -20.2 (multiple pops.)	Lyngsø et al. (2014)
	Length =	Med. 3.6	Kristensen et al. (2013)

Table 25. Summary of Epidemiology Studies of Reproductive Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study references**
Polycystic ovary syndrome	OR ↑	Geo. mean cases = 8.2 controls = 4.9	Vagi et al. (2014)
	Follicles/ovary =	Med. 3.6	Kristensen et al. (2013)
Menopause	Achieved menopause (age adj.) =	Med. 10.3-17.5 (diff. pops. for each endpoint)	Taylor et al. (2014)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association			

Table 26. Summary of Epidemiology Studies of Developmental Effects			
Endpoint	**Effect and Direction**	**Serum PFOS concentration (ng/ml) (mean, median, etc.)**	**Study references**
Neurobehavioral	Neurobehv. Scale =	Geo. mean 13.25 (maternal)	Donauer et al. (2015)
	SDQ (behav. Difficulties) =	Med. 34.4	Fei and Olsen (2011)
Neuromotor	Gross motor ↓	Mean 7.0 (cord)	Chen et al. (2013)
	DCDQ (coordination) ↓	Med. 34.4	Fei and Olsen (2011)
Cerebral palsy	↑ (boys only)	Med. 26-29	Liew et al. (2014)
Morphogenic	BMI (offspring at 20 yrs old) =	Med. 21.5 (maternal)	Halldorsson et al. (2012)
	Waist circum. (offspring at 20 yrs old) =	Med. 21.5 (maternal)	Halldorsson et al. (2012)
	Overweight (offspring at 20 yrs old) =	Med. 21.5 (maternal)	Halldorsson et al. (2012)
Genital	Cryptorchidism =	Med. 9.1	Versterholm-Jensen et al. (2014)
↑ statistically significant positive association ↓ statistically significant negative association = no significant association/equivocal association DCDQ: Developmental Coordination Disorder Questionnaire SDQ: Strengths and Difficulties Questionnaire			

Overall summary for reproductive and developmental effects

Animal data demonstrate that gestational PFOS exposure causes adverse effects in offspring including increases in offspring mortality, decreases in offspring body weight, and structural deformities. Additionally, animal data indicate that gestational PFOS exposure may cause endocrine and metabolic effects such as changes in thyroid hormone levels and in parameters associated with glucose metabolism. Human data do not provide clear, consistent evidence for reproductive effects following PFOS exposure. However, there is an indication of decreased birthweight and delays in developmental milestones in humans. Some human data suggest that PFOS may have developmental neurological effects. The overall weight of evidence appears to justify the inclusion of reproductive/developmental endpoints for dose-response evaluation.

Overall summary for non-cancer hazard identification

PFOS causes a number of different types of toxicological effects in animals including endocrine, hepatic, immune system, and developmental toxicity. In humans, epidemiology studies suggest an association of PFOS exposure with decreased vaccine response, elevated serum uric acid/hyperuricemia, and increased total cholesterol.

Carcinogenicity

Animal studies

Butenhoff et al. (2012) conducted the only chronic animal bioassay of PFOS. Their study exposed Sprague-Dawley rats of both sexes to PFOS by diet for up to 104 weeks. The study included a recovery group exposed to the highest concentration for 52 weeks and then kept on regular diet for the remaining study period. The data showing statistically significant incidence of tumors are summarized in Table 27 below.

Table 27. Summary of select tumor data from Butenhoff et al. (2012)

	sex	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm (recovery)	p-trend
Liver								
Hepatocellular Adenoma	M F	0/60 0/50	3/50 1/50	3/50 1/49	1/50 1/50	7/60 * 5/60 *	0/40 2/40	* *
Hepatocellular adenoma + carcinoma	F	0/60	1/50	1/49	1/50	6/60 *	2/40	**
Thyroid								
Follicular cell adenoma	M	3/60	5/49	4/50	4/49	4/59	9/39 *	
Mammary								
Fibroadenoma + adenoma	F	23/60	30/50 *	22/48	26/50	15/60 * [a]	16/40	* [b]

* $p \leq 0.05$ compared to controls or trend as indicated. ** $p \leq 0.01$ compared to controls or trend as indicated

a. Note that the significance is for a decreased incidence compared to controls.

b. Note that the significance is for an overall negative trend

It should be noted that the denominators of the incidence ratios, as reported in Butenhoff et al. (2012), apparently include animals with unscheduled mortality as well as interim and terminal sacrifices. Interim and unscheduled sacrifices, if conducted prior to the appearance of the first tumor, would have the effect of artificially increasing the presumed number of animals at risk of developing a tumor, thus increasing the denominator and thus, decreasing the incidence ratio (this issue is addressed in the Dose-Response section). Nonetheless, it is clear from the data as reported that both male and female rats exposed to 20 ppm dietary PFOS experienced statistically elevated hepatocellular tumor incidence.

Male rats also experienced a statistically elevated incidence of thyroid follicular tumors in the 20 ppm recovery group (Butenhoff et al., 2012). With respect to the statistically significant elevation in the incidence of thyroid follicular cell tumors observed in males in the 20 ppm recovery group, the authors consider this observation to be "paradoxical" given the absence of histopathological changes in the thyroid and the lack of a significantly elevated tumor incidence in the full term 20 ppm exposure group. Chang et al. (2009) exposed maternal Sprague-Dawley rats to PFOS from GD 1-20 or GD 1-PND 21, and several thyroid parameters potentially relevant to carcinogenicity were analyzed. No significant differences between PFOS exposed (maternal dose, 1.0 mg/kg/day) and control fetuses or pups were observed with respect to thyroid histology. Morphometric analysis of follicular epithelial height (a measure of increased thyroid activity) found a significant increase in PFOS treated female pups compared to controls at PND 21. However, the authors question the relevance of this observation due to an abnormally low follicular epithelial height in the relevant controls. In addition, thyroid follicular epithelial proliferation (cell counts) was significantly increased in 1 mg/kg/day PFOS maternally exposed GD 20 female fetuses at a level twice that of controls. Thus, the origin of these tumors and their potential relevance to human cancer risk is unclear.

Statistically significant increases were reported for mammary fibroadenomas and for combined mammary fibroadenomas/adenomas only in the low dose (0.5 ppm) group. The percent incidence of these tumors in each dose group was: Control – 38%; 0.5 ppm – 60%; 2 ppm – 45%; 5 ppm –52%; 20 ppm recovery – 40%; 20 ppm - 25%. When the incidence data were considered across all the dose groups for both categories of tumors, a statistically significant decreased trend was observed for these endpoints. This is due to the statistically significant decreases in the incidence of these tumors in the highest dose group compared to controls. No statistically significant changes in mammary carcinomas or adenomas alone were reported in any dose group. Based on these limited data, conclusions cannot be made about the potential for PFOS to cause mammary tumors.

Human epidemiology studies

There are a limited number of epidemiological studies assessing cancer risk from PFOS exposure. As reviewed below, these studies assessed cancer risk in occupationally exposed populations or in the general population.

Occupational studies

Studies of occupational PFOS exposure are all based on workers from a single facility (Decatur, AL) with high PFOS exposure (Alexander et al., 2003, 2007; Olsen et al., 2004; Grice et al., 2007). These studies have several drawbacks in identifying potential associations between PFOS exposure and cancer. Exposure assessment was indirect and involved job location/category linked with location-specific measurements of PFOS air concentration, or serum PFOS concentration from a relatively small sample of workers. For those studies utilizing serum PFOS concentrations from this sample, the "no" or "minimal" exposure category were approximately two orders of magnitude higher than that of the US median as reported by CDC (2017). This could potentially obscure an exposure-response relationship. Ascertainment of cancer cases, was generally indirect, or based on mortality rather than incidence. Finally, the cohorts contained relatively few women.

Alexander et al. (2003) found no association between estimated PFOS exposure and all cancer mortality. For liver cancer mortality, the standardized mortality ratio (SMR) was slightly elevated (1.61 observed versus 1.24 expected) but not statistically significant. For bladder cancer, the SMR was elevated (4.81 observed versus 0.62 expected) and borderline statistically significant. The SMR was slightly increased when the analysis was confined to workers employed for ≥ 5 years.

Alexander et al. (2007) followed up on the previous study (Alexander et al., 2003), focusing on bladder cancer. This study collected information on current and deceased bladder cancer cases and from current and former employees. Self reporting (n = 1,400, 67% of eligible) was combined with physician follow-up or death certification acquisition (n = 185, 98% of eligible). The bladder cancer incidence was elevated (standardized incidence ratio (SIR) = 1.28) but was not statistically significant. There did not appear to be a relevant exposure-response relationship. The SIR was also elevated, but not statistically significant when the analysis was confined to the high exposure category or to workers employed for 5-10, or > 10 years.

Olsen et al. (2004) reviewed employee health claims for treatment through the company's health insurance and compared exposed workers to "unexposed" workers. Malignancies of the colon (risk ratio; RR = 5.4), lower respiratory tract (RR = 2.7), skin (RR = 12) and prostate (RR = 79) were elevated but not statistically significant. Since "unexposed" workers were classified by job location/duties, and not serum concentrations, it is likely that these workers have at least general population level exposures to PFOS.

Grice et al. (2007) employed self-reported cancer diagnosis (n = 1,400, 74% of eligible). Estimated PFOS exposure was not associated with any cancer type.

Overall, studies of this worker population did not show consistent evidence of cancer in general or of cancer of any specific type.

General population studies

Eriksen et al. (2009) conducted a case (n = 67-713 depending on cancer type) control (n = 680) study nested in a prospective cohort (age: 50-65 years old, n = 57,051) using the Danish National Cancer Registry. The incident rate ratio (IRR) was not significant for cancer of any type for any quartile of serum PFOS concentration. Prostate cancer was elevated for quartiles 2-4 of serum PFOS (relative to the first quartile) and this elevation was borderline statistically significant at each quartile. However, there was no clear evidence of a trend across quartiles.

Bonefeld-Jorgensen et al. (2011) conducted a case (n = 31)-control (n = 115) study of breastcancer and PFOS exposure among Greenland Inuit. This population had a relatively high PFOS exposure (median concentration among cases = 45.6 ng/ml). The OR relative to a unit increase (ng/ml) of serum PFOS was small (1.03), but statistically significant. As a follow up, Ghisari et al. (2014) examined the relationship of single nucleotide polymorphisms (SNPs) in a number of cytochrome P450 (CYP) isoforms as a function of serum PFOS in the same cases and controls studied in Bonefeld-Jorgensen et al. (2011). For all CYP genes tested, the OR was significantly > 1.0 for the (dichotomous) high PFOS category for at least one SNP. While this is largely a population-based mechanistic study, it adds some weight to the association of PFOS exposure and breast cancer from the Bonefeld-Jorgensen et al. (2011) study in providing evidence that cases differed from controls in a biochemical characteristic that is potentially causal with respect to breast cancer.

Hardell et al. (2014) examined the association of PFOS with prostate cancer in a case (n = 201)- control (n = 186) study in Sweden. No significant association was detected between serum PFOS concentration and the OR for prostate cancer, the stage of prostate cancer (Gleason score), and the PSA (prostate-specific antigen) level. There was a significant OR for PFOS serum concentration and having a first order relative with prostate cancer. This significance of this observation is not entirely clear, however.

Summary of epidemiological evidence for cancer

Although individual studies have shown borderline or weak (albeit statistically significant) associations between PFOS exposure and specific cancer types, there is no consistent indication of an association between PFOS exposure and cancer in general, or any specific form of cancer. Nonetheless, the database cannot be considered strong. In contrast to PFOA (DWQI, 2017), there are no studies of communities with elevated exposures from contaminated drinking water or other environmental media. Exposure characterization and case ascertainment was problematic in the occupational studies with high levels of exposure, and the non-occupational studies generally had small sample sizes.

Overall conclusions regarding the potential for human cancer risk from PFOS

Based on the liver and thyroid tumors reported by Butenhoff et al. (2012), the designation of "Suggestive Evidence of Carcinogenic Potential" in the 2005 USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a) is appropriate. In particular, this determination is consistent with the descriptor: "*A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system*." USEPA Office of Water (2016b) also concluded that the descriptor "Suggestive Evidence of Carcinogenic Potential" as appropriate for PFOS. A discussion of the potential human relevance of the tumors observed in Butenhoff et al. (2012) is found in the Mode of action for carcinogenicity section (below).

MODE OF ACTION

General

As discussed in the Hazard Identification section, PFOS produces effects in multiple organ systems and tissues. At a minimum, strong evidence exists from animal and/or epidemiological studies for effects on the liver, the immune system, birth weight, and neonatal survival. In addition, PFOS causes liver tumors, and possibly thyroid tumors in rats. The breadth of these effects suggests that PFOS may cause toxicity through multiple modes of action (MOAs). However, as discussed below for hepatic, immune, and developmental effects, there is insufficient evidence to fully support a definitive MOA for any of the tissue/organ-specific effects of PFOS.

Role of PPARα and other receptors in hepatic effects of PFOS

While mode-of action data are most abundant for PFOS effects on the liver, most of the evidence relates to evaluation of the role of peroxisome proliferator-activated receptor-alpha (PPARα) in its hepatic effects.

Some hepatic effects (e.g., increased liver weight) of PFOS in rodents are similar to those caused by known and potent PPARα activators (e.g., Corton et al., 2014). On this basis, carcinogenic and non-carcinogenic hepatic effects of PFOS have sometimes been assumed to occur through activation of PPARα. However, several lines of evidence do not support a conclusion that liver effects due to PFOS exposure are PPARα-dependent.

PPARα is a member of the soluble nuclear receptor hormone superfamily (Peraza et al., 2006). There is evidence that endogenous fatty acid derivatives are the natural ligands for PPARα and that under normal circumstances, PPARα is involved with lipid homeostasis. It also appears that PPARα is involved (at least in some tissues) with cell proliferation, apoptosis, inflammation and oxidative stress (Peters et al., 2005).

The functioning of PPARα in response to exogenous chemicals has been most thoroughly documented in the liver. Compared to adult rodent liver, the abundance of PPARα mRNA in adult human liver is only about 10% (Abbott et al., 2009b). Also, for at least some exogenous agonists, the magnitude of response of rodent PPARα is greater than human PPARα (Peters and Gonzalez, 2011). The role played by PPARα in adverse hepatic effects has historically been largely derived from observation of the effects of model PPARα agonists such as WY-14,643, bezafibrate and ciprofibrate, which are assumed to be "pure" PPARα agonists (i.e., substances whose significant effects occur only as a result of PPARα binding). Bezafibrate and ciprofibrate are hypolipidemic pharmaceuticals with known peroxisome proliferation activity. WY-14,643 is a strong PPAR agonist and peroxisome proliferator used experimentally as a model PPARα agonist. Hays et al. (2005) found that exposure of wild-type (WT) Sv/129 mice to bezafibrate for one year resulted in the liver weight increase characteristic of PPARα agonists. In addition, they found altered liver foci in 100% of exposed mice, as well as occurrence of single adenomas and multiple adenomas and one carcinoma, with no neoplasms in the control WT mice. In contrast, PPARα-null mice exposed to bezafibrate for 1 year exhibited no clear treatment-related tumors. Peters et al. (1998) compared the responses of hepatic tissue from wild-type (WT) and PPARα -null mice treated for 11 months with WY-14,643. Exposure of the WT mice to WY resulted in increased production of proteins (and their corresponding mRNAs) involved in cell cycle regulation and cell proliferation. These included, cyclin-dependent kinases, c-myc, and PCNA (proliferating cell nuclear antigen). These responses, consistent with a cancer mode of action, were not seen in the PPARα-null mice.

In *in vitro* binding assays (Vanden Heuvel et al., 2006), PFOS bound to mouse, rat and human PPARα much less than ciprofibrate, the model PPARα agonist used a positive control in this study. Relative to the concentration producing the maximum reporter assay response for PPARα binding, PFOS produced only about 25% response for mouse PPARα, no significant response for rat PPARα, and an 8% response for human PPARα. In a PPARα binding assay in cultured cells transfected with mouse PPARα, the lowest observed effective concentration for PFOS was 113 times greater than that for PFOA and 21 times that for PFNA (Wolf et al., 2008). Such data show a lack of a robust PPARα response by PFOS and suggest that effects following PFOS exposure are independent of PPARα.

In contrast to the characteristic linkage between PPARα activation and liver weight increase seen with PPARα agonists such as bezafibrate and the WY compound, PFOS causes liver weight increases in PPARα-null mice (Qazi et al., 2009b; Rosen et al., 2010). In addition, Rosen et al. (2010) dosed WT and PPARα-null mice with WY or PFOS for 7 days. Both WT and PPARα-null mice exposed to PFOS showed hepatomegaly and increased incidence of hepatic vacuole formation. Profiling of gene expression was conducted with microarray analysis. Gross qualitative and quantitative differences in gene expression for fatty acid metabolism, inflammatory response, xenobiotic metabolism and ribosome biogenesis, as well as markers of PPARα activation, were found between WY and PFOS treated WT mice. These observations provide evidence that prototypical PPARα agonists (e.g., the WY compound) are not appropriate surrogates to predict the molecular and apical hepatic effects following PFOS exposure.

Additionally, hepatic effects, including tumors, have been observed in rodents exposed to PFOS without evidence of peroxisome proliferating activity. For example, Butenhoff et al. (2012) reported that chronic dietary exposure to 20 ppm PFOS resulted in liver tumors as well as hepatocellular hypertrophy and necrosis in male and female rats. However, an increase in hepatic peroxisomal bodies was not observed based on transmission electron

microscopy.

Further, increased palmitoyl CoA oxidase activity, a generally accepted marker of peroxisome proliferation induction and overall PPARα activation (Klaunig et al., 2003), has not been observed when hepatic effects were reported in PFOS-exposed rats. As part of the 2-year bioassay reported in Butenhoff et al. (2012), Seacat et al. (2003) reported on interim sacrifices following 4 and 14 weeks of dietary exposure. When assessing the 20 ppm group, the dose that caused liver tumors in Butenhoff et al. (2012), liver effects were limited to an increase in relative liver weight in male rats after 4 weeks of exposure. However, no significant increase in hepatic palmitoyl CoA oxidase activity was observed. Following 14 weeks of exposure, liver effects in the 20 ppm group included hepatocellular hypertrophy and vacuolation in males and females as well as increased relative liver weight in males with no observed significant increase in hepatic palmitoyl CoA oxidase activity.

Studies with shorter durations of exposure in rats by Elcombe et al. (2012a, 2012b) provide similar hepatic observations as those following chronic and subchronic PFOS exposures in rats as reported in Seacat et al. (2003) and Butenhoff et al. (2012). Following cessation (i.e., on recovery day 1) of 7 days of dietary PFOS exposure at 20 ppm, increases in relative liver weight and hepatocellular hypertrophy along with changes in alanine aminotransferase, aspartate aminotransferase, and cholesterol were observed (Elcombe et al., 2012b). However, no increase was observed for hepatic palmitoyl CoA oxidase activity. Following 28 days of exposure to 20 ppm PFOS, Elcombe et al (2012a) observed increased relative liver weight and hepatocellular hypertrophy along with a decrease in cholesterol. These hepatic observations were accompanied with only a marginal (i.e., 1.4-fold) increase in hepatic palmitoyl CoA oxidase activity.

To the extent that there is a relatively small amount of interaction with PFOS, PPARα may make a minor contribution to PFOS liver effects. This is in contrast to PPARα activators/peroxisome proliferators such as WY and the fibrates, for which liver effects, including carcinogenicity are clearly linked to PPARα activation. In summary, PFOS effects on the rodent liver do not appear to primarily operate through a PPAR-dependent mode of action, including at doses resulting in liver tumors as in Butenhoff et al. (2012). Thus, the lower abundance of PPARα and lower response to model PPARα activators in human liver as compared to rodent liver is not clearly relevant to the potential for PFOS to cause human hepatic effects including cancer.

Other receptors whose activities overlap to some extent with those of PPARα may also be activated by PFOS, suggesting alternative, non-PPARα modes of action. These other receptors include: CAR, PPARβ/δ, PPARγ, PXR, HNF-4α and possibly, ERα [Corton et al. (2014); Peters and Gonzalez (2011); Kobayashi et al. (2015)]. CAR appears to be involved in liver tumorigenesis in PPARα-null mice for di(2-ethylhexyl)phthalate (DEHP), an activator of PPARα (Corton et al., 2014). The set of genes expressed following CAR activation in PPARα- null mice overlap with those genes expressed following PPARα activation in WT mice. CAR- specific gene expression in WT mice is minor compared to its expression in PPARα-null mice. It is hypothesized that in WT mice, chemicals such as PFOA and DEHP that are relatively strong PPARα activators, suppress CAR (Corton et al., 2014). However, since PFOS appears to be a relatively weak PPARα agonist compared to PFOA, PFOS may preferentially activate CAR or other nuclear receptors rather than PPARα. Hepatocyte nuclear factor 4-α (HNF-4α) is considered "the master regulator of hepatic differentiation." (Beggs et al., 2016). It regulates liver development, transcriptional regulation of liver-specific genes, regulation of lipid metabolism, and maintenance of hepatocellular quiescence and differentiation. Human hepatocytes in primary culture exposed (*in vitro*) to PFOS at "occupationally relevant" concentrations resulted in downregulation of HNF-4α protein levels (but not HNF-4α mRNA). There were, however, changes in mRNA expression in genes regulated by HNF-4α, including those related to hepatic steatosis, proliferation, and tumorogenesis. HNF-4α was the upstream regulator of 90 of 681 genes with altered expression due to PFOS exposure. Beggs et al. (2016) hypothesize that PFOS causes downregulation of HNF-4α in human hepatocytes leading to hepatomegaly and steatosis.

MOA for immune effects

Following PFOS exposure in animals, immunosuppression as well as effects on immune organs, cell populations, and mediators have been observed. In humans, an association with suppression of vaccine response has been reported. Despite research efforts, reviewed in part below, the mode(s) of action by which PFOS exposure results in immune effects is unclear (DeWitt et al, 2009, 2012; Corsini et al., 2014; Chang et al., 2016).

As discussed below, based on rodent studies, it appears that PPARα may play a role in some immune effects caused by PFOS. Unlike the case for the liver, there are no data to suggest that PPARα is less active in the human immune system than in rodents. Therefore, both PPARα dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment.

The role of PPARα in PFOS-mediated immunotoxicity has been reviewed by DeWitt et al. (2009; 2012) and Corsini et al. (2014). Some data suggest that PFOS-mediated immunosuppression is not dependent on PPARα. As reviewed in DeWitt et al. (2012), research by Peden-Adams et al. (2010) reported that 28 days of PFOS exposure resulted in a similar degree of plaque forming cell response suppression in WT and PPARα-null mice. Some evidence, however, suggests a partial role for PPARα in PFOS immunotoxicity. Qazi et al. (2009b) observed that PFOS exposure (10 days) resulted in a similar change in spleen weights in WT (22% decrease) and PPARα-null (24% decrease) mice. However, for thymus weight, the extent of decrease was different between WT (34%) and PPARα-null (17%) mice. Additionally, decreases in splenocytes and thymocytes were observed in WT mice following PFOS exposure. The number of splenocytes and thymocytes were also reduced in PPARα-null mice, with differential effects for different sub-populations, although, this reduction was not to the same level of as observed in WT mice. However, in Dong et al. (2009), decreased spleen and thymus cellularity occurred at a three-fold higher serum concentration than the inhibition of plaque forming cell response. Therefore, it is not clear that the decreased spleen and thymus cellularity that appears to be partially mediated by PPARα is necessarily linked to the PFOS mediated decrease in plaque forming cell response.

Immunotoxicity data following PFOA exposure may also inform the role of PPARα in immunotoxicity following PFOS exposure. As reviewed in Corsini et al. (2014), PPARα may mediate immune suppression following PFOA in some strains of mice, based on studies in PPARα null mice. However, Corsini et al. (2014) note the much smaller affinity of PFOS for PPARα compared to PFOA and therefore hypothesize a significant role for non-PPARα mechanisms in PFOS-mediated immunotoxicity. This hypothesis for non-PPARα mechanisms is consistent with the observation of Peden-Adams et al. (2010) of suppression of IgM T-cell dependent immune response by PFOS as reflected in inhibition of the plaque-forming response in PPARα-null mice. As reviewed by DeWitt et al. (2009), this hypothesis is also consistent with the observation of Yang et al. (2002) that in PPARα-null mice exposed to PFOA, lymphoid organ weight is decreased relative to WT mice. DeWitt et al. (2009) suggest that this points to a non-PPARα mechanism for immune effects originating in the spleen/thyroid.

In addition to the extent of PPARα involvement, other mechanistic considerations may inform the mode of action for PFOS-mediated immunotoxicity. Incubation with PFOS inhibited the release of pro-inflammatory cytokines from human peripheral blood leukocytes that had been stimulated with the mitogen, phytohemagglutinin, or the endotoxin, lipopolysaccharide (Corsini et al., 2011; Corsini et al, 2012). For some of the cytokines evaluated, the LOAEL for this effect was 100 ng/L, the lowest PFOS concentration tested. Notably, this PFOS concentration is within the range of found in in the blood of highly exposed individuals.

Additionally, Corsini et al. (2014) suggest the possible involvement of an alteration of cell signaling response in PFOS mediated immune suppression since this suppression occurs without a change in the number of relevant leukocyte populations in response to PFOS exposure. Specifically, Corsini et al. (2014) cite research by Peden-Adams et al. (2010) where there was an observed suppression of IL-6 in B-cells, and translocation of NF-κB in

splenic nuclear extracts following 28 days of PFOS exposure, consistent with alterations in cell signaling. This hypothesis of altered cell signaling is also consistent with the observation by Peden-Adams et al. (2007) of a decreased response in mice to sheep red blood cells in response to the pesticide sulfuramid (rapidly metabolized to PFOS), which occurred in the absence of a related decrease in the number of T helper cells or B cells. Aside from alterations in cell signaling, DeWitt et al. (2012) note that PFOS appears to suppress both T-cell dependent, and T-cell independent antigen response. They suggest that B cells and/or macrophages might be involved in the mode of action of PFOS immunosuppression.

In general, stress may influence immune effects following chemical exposure. However, Dong et al. (2009) observed that increases in serum corticosterone, a marker for stress, in response to PFOS exposure in mice occurred only at high PFOS doses (≥ 0.8 mg/kg/day), whereas a decrease in plaque forming cell response occurred at all but the lowest dose tested (> 0.008 mg/kg/day). Corsini et al. (2014) also suggest the possibility that changes in lipid balance resulting from PFOS activity in the liver could affect the immune response. However, there does not appear to be specific evidence to support this hypothesis. Finally, although speculative, we note that in discussing the apparent effect of PFOS on serum T4 levels, Chang et al. (2007) present evidence that serum PFOS may interfere with standard immunoassays for T4 by competitively binding with antibodies in the assays. If PFOS is capable of interfering with specific immune reactions to T4 in these *in vitro* assays, it may also be capable of similarly interfering with immune responses *in vivo* such as anti-vaccine immune responses in humans.

MOA for developmental/fetal effects

Gestational exposure to PFOS is associated with several different endpoints, including decreased birth weight, malformations, and most notably, neonatal mortality. The modes of action for these effects are not known. However, it appears that the various types of developmental effects do not necessarily share similar modes of action.

Research in WT and PPARα-null mice suggests that developmental effects following gestational PFOS exposure are PPARα independent. Abbott et al. (2009b) compared the developmental effects of maternal PFOS exposure in WT and PPARα-null mouse pups exposed during GD 15-18. The effects of PFOS included increased pup relative liver weight, decreased pup survival (mostly on PND 1-2), and increased time for opening of both eyes. For each of these effects, the extent and the dose-response were comparable for the WT and PPARα-null mice. This strongly argues that these offspring effects following gestational PFOS exposure are PPARα independent. In contrast, following gestational PFOA exposure, neonatal mortality appears to be PPARα dependent (Abbott et al., 2007). Neonatal mortality following gestational PFOS exposure has been noted in several rodent studies (Abbott et al., 2009a; Luebker et al., 2005a, 2005b; Lau et al., 2003; Rosen et al., 2009) and is a striking and salient effect. The underlying toxicity resulting in this effect occurs with maternal exposure during late gestation (after GD 19) (Grasty et al., 2003, 2005). Due to the observation of labored breathing associated with this mortality and the late developmental nature of the toxicity, immature lung development, possibly related to PFOS interference with lung surfactant was suggested as a possible mode of action (Grasty et al., 2005). Lung development in rats is characterized by thinning of septal walls of the distal airway epithelium following GD 21 consistent with the maturation of this tissue into alveolar epithelial cells.

Grasty et al. (2005) dosed pregnant Sprague-Dawley rats by oral gavage on GD 19-20 at 25 or 50 mg/kg/day. On PND 0, approximately 50% of newborn rat pups exposed gestationally to 50 mg/kg/day and a smaller proportion exposed to 25 mg/kg/day PFOS had distal lung tissue morphology with the appearance of (relatively undifferentiated) GD 21 control fetuses. Although the severity of undifferentiated morphology in distal airway epithelium was the same in affected pups at both PFOS doses, mortality was greater at the higher dose. Additionally, the use of rescue agents (i.e., dexamethasone and retinyl palmitate) that accelerate lung maturation and lung surfactant production did not increase neonatal survival following gestational PFOS exposure. Grasty et

al. (2005) therefore suggest that the delay in morphological development was not the primary cause of the mortality. Further, PFOS did not affect the phospholipid concentration, and had only a minor effect on the phospholipid profile, in whole lungs of newborns or in amniotic fluid at GD 21. No overall pattern was observed in lung RNA microarray analysis from newborn lungs. In particular, there was no indication of changes in cell signaling pathway gene expression or expression of lung maturation markers. As a result, Grasty et al. (2005) ultimately hypothesized that PFOS could have interfered with the release of surfactant onto alveolar surfaces.

Rosen et al. (2009) hypothesize that PFOS may exert a physical interaction (i.e, PPARα independent) with lung surfactant, which may be an underlying cause of the neonatal mortality. Such a physical interaction is plausible, as PFOS has been detected in the lungs of perinatal offspring following gestational exposure (Borg et al., 2010). Oxidative stress and apoptosis have also been implicated in offspring lung injury that may be responsible for neonatal mortality (Chen et al., 2012a). Additionally, defects in cardiopulmonary function, such as the intracranial blood vessel dilation or enlarged right atria observed following gestational PFOS exposure, have been postulated as possible contributors to neonatal mortality (Lau et al., 2003; Yahia et al., 2008). Even with these hypotheses and observations, there is no clear mode of action responsible for PFOS-mediated newborn mortality.

MOA for carcinogenicity

Genotoxicity and mutagencity

As reviewed by USEPA (2016b), PFOS does not appear to be genotoxic or mutagenic. This conclusion is based on the results from numerous *in vitro* and *in vivo* genotoxicity assays. PFOS did not cause gene mutations in *Salmonella* strains, *Saccharomyces cerevisiae*, or *Escherichia coli*, either in the presence or absence of metabolic activation. In eukaryotic cellular systems, PFOS did not cause chromosomal aberrations in human lymphocytes and was negative for unscheduled DNA synthesis in rat hepatocytes. PFOS did not induce micronuclei in the bone marrow of exposed mice.

MOA for rodent hepatic tumors and relevance to human risk

Elcombe et al. (2012b) exposed Sprague Dawley rats to dietary PFOS for 7 days at concentrations of 20 or 100 ppm in feed, followed by up to 84 days of recovery (i.e., exposure to regular feed). They observed significant hepatic cell proliferation at both concentrations on day 1 of recovery, but not after 28 days of recovery. They also observed a significantly decreased percentage of hepatocellular apoptosis at both concentrations that persisted through the recovery period. These observations suggest a mode of action for hepatic tumors with chronic exposure to PFOS in rats that combines sustained cell proliferation with inhibition of apoptosis. However, the available data do not permit a firm conclusion as to the relevant cancer mode(s) of action.

Mode of action data relevant to the role of PPARα in the hepatic toxicity and tumorogenicity of PFOS is discussed in detail above. As discussed above, PFOS liver carcinogenicity has sometimes been considered in the context of a mode of action dependent on activation of PPARα based on some hepatic effects in rodents that are similar to those caused by known and potent PPARα activators such as benzofibrate and WY-14,643. The studies of these two compounds reviewed above indicate that they cause liver tumors in mice through a PPARα MOA. In contrast, data on PFOS reviewed above indicate that hepatic toxicity and tumorigenesis of PFOS does not occur through the same MOA as benzofibrate and WY-14,643 and is not dependent on PPARα.

Additionally, in rats, many (but not all) PPARα activators produce Leydig cell and pancreatic acinar cell tumors in addition to hepatic tumors, commonly referred to as the tumor triad (Corton et al., 2014; Klaunig et al., 2003). Although data on tumors caused by PFOS is limited to the study of Butenhoff et al. (2012), that study did not report significantly increased incidence of either Leydig cell or pancreatic acinar cell tumors. This is additionally

consistent with a non-PPARα-mediated hepatic cancer MOA.
Finally, as discussed above, there is good evidence that PFOS activates other nuclear receptors, including, PPARβ/δ, γ, and, CAR and PXR (Ren et al., 2009) and that there is evidence for the involvement of PXR (Qiao et al., 2013) and CAR (Kobayashi et al., 2015) in liver cancer.

It is generally accepted that humans are less susceptible than rodents to liver tumors that occur via activation of the PPARα receptor, due to lower intrinsic activity and/or lower number of PPARα receptors in human liver as compared to rodents. This observation has been the basis for the suggestion that rodent liver tumors and other adverse liver effects caused by environmental contaminants through PPARα activation may not be relevant to humans exposed to PFOS at environmental levels of exposure. However, as discussed above, available data do not support the conclusion that PFOS causes liver effects through a PPARα-dependent mode of action at the doses that resulted in tumors in Butenhoff et al. (2012).

There does not appear to be any data to suggest that the PFOS hepatic carcinogenicity observed in rodents is not relevant for consideration of human cancer risk. It should be noted that under the USEPA (2005a) Guidelines for Carcinogen Risk Assessment, identification of a mode of action is not required to characterize a chemical as posing a relevant risk of cancer to humans.

Mode of action (MOA) for rodent thyroid tumors and relevance to human risk
Butenhoff et al. (2012) observed evidence of thyroid follicular cell tumors in male rats at the high dose following recovery from dosing. As discussed in the Cancer Hazard Identification section, the relevance of these tumors to PFOS exposure is not clear due to lack of accompanying histopathological changes and the absence of tumors in the high dose, non- recovery group. Thus, there is limited evidence supporting the scientific reasonableness of thyroid follicular epithelial cell proliferation consistent with thyroid follicular epithelial cell tumors. A possible MOA for the PFOS-mediated thyroid follicular cell tumors observed by Butenhoff et al. (2012) is not known and there is no evidence to support a reasonable assumption of a MOA. The absence of an identifiable MOA for these tumors does not, in itself, decrease their potential human relevance. However, as discussed in the Cancer Hazard Identification section, other factors make the assumption of human relevance of these tumors from Butenhoff et al. (2012) problematic.

POINTS OF DEPARTURE FOR NON-CANCER AND CANCER ENDPOINTS

Identification of most sensitive endpoints

Dose-response analysis focused on health endpoints from animal studies with exposure durations greater than 30 days, as well as on shorter-term reproductive and developmental endpoints from animal studies involving exposures during gestation and/or the immediate post-natal period (i.e., reproductive/developmental studies). Endpoints were selected for dose-response analysis based on their reporting of serum PFOS concentrations associated with exposure. Serum concentrations are preferable to external administered doses (e.g., mg /kg body weight/day) for use in dose-response evaluation for PFOS because they represent the internal dose and account for pharmacokinetic differences between species and strains. Since a given administered dose of PFOS will result in a much higher internal dose (as indicated by serum level) in humans than in experimental animals, interspecies comparison on the basis of serum PFOS concentration reduces uncertainty when extrapolating from health effects in animals to health effects and equivalent daily intake doses in humans.

Numerous adverse endpoints that were reported from animal studies have corresponding serum PFOS concentrations. Endpoints with Lowest Observed Adverse Effect Levels (LOAELs) at the higher end of the range of reported serum PFOS concentrations in the identified animal database are useful for hazard identification, but are not necessarily useful for deriving an RfD intended to provide protection for the most sensitive relevant effects.

Therefore, only the most sensitive endpoints in the animal studies (i.e., those associated with LOAELs in the lower end of the range of serum PFOS concentrations) reported in the identified literature were considered for dose-response modeling, and potentially for RfD derivation. These most sensitive endpoints were identified by stratifying the endpoints from animal studies into quartiles based on serum PFOS concentrations corresponding to the LOAEL. Figure 8 below outlines the approach taken for identifying the most sensitive endpoint.

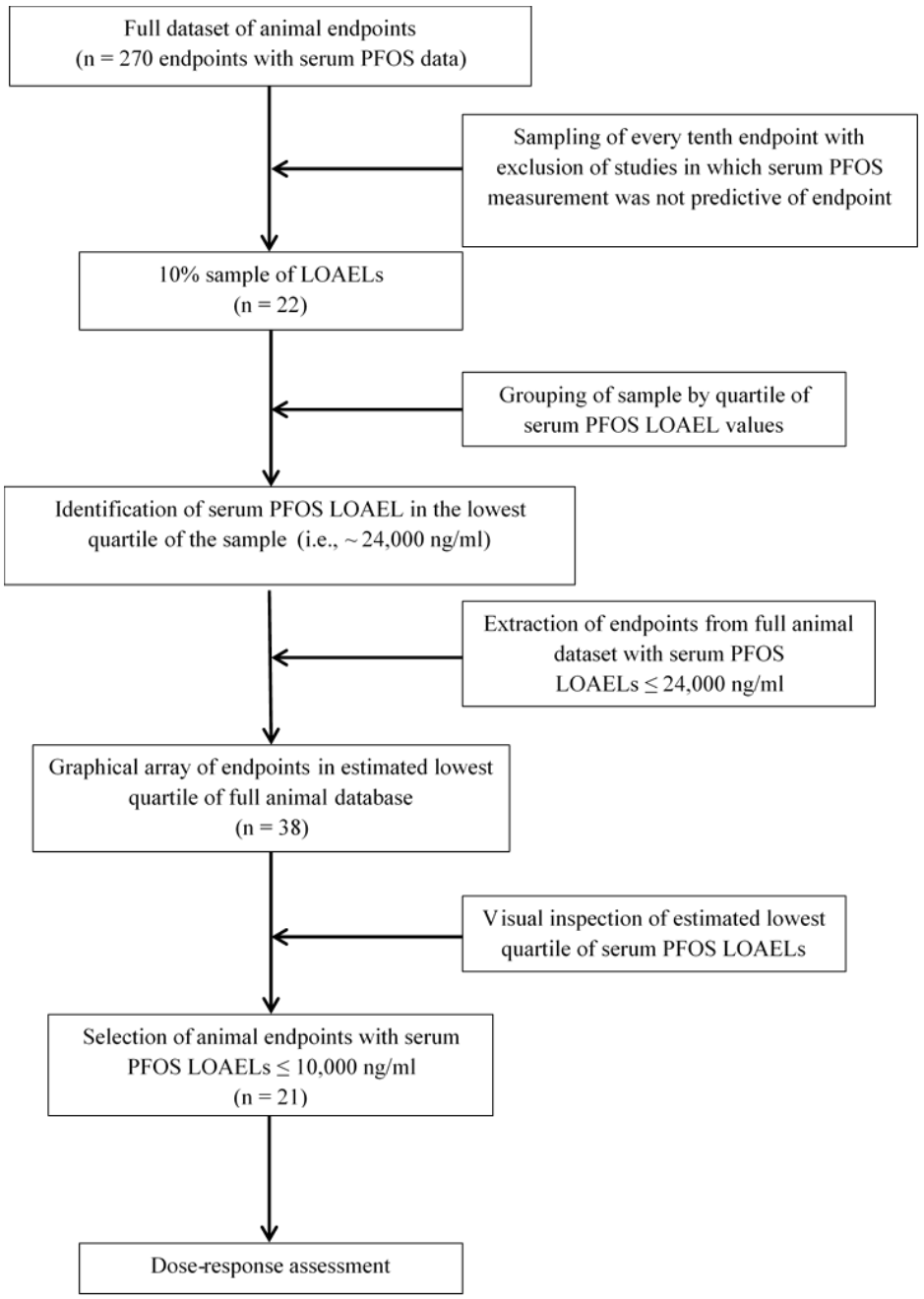

Figure 8. Graphical representation of approach taken to identify most sensitive endpoints

As the first step in generating these quartiles, the hazard identification data for all animal endpoints included in evidence tables were compiled using the Study Summary Tables (see Hazard Identification section). Studies in which serum PFOS would have substantially decreased prior to serum PFOS measurement at the time of the endpoint ascertainment (e.g. substantial time interval between end of dosing and measurement of serum PFOS and

endpoint ascertaintment) were excluded. This yielded approximately 270 endpoints with LOAELS and corresponding serum PFOS measurements from the 34 animal studies meeting the criteria for inclusion in evidence tables (see *Reviewing animal toxicology studies* in the Hazard Identification section). To estimate the numerical ranges for the quartiles in the full animal dataset, a 10% sample of the full dataset was generated by extracting every tenth LOAEL from the endpoints listed in the full dataset. If an endpoint yielded two LOAELs (i.e., male and female), each LOAEL was counted separately. This list, based on selection of every 10th LOAEL, included 22 endpoints from animal studies. The LOAELs based on serum PFOS concentration in this sample ranged from 4,460 to 223,000 ng/mL with a median concentration of approximately 45,000 ng/mL. In the lowest quartile, the maximum LOAEL serum PFOS concentration was approximately 24,000 ng/mL.

Based on this estimate generated from the sample, the lowest quartile of LOAELs in the full animal dataset of all endpoints with LOAELs ≤ 24,000 ng/ml were extracted and graphically arrayed by endpoint (Figures 9 to 13). Visual inspection across arrays revealed a general clustering of animal endpoints occurring with a LOAEL where the serum PFOS concentration was ≤ 10,000 ng/mL. Endpoints occurring at or below this serum PFOS concentration were thus considered to be within the group of most sensitive animal endpoints. Not all of these endpoints were considered for dose-response modeling due to study-specific concerns and/or lack of biological significance.

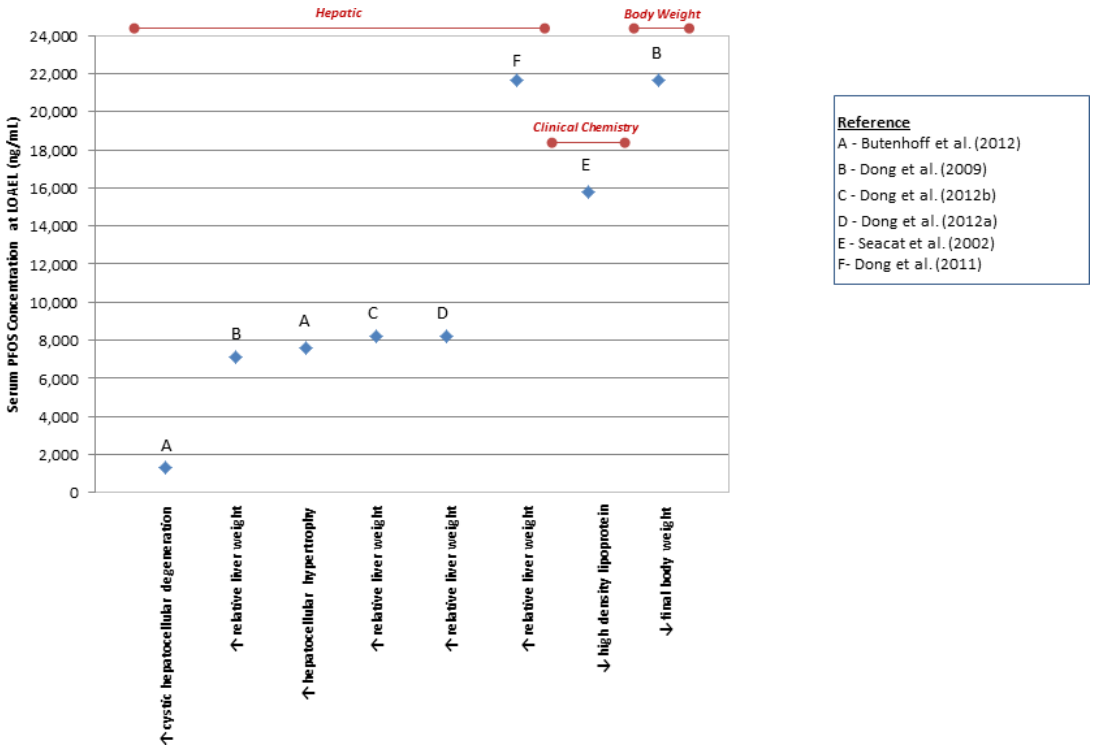

Figure 9. Graphical array of body weight, clinical chemistry, and hepatic effects in adult animals within the first quartile of serum PFOS concentrations.

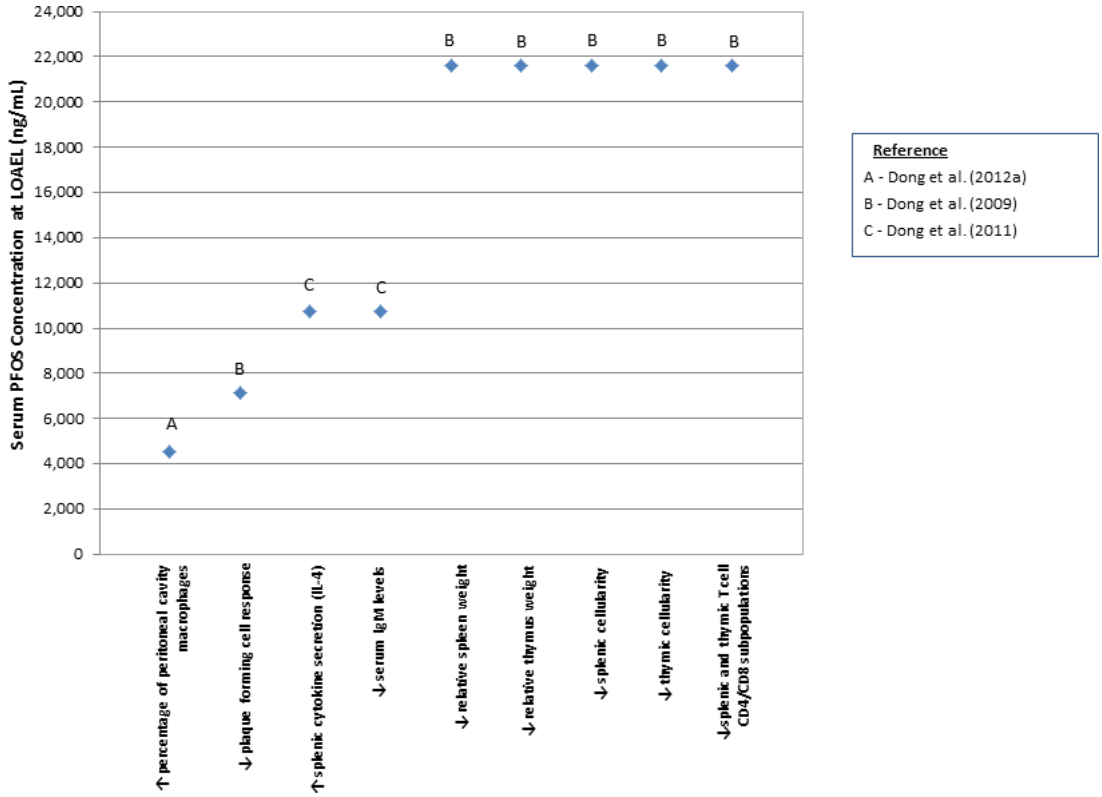

Figure 10. Graphical array of immune effects in adult animals within the first quartile of serum PFOS concentrations.

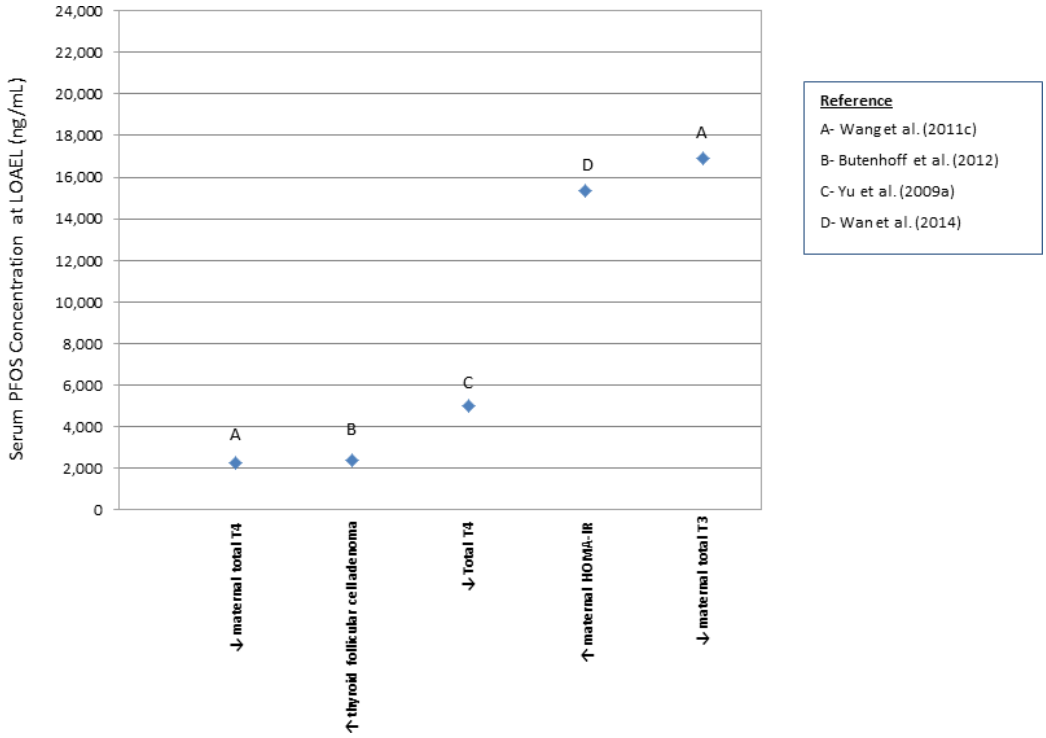

Figure 11. Graphical array of endocrine/metabolic effects in adult animals within the first quartile of serum PFOS concentrations.

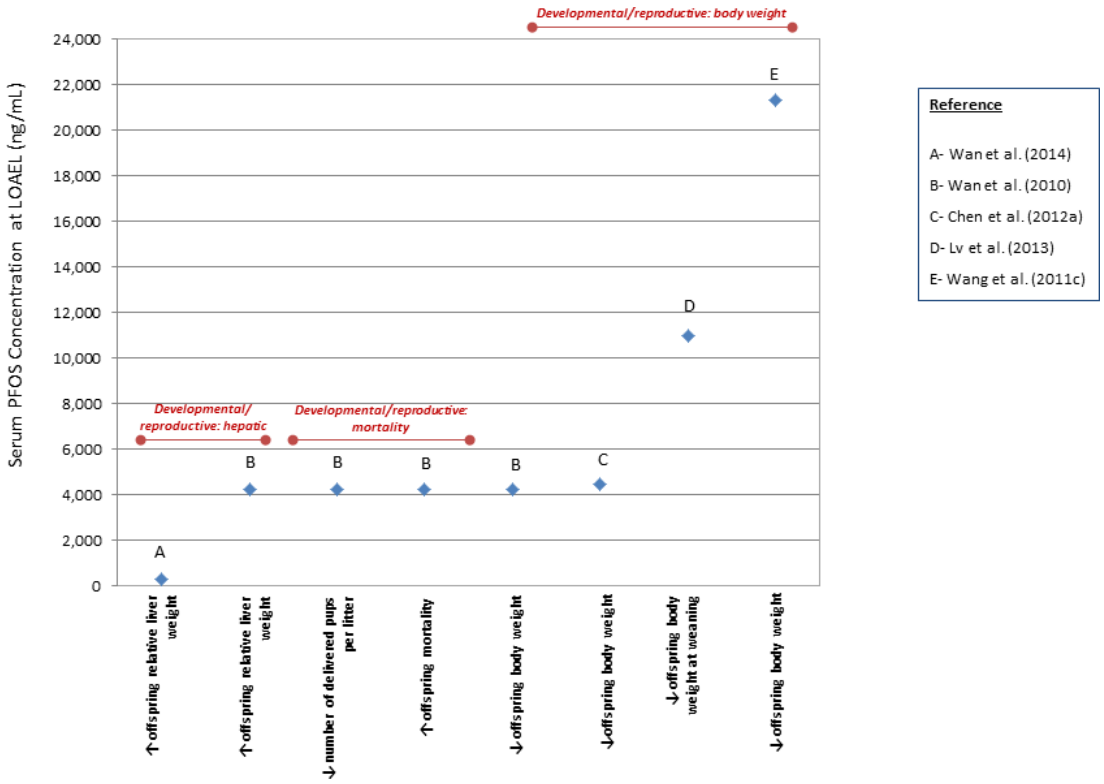

Figure 12. Graphical array of body weight, hepatic, and mortality effects in offspring animals within the first quartile of serum PFOS concentrations.

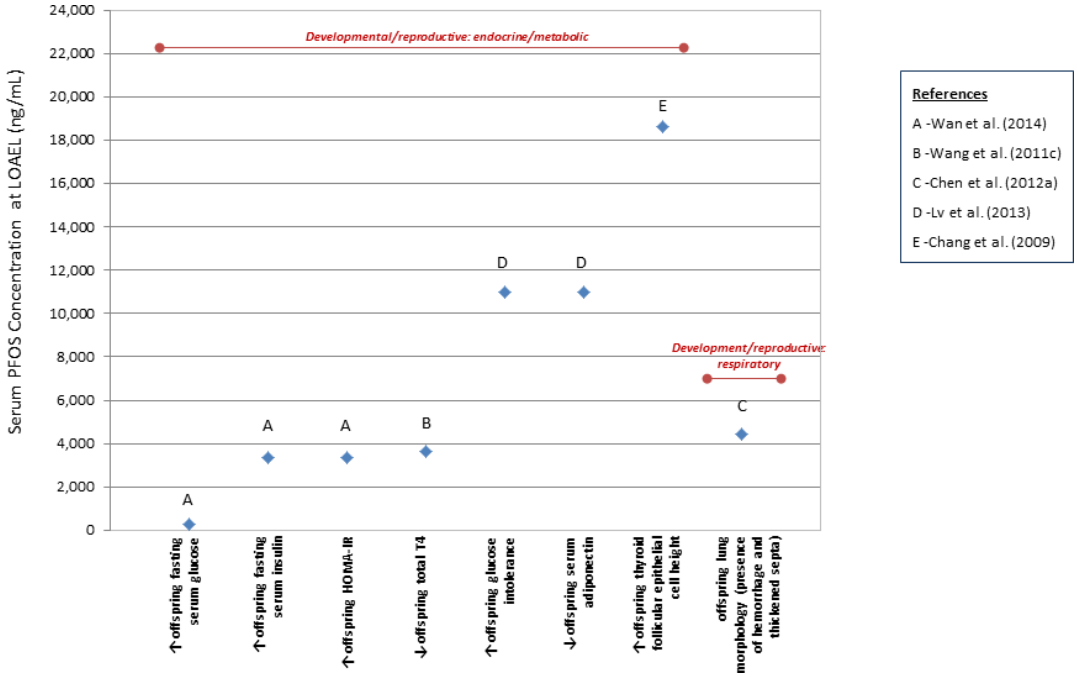

Figure 13. Graphical array of endocrine/metabolic and respiratory effects in offspring animals within the first quartile of serum PFOS concentrations.

Table 28 lists those endpoints for which the serum PFOS concentration at the LOAEL was 10,000 ng/mL or lower, sorted from lowest to highest serum PFOS concentration. Although a total of 21 endpoints with a LOAEL ≤ 10,000 ng/mL were identified, as depicted in Figures 7 to 11 above, only 20 endpoints are listed in Table 28 as the increased relative liver weight data presented in Dong et al. (2012a) and Dong et al. (2012b) were similar. Because Dong et al. (2012a) included data on additional dose groups, data from this study were considered for dose-response analysis.

Table 28. List of endpoints with serum PFOS concentration of ≤ 10,000 ng/mL at the LOAEL.

Endpoint	*Serum PFOS concentration at the LOAEL (ng/mL)*	*Reference*
↑ offspring fasting serum glucose, mouse offspring	300	Wan et al. 2014
↑ cystic hepatocellular degeneration, adult rats	1,310	Butenhoff et al. 2012
↓ maternal total thyroxine, adult rats	2,290	Wang et al. 2011c
↑ thyroid follicular cell adenoma, adult rats	2,420	Butenhoff et al. 2012
↑ offspring fasting serum insulin, mouse offspring	3,360	Wan et al. 2014
↑ offspring HOMA-IR, mouse offspring	3,360	Wan et al. 2014
↑ offspring relative liver weight, mouse offspring	3,360	Wan et al. 2014
↓ offspring total thyroxine, rat offspring	3,650	Wang et al. 2011c
↓ number of delivered pups per litter, rat offspring	4,260	Wan et al. 2010
↑ offspring mortality, rat offspring	4,260	Wan et al. 2010
↓ offspring body weight, rat offspring	4,260	Wan et al. 2010
↑ offspring relative liver weight, rat offspring	4,260	Wan et al. 2010
↓offspring body weight, rat offspring	4,460	Chen et al. 2012a
altered offspring lung morphology, rat offspring	4,460	Chen et al. 2012a
↑ percentage of peritoneal cavity macrophages, adult mice	4,350	Dong et al. 2012a
↓ total thyroxine, adult rats	5,000	Yu et al. 2009a

↑ relative liver weight, adult mice	7,130	Dong et al. 2009
↓ plaque forming cell response, adult mice	7,130	Dong et al. 2009
↑ hepatocellular hypertrophy, adult rats	7,600	Butenhoff et al. 2012
↑ relative liver weight, adult mice	8,210	Dong et al. 2012a, Dong et al. 2012b

In adult animals, the most sensitive endpoints (i.e., those with the lowest LOAELs based on serum PFOS concentrations; 9 in total) included: endocrine/metabolic effects (e.g., decreases in thyroid hormone and increased incidence of thyroid follicular cell adenomas), changes in immune parameters (e.g., increased relative number of macrophages and decreased plaque forming cell response), and increased liver weight and liver histopathology.

In perinatal or adult offspring, the most sensitive endpoints (i.e., those with the lowest LOAELs based on serum PFOS concentrations; 11 in total) included: decreased body weight, changes in endocrine/metabolic parameters (i.e., fasting levels of serum glucose and insulin, markers of insulin resistance, and thyroid hormone levels), increased liver weight, changes in lung morphology, and increased mortality. These endpoints resulted from gestational and/or post-natal exposures (e.g., via lactation).

These 20 endpoints were given further examination in terms of timing of endpoint ascertainment, biological significance, and suitability for dose-response analysis (e.g., incomplete quantitative reporting of dose-response data such as descriptions of morphological presentation at each dose). For offspring endpoints observed following gestational exposure, the effective exposures were taken to be represented by the maternal serum PFOS concentration at or near birth.

Selection of endpoints for dose-response analysis

Non-cancer endpoints

The following discussion provides the rationale for exclusion of the non-cancer endpoints and studies for which the LOAEL PFOS serum concentration was ≤ 10,000 ng/mL (Table 28) that were not considered for dose-response analysis.

Following gestational PFOS dosing (GD3 to birth) and then lactational exposure (via continued materinal dosing to PND21) in mice, Wan et al. (2014) observed at PND 63 increases in the following offspring endpoints: fasting serum glucose, fasting serum insulin, HOMA-IR, and relative liver weight. Of these, the increase in offspring fasting serum glucose was identified as the most sensitive endpoint with a serum PFOS concentration of 300 ng/mL at the LOAEL. For the three other offspring endpoints, the serum PFOS concentration was 3,360 ng/mL at the LOAEL. Both the offspring endpoints and offspring serum PFOS concentrations were determined at PND 63. However, these serum PFOS concentrations at PND63 do not reflect the higher serum PFOS concentrations that were achieved during gestational exposure and are presumed to be responsible for the observed offspring effects at PND 63. Serum PFOS concentrations were also determined at PND21 for the offspring mice and their dams. However as with the PND 63 serum concentration measurement, these determinations at PND 21 may not accurately reflect the serum PFOS concentration leading to the offspring effects occurring at PND 63. Therefore, due to a lack of an appropriate measurement of serum PFOS concentration (e.g., at PND 0), the four endpoints listed for Wan et al. (2014) were excluded from dose- response analyses.

In Wang et al. (2011c), pregnant rats were exposed to PFOS from GD 3 to PND 14. At PND 1, the authors observed

a decrease in maternal total thyroxine levels with a corresponding serum PFOS concentration of 2,290 ng/mL, making this endpoint the most sensitive maternal effect observed in this study. Decreased total triiodothyronine levels were also observed in the dams but only at higher administered doses. The biological significance of these decreases in maternal thyroxine and triiodothyronine is unclear since no other thyroid endpoints, such as thyroid stimulating hormone or thyroid histopathology and relative weight, were assessed to corroborate these observations. Therefore, the maternal effect on total thyroxine as reported in Wang et al. (2011c) was excluded from dose-response analysis.

Wang et al. (2011c) found a significant decrease in offspring serum total thyroxine on PND7 following gestational and lactational exposure as a function of maternal serum PFOS concentration measured on PND1. Wang et al. (2011c), like the Yu et al. (2009a) study, measured total T4 using an immunoassay. This type of assay is subject to the same uncertainties about method artifact in the measurement of T4 using this immunoassay method discussed in the description of the Yu et al. (2009a) study above. Further, lack of an observed association between PFOS exposure and decreased T4 (total or free) among 16 epidemiologic studies raises concerns as to the human relevance of this endpoint. Additionally, even if this were to be considered a valid endpoint, as discussed in the Toxicokinetics section, differences exist between rats and humans in maternal-fetal transfer of PFOS making identification of the corresponding human serum concentration problematic. For these reasons, the Wang et al. (2011c) study was not considered further for dose-response analysis.

In Wan et al. (2010), pregnant rats were exposed to PFOS from GD 2 to GD 21. Following parturition, a decrease in the number of delivered pups per litter and an increase in pup mortality were observed at PND 3. At PND 21, a decrease in pup body weight and an increase in pup relative liver weight were also observed. Serum PFOS concentrations in this study were only determined for the offspring at PND 21 and were reported to be 4,260 ng/mL at the LOAEL. However, this serum PFOS concentration at PND 21 is unlikely to reflect the higher serum PFOS concentration that was achieved during gestational exposure and responsible for the effects on the number of pups delivered and on pup mortality observed at PND3. Similarly, the offspring body weight and liver weight effects likely resulted from higher serum PFOS concentrations achieved during or immediately following gestational exposure, not at the serum concentration at PND 21. Therefore, due to a lack of an appropriate measurement of serum PFOS concentration (e.g., at PND 0), the four endpoints listed for Wan et al. (2010) were excluded from dose- response analyses.

In Chen et al. (2012a), pregnant rats were exposed to PFOS from GD 1 to GD 21. A decrease in offspring body weight was observed in the high dose group starting on PND 0 through PND 21. Offspring LOAEL serum PFOS concentrations at PND 0 and PND 21 were > 47,000 ng/mL and 4,460 ng/mL, respectively. While a decrease in offspring body weight at PND 0 is a biologically significant effect, the corresponding serum PFOS concentration (> 47,000 ng/mL) at PND 0 was in excess of the 10,000 ng/mL cut off concentration that is applied here for identifying endpoints for dose-response analysis. As stated above, it is assumed that effects observed in offspring exposed during gestation were all or mostly attributable to gestational exposure, even if lactational exposure from the previously exposed dams occurred. Therefore, the PND 21 serum PFOS concentrations measured in Chen et al. (2012a) are not considered to be appropriate predictors of the dose-response for endpoints observed in this study. Thus, given that the LOAEL serum PFOS concentration based on the PND0 measurements exceeded the 10,000 ng/ml cutoff, the decreased offspring body weight and changes in offspring lung morphology endpoints reported in Chen et al. (2012a), was not further considered for dose-response modeling.

In Dong et al. (2012a) adult male rats were exposed to PFOS for 60 days. After this exposure, the authors observed a statistically significant increase in the percentage of macrophages in the peritoneal cavity (i.e., the relative proportion of macrophages among all other cells isolated). The corresponding serum PFOS concentration at the LOAEL was 4,350 ng/mL. The biological significance of this observation is unclear because there was no change

in the absolute number of macrophages. Rather, the increase in the percentage of macrophages was driven by a non- statistically significant decrease in the total number of cells collected from the peritoneal cavity. Therefore, the increase in the percentage of macrophages in the peritoneal cavity was excluded from dose-response analysis.

Butenhoff et al. (2012) identified cystic hepatocellular degeneration as a sensitive endpoint for PFOS in adult rats. However, several factors argue against carrying this endpoint forward to dose-response analysis. Although the dose response was quite steep for the two lowest doses, it plateaued for the two highest doses. Since this endpoint ostensibly results from disruption of hepatocellular architecture, the lack of progression with increasing dose would not seem to be explainable by receptor saturation, and the mode of action is, thus, unclear. Cystic hepatocellular degeneration, also referred to as spongiosis hepatis, in rats is known to be most prevalent in males, spontaneous and age-related (Karbe and Kerlin, 2002; Thoolin et al., 2010), and the lack of continuous dose-response in the chronic Butenhoff et al. (2012) study may indicate that PFOS makes a small contribution to the spontaneous occurrence of this effect. There is a disagreement in the literature as to whether cystic hepatocellular degeneration is pre-neoplastic (Karbe and Kerlin, 2002; Bannasch, 2003; Kerlin and Karbe, 2004), but there is some speculation that it may, instead, be reparative, or simply due to the overproduction of proteoglycans (Karbe and Kerlin, 2002). Finally, Karbe and Kerlin (2002) and Thoolen et al. (2010) state that cystic hepatocellular degeneration is either not seen, or is very rarely seen in humans. While this observation does not preclude that this effect could be induced by a xenobiotic, or that PFOS could produce other liver toxicity through the same mode of action responsible for this effect in rats, the overall weight of evidence indicates that the toxicological significance of cystic hepatocellular degeneration to humans is unclear. Therefore, the cystic hepatocellular degeneration endpoint from Butenhoff et al. (2012) was not further considered for dose-response analysis.

Yu et al. (2009a) identified reduced total T4 in adult rats dosed with PFOS. However, thyroid stimulating hormone (TSH) was not increased in this study. Reduced total T4 might be interpreted as hypothyroidism. However, T4 and TSH are closely linked by a negative feedback loop such that a functional decrease of T4 triggers a compensatory upregulation of TSH in an attempt to increase T4 production (DeVito et al, 1999; Chang et al., 2007). Therefore, the lack of observed TSH increase in response to PFOS exposure raises questions about the significance of the observed decrease in T4. Chang et al. (2007) suggest that the observed decrease in T4 in response to PFOS exposure is an artifact of immunoassays for T4. They suggest that free PFOS in serum binds to the proteins added to the serum in the immunoassay, reducing their availability to react with T4, and thus giving the appearance of reduced T4 in the serum. They compared total T4 in rat serum measured with two immunoassays and an alternate, non-immunoassay (LC-MS/MS) assay. They found significantly lower total T4 and free T4 (FT4) in rats exposed to 5 mg/kg/day PFOS compared to controls when using the immunoassays, but no significant difference when using the LC-MS/MS assay. Lopez-Espinosa et al. (2012b), however, did not find a difference in total T4 in human serum in a population with general population level PFOS exposures when comparing immuno- and non-immunoassays for T4. They suggested that the difference between their observation and that of Chang et al. (2007) may be due to the lower serum PFOS concentrations in the human population. Thus, the exclusive use of an immunoassay for T4 by Yu et al. (2009a) raises the possibility that observed decrease in total T4 as a function of PFOS exposure could have been an artifact of the assay. Additionally, the absence of an observed association between PFOS exposure and decreased T4 (total or free) across the 16 available epidemiology studies raises questions about the human relevance of the effect observed by Yu et al. (2009a). Given the uncertainties about its toxicological significance, the endpoint of decreased total T4 in adult rats from the Yu et al. study was not considered further for dose-response analysis.

Based on the preceding exclusions, the following endpoints were selected for further consideration in non-cancer dose-response analyses:

- increased relative liver weight, adult mice (Dong et al., 2009)

- decreased plaque forming cell response, adult mice (Dong et al., 2009)
- increased hepatocellular hypertrophy, adult rats (Butenhoff et al., 2012)
- increased relative liver weight, adult mice (Dong et al., 2012a)

Tumor endpoint

As discussed above, increases in hepatic and thyroid follicular tumors were observed in rats in the only chronic study of PFOS (Butenhoff et al., 2012). As discussed above, the origin of the thyroid tumors is unclear, and they do not occur in a clear dose-related manner. In contrast, mode of action information indicates that the hepatic tumors should be considered relevant to humans for the purposes of risk assessment, and their incidence increased with dose. Therefore, dose- response analysis was conducted on the hepatocellular tumors in male and female rats. This is presented in the section on Estimation of Cancer Risk from PFOS in Drinking Water, below.

Dose-response Analysis

As discussed above, four non-cancer endpoints from three studies and one cancer endpoint were identified for consideration for dose-response assessment. The four non-cancer endpoints were selected from the larger group of non-cancer endpoints from animal studies that were observed at PFOS serum levels ≤ 10,000 ng/ml. These endpoints and their respective studies are listed in Table 29 below.

Table 29. List of cancer and non-cancer endpoints carried forward into dose-response assessment

Butenhoff et al. (2012) Male rats	hepatocellular hypertrophy
	hepatocellular tumors
Dong et al. (2009) Male mice	relative liver weight
	plaque-forming cell response
Dong et al. (2012a) Male mice	relative liver weight

Identification of Points of Departure (PODs) for non-cancer endpoints

The first step in dose-response analysis is identification of a Point of Departure (POD), which is the dose within or close to the dose range used in the study from which extrapolation begins. As described below, if a Benchmark Dose can be developed, it is preferred for use as the POD. If BMD modeling does not give an acceptable fit to the data, the NOAEL (or LOAEL, if a NOAEL is not identified) is used as the POD.

The dose-response for each of these five endpoints was investigated using the USEPA benchmark dose software, BMD software (ver. 2.6.0.1) accessed at: https://www.epa.gov/bmds/download-benchmark-dose-software-bmds. The results of the BMD modeling for the non-cancer endpoints are presented in this section. The BMD modeling of the hepatocellular tumor data is presented in the section on Estimation of Cancer Risk from PFOS in Drinking Water later in this document.

Benchmark dose (BMD) modeling is a quantitative approach commonly used to estimate the lower 95% confidence limit (the BMDL) on the dose corresponding to a pre-determined minimal response (the benchmark response, BMR) that is consistent with the observed data. The BMDL is considered to be an estimate of the

NOAEL. However, because it is based on the entire dose- response curve for the endpoint of interest rather than just the fixed doses administered in the study, it provides a generalizable estimate of the no-observed adverse effect dose that is not linked to specific administered doses in the original study. Benchmark dose modeling is identified by the USEPA (2012) as the preferred approach for dose-response modeling when the available data are sufficient to support it.

When the necessary data are available and appropriate, BMD modeling can be performed using the serum concentrations of a chemical instead of administered doses. Serum concentrations are preferable to administered doses as the basis for BMD modeling because they better represent the shape of the internal dose-response curve and reflect interspecies pharmacokinetic differences. BMD modeling was performed on serum PFOS data in order to determine whether BMDLs for serum PFOS concentrations could be used as the points of departure (PODs) to develop RfDs. If BMD modeling did not give an acceptable fit to the data, the NOAEL (or LOAEL, if a NOAEL was not identified) based on serum PFOS concentration was used as the POD.

Criteria for BMDL selection

The appropriate BMDL (if any) for each endpoint was determined based on all of the following criteria:

- A scaled residual at each input serum PFOS concentration < $\left|2\right|$.
- An acceptable fit based on chi-squared goodness of fit statistics ($p > 0.1$).
- A relatively small Akaike information criterion (AIC) statistic – generally within 1% of
- the lowest AIC value among the available models.
- A biologically appropriate model fit. This criterion applies most specifically to the
- portion of the dose-response near the BMR. Models with non-monotonic fits at the
- highest dose, but biologically reasonable fits at all other doses would not necessarily be
- excluded from consideration. In addition, if models gave an unacceptable fit to the data
- using the full dataset, but an acceptable fit after excluding the highest dose, benchmark
- dose modeling could be attempted after excluding the response at the highest dose from
- the modeling.
- The smallest BMDL meeting all of these criteria, or:
- If several models for a given endpoint all met the preceding criteria, with AIC values
- differing by < 1%, and their BMDL values differing by < 10%, their BMDLs can be
- averaged to give a summary BMDL.

Use of serum PFOS data in dose-response analysis

Male mouse studies

As discussed above, dose-response analysis was based on serum PFOS levels (internal dose) rather than administered dose. For the two male mouse studies (Dong et al., 2009; Dong et al., 2012a) for which dose-response analysis was conducted, animals were dosed for 60 days and serum PFOS levels were measured at sacrifice, one day after dosing ended.

Since the half-life for PFOS in male mice is approximately 40 days (~6 wks) (USEPA, 2016b), it is likely that the PFOS serum concentrations were increasing at the end of the 60 days of dosing. Therefore, the serum concentration

at terminal sacrifice may overestimate the dose at the onset of the adverse effect. Thus, the use of the terminal sacrifice serum PFOS concentration in the derivation of the PODs would tend to bias the PODs toward higher values. This is a non-conservative bias in that it, ultimately, has the effect of resulting in higher criteria levels.

Area under the curve (AUC) for serum PFOS data from chronic rat study (Butenhoff et al., 2012)

Dose-response analysis was also conducted for two endpoints from the chronic rat study (Butenhoff et al., 2012), hepatocellular hypertrophy and hepatocellular tumors (presented in a later section of this document). Since the serum PFOS concentrations changed greatly over time in Butenhoff et al. (2012, it is appropriate to consider the available serum PFOS data over the course of the entire 105-week study. Therefore, for the endpoints from Butenhoff et al. (2012), the serum PFOS concentrations used in dose-response analysis are based on the area under the curve (AUC) for serum PFOS, as described below.

The maximum serum concentration in males was reached by approximately 14 weeks of dosing and declined after that time point in all dose groups. The authors suggest that this decrease was due to chronic progressive nephritis, resulting in increased urinary elimination of PFOS. As shown in Figure 14, use of the serum PFOS concentration at terminal sacrifice (105 wks) would substantially underestimate the serum concentration during a significant portion of the study. To address this, the area under the curve (AUC) was calculated for each dose group. The relative lack of data precluded fitting smooth functions to these data and the AUC was, therefore, calculated using linear interpolation.

For females, the serum concentration remained relatively constant or increased slightly after 14 weeks of dosing, except for the 20 ppm recovery group for which, as anticipated, the serum PFOS concentration decreased following the cessation of dosing at 52 weeks. The AUC was calculated for the females in each dose group including the 20 ppm recovery group.

Table 30 presents the results of the AUC calculations. To obtain the time-weighted average serum concentration for each dose, the AUC was divided by the timepoint at which the final serum PFOS concentration was determined (e.g., 102, 105, or 106 wks).

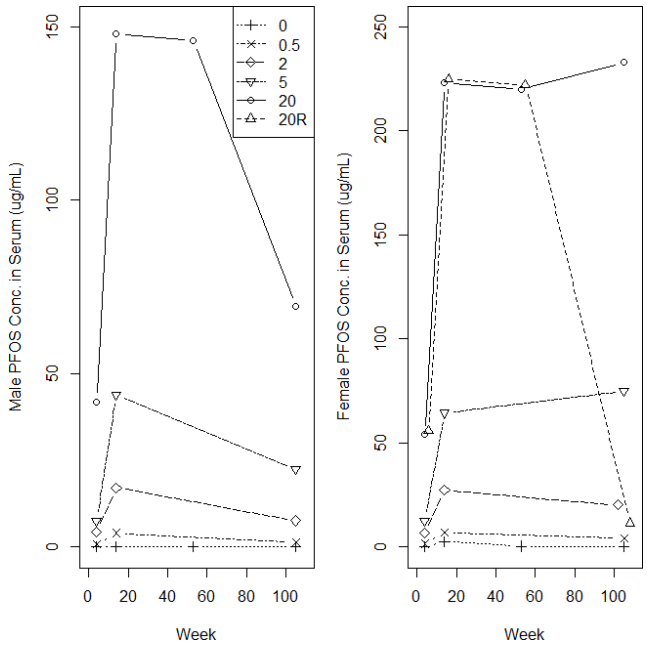

Figure 14. PFOS - Area Under Curve (AUC) (data from Table 7 of Butenhoff et al., 2012) and 3M Environmental Laboratory (2001; week 53 female serum PFOS concentration in the 20 ppm group).

Table 30. Summary of AUC and time-weighted average serum concentration for male and female rats from Butenhoff et al. (2012) and 3M Environmental Laboratory (2001).				
Dietary K^+PFOS Conc. (μg K^+PFOS/g diet)	*Male AUC (ng*wk/mL)*	*Time-weighted average serum conc. (ng/ml)*	*Female AUC (ng*wk/mL)*	*Time weighted average serum conc. (ng/ml)*
0	2.6×10^3	24.8	8.57×10^4	816
0.5	2.682×10^5	2,554.3	5.575×10^5	5,309
2	1.231×10^6	11,723.8	2.2596×10^6	22,153
5	3.2786×10^6	31,224.8	6.7277×10^6	64,073
20	1.22798×10^7	116,950.5	2.1802×10^7	210,790
20 recovery (dosing ended at 52 weeks)	16,105.5	1.6106×10^7	106	151,939

Benchmark dose modeling for non-cancer endpoints

For comparison among endpoints, a summary of serum PFOS and endpoint data used for benchmark dose modeling of non-cancer endpoints are listed below in Table 31. Benchmark dose-modeling for the cancer endpoint (hepatocellular tumors from Butenhoff et al., 2012) is presented in the section on Estimation of Cancer Risk from PFOS in Drinking Water below.

Table 31. Summary of dose-response data for the four non-cancer endpoints that underwent benchmark dose modeling.				
Study	***Endpoint***	***Administered dose (mg/kg/day, unless noted otherwise)***	***Serum PFOS concentration (ng/ml)***	***Endpoint data[a]***
Butenhoff et al. (2012)	Increased hepatocellular hypertrophy (male rats)	0	24.8[b]	0/65
		0.024	2,554.3	2/55
		0.098	11,723.8	4/55
		0.242	31,224.8	22/55
		0.984	116,950.5	42/65
Dong et al. (2009)	Increased relative liver weight (male mice)	0	48	5.17 ± 0.12 (10)
		0.0083	674	5.21 ± 0.17 (10)
		0.083	7132	5.78 ± 0.13 (10)
		0.417	21638	6.67 ± 0.11 (10)
		0.833	65426	8.17 ± 0.21 (10)
		2.1	120670	11.47 ± 0.12 (10)
Dong et al. (2009)	Decreased plaque-forming cell response (male mice)	0	48	597 ± 64 (10)[c]
		0.0083	674	538 ± 52 (10)
		0.083	7132	416 ± 43 (10)
		0.417	21638	309 ± 27 (10)

Table 31. Summary of dose-response data for the four non-cancer endpoints that underwent benchmark dose modeling.

Study	*Endpoint*	*Administered dose (mg/kg/day, unless noted otherwise)*	*Serum PFOS concentration (ng/ml)*	*Endpoint data*[a]
		0.833	65426	253 ± 21 (10)
		2.08	120670	137 ± 16 (10)
Dong et al. (2012a)	Increased relative liver weight (male mice)	0	40	4.87 ± 0.13 (6)
		0.0083	580	5.13 ± 0.15 (6)
		0.0167	4350	5.09 ± 0.12 (6)
		0.0833	8210	5.39 ± 0.15 (6)
		0.417	24530	6.48 ± 0.14 (6)
		0.833	59740	9.03 ± 0.27 (6)
		2.08	114190	12.11 ± 0.25 (6)

a = data reported as either incidence (number of animal affected/number of animals observed) or mean ± standard deviation or standard error. For data reported as mean value, number in parenthesis is sample size.
b = serum PFOS concentrations for Butenhoff et al. (2012) based on AUC analysis described in Dose-Response section.
c = plaque forming cell response data presented graphically in Dong et al. (2009). Numerical data for plaque forming cell response obtained via personal communication with G-H Dong, May 2016.

The summary benchmark dose statistics for each of the four non-cancer endpoints are presented below. Detailed model outputs are presented in Appendix 7.

Butenhoff et al. (2012) - Hepatocellular hypertrophy (male rats)

Hepatocellular hypertrophy was treated as a quantal endpoint (i.e., for each animal, the outcome was either positive or negative for the condition). The dose-response was, therefore, modeled as a quantal response. The recommended BMR for quantal dose-response modeling in the BMDS software is a 10% change from the control response. The summary results of the benchmark dose modeling for this study are presented in Table 32 below.

Table 32. Summary of BMD modeling results for hepatocellular hypertrophy in male rats (Butenhoff et al., 2012); BMR = 10% change from the control response						
Model (BMR = 0.1)	*Beta/Power/Slope*	*Poly-nomial degree*	*Chi-square p-value*	*AIC*	*BMD (ng/mL)*	*BMDL (ng/mL)*
Gamma	**Restrict Power ≥ 1**	**-**	**0.173**	**212.51**	**10203.40**	**8368.92**
Gamma	**No Power Restriction**	**-**	**0.147**	**213.86**	**8291.14**	**4550.43**
Logistic	-	-	0.000	238.66	31419.00	26497.40
Log Logistic	**Restrict Slope ≥ 1**	**-**	**0.274**	**212.48**	**8699.10**	**5699.63**
Log Logistic	**No Slope Restriction**	**-**	**0.274**	**212.48**	**8699.12**	**5225.39**
Log Probit	**No Slope Restriction**	**-**	**0.246**	**212.76**	**8370.95**	**5213.28**
Log Probit	Restrict Slope ≥ 1	-	0.014	219.42	16623.90	13644.30
Multistage	**Restrict Betas ≥ 0**	**1st**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage	**Restrict Betas ≥ 0**	**2nd**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage	**Restrict Betas ≥ 0**	**3rd**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage	**No Beta Restriction**	**1st**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage	**No Beta Restriction**	**2nd**	**0.287**	**212.56**	**7737.04**	**5485.69**
Multistage	**No Beta Restriction**	**3rd**	**0.353**	**212.32**	**10641.20**	**6596.30**
Multistage - Cancer	**-**	**1st**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage - Cancer	**-**	**2nd**	**0.173**	**212.51**	**10203.40**	**8368.92**
Multistage - Cancer	**-**	**3rd**	**0.173**	**212.51**	**10203.40**	**8368.92**
Probit	-	-	0.000	236.38	28960.60	24709.50
Weibull	**Restrict Power ≥ 1**	**-**	**0.173**	**212.51**	**10203.40**	**8368.92**
Weibull	**No Power Restriction**	**-**	**0.163**	**213.68**	**8105.33**	**4571.23**
Quantal-Linear	**-**	**-**	**0.173**	**212.51**	**10203.40**	**8368.92**

Of the 20 different dose-response models or variants of models (i.e., with and without slope, power, or beta restrictions), 17 gave acceptable fits to the data. The lowest BMDLs all clustered closely. These are presented with their AIC values in Table 33 below.

Table 33. Summary of BMDLs and AIC values for hepatocellular hypertrophy in male rats (Butenhoff et al., 2012)

Model	***BMDL (ng/ml)***	***AIC***
Gamma No power restriction	4550.43	213.86
Weibull No power restrictions	4571.23	213.68
Log probit No slope restrictions	5213.28	212.76
Log logistic No slope restrictions	5225.39	212.48

The next highest BMDL value among the other models was 5485.69 ng/ml. The highest and lowest of the BMDL values among these four models differ by 13.8%. The two lowest of these BMDL values differ by less than 0.5%, and their AIC values differ by only 0.08%. It is, therefore most appropriate to average the two lowest of these four BMDLs. **This gave a value of 4,561 ng/ml, and this is identified as the point-of departure (POD) for hepatocellular hypertrophy.**

Dong et al. (2009) – Relative liver weight (male mice)

Relative liver weight change in mice was treated as a continuous endpoint (i.e., the observed mean value for relative liver weight at each dose and the control value was used in the benchmark dose modeling). Althought the default BMR in the BMDS software for continuous data is 1 S.D. from the mean control value, from a biological standpoint, a BMR of 10% is considered to be more appropriate for relative liver weight increase and has been used in previous BMD modeling of this endpoint for other PFCs (Butenhoff et al., 2004; EFSA, 2008; DWQI, 2015a; DWQI, 2017). Therefore, a BMR of 10% is chosen for this endpoint. Furthermore, the LOAEL for increased relative liver weight in this study corresponds to a 12% increase over the relative liver weight in the controls. Thus, a BMR of 10% is statistically appropriate relative to the distribution of the responses for this endpoint. The summary results of the benchmark dose modeling for this study are presented in Table 34 below.

Table 34. Summary of BMD modeling results for relative liver weight in male mice (Dong et al., 2009); BMR = 10% change from the control response								
Model	*Variance*	*Beta/Power/Slope*	*Distribution*	*Poly*	*Chi-square p- value*	*AIC*	*BMD (ng/mL)*	*BMDL (ng/mL)*
Exponential (Model 4)	Constant (Rho=0)	Restrict Power ≥ 1	Normal	-	< 0.0001	-90.65	10,534.5	10,159.5
Exponential (Models 2&3)	Not Constant	Restrict Power ≥ 1	Normal	-	< 0.0001	-95.17	15,553.5	15,217.0
Exponential (Model 4)	Constant (Rho=0)	Restrict Power ≥ 1	Lognormal	-	< 0.0001	-323.09	10,557.7	9,399.3
Exponential (Model 4)	Not Constant	Restrict Power ≥ 1	Lognormal	-	< 0.0001	-323.09	10,557.7	9,399.3
Hill	-	-	-	-	-	-	-	-
Linear	Constant (Rho=0)	-	-	1st	< 0.0001	-92.66	10,535.0	10,160.0
Linear	Not Constant	-	-	1st	< 0.0001	-94.18	10,585.3	10,175.0
Polynomial	Constant (Rho=0)	-	-	2nd	< 0.0001	-96.06	12,122.8	10,904.9
Polynomial	**Constant (Rho=0)**	**-**	**-**	**3rd**	**0.84**	**-165.53**	**6,086.2**	**5,584.3**
Polynomial	Not Constant	-	-	2nd	< 0.0001	-95.53	13,461.1	11,093.4
Polynomial	**Not Constant**	**-**	**-**	**3rd**	**0.84**	**-163.56**	**6,085.3**	**5,586.7**
Power	Constant (Rho=0)	Restrict Power ≥ 1	-	-	< 0.0001	-90.89	11,158.7	10,176.7
Power	Not Constant	Restrict Power ≥ 1	-	-	< 0.0001	-94.18	10,585.3	10,175.0
Power	Constant (Rho=0)	No Power Restriction	-	-	< 0.0001	-90.89	11,158.7	9,085.9
Power	Not Constant	No Power Restriction	-	-	< 0.0001	-106.45	6,209.8	5,121.9

Only two closely related models provided an acceptable fit to these data, the polynomial (3rd degree), constant variance and rho = 0 model, and the polynomial (3rd degree) non-constant variance model. Although the 3rd degree polynomial function allowed a response in the high dose range that was somewhat biologically unrealistic (see Appendix 7), the BMD for this function falls in between the control and first dose group. In this range and up to the third dose, the dose-response is entirely plausible. These two models gave nearly identical fits (AIC percent difference = 1.2%) and nearly identical BMDLs (percent difference = 0.04%). **It was, therefore, judged appropriate to average these BMDLs to give a composite BMDL of 5,586 ng/ml. This is identified as the POD for increased relative liver weight from the Dong et al. (2009) study.**

Dong et al. (2012a) – Relative liver weight

Change in relative liver weight resulting from PFOS exposure was treated as a continuous response (i.e., the observed mean values for relative liver weight at each dose and the control value was used in the benchmark dose modeling). As discussed for the closely related Dong et al. (2009) study, a BMR of 10% was used for relative liver weight in this study. The summary results of the benchmark dose modeling for this dataset are presented in Table 35 below.

Table 35. Summary of BMD modeling results for relative liver weight in male mice (Dong et al., 2012a); BMR = 10% change from the control response

Model	*Variance*	*Beta/Power/Slope/n*	*Distribution*	*Poly*	*Chi-square p-value*	*AIC*	*BMD (ng/mL)*	*BMDL (ng/mL)*
Exponential (Model 5)	Constant (Rho=0)	Restrict Power ≥ 1	Normal	-	0.070	-91.8	9,973.7	8,182.2
Exponential (Model 5)	Not Constant	Restrict Power ≥ 1	Normal	-	0.010	-92.4	10,011.4	8,357.7
Exponential (Model 5)	Constant (Rho=0)	Restrict Power ≥ 1	Lognormal	-	0.005	-249.8	9,958.04	8,365.6
Exponential (Model 5)	Not Constant	Restrict Power ≥ 1	Lognormal	-	0.005	-249.8	9,958.0	8,365.6
Hill	Constant (Rho=0)	Restrict n > 1	-	-	0.070	-91.8	10,116.5	8,252.3
Hill	Constant (Rho=0)	No Restriction	-	-	0.070	-91.8	10,116.5	8,252.3
Linear	Constant (Rho=0)	-	-	1st	0.0003	-79.7	7,727.3	7,476.6
Linear	Not Constant	-	-	1st	0.0002	-83.8	7,622.3	7,343.8
Polynomial	Constant (Rho=0)	-	-	2nd	0.003	-85.1	6,801.1	6,305.2
Polynomial	Constant (Rho=0)	-	-	3rd	0.05	-91.2	8,909.6	7,501.2
Polynomial	Not Constant	-	-	2nd	0.0003	-84.9	6,962.7	6,413.1
Polynomial	Not Constant	-	-	3rd	0.007	-91.7	9,012.4	7,673.2
Power	Constant (Rho=0)	Restrict Power ≥ 1	-	-	0.0003	-79.7	7,727.3	7,476.6
Power	Not Constant	Restrict Power ≥ 1	-	-	0.0002	-83.8	7,622.3	7,343.8
Power	Constant (Rho=0)	No Power Restriction	-	-	0.0005	-80.8	6,520.7	5,487.8
Power	Not Constant	No Power Restriction	-	-	< 0.0001	-82.1	7,182.1	5,968.9

None of the models gave an acceptable fit to these data, as all of the chi-squared p-values were < 0.1. Alternatively, the LOAEL from this study is 8,210 ng/ml, and the NOAEL is 4,350 ng/ml. **Therefore, the POD for relative liver weight increase from the Dong et al. (2012a) study is identified as the NOAEL of 4,350 ng/ml.**

Dong et al. (2009) – Plaque-forming cell response (male mice)

Change in plaque forming cell response to antigen challenge in mice was treated as a continuous endpoint (i.e., the observed mean response at each dose and the control value was used in the benchmark dose modeling). The default BMR in the BMDS software for continuous data is 1 S.D. from the mean control value. The summary results of the benchmark dose modeling for this study are presented in Table 36 below. Note that the plaque-forming cell response data were reported graphically in Dong et al. (2009, Figure 7 therein). The study authors provided the actual numerical data (mean ± standard error of the mean), which for the control group to the highest dose group were: 597±64, 538±52, 416±43, 309±27, 253±21, and 137±16 (personal communication with G. Dong, 2016).

Table 36. Summary of BMD modeling results for plaque forming cell response in male mice (Dong et al., 2009); BMR = 1 S.D. change from the control response								
Model (BMR = 1 S.D.)	*Variance*	*Beta/Power/Slope/n*	*Ln-transformation of dose*	*Poly*	*Chi-square p-value*	*AIC*	*BMD (ng/mL)*	*BMDL (ng/mL)*
Exponential	Constant (Rho=0)	Restrict Power ≥ 1	N	-	-	-	-	-
Exponential	Not Constant	Restrict Power ≥ 1	N	-	-	-	-	-
Exponential	Constant (Rho=0)	Restrict Power ≥ 1	Y	-	-	-	-	-
Exponential	Not Constant	Restrict Power ≥ 1	Y	-	-	-	-	-
Hill	Constant (Rho=0)	Restrict n > 1	-	-	< 0.0001	531.04	1722.11	1251.23
Hill	Constant (Rho=0)	No Restriction	-	-	0.0066	519.29	27.27	3.17
Linear	Constant (Rho=0)	-	-	1st	< 0.0001	594.31	25147.70	21038.90
Linear	Not Constant	-	-	1st	< 0.0001	566.19	39674.70	32215.50
Polynomial	Constant (Rho=0)	-	-	1st	< 0.0001	594.31	25147.70	21038.90
Polynomial	Constant (Rho=0)	-	-	2nd	< 0.0001	572.70	9628.70	7761.42
Polynomial	Constant (Rho=0)	-	-	3rd	0.0006	524.01	2440.00	2028.48
Polynomial	Not Constant	-	-	1st	< 0.0001	566.19	39674.70	32215.50
Polynomial	Not Constant	-	-	2nd	< 0.0001	547.78	19843.10	15292.70
Polynomial	Not Constant	-	-	3rd	0.0037	498.09	3650.90	2884.27
Power	Constant (Rho=0)	Restrict Power ≥ 1	-	-	< 0.0001	594.31	25147.60	21038.90
Power	Not Constant	Restrict Power ≥ 1	-	-	< 0.0001	566.19	39674.70	32215.50
Power	Constant (Rho=0)	No Power Restriction	-	-	0.0196	517.12	4.20	0.11
Power	Not Constant	No Power Restriction	-	-	< 0.0001	507.30	59.08	3.08

None of the available models gave an acceptable fit to these data. Specifically, the chi-squared p-value was < 0.1 for all of the models and each model had at least one dose for which the scaled residual was > $\left| 2 \right|$. As can be seen in Appendix 7, this appears to be due to a disproportionately large decrease in plaque-forming response at the highest dose. Therefore, additional benchmark dose analysis was carried out excluding the high dose. This gave a reduced dataset with four doses plus the control. The summary results of the benchmark dose modeling for this reduced dataset are presented in Table 37 below.

Table 37. Summary of BMD modeling results for plaque forming cell response in male mice, excluding the highest dose (Dong et al., 2009); BMR = 1 S.D. change from the control response

Model	*Variance*	*Beta/Power/Slope/n*	*Distribution*	*Poly*	*Chi-square p-value*	*AIC*	*BMD (ng/mL)*	*BMDL (ng/mL)*
Exponential [a]	Constant (Rho=0)	Restrict Power ≥ 1	Normal	-	-	-	-	-
Exponential [a]	Not Constant	Restrict Power ≥ 1	Normal	-	-	-	-	-
Exponential [a]	Constant (Rho=0)	Restrict Power ≥ 1	Lognormal	-	-	-	-	-
Exponential [a]	Not Constant	Restrict Power ≥ 1	Lognormal	-	-	-	-	-
Hill	**Constant (Rho=0)**	**Restrict n > 1**	**-**	**-**	**0.2008**	**435.07**	**1040.97**	**717.23**
Hill	**Not Constant**	**Restrict n > 1**	**-**	**-**	**0.3049**	**421.5**	**1574.6**	**NA [b]**
Hill	**Constant (Rho=0)**	**No Restriction**	**-**	**-**	**0.1995**	**435.51**	**375.08**	**11.85**
Hill	**Not Constant**	**No Restriction**	**-**	**-**	**0.1273**	**423.5**	**1346.94**	**NA [b]**
Linear	Constant (Rho=0)	-	-	1st	< 0.0001	496.28	18119.90	14610.50
Linear	Not Constant	-	-	1st	< 0.0001	484.49	31885.20	23977.00
Polynomial	Constant (Rho=0)	-	-	2nd	0.0004	447.46	3110.14	2550.69
Polynomial	Constant (Rho=0)	-	-	3rd	0.0336	438.38	1534.12	1189.84
Polynomial	Not Constant	-	-	2nd	0.0016	432.06	4821.99	3667.36
Polynomial	Not Constant	-	-	3rd	0.0979	423.89	2239.22	1630.89
Power	Constant (Rho=0)	Restrict Power ≥ 1	-	-	< 0.0001	496.28	18119.90	14610.50
Power	Not Constant	Restrict Power ≥ 1	-	-	< 0.0001	484.49	31885.20	23977.00
Power	Constant (Rho=0)	No Power Restriction	-	-	0.0606	437.47	0.28	0.28
Power	Not Constant	No Power Restriction	-	-	0.0093	428.52	0.24	0.24

Scaled residuals for one or more doses/serum concentrations for each of the four exponential models were> |2|. The fit was inadequate for benchmark does modeling, and the model failed to calculate BMD and BMDL.
BMDL computation failed.

Only four closely related models (the Hill model with and without the power function restricted to > 1, and with and without constant variance) gave acceptable fits to the data based on the criteria of scaled residuals, and chi-square, and AIC statistics. All four of these versions of the Hill model gave similar AIC values (maximum difference = 3%). However, the BMDS software identified that the data did not meet the requirements for the assumption of constant variance across doses using the Hill model even though the models run under that assumption yielded BMDL values. Further, the BMDS software was unable to calculate BMDL values for the models run under the assumption of non-constant variance. It seems likely that the failure to calculate BMDL values resulted from the steepness of the dose-response data in the neighborhood of the BMD. Thus, the dose-response of the Dong et al. (2009) data for plaque forming cell response are not amenable to benchmark dose modeling. However, in the absence of a BMDL a valid NOAEL is an appropriate POD. **The NOAEL of 674 ng/ml is identified as the POD for decreased plaque forming cell response from the Dong et al. (2009) study.**

DEVELOPMENT OF POTENTIAL ISGWQCs FOR NON-CANCER ENDPOINTS

The overall process used to develop potential ISGWQCs from PODs for non-cancer endpoints is shown in Figure 15 and is discussed in detail below. In summary, the PODs for PFOS are based on serum PFOS levels rather than administered doses. Uncertainty factors are applied to the serum level PODs to develop Target Human Serum levels that are analogous to Reference Doses (RfDs) but in terms of serum level rather than administered dose. The Target Human Serum Levels are converted to Reference Dose with a clearance factor that relates administered doses to human serum levels. ISGWQCs are developed from the RfDs by application of exposure factors for body weight and daily drinking water consumption, and a Relative Source Contribution factor to account for non-drinking water exposure sources.

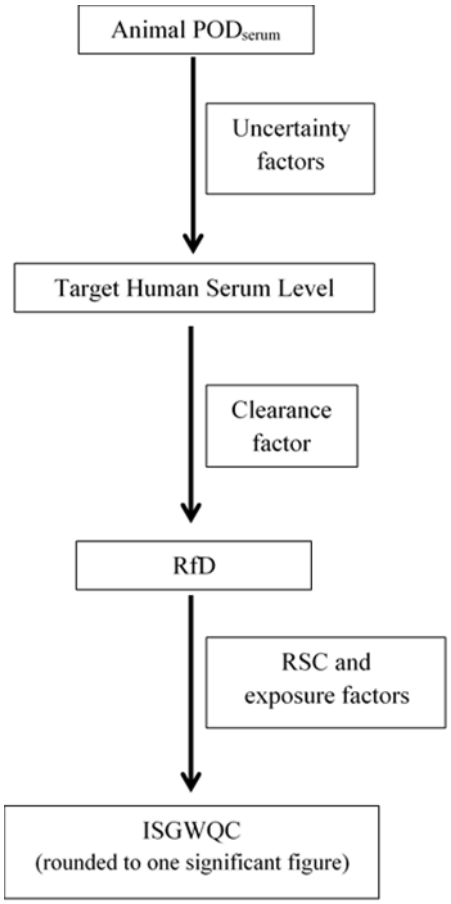

Figure 15. Graphical representation of the approach used to derive the ISGWQC

Target Human Serum Level and RfD development

Selection of PODs for Target Human Serum Level and RfD development

The PODs (NOAELs or BMDLs) for the four non-cancer endpoints for which dose-response analysis was performed above are shown in Table 38.

Table 38. PODs, NOAELs and LOAELs (based on serum PFOS concentration) for endpoints identified for dose-response assessment				
Study	***Endpoint***	***POD (ng/ml)***	***NOAEL (ng/ml)***	***LOAEL (ng/ml)***
Butenhoff et al. (2012)	Hepatocellular hypertrophy (male rats)	4,560.8 (BMDL)	2,554 [a]	11,724 [a]
Dong et al. (2009)	Relative liver weight increase (male mice)	5,585.5 (BMDL)	674	7,132
Dong et al. (2012a)	Relative liver weight increase (male mice)	4,350 (NOAEL)	4,350	8,210
Dong et al. (2009)	Decreased plaque-forming immune response (male mice)	674 (NOAEL)	674	7,132

[a] Based on AUC

Of the PODs in Table 39, the POD for increased relative liver weight based on the NOAEL of 4,350 ng/ml from Dong et al. (2012a) study was lower than the the POD of 5,585.5 ng/ml based on the BMDL for the same endpoint from Dong et al. (2009). Therefore, the the POD for increased relative liver weight from Dong et al. (2009) was not further considered for RfD development, and Target Human Serum Levels and RfDs were developed for the three the non- cancer endpoints shown in Table 39.

Table 39. PODs for endpoints selected for criterion development			
Study	***Species***	***Endpoint***	***Animal POD $_{serum}$ (ng PFOS/ml serum)***
Butenhoff et al. (2012)	Rat (male)	Hepatocellular hypertrophy	4,561 BMDL
Dong et al. (2012a)	Mice (male)	Increased relative liver weight	4,350 NOAEL
Dong et al. (2009)	Mice (male)	Decreased plaque forming cell response	674 NOAEL

Development of Target Human Serum Levels from PODs

Target Human Serum Levels are analogous to RfDs but based on serum concentration rather than administered dose. They are developed by application of uncertainty factors (UFs) to the PODs based on the serum concentration from the animal study (animal POD_{serum}). The UFs address specific factors for which there is uncertainty about the relationship of the POD to the protection of sensitive human sub-populations over a lifetime of exposure. UFs are generally applied as factors of 1 (no adjustment), 3 or 10, with 3 and 10 representing 0.5 and 1.0 log-unit. Because individual UFs represent log-units, the product of two UFs of 3 is taken to be 10. The following UFs are

considered in all cases:

$UF_{sub-chronic}$ – Applied to a sub-chronic animal POD_{serum} to estimate the corresponding NOAEL for a chronic duration study. Herein, a sub-chronic study duration is defined as an exposure of > 30 day to ≤ 90 days.

UF_{LOAEL} – Applied to an animal POD_{serum} based on a LOAEL to estimate the corresponding NOAEL, when no NOAEL is identified in the study under consideration. The UF_{LOAEL} has the value of 1 in the case of an animal POD_{serum} based on a BMDL since the BMDL is considered to be an estimate of the NOAEL.

UF_{animal} – Applied to an animal POD_{serum} to address differences between humans and animals in both toxicokinetics and toxicodynamics. A factor of 3 (i.e. one half on a log scale of the full default UF of 10) is normally applied to each. In the case of PFOS, however, the animal POD_{serum} is based serum PFOS concentration, and the use of this metric is assumed to account for the toxicokinetic differences between rodents and humans. Therefore, the UF_{animal} is assigned a value of 3 (rather than a full value of 10) to account for potential toxicodynamic differences between rodents and humans.

UF_{human} – Applied to the animal POD_{serum} to estimate the potential increased sensitivity of sensitive human sub-populations compared to the average human population. A full value of 10 is typically applied unless the endpoint is based on human data that includes sensitive sub-populations.

$UF_{database}$ – Applied to address insufficiencies in the toxicological database such as the absence of useful data on possible reproductive, developmental or neurological endpoints. For PFOS, the database is considered to be relatively complete and a value of 1 is applied.

The UFs were applied to each of the endpoints in Table 39 as follows:

<u>Hepatocellular hypertrophy (male rats; Butenhoff et al., 2012)</u>

$UF_{sub-chronic}$ = 1 – This study was a chronic duration study.

UF_{LOAEL} = 1 – The animal POD_{serum} is based on a BMDL.

UF_{animal} = 3 – To account for interspecies toxicodynamic differences as discussed above.

UF_{human} = 10

$UF_{database}$ = 1

UF_{TOTAL} = 30

<u>Increased relative liver weight (male mice; Dong et al., 2012a)</u>

$UF_{sub-chronic}$ = 3

This study was a sub-chronic duration study (60 days). There is only one chronic duration study of PFOS, the 104-week rat study of Butenhoff et al. (2012). That study showed progression of adverse effects. Following 98 days of exposure to PFOS, the interim sacrifice of the rats in Butenhoff et al. study (as reported in Seacat et al., 2003), exhibited increased relative liver weights, liver histopathology (i.e., centrilobular hypertrophy and mid-zonal to centrilobular vacuolation), increased alanine aminotransferase, and decrease serum cholesterol. At final sacrifice as reported

in Butenhoff et al. (2012), these effects generally continued to be observed, and there was emergence of hepatocyte necrosis and hepatocellular tumors, with prolonged exposure to PFOS (≤ 104 weeks) in this same cohort of rats as examined in the interim sacrifice. There are no chronic duration exposure studies in mice. However, adverse endpoints that were observed in mice with subchronic exposures (e.g., decreases in relative spleen and thymus weight and cellularity; Dong et al., 2009), and increased liver weight (Dong et al., 2012a) have the potential to quantitatively and qualitatively progress to more severe effects with longer duration of exposure, thus, given that the lone chronic study showed progression of liver effects in rats. It is possible that liver and other adverse effects would be observed in mice at lower serum concentrations with chronic exposure. Furthermore, it is possible, but unknown whether adverse effects in mice that may occur with chronic exposure would have PODs that would be lower than the critical effect (see below).

UF_{LOAEL} = 1 – The animal POD_{serum} is based on a NOAEL.

UF_{animal}= 3 – To account for interspecies toxicodynamic differences as discussed above.

UF_{human} = 10

$UF_{database}$ = 1

UF_{TOTAL} = 100

Decreased plaque forming cell response (male mice; Dong et al., 2009)

$UF_{sub\text{-}chronic}$ = 1

A sub-chronic to chronic uncertainty factor ($UF_{sub\text{-}chronic}$) of 3 or 10 may be applied to a sub-chronic POD to account for effects that may occur at lower doses with longer exposure durations. The mice in Dong et al. (2009) were exposed for 60 days, which is considered a subchronic duration (i.e., > 30 day to ≤ 90 days). However, a UF of 1 was used because, as discussed in detail below, dose-response for decreased plaque forming cell response based on serum concentration (internal dose) in studies of durations from 7 to 60 days did not show a greater effect with longer exposure duration (see Figure 16, below). In summary, this independence from exposure duration suggests that longer durations of exposure to lower concentrations of PFOS would not produce more severe decreases in plaque forming cell response.

The selection of a factor of 1 for the $UF_{sub\text{-}chronic}$ is supported by a lack of progression of the plaque forming cell response over a wide range of doses and various lengths of duration. As depicted in Figure 16, PFOS caused decreased plaque forming cell response in three studies of adult mice, while no effect was observed in only one study that included only one PFOS dose level (Qazi et al., 201a). The maximum decrease in plaque forming cell response was between approximately 70% and 85% compared to controls, regardless of the length of PFOS exposure, which ranged from 7 days to 60 days. Specifically, the maximum decrease in plaque forming cell response from Peden-Adams et al. (2008) was ~70% following 28 days of exposure with a serum PFOS concentration of 131 ng/ml. For Zheng et al. (2009), the maximum decrease in plaque forming cell response was ~85% following 7 days of exposure with a serum PFOS concentration of 3.4×10^5 ng/ml. The maximum decrease in plaque forming cell response for Dong et al. (2009) was ~80% following 60 days of exposure with a serum PFOS concentration of 1.2×10^5 ng/ml. Additionally, and importantly, in both Dong et al. (2009) and Zheng et al. (2009), a decrease of approximately 60% occurred at a serum PFOS concentration of approximately 1×10^5 ng/ml

despite the difference in exposure duration (Dong et al. (2009) = 60 days; Zheng et al. (2009) = 7 days). This further suggests that the decrease in plaque-forming cell response does not progress with longer exposure duration.

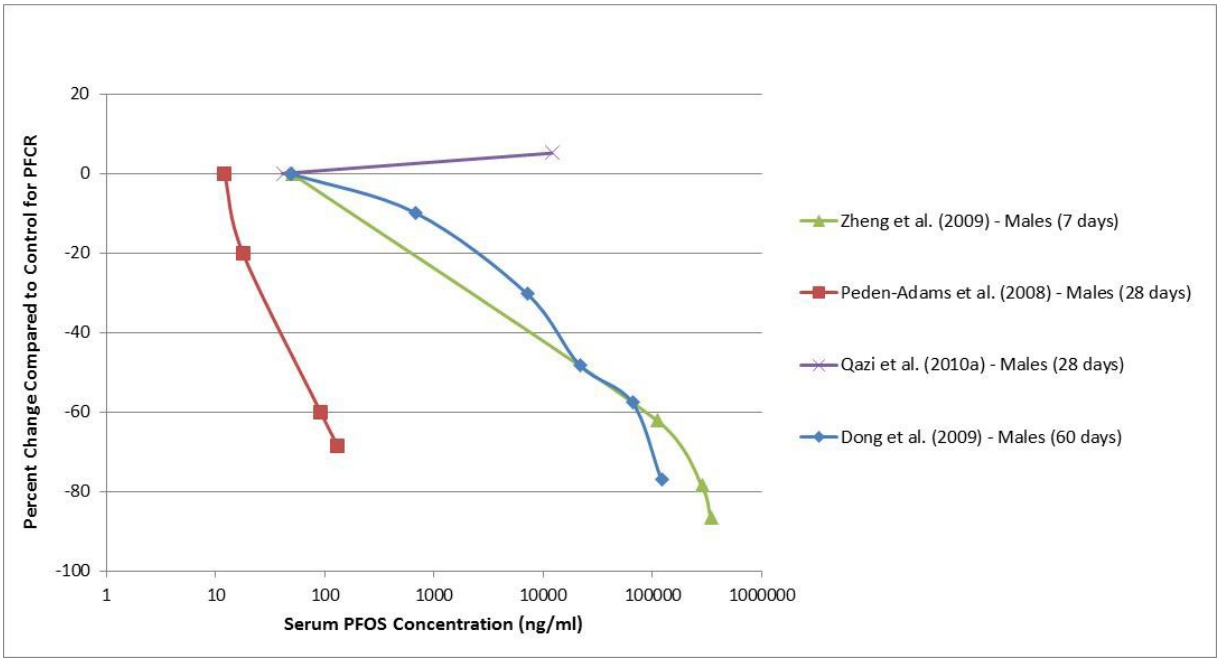

Figure 16. Comparison of plaque forming cell response studies. Percent change from controls was calculated for the studies represented in Table 40 (below), with the exception of the Keil et al (2008) study that did not report serum PFOS concentrations and the female mice from Peden-Adam et al. (2008) as the male response occurred at lower serum PFOS concentrations. Plaque forming cell response values were visually estimated from the original studies as necessary and percent change from controls was calculated as: [(treated value – control value)/control value] x 100.

UF_{LOAEL} = 1 – The animal POD_{serum} is based on a NOAEL.

UF_{animal}= 3 – To account for interspecies toxicodynamic differences as discussed above.

UF_{human} = 10

$UF_{database}$ = 1

UF_{TOTAL} = 30

Table 40. Calculation of Target Human Serum Levels			
Study	***Animal POD$_{serum}$*** *(ng/ml serum)*	***UF$_{TOTAL}$***	***Target Human Serum Level (ng/ml serum)***
Butenhoff et al. (2012) (Hepatocellular hypertrophy)	4,561	30	152
Dong et al. (2012a) (Increased relative liver weight)	4,350	100	43.5
Dong et al. (2009) (Decreased plaque forming cell response)	674	30	22.5

Table 40 presents the total UFs applied to each of the selected PODs and the resulting Target Human Serum Level.

Calculation of RfDs from Target Human Serum Levels

The RfD (as an intake dose; mg/kg/day) is calculated from the Target Human Serum Level (internal dose; ng/L) using the chemical-specific clearance factor (CL) developed by the USEPA (2016b). As discussed in the Toxicokinetics section (above), the CL relates the Target Human Serum Level to the RfD as follows:

RfD (ng/kg/day) = Target Human Serum Level (in ng/ml) x CL (ml/kg/day)

Table 41. RfDs derived from Target Human Serum Levels			
Study	***Target Human Serum Level (ng PFOS/ml serum)***	***RfD (ng/kg/day)***	***RfD (mg/kg/day)***
Butenhoff et al. (2012) (Hepatocellular hypertrophy)	152	12.3	1.23×10^{-5}
Dong et al. (2012a) (Increased relative liver weight)	43.5	3.5	3.5×10^{-6}
Dong et al. (2009) (Decreased plaque forming cell response)	22.5	1.8	1.8×10^{-6}

Table 41 presents the RfD calculated for the Target Human Serum Level for each study carried forward to criterion development.

Exposure factors for ISGWQCs based on non-cancer endpoints

The ISGWQC is a PFOS drinking water concentration intended to be protective for drinking water consumption over a lifetime. The ISGWQC was calculated from the RfD for decreased plaque forming cell response using NJDEP default values for body weight (70 kg), daily drinking water ingestion (2 L/day), and Relative Source Contribution (RSC) factor (20%; discussed below).

Relative Source Contribution (RSC) Factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used by the NJDEP, USEPA, and other states in the development of health-based drinking water concentrations based on non- carcinogenic effects. The RSC is intended to prevent total exposure from all sources from exceeding the RfD (USEPA, 2000b). When sufficient chemical-specific information on non-drinking water exposures is not available, a default RSC of 0.2 (20%) is used (i.e. it is assumed that 20% of exposure comes from drinking water and 80% from other sources). When sufficient chemical-specific exposure data are available, a less stringent chemical-specific RSC may be derived, with floor and ceiling RSC values of 20% and 80% (USEPA, 2000).

We conclude that there are insufficient data to develop a chemical-specific RSC for PFOS. Elevated levels of PFOS were detected in several PWS located throughout NJ in USEPA UCMR3 and other monitoring studies; PFOS was detected more frequently at 40 ng/L in NJ PWS (3.4%) than nationwide (1.9%) in UCMR3 (discussed in the Drinking Water Occurrence section). Potential sources of this contamination have been identified in some instances, while sources are unknown in other locations. There are no New Jersey-specific biomonitoring data for PFOS, and its more frequent occurrence in NJ PWS as compared to the U.S. as a whole suggests that New Jersey residents may also have higher exposure from non-drinking sources than the U.S. general population (e.g. NHANES). Environmental contamination with PFOS that results in its presence in drinking water can arise from a number of different types of sources (reviewed in Fate and Transport Relevant to Drinking Water Contamination), particularly releases of AFFF at civilian and military fire fighting and training sites. In communities with drinking water contaminated by environmental discharge of PFOS, exposure to PFOS may also result from contamination of other media such as soil and house dust. It is especially noteworthy that PFOS (unlike PFOA) bioaccumulates in fish, and consumption of recreationally caught fish from contaminated waters may be a major source of PFOS exposure.

Additionally, the exposure factors used to develop the ISGWQC (below) are based on an adult drinking water consumption rate and body weight. The default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the higher PFOS exposures in young infants who would not be exposed to PFOS through other sources such as food. Although serum levels in infants are lower than their mothers at birth, several studies demonstrate that infant serum levels increase rapidly by several-fold shortly after birth to levels higher than maternal levels (dicussed in detail in Toxicokinetics section). PFOS exposures to infants, both breastfed and consuming formula prepared with contaminated drinking water, are higher than in than older individuals. Infants consume much more fluid (breast milk or formula) than older individuals on a body weight basis and, PFOS concentrations in breast milk are expected to be similar or higher than in the mother's drinking water source.

These higher infant exposures must be considered because, as discussed above, the most sensitive toxicological effect occurred from short term exposures relevant to elevated short-term exposures in infancy. The dose-response for the most sensitive toxicological effect, decreased plaque forming cells in mice (an indicator of decreased immune response relevant to decreased vaccine response in humans) was similar in studies of short (7 day) and longer (60 day) durations, indicating that the Reference Dose for this effect is relevant to short-term exposures as well as chronic exposures. For the reasons discussed above, the default RSC of 20% (0.2) is used to develop the

Health- based ISGWQC.

Derivation of potential ISGWQCs for non-cancer endpoints

The equation used to derive the ISGWQC is:

$$Health - based\ MCL\ (ng/L) = \left(\frac{RfD\ (ng/kg/day)\ \times 70\ kg}{2\ L}\right) \times 0.2$$

Where:

2 L/day = assumed daily drinking water intake
70 kg = assumed adult body weight
0.2 = Relative Source Contribution (20%)

The potential ISGWQCs based on the RfDs developed above are shown in Table 42. The ISGWQC of 10 ng/L for decreased plaque forming cell response from Dong et al. (2009) is the most stringent of the three potential ISGWQCs. Information that further supports use of this study and endpoint as the basis for the ISGWQC is presented below.

Table 42. Calculation of potential ISGWQCs

Study	***Endpoint***	***RfD*** *(ng/kg/day)*	***Water Concentration*** *(ng/L = ppt)*	***Potential ISGWQC**** *(ng/L=ppt)*
Butenhoff et al. (2012)	Hepatocellular hypertrophy	12.0	84	80
Dong et al. (2012a)	Increased relative liver weight	3.5	25	30
Dong et al. (2009)	**Decreased plaque forming cell response**	**1.8**	**13**	**10**

*ISGWQC are rounded to one significant figure.

Supporting information for decreased plaque forming cell response from Dong et al. (2013) as basis for ISGWQC

As discussed above, the most stringent potential ISGWQC is based on decreased plaque forming cell response in mice (Dong et al., 2009). We note that USEPA IRIS has used decreased plaque-forming cell response as the basis for the RfDs for at least two chemicals, trans-1,2-dichloroethylene and trichloroethylene (USEPA 2010, 2011c). This endpoint has also recently been identified as a sensitive toxicological endpoint that should be considered in risk assessment of PFOS in evaluations by several other scientific groups.

The National Toxicology Program (NTP) recently completed a systematic review of immunotoxicity of PFOS, based on consideration of human and animal studies, along with mechanistic data (NTP, 2016). NTP (2016) concludes that exposure to PFOS is presumed to be an immune hazard to humans based on: 1) a high level of evidence that PFOS suppressed the antibody response from animal studies, and 2) a moderate level of evidence from studies in humans. NTP also considered additional, although weaker, evidence from laboratory animal studies suggesting PFOS may suppress infectious disease resistance and natural killer cell activity in humans. NTP stated that "the bodies of evidence indicating that PFOS suppresses multiple aspects of the immune system add to the overall confidence that PFOS alters immune function in humans."

Additionally, the Minnesota Department of Health (MDH, 2017) updated Reference Dose for PFOS and the ATSDR (2018) Minimum Risk Level (MRL) for PFOS both incorporate an additional uncertainty factor for potentially more sensitive immune system toxicity.

Finally, two recent peer reviewed publications have identified immunotoxicity as a sensitive toxicological endpoint for PFOS. Both Lilienthal et al. (2017) and Dong et al. (2017) noted that immune system toxicity is a more sensitive endpoint than the developmental effects used as the basis for the USEPA (2016a) PFOS Reference dose, and Lilienthal et al. (2017) states that decreased immune system response from PFOS and (low-dose developmental effects of PFOA) "likely constitute a sound basis for ongoing and future regulations."

Consideration of human epidemiology data

Both the human epidemiology data and the animal toxicology data were considered as part of the overall weight of evidence for the potential human health effects of PFOS. The decrease of plaque forming cell response in mice is an indicator that PFOS is able to cause immune suppression in laboratory animals. In humans, an analogous indicator of immune suppression is antibody response to vaccination. As summarized below, epidemiologic studies have demonstrated associations between PFOS exposure and decreased levels of antibodies to several vaccines at PFOS exposure levels prevalent in the general population. The epidemiologic data for this effect is notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings in experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of this endpoint, and the observation of associations within the exposure range of the general population.

However, the human epidemiology data have limitations and are therefore not used as the quantitative basis for the ISGWQC. Instead, the ISGWQC is based on a sensitive and well-established animal toxicology endpoint, plaque forming cell response, that is considered analogous to decreased vaccine response observed in humans. Importantly, continued exposure to even relatively low levels of PFOS in drinking water is known substantially increase concentrations of PFOS in blood serum. The evidence for increased risk of decreased immune response, from low-level PFOS exposures prevalent in the general population suggests a need for caution about additional exposure to PFOA from drinking water.

Relevant to this point, it is noted that the German Human Biomonitoring Commission recently developed a Human Biomonitoring Level I ((HBM I) the serum level below which adverse health effects are not expected) for PFOS of 5 ng/ml which is close to the current median PFOS serum level in the U.S. general population. This HBM I is based on the serum PFOS levels associated with health effects in human and animal studies (Apel et al., 2016). The human epidemiological data thus support the use of a public health-protective approach in developing a ISGWQC based on animal toxicology data.

Summary of epidemiology studies of PFOS and vaccine response

As discussed in the section on human epidemiology studies of vaccine response/antibody titers in the Hazard Identification section above, five studies evaluated associations of serum PFOS concentrations and antibody concentrations following vaccination for measles, mumps, rubella, diphtheria, tetanus and/or influenza (Grandjean et al., 2012, Granum et al., 2013, Stein et al., 2016, Kielsen et al., 2016, and Looker et al., 2014). These studies are summarized in Table 43 below. The total number of epidemiology studies examining antibody response to vaccines is relatively small and each type of vaccine was included only in a few (and often in only one or two) studies. Nonetheless, the study findings are consistent and support a potential for PFOS to reduce vaccine response, particularly for some vaccine types in children. The effects of PFOS on suppression of vaccine response appears to occur at or close to levels of PFOS exposure prevalent in the general population. However, there is not sufficient information to evaluate associations of PFOS and vaccine response in adults. The sole study that did not show a significant association between PFOS exposure and any antibody response (Looker et al., 2014) was conducted in adults and assessed influenza vaccine response only. Consistent with this finding, the only other study that evaluated influenza vaccine response (Granum et al., 2013) also did not find a statistically significant association between influenza vaccine response and PFOS exposure in children, although it did find a significant association of rubella vaccine response and PFOS exposure. It may be the case that PFOS affects antibody response differentially for different vaccine challenges.

It is noted that these studies did not statistically separate the relative contribution of PFOS to reduced antibody response compared to other perfluorinated compounds detected in serum. Therefore, it is possible that the observed association was due to one or more other perfluorinated compounds or due to a common effect of perfluorinated chemicals at the serum concentrations detected in these studies. Alternatively, it is also possible that this effect is primarily due to PFOS.

Table 43. Summarized results of epidemiology of serum PFOS concentration and vaccine response.

Study	***Age of population***	***PFOS concentration (central tendency)*** [1]	***Outcome by Vaccine type***					
			Tetanus	Diphtheria	Rubella	Measles	Influenza [2]	Mumps
Grandjean et al. (2012)	5 yrs old Pre- and post-booster	27.0 ng/ml (maternal) 16.7 ng/ml (5 yrs old)	↓	↓	ND [3]	ND	ND	ND
	7 years old Post-booster		-	↓	ND	ND	ND	ND
Granum et al., (2013)	3 yrs old	5.6 ng/ml (maternal)	-	ND	↓	-	-	ND
Stein et al. (2016)	12-19 yrs old	20.9 ng/ml	ND	ND	↓	-	ND	↓

Kielsen et al., (2016)	Adults (mean 37.9 yrs old)	9.52 ng/ml	- [4]	↓	ND	ND	ND	ND
Looker et al. (2014)	Adults (> 18 yrs old)	9.12 ng/ml	ND	ND	ND	ND	-	ND

1. Reported as median, mean, or geometric mean
2. For Granum et al. (2013), influenza B (Hib); for Looker et al. (2014), A/H3N2, A/H1N1 and influenza B
3. ND – Not determined
4. - No significant response observed

The observation of decreased resistance to childhood diseases in association with low, general population levels of PFOS exposure, and the consistency of this effect with a directly analogous outcome from animal studies, decreased plaque forming response, emphasizes the practical public health significance of PFOS-mediated immunosuppression. These findings lend additional support to the identification of decreased plaque forming cell response as the critical endpoint for derivation of a ISGWQC.

Selection of decreased plaque-forming cell response in mice as critical endpoint

Immunosuppression in the form of a decrease in antibody (e.g., IgM) production in response to an immune challenge (e.g., sheep red blood cells) is a well-accepted indicator of immune function and potential disease risk. Accordingly, many immunotoxicity guidelines and testing requirements include measures of the development of specific antibodies in response to an immune challenge (NTP, 2016). As noted above, the USEPA IRIS program has used decreased plaque forming cell response as the basis for the RfDs for at least two chemicals, trans-1,2-dichloroethylene and trichloroethylene (USEPA 2010, 2011c), and it has also recently been identified as a sensitive toxicological endpoint that should be considered in risk assessment of PFOS in evaluations by several other scientific groups (NTP, 2016; Dong et al., 2017; Lilienthal et al., 2017; MDH, 2017).

The reduction in IgM response, as measured by the plaque forming cell response assay, resulting from PFOS exposure was investigated in five separate studies in mice (Dong et al., 2009; Peden- Adams et al., 2008; Zheng et al., 2009; Keil et al, 2008; and Qazi et al., 2010a; Table 44). A statistically significant decrease was observed in four of these studies. As discussed below, the failure to observe a significant PFOS-mediated reduction in the Qazi et al. (2010a) study may be explainable on the basis of methodological differences between that study and the other four studies. In each of the four studies showing a PFOS-mediated reduction in plaque forming cell response, a monotonic serum PFOS concentration-response relationship was observed.

As summarized above, the reduction in plaque forming cell response is supported by several epidemiological studies of the association of decreased vaccine response with PFOS exposures in the general population. The association of PFOS exposure with reduced response to vaccination is directly analogous to the reduction in plaque forming cell response in mice following inoculation with a foreign protein (i.e., sheep red blood cell). Thus, the animal data and epidemiology data are mutually supportive of an effect of PFOS on immune suppression. This endpoint has a direct relationship to public health as it is predictive of reduced resistance to infection and reduced ability to respond to vaccination.

Selection of Dong et al. (2009) as critical study

The Dong et al. (2009) study was among the group of studies with the lowest serum PFOS LOAELs of the available studies with exposure duration of > 30 days. The study was a 60-day exposure study that employed standard methodology and produced a clear dose response with a NOAEL and a LOAEL. The animals in the LOAEL dose group were otherwise healthy, with no significant decrease in weight gain, and no significant change in spleen, thymus, or kidney weight. The animals in the LOAEL dose group did, however, have a significant 12% increase in liver weight, which is typical of PFOS exposure. In addition, the animals in the LOAEL dose group did not

have a significant elevation in serum corticosterone, a marker of stress that can decrease immune function. A significant increase in serum corticosterone was not seen until the dose of PFOS was ten times the LOAEL dose.

This study determined serum PFOS concentrations and employed an adequate number of exposure levels to demonstrate the relationship between dose and response. Although data for plaque forming cell response were reported graphically (Figure 7), the relevant numerical data were provided by Dong et al. (2009) via personal communication.

Figure 16 shows the dose-response data for the four studies of plaque forming cell response in adult mice, and Table 44 provides the details of all five plaque forming cell response studies including the developmental study. As discussed in detail below, the lower plaque forming cell response in the control group in Dong et al. (2009) compared to the control groups in the other studies suggests that the mice in the Dong et al. (2009) study and/or the plaque forming cell response assay in that study may have had a decreased sensitivity for this effect. Additionally, the data presented in Figure 17 (below) suggest that all of the doses in Dong et al. (2009) may have fallen beyond the most sensitive portion of the dose-response curve for plaque forming cell response. All of these issues could have influenced the resulting ISGWQC toward a higher value.

Table 44. Comparison among studies of plaque-forming cell response with PFOS exposure with respect to uncertainties in the interpretation of Dong et al. (2009)

Study	*Species/ strain/ sex/ age*	*PFOS cation used*	*Duration and route of exposure*	*Animals per dose group*	*Method for plaque forming cell response*	*Serum PFOS in control animals (ng/ml)*	*Administered PFOS Dose (mg/kg/d)*	*Serum [PFOS] (ng/ml)*	*PFCR in control animals (per 10^6 splenocytes)*	*LOAEL Serum [PFOS] (ng/ml)*
Dong et al. (2009)	Mice C57BL/6 M Adult (8-10 wks)	K^+	60 d Gavage	10	Jerne and Nordin (1963) as modified by Cunningham and Szenberg (1968) [a]	48	0	48	597[b]	7,132
							0.008	674		
							0.08	7,132		
							0.42	21,638		
							0.83	65, 426		
							2.1	120,670		
Peden-Adams et al. (2008)	Mice B6C3F1 M and F Adults (7-8 wks)	K^+	28 d Gavage	5/sex	Jerne and Nordin (1963) as modified by Cunningham and Szenberg (1968)	12.1 (M) 16.8 (F)	0	M - 12.1 [c] F - 16.8	M ~ 3,500 [d] F ~ 3,000 [d]	91.5 (M) 666 (F)
							0.00017	M - 17.8 F - ND		
							0.0017	M - 91.5 F - 88.1		
							0.0033	M - 131 F - 123		
							0.02	M - ND F - 666		
							0.03	M - ND F - ND		
							0.17	M - NR F - NR		

Table 44. Comparison among studies of plaque-forming cell response with PFOS exposure with respect to uncertainties in the interpretation of Dong et al. (2009)

Study	*Species/ strain/ sex/ age*	*PFOS cation used*	*Duration and route of exposure*	*Animals per dose group*	*Method for plaque forming cell response*	*Serum PFOS in control animals (ng/ml)*	*Administered PFOS Dose (mg/kg/d)*	*Serum [PFOS] (ng/ml)*	*PFCR in control animals (per 10^6 splenocytes)*	*LOAEL Serum [PFOS] (ng/ml)*
Keil et al. (2008)	Mice B6C3F 1 M and F Challenged as adults (8 wks)	K^+	GD 1-17 (Gestational exposure) Gavage	6/sex (1 /litter)	Jerne and Nordin (1963)	ND	0.0	ND	~2,300 [d] (for M and F)	ND
							0.1	ND		
							1	ND		
							5 (LOAEL M; NOAEL F)	ND		
Zheng et al. (2009)	Mice C57BL/ 6 M Adults (8-10 wks)	K^+	7 d Gavage	12	Jerne and Nordin (1963) as modified by Cunningham and Szenberg (1968)	≤ 50 [e]	0	≤ 50 [e]	~3,700 [d]	110,000
							5	110,000		
							20	280,000		
							40	340,000		
Qazi et al. (2010a)	Mice B6C3F1 M Adults (7-8 wks)	TEA	28 d Dietary	5	Jerne and Nordin (1963) as modified by Cunningham and Szenberg (1968)[e]	41	0	41	~7,500[d]	No LOAEL
							0.25	12,000		

ND – Not determined; NR – Not reported (exceeded calibration); PFCR – plaque forming cell response; TEA – tetraethylammoniuma. Although Dong et al. (2009) cite the use of both the original Jerne and Nordin (1963) and Cunningham and Szenberg (1968) modification of the original method, personal communications with G-H Dong (Feb., 2017) has clarified that only the latter method was used.; b. G-H Dong, personal communication May, 2016; c. Authors reported measured serum PFOS concentrations in ng/g and stated that this concentration is approximately equivalent to ng/ml; d. Visually estimated from graphic presentation in respective studies; e. Reported as below detection. Detection limit reported as 0.05 mg/L (50 ng/ml); e. Stated by authors as "Cunningham and Szenberg (1968)", which refers to mofication of Jorne and Nordin (1963).

Compared to Dong et al. (2009) study, Peden-Adams et al. (2008) administered lower doses of PFOS and consequently achieved lower serum PFOS concentrations at all doses than any of the dose groups except the control animals in the Dong et al. (2009). Notwithstanding the lower serum PFOS concentrations, Peden-Adams et al. (2008) reported a significant PFOS serum-response (i.e., decrease) in the plaque-forming cell response assay. Thus, if Peden-Adams et al. (2008) had been chosen as the critical study for the derivation of the ISGWQC, a more stringent criterion would have resulted.

In four of these studies (Peden-Adams et al., 2008; Dong et al., 2009; Zheng et al., 2009; Qazi et al., 2010a), PFOS was administered to adult animals and serum PFOS levels are reported. Keil et al. (2008) is not directly comparable to the other studies because it reflects effects of developmental exposure to PFOS and because serum PFOS levels are not reported. Zheng et al. (2009) administered substantially higher doses of PFOS than the other studies in adult animals, resulting in a substantially greater serum PFOS LOAEL. Qazi et al. (2010a) reported no effect on plaque forming cell response at a serum PFOS concentrations higher than the LOAELs in Dong et al. (2009) and Peden-Adams et al. (2008). The serum PFOS LOAEL in Dong et al. (2009) was almost two orders of magnitude higher than the serum PFOS LOAEL in Peden-Adams et al. (2008). However, it should also be noted that the statistically significant effect on plaque forming cell response was not found at the lowest dose in Dong et al. (2009), at a PFOS serum concentration almost an order of magnitude higher than the LOAEL serum PFOS concentration in Peden-Adams et al. (2008). In summary, decreased plaque forming cell response was reported by Peden-Adams et al. (2008) at serum PFOS levels far below the LOAELs in the other comparable studies.

In addition, stress, as measured by corticosterone levels in serum, is known to decrease immune function. Dong et al. (2009) measured corticosterone levels. Corticosterone levels were not significantly elevated at the LOAEL dose for plaque forming cell response and were only found to be significantly elevated at a dose 10 times the LOAEL dose. In contrast, Peden-Adams et al. (2008) did not measure corticosterone. Therefore, it is not known whether the greater sensitivity in plaque forming cell response reduction in the Peden-Adams et al. (2008) study could have been influenced by increased stress of the male mice.

In summary, for the reasons discussed above, although Peden-Adams et al. (2008) reported a more sensitive response for decreased plaque forming cell response, Dong et al. (2009) was judged to be the most appropriate study for use as the basis for risk assessment.

Species and strain

Each of the five studies listed in Table 44 above, was conducted on mice. Two strains of mice were used. Dong et al. (2009) that is the critical study for the ISGWQC used C57BL/6 mice, as did Zheng et al. (2009). Peden-Adams et al. (2008), Keil et al. (2008), and Qazi et al. (2010a) used the B6C3F1 strain, which is a cross between female C57BL/6 mice and male C3H mice. We are not aware of a known difference in immune competency or sensitivity to immunotoxicants between these strains. We note, however, that both the study showing the lowest serum PFOS concentration LOAEL for plaque forming cell response (Peden-Adams et al., 2008) and the study showing no response (Qazi et al., 2010a) used the B6C3F1 strain. Based on the information above, the use of the C57BL/6 strain by Dong et al. (2009) appears to be appropriate for the derivation of a ISGWQC.

Sex

Dong et al. (2009) used only male mice, as did Zheng et al. (2009) and Qazi et al. (2010a). Peden-Adams et al. (2008) used both male and female mice, and Keil et al. (2008) assessed immunocompetency in male and female offspring of exposed dams. In both of these studies, male mice were more sensitive to the immunotoxic effects of PFOS. These limited results suggest that male mice are more sensitive than females for this effect of PFOS.

Issues related to dietary exposure study (Qazi et al., 2010a)

With the exception of Qazi et al. (2010a) in which mice were exposed to PFOS through the diet, the other studies all exposed mice through gavage. Qazi et al. (2010a) was specifically designed to contrast the effects on immunotoxicity of dietary versus gavage exposure to PFOS. Gavage exposure differs from dietary exposure by providing a concentrated dose over a short period of time. With dietary exposure, mice consume their feed in multiple feedings over an extended period of time and the rate of absorption of the toxicant tends to be reduced by the physical and chemical aspects of the feed. In general, this difference can influence the toxicokinetics of exposure such that the target tissues may experience a higher concentration of the toxicant during the period immediately following gavage dosing, even when the AUC of serum concentration versus time for a gavage and a dietary study is identical. Howeveer, the route of exposure is not expected to influence the average serum concentration over time (i.e. the AUC).

There are other differences between the Qazi et al. (2010a) study and the other four plaque forming cell response studies that could potentially explain the difference in response. Qazi et al. (2010a) used the tetraethylammonium salt of PFOS while the other studies used the potassium salt. Also, Qazi et al. (2010a) administered PFOS at a single concentration in feed, resulting in a single average intake dose. The resulting serum PFOS concentration (1.2×10^4 ng/ml) was 1.7 times the LOAEL serum PFOS concentration in Dong et al. (2009) (7.1×10^3 ng/ml) and almost identical to the serum LOAEL in Zheng et al. (1.1×10^4). Thus, in the absence of other doses to establish a dose-response relationship in the Qazi et al. (2010a) study, it is uncertain to what extent the Qazi et al. (2010a) study might have shown a different dose-response compared to the other adult dosing studies if additional doses had been included.

Serum PFOS in control animals

Dong et al. (2009), Peden-Adams et al. (2008), and Qazi et al. (2010a) found potentially significant levels of PFOS in the control (no intentional PFOS exposure) mice. Similarly, measurable levels of PFOA were detected in the serum of animals in untreated control groups in some studies of PFOA. As discussed in DWQI (2017), these exposures are likely due to a combination of two factors. First, there is likely some level of unavoidable background exposure to PFOS in laboratory animals, just as in the general human population, due to the ubiquitous presence of PFOS at low levels in the environment. Second, in some studies, the controls may have experienced some level of inadvertent exposure to the PFOS used to dose the treated animals.

Zheng et al (2009) reported the PFOS concentration in the control mice as below the detection limit (i.e., ≤ 50 ng/ml). However, as the PFOS detection limit in Zheng et al. (2009) is in the range of the serum PFOS concentrations detected in control animals in the other studies that did report PFOS concentrations in control serum, it is not clear to what extent the PFOS exposure in control animals in Zheng et al. (2009) may have differed from these other studies. As shown in Table 44, the reported concentrations of PFOS in control animals in the Peden-Adams et al. (2008) study (12.1 ng/ml) was about 25% that in Dong et al. (2009) (48 ng/ml) or Qazi et al. (2010a) (40.9 ng/ml). This is potentially significant because the Peden-Adams et al. (2008) study had a serum PFOS LOAEL for plaque forming cell response that was only about 1% of the Dong et al. (2009) serum PFOS LOAEL. Figure 17 shows the serum PFOS- plaque forming cell response data from Peden-Adams et al. (2008) (Note that the serum PFOS concentrations in this figure were visually estimated from the graphic data presented by the authors). Also shown in this figure is the PFOS serum concentration in the control (male) mice from Dong et al. (2009) (48 ng/ml).

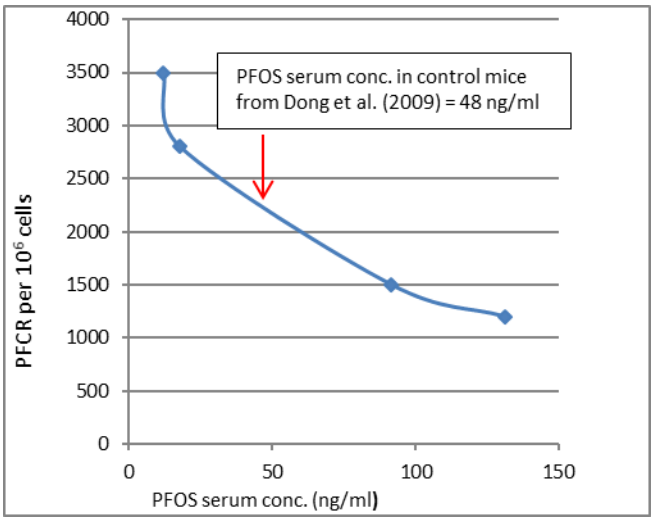

Figure 17. Serum PFOS- plaque forming cell response response (PFCR) (male mice; diamonds) from Peden-Adams et al. (2008) and serum PFOS concentration in control animals (arrow) from Dong et al. (2009). Plaque forming cell response data were visually estimated from the graphic presentation in Peden-Adams et al. (2008). (Note: Serum PFOS concentration at the NOAEL and LOAEL in male mice from Peden-Adams et al. (2008) was 17.8 and 91.5 ng/ml, respectively.)

As suggested in Figure 17, if the mice in Dong et al. (2009) followed the same serum concentration- plaque forming cell response relationship as the male mice in Peden-Adams et al. (2008), then the plaque forming cell response inhibition already occurring in these control mice (in the absence of added PFOS exposure) would fall well within the linear descending portion of the Peden-Adams et al. (2008) PFOS serum concentration- plaque forming cell response curve, but not in the steepest portion of the curve (i.e., serum PFOS concentration in the range of 12.1-17.8 ng/ml). This suggests that the control mice in Dong et al. (2009) may have already experienced decreased plaque forming cell response due to their background PFOS exposure. If this were the case, then the serum LOAEL from Dong et al. (2009) from *intentional* PFOS exposure might have occurred in a portion of the concentration-response curve in which the response was attenuated (i.e., less steep) compared to the portion of the concentration-response curve described by the Peden-Adams et al. (2008) data. This could have resulted in Dong et al. (2009) overestimating the serum PFOS concentration at which significant decreases in plaque forming cell response first occur. It is, therefore, possible that a lower serum PFOS concentration in the mice in Dong et al. (2009) prior to PFOS exposure would have resulted in a lower ISGWQC value.

<u>Plaque forming cell response to SRBC inoculation in control animals not dosed with PFOS</u>

In the plaque forming cell response assay, the response of the control animals (i.e., those animals inoculated with SRBC antigen, but not intentionally exposed to PFOS) is the baseline for determining possible suppression of immunological response. The plaque forming cell response in the control animals in Dong et al. (2009) ($597/10^6$ splenocytes) is lower than the response in any of the four remaining studies (range 2,300-7,500/10^6 splenocytes). The reason for this is not clear, but may include factors such as inter-individual differences in SRBC antigenicity among sheep that were the source of the SRBC, different suppliers of mice, different animal husbandry, different diets, and intra-strain genetic drift. Although Peden-Adams et al. (2008), Keil et al. (2008), and Qazi et al (2010a) all used B6C3F1 mice while Dong et al. (2009) used C57BL/6 mice, this is not likely to be the explanation for the decreased plaque forming cell response response in control mice in Dong et al. (2009) since Zheng et al. (2009) also used C57BL/6 mice and achieved a plaque forming cell response in control mice of ~$3,700/10^6$ splenocytes.

Although the reason for the lower plaque forming cell response among control animals in Dong et al. (2009) is not clear, it suggests the possibility that the performance in the plaque forming cell response assay in the mice used by Dong et al. (2009) may have been generally attenuated, resulting in overestimating the true serum PFOS LOAEL from that study, and ultimately resulting in a higher RfD and ISGWQC.

Summary of basis for use of Dong et al. (2009) for derivation of the ISGWQC

A number of factors related to the selection of Dong et al. (2009) as the critical study for Health- based ISGWQC development are discussed above. Those factors with the greatest potential to affect the ISGWQC are: choice of Dong et al. (2009) as the most appropriate study from the standpoint of sensitivity of response, impact of the background serum PFOS concentration in control animals, and the possible attenuation of the plaque forming cell response assay in Dong et al. (2009) as suggested by the relatively low plaque forming cell response in the control animals. However, each of these factors has the potential to influence the ISGWQC to a higher (less protective) value than might have been derived otherwise.

Relationship of the Target Human Serum Level and ISGWQC to exposures associated with decreased vaccine response

The Target Human Serum Level of 23 ng/ml in serum and the ISGWQC of 10 ng/L in drinking water were derived from the most sensitive and relevant toxicological endpoint identified in the scientific literature. This endpoint is immunotoxicity, specifically decreased plaque-forming cell response. The Target Human Serum Level (23 ng/ml) is analogous to a Reference Dose, but in terms of serum level rather than administered dose. It was develop using a risk assessment approach intended to be protective for chronic (lifetime) exposure, including to susceptible subpopulations. The potential risk of immunotoxicity with PFOS exposure at the Target Human Serum Level can be evaluated by comparison to serum PFOS concentrations associated with immunotoxicity in the epidemiology literature.

Decreases in vaccine response in humans have been observed in study populations with measures of PFOS serum concentration central tendency ranging from 6 to 27 ng/mL (Grandjean et al., 2012; Granum et al., 2013; Kielsen et al., 2016; Stein et al., 2016). For comparison to general population serum PFOS concentrations, the median and the 95th percentile serum PFOS concentrations as reported in the NHANES database for 2013-2014 are 5.2 and 19 ng/mL, respectively (CDC, 2017). Therefore, serum PFOS levels in the general U.S. population are currently near or within the range of central tendency serum PFOS levels in the studies which found associations with decreased immune response.

The ISGWQC was developed using a risk assessment approach intended to be protective for lifetime exposure. It is derived as a PFOS drinking water concentration that will result in an increase in PFOS serum level that is equal to 20% of the Target Human Serum Level (23 ng/ml), or 4.7 ng/L.

As discussed above (Sources of Human Exposure), drinking water is not a substantial contributor to the PFOS exposures prevalent in the general population. Food, consumer products and possibly house dust are major sources of human exposure because most sources of drinking water are not contaminated by PFOS. Therefore, ingestion of drinking water contaminated with PFOS adds to the body burden from other exposure sources.

Assuming the conservative (i.e. health protective) default drinking water consumption rate of 0.029 L/kg/day (an upper percentile estimate based on 2 L/day/70 kg body-weight), the increase in serum PFOS concentration would be 4.7 ng/ml (i.e., 20% of the Target Human Serum Level). This additional contribution would, therefore, on average, increase the median serum PFOS concentration from 5.2 to 9.9 ng/ml and the 95th percentile serum PFOS concentration from 19 to 23.7 ng/ml. This contribution from drinking water exposure at the ISGWQC represents a 1.9-fold increase above the median level of PFOS exposure in the U.S. and a 1.2-fold increase above the 95th

percentile of PFOS exposure in the U.S. population. As summarized above, health effects have been observed in epidemiologic studies with PFOS serum concentrations comparable to the general population. With expected increases from drinking water exposure to serum PFOS level substantially higher than those found in the general population, it cannot be definitively concluded that lifetime exposure at the proposed Target Human Serum level is protective for the most sensitive effects, including in sensitive subpopulations. Therefore, there is uncertainty regarding the extent of protectiveness provided by the ISGWQC.

ESTIMATION OF CANCER RISK FOR PFOS IN DRINKING WATER

We conclude that a ISGWQC for PFOS based on carcinogenicity would be much more uncertain than one based on the non-cancer endpoint, decreased immune response as assessed by plaque forming cell response in mice. As discussed above, decreased plaque forming cell response is a sensitive and well-established animal toxicology endpoint which is an indicator of decreased immune response. This effect was reported in multiple toxicological studies, and it is considered relevant to humans based on epidemiological and mode of action data. In contrast, carcinogenicity of PFOS has been studied only in a single chronic duration rat study (Butenhoff et al., 2012). For this and other reasons discussed below, the cancer risk assessment for PFOS is highly uncertain as compared to the non-cancer risk assessment. Accordingly, the quantitative estimate of cancer risk for PFOS in drinking water is presented below to provide context and for informational purposes and is not used as the basis for a potential ISGWQC.

The dietary rat study conducted by Butenhoff et al. (2012) is the only chronic study of PFOS. As discussed above, PFOS is most appropriately described as having "*Suggestive Evidence of Carcinogenic Potential*" based on the USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a). This descriptor is consistent with USEPA (2005a) which states that "*Suggestive Evidence*" should be used when there is "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor *'Likely to Be Carcinogenic to Humans*'. USEPA Office of Water (2016b) also concluded that the descriptor "*Suggestive Evidence of Carcinogenic Potential*" is appropriate for PFOS.

An increased incidence of hepatocellular and thyroid tumors was reported by Butenhoff et al. (2012). The hepatocellular tumor data are appropriate for dose-response analysis, while the thyroid tumor data do not follow a dose-response pattern that can be used for estimation of cancer risk. Therefore, hepatocellular tumor data from the chronic rat study (Butenhoff et al., 2012) were selected for dose-response modelling and estimation of the cancer risk from PFOS in drinking water.

The mode of action for the rat hepatoceullular tumors caused by PFOS has not been established, and they are considered relevant to humans for the purposes of risk assessment (See discussion in Mode of Action section.) USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a) state that linear low-dose extrapolation should be used for dose-response modeling if the mode of action has not been established. Therefore, the linear low-dose extrapolation was used for dose-response modeling of these tumors. The linear low dose extrapolation approach is based on the assumption that exposure to any dose of a carcinogen results in some risk of cancer and is presented below:

Benchmark dose modeling for hepatocellular tumors

Butenhoff et al. (2012) presents the summary data for the occurrence of hepatocellular tumors, and Thomford et al. (2002), a contract laboratory report not from the peer-reviewed literature, presents the detailed, individual animal data that are summarized in Butenhoff et al. (2012). The data for both males and females from Thomford et al. (2002) were reviewed to determine the animals at risk for PFOS-mediated tumors (i.e., those animals alive after 52 weeks of exposure) and to confirm the occurrence and nature of the tumor data presented in Butenhoff et al., 2012).

In addition to hepatocellular tumors, Thomford et al. (2002) also reported a liver sarcoma in a male in the high exposure-recovery group, a cholangioma in a female in the 5 ppm PFOS dose group, and a number of neoplasms in the liver identified as having origins in other tissue that were not considered to be related to PFOS exposure. Based on guidance suggested by McConnell et al. (1986) and generally followed by the USEPA IRIS, these tumors were not included in the dose-response modeling presented below. However, we note that the occurrence of the liver sarcoma and the cholangioma are not necessarily inconsistent with the mode of action that resulted in the hepatocellular tumors.

It should be noted that the hepatocellular tumor incidence-by-exposure group employed here differs somewhat from the incidence presented by Butenhoff et al. (2012). Butenhoff et al., calculated the number of rats at-risk in each exposure group using the "Poly-3" approach. This approach estimates the number of animals at-risk as a modeled function of the animals surviving at any given time point up to the end of the study based on the assumption that tumors appear as a third-degree polynomial with respect to time. In contrast, as noted above, the approach employed here follows the approach used by USEPA IRIS.

Males

The occurrence of hepatocellular tumors in the male rats is summarized in Table 45.

Table 45. Summary of hepatocellular tumor data in male rats from Butenhoff et al. (2012)

Concentration in Feed (ppm)	***0 (controls)***	***0.5***	***2***	***5***	***20***	***20 Recovery group***
Serum concentration (calculated on the basis of the area under the curve (AUC) (ng/ml) [1]	25	2,554	11,724	31,225	116,950	-
Number of rats with observed tumors [2]	0	3	3	1	7	0
Number of animals in original exposure group	70	60	60	60	70	40
Number of animals with mortality ≤ 52 weeks [3]	11	12	10	10	12	0
Animals assumed to be at-risk of developing a tumor [4]	59	48	50	50	58	40
Hepatocellular tumor incidence	0	0.063	0.060	0.020	0.121	0

1. AUC was calculated as described in the text at the beginning of the dose-response section.
2. For males, all hepatocellular tumors were adenomas.
3. Includes scheduled sacrifices and spontaneous deaths (data from Thomford (2002).
4. Number of animals in original exposure group minus animals with mortality ≤ 52 weeks.

Dose-Response Considerations

For hepatocellular tumors in males (all adenomas), there is one exposure group with a significant elevation in tumor incidence (20 ppm PFOS in feed). Figure 18 is an example of the fitting of a parametric dose-response function to these data using the USEPA BMDS software.

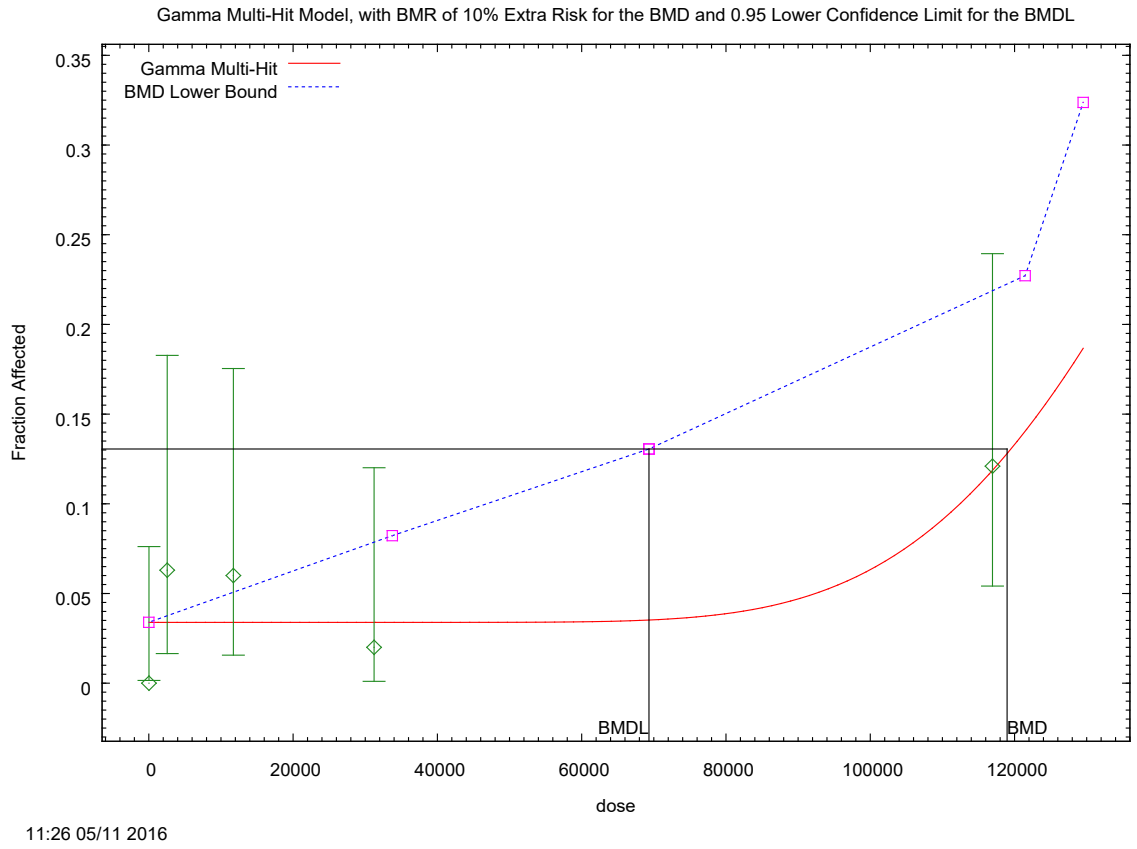

Figure 18. Fit of gamma multi-hit model to data on increased hepatocellular tumors in male rats (Butenhoff et al., 2012); data on x-axis represent serum PFOS concentration in ng/ml as summarized in Table 45 above.

As demonstrated in this figure, there are effectively only two points that determine the fit of these dose response models, the control, and the response of the 20-ppm group (corresponding to 120,000 ng/ml serum PFOS concentration). Therefore, all models have an equal likelihood of modeling the response between these two points and benchmark dose modeling is not informative for deriving a point of departure. The more appropriate approach to estimation of the hepatocellular cancer potency in males is to calculate the linear slope of the line between the response of the 20-ppm exposure group and the origin using the incidence data as given in Table 45 above.

It should be noted that there were no hepatocellular tumors in the male recovery group (in contrast to females, which did have tumors in the recovery group). The recovery group was not included in the BMD modeling of these tumors in males, while it was included in the modeling of data from females (below). However, inclusion of the recovery group in the dose-response evaluation for males would not have changed the result since the cancer slope factor is based on the slope of the line between the origin and the high dose group.

Cancer Potency Calculation

The cancer potency for hepatocellular tumors in male rats was calculated in terms of serum PFOS concentration

rather than the PFOS concentration in the feed (i.e., the administered dose). Therefore, based on the area-under the-curve (AUC) calculations, the average serum concentration over the 105 weeks of exposure (116,950 ng/ml) is used to define the (internal) exposure of this group. As given in Table 45 above, the hepatocellular tumor incidence for the 20-ppm exposure group is 0.121. Therefore, the cancer potency is the slope of the line from this exposure group to the origin (0 ng/ml serum concentration; 0 tumor incidence). This is calculated as: 0.121/ 116,950 ng/ml = 1 x 10^{-6} $(ng/ml)^{-1}$.

Females

The occurrence of hepatocellular tumors in the female rats is summarized in Table 46.

Table 46. Summary of hepatocellular tumor data in female rats from Butenhoff et al. (2012)

Concentration in Feed (ppm)	***0 (controls)***	***0.5***	***2***	***5***	***20 recovery group*** [2]	***20***
Serum concentration (calculated on the basis of the area under the curve (AUC)) (ng/ml) [1]	816	5,309	22,153	64,073	151,939	207,633
Number of rats with observed tumors [3]	0	1	1	1	2	6 (includes 1 carcinoma)
Number of animals in original exposure group	70	60	60	60	40	70
Number of animals with mortality ≤ 52 weeks [4]	10	13	12	11	1	11
Animals assumed to be at-risk of developing a tumor [5]	60	47	48	49	39	59
Hepatocellular tumor incidence	0	0.021	0.021	0.020	0.051	0.102

1.AUC was calculated as described in the text at the beginning of the dose-response section.

2. The 20-ppm recovery group was exposed to 20 ppm dietary PFOS for 53 weeks and then removed from exposure (i.e., was fed a control diet).

3. Except as indicated, all hepatocellular tumors were adenomas.

4. Includes scheduled sacrifices and spontaneous deaths (data from Thomford (2002).

5. Number of animals in original exposure group minus animals with mortality ≤ 52 weeks.

Benchmark dose modeling of hepatocellular tumors

Benchmark dose modeling was conducted on the incidence of hepatocellular adenomas plus carcinomas in female rats. For each dose group, the PFOS serum concentrations over the entire exposure period were estimated as the area-under-the-curve (AUC) of serum concentration versus time. It was assumed that internal exposure to PFOS in the recovery group (i.e., termination of 20 ppm dietary exposure at 52 weeks) continued (but decreased) after the termination of dietary exposure. Benchmark dose modeling was carried out using all available dichotomous models and a BMR of 10% in the USEPA BMDS software (version 2.6.0.1). The use of a BMR of 10% is supported by the observation that the tumor incidence in the high dose group was 10%. Therefore, a BMR of 10% is appropriate for modeling these data. Table 47 gives the results of the benchmark dose modeling. Detailed model outputs are presented in Appendix 7.

Table 47. Benchmark Dose modeling of hepatocellular adenomas plus carcinomas in female rats (data from Butenhoff et al. (2012) and Thomford et al. (2002)

Model	***Parameter Restrictions***	***Poly***	***Chi-square p-value***	***AIC***	***BMD (ng/ml)***	***BMDL (ng/ml)***
Gamma	**No Power Restriction**	**-**	**0.7254**	**91.72**	**223,921**	**136,931**
Gamma	Restrict Power ≥ 1	-	0.7254	91.72	223,921	146,863
Log Logistic [1]	No Slope Restriction	-	0.7252	89.78	293,786	135,695
Log Logistic	Restrict Slope ≥ 1	-	0.7278	91.71	222,762	145,871
Log Probit [1]	No Slope Restriction	-	0.7065	89.89	341,864	134,024
Log Probit	Restrict Slope ≥ 1	-	0.7297	91.77	224,375	163,078
Logistic [1]	-	-	0.8680	89.54	217,195	172,669
Multistage [2]	No Beta Restriction	3rd	0.5175	93.16	207,177	144,054
Multistage [3]	Restrict Betas ≥ 0	3rd	0.7266	91.52	219,137	149,798
Multistage	Restrict Betas ≥ 0	2nd	0.6971	91.64	228,610	148,097
Multistage [2]	No Beta Restriction	2nd	0.6971	91.64	228,610	135,207
Probit [1]	-	-	0.8582	89.57	220,249	168,550
Quantal-Linear [4]	-	-	0.7698	89.81	257,440	145,713
Weibull [5]	No Power Restriction	-	0.7272	91.70	222,462	137,093
Weibull [5]	Restrict Power ≥ 1	-	0.7272	91.70	222,462	147,127

[1] Background parameter estimate hit a boundary.

[2] BMDU did not converge, so BMDU calculation failed.

[3] The beta2 parameter estimate hit a boundary.

[4] Power parameter estimate hit a boundary.

[5] Background, slope, and power parameter estimates hit boundaries

Model Selection

Upon initial inspection, all models appeared to give acceptable fits as judged by the chi-square p- value and the scaled residuals. USEPA Benchmark Dose technical guidance (USEPA, 2012) calls for selection of an overall BMDL based on consideration of several factors including, the relative magnitude of the available BMDLs and the quality of the available models as assessed by the Akaike information criterion (AIC). As noted in Table 47, for several of the models, estimation of various model parameters hit a boundary and that parameter could not be integrated into the fit of the model to the data. Although the BMDS software still fit these models to the data, the resulting fit did not reflect the full structure of the model. In addition, because the AIC parameter is partially determined by the number of parameters in each model, those models in which parameters were dropped because of boundary problems had artificially reduced AIC values. Thus, those models cannot be compared to the other models on the basis of their AIC values. Excluding all models for which parameter estimates hit a boundary, five models remained. The BMDLs for these models ranged from 136,931 to 163,078 ng/ml, and the AIC values ranged from 91.64 to 91.77. Both BMDLs and AIC values for these models, therefore, fell into a relatively narrow range. The two models with the smallest BMDL values (Gamma- no power restriction, BMDL = 136,931 ng/ml; and Log-logistic – slope restricted to ≥ 1, BMDL =145,871 ng/ml) had nearly identical AIC values (91.72 and 9.71, respectively), and both had nearly identical scaled residuals at the serum concentration closest to the BMD. Although these BMDLs are close (6% difference), the smallest BMDL is sufficiently distinct to be used independently for calculating the cancer slope factor (CSF). **Therefore, the POD for calculation of the CSF is 136,931 ng/ml.**

Cancer potency factor (cancer slope factor)

The cancer potency slope (cancer slope factor) based on serum concentration from the hepatocellular tumor incidence in the female rats in the Butenhoff et al. (2012) study is derived as the linear slope of the line between the POD (148,160 ng/ml; 10% response) and the origin (0 ng/ml; 0% response) as 0.1/148,088 ng/ml =7.3×10^{-7} $(ng/ml)^{-1}$. Based on the clearance factor that relates human serum PFOS serum levels (ng/ml) to intake dose (ng/kg/day) of 8.1×10^{-5} L/kg/day (8.1×10^{-2} ml/kg/day), the human cancer potency factor based on intake dose is **9.0×10^{-6} $(ng/kg/day)^{-1}$.**

As discussed above, the cancer potency estimated from the hepatocellular tumor incidence in the male rats in the Butenhoff et al. (2012) is 1×10^{-6} $(ng/ml)^{-1}$.

The two cancer potency estimates are close, and the potency estimate based on male rat data is slightly higher than the estimate from the female rat data. However, the estimate from the female rats is based on a more robust and more informative data set, since liver tumors occurred only in the high dose group in males but occurred in all dosed groups in females. Therefore, data from female rats is more appropriate for estimating the cancer risk of PFOS in drinking water.

Estimated cancer risk at ISGWQC

As above, the cancer potency factor (slope factor) for liver tumors in female rats, 9.0×10^{-6} $(ng/kg/day)^{-1}$, was used to estimate cancer risk. Uncertainties associated with this cancer slope factor include uncertainties regarding inclusion of the recovery group data in dose-response analysis and uncertainties about the dose metric based on AUC serum levels. The BMD modeling of liver tumors in females included tumor incidence data from the 20 ppm recovery group (dosed with PFOA for one year followed by one year without dosing until sacrifice at 2 years) While inclusion of the recovery group females helps to inform the shape of the dose-response curve, there is uncertainty about including these data in dose-response modeling withother dose groups exposed for the full 2-

year study duration, due to differences in the time course of exposure in the recovery group. Additionally, the dose-response modeling was based on AUC of serum PFOS data. Since the AUCs were developed using linear interpolation from data for a relatively small number of time points, and data for some time points were not available for all dose groups, there is considerable uncertainty in the AUC estimates.

Cancer risk (unitless) is calculated from the cancer potency factor and dose as follows:

Risk = Potency Factor $(ng/kg/day)^{-1}$ x Dose (ng/kg/day)

From above, the cancer potency factor for hepatocellular tumors in female rats is 9.0×10^{-6} $(ng/kg/day)^{-1}$.

The dose at the ISGWQC of 10 ng/L can be calculated using default assumptions for body weight (70 kg) and drinking water consumption (2 L/day).

$$\text{Dose (ng/kg/day) from 10 ng/L} = \frac{10 \text{ ng/L} \times 2 \text{ L/day}}{70 \text{ kg}} = 0.29 \text{ ng/kg/day}$$

The lifetime cancer risk is therefore calculated as:

9.0×10^{-6} $(ng/kg/day)^{-1}$ x 0.29 ng/kg/day = 3×10^{-6} (3 in one million)

The estimated cancer risk of 3 in one million is slightly above the cancer risk goal for New Jersey ISGWQCs of one in one million. It is the general policy of the NJDEP, and USEPA Office of Water to apply an additional uncertainty factor of 10 to an RfD for a non-cancer endpoint to account for potential cancer risk of Suggestive Carcinogens when a cancer potency factor (slope factor) is not available or is considered uninformative. However, since the estimated cancer risk at the ISGWQC based on a sensitive non-carcinogenic effect is close to the New Jersey cancer risk goal of one in one million, application of this uncertainty factor is not necessary.

ISGWQC

The ISGWQC of 10 ng/L based on decreased plaque forming cell response from Dong et al. (2009) is the lowest of the three potential ISGWQCs based on non-cancer endpoints. In addition to yielding the lowest ISGWQC value, this endpoint is an appropriate basis for the ISGWQC because of the clear toxicological relevance of decreased response to foreign antigens and evidence for the association of decreased vaccine response in humans with general population level exposure to PFOS. The estimated cancer risk at the ISGWQC of 10 ng/L is close to the New Jersey cancer risk goal of one in one million. Thus, a ISGWQC of 10 ng/L based on immune system toxicity is considered to be both scientifically appropriate and health protective.

Therefore, the ISGWQC is 10 ng/L.

DISCUSSION OF UNCERTAINTIES

- PFOS is associated with several human health effects in epidemiology studies of the general population, most notably decreased vaccine response. Although causality cannot be definitively proven for these associations due to the design of the epidemiology studies and limitations in the results, these findings indicate the need for caution about drinking water exposures that will increase serum PFOS to levels

substantially higher than in the general population. This is particularly true because elevated serum PFOS levels persist for many years after exposure ends, due to its long human half-life (several years).

Ongoing exposure to the ISGWQC of 10 ng/L is expected to increase serum PFOS levels, on average, by about 2.0 ng/ml (ppb) with average daily water consumption and 3.6 ng/ml (ppb) with upper percentile daily water consumption in adults. Increases in serum PFOS levels are predicted to be substantially higher in infants than in adults, including both breastfed infants whose mothers ingest PFOS in drinking water or from formula prepared with water contaminated with PFOS.

- Human epidemiology studies of PFOS have been conducted in the general population and in workers with higher occupational exposures, but there are no studies of associations of PFOS with health effects in communities exposed to contaminated drinking water. Associations of the related compound PFOA with multiple health effects, including two types of cancer, have been identified in studies of communities with contaminated drinking water (DWQI, 2017). It is unknown whether such studies of PFOS would reveal associations with additional health effects that have not yet been identified.

- Chronic toxicity and carcinogenicity of PFOS have been studied only in a single rat study. There is uncertainty about chronic effects including carcinogenicity in other species. Furthermore, the chronic studies did not assess effects including carcinogenicity which might result from exposures during the critical developmental stages which are known to be sensitive periods for PFOS toxicity.

  Uncertainties about the human relevance of effects seen in animals are inherent to all risk assessments based on animal data. As reviewed in detail in this document, the available information indicates that the effects of PFOS observed in experimental animals are relevant to humans for the purposes of risk assessment.

- A number of reproductive and development effects were reported from gestational and/or lactational PFOS exposure in animals including increased mortality, decreased body weight, structural abnormalities, and endocrine/metabolism effects such as changes in thyroid hormone levels and glucose metabolism. From epidemiologic studies, there is some suggestion that PFOS may have developmental neurological effects. Therefore, early lifestages may represent a window of susceptibility following PFOS exposure. As reviewed above, decreased offspring total thyroxine levels (Wang et al., 2011c) was the only reproductive/developmental endpoint identified as one of the most sensitive for PFOS. This endpoint was excluded from ISGWQC derivation due to uncertainties in measuring total thyroxine and uncertain human relevance given the lack of epidemiologic support for an association of PFOS with this effect. However, for comparison, BMD modeling was conducted (Appendix 7) on these data but did not provide a stable fit to any of the available BMD models. As a point of reference, however, if a criterion were to be derived for this effect, the POD as a maternal serum PFOS LOAEL (PND 1) of 2,290 ng/ml would be modified by the application of: a UF_{human} of 10; a UF_{animal} of 3; a UF_{LOAEL} of 3(due to a lack of a NOAEL); a $UF_{sub\text{-}chronic}$ of 1 (because exposure was of short duration during gestation); and a $UF_{database}$ of 1, yielding a total UF of 100. This would correspond to a drinking water concentration of 13 ng/L which rounds to an ISGWQC of 10 ng/L, which is identical to the drinking water concentration of 13 ng/L for decreased plaque forming cell response (Dong et al., 2009). Based on the above, the ISGWQC of 10 ng/L is protective of the reproductive and developmental effects identified in this assessment.

- Available information indicates that the toxicological effects are generally similar for PFOS and some other PFCs, including PFOA (DWQI, 2017). Additionally, the health effects

associated with PFOS in epidemiology studies are also associated with PFOA. Therefore, the toxicity of PFOS and other PFCs may be additive. Although PFOS and other PFCs, including PFOA, are known to co-occur in some NJ public water supplies, the potential for additive toxicity of PFOS and other PFCs was not considered in development of the ISGWQC.

In conclusion, the health-based drinking water concentration for PFOS is 13 ng/L. As interim ground water criteria are rounded to one significant figure, **the Interim Specific Ground Water Criterion for PFOS is 10 ng/L (0.01 µg/L).**

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Appendix 1: Literature search strategy and results

Table A-1. Summary of PubMed and Toxline database search strategies

Database or website (date of search)	Search term string
PubMed (3/24/15) Limitations Publication dates, custom range = 1900/01/01 to 2014/12/31	Perfluoroalkyl OR PFOS OR 1763-23-1[rn] OR 2795-39-3[rn] OR 29081-56-9[rn] OR 29457-72-5[rn] OR 4021-47-0[rn] OR 70225-14-8[rn] OR "1-octanesulfonic acid"[tiab] OR "1-octanesulphonic acid"[tiab] OR "1-perfluoroctanesulfonic"[tiab] OR "1-perfluorooctanesulfonic"[tiab] OR "heptadecafluoro-1-octane sulfonic"[tiab] OR "heptadecafluoro-1-octanesulfonic"[tiab] OR "heptadecafluoroctane sulfonic"[tiab] OR "heptadecafluorooctane sulfonic"[tiab] OR "heptadecafluorooctane sulphonic"[tiab] OR heptadecafluorooctanesulfonic[tiab] OR "octanesulfonic acid"[tiab] OR "octanesulphonic acid"[tiab] OR "perfluoroalkyl sulfonate"[tiab] OR "perfluoroalkyl sulphonate"[tiab] OR "perfluoroctane sulfonate"[tiab] OR "perfluoroctane sulfonic"[tiab] OR "perfluoroctane sulphonate"[tiab] OR "perfluoroctane sulphonic"[tiab] OR perfluoroctanesulfonate[tiab] OR perfluoroctanesulfonic[tiab] OR perfluoroctanesulphonate[tiab] OR perfluoroctanesulphonic[tiab] OR perfluoroctylsulfonic[tiab] OR "perfluoro-n-octanesulfonic"[tiab] OR "perfluorooctane sulfonate"[tiab] OR "perfluorooctane sulfonic acid"[tiab] OR "perfluorooctane sulphonate"[tiab] OR "perfluorooctane sulphonic"[tiab] OR perfluorooctanesulfonate[tiab] OR perfluorooctanesulfonic[tiab] OR perfluorooctanesulphonate[tiab] OR perfluorooctanesulphonic[tiab] OR "perfluorooctanyl sulfonate"[tiab] OR "perfluorooctanyl sulphonate"[tiab] OR "perfluorooctylsulfonic acid"[tiab]
Toxline (3/24/15) Limitations Include PubMed records = no (box unchecked); Advanced search, Year of Publication = 1900 through 2014	Perfluoroalkyl OR PFOS OR 1763-23-1 OR 2795-39-3 OR 29081-56-9 OR 29457-72-5 OR 4021-47-0 OR 70225-14-8 OR "1-octanesulfonic acid" OR "1-octanesulphonic acid" OR "1-perfluoroctanesulfonic" OR "1-perfluorooctanesulfonic" OR "heptadecafluoro-1-octane sulfonic" OR "heptadecafluoro-1-octanesulfonic" OR "heptadecafluoroctane sulfonic" OR "heptadecafluorooctane sulfonic" OR "heptadecafluorooctane sulphonic" OR heptadecafluorooctanesulfonic OR "octanesulfonic acid" OR "octanesulphonic acid" OR "perfluoroalkyl sulfonate" OR "perfluoroalkyl sulphonate" OR "perfluoroctane sulfonate" OR "perfluoroctane sulfonic" OR "perfluoroctane sulphonate" OR "perfluoroctane sulphonic" OR perfluoroctanesulfonate OR perfluoroctanesulfonic OR perfluoroctanesulphonate OR perfluoroctanesulphonic OR perfluoroctylsulfonic OR "perfluoro-n-octanesulfonic" OR "perfluorooctane sulfonate" OR "perfluorooctane sulfonic acid" OR "perfluorooctane sulphonate" OR "perfluorooctane sulphonic" OR perfluorooctanesulfonate OR perfluorooctanesulfonic OR perfluorooctanesulphonate OR perfluorooctanesulphonic OR "perfluorooctanyl sulfonate" OR "perfluorooctanyl sulphonate" OR "perfluorooctylsulfonic acid"

Table A-2. Summary of additional databases and website searched		
Database or website	**Date searched**	**Search terms**
Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles http://www.atsdr.cdc.gov/toxprofiles/index.asp California Environmental Protection Agency (CalEPA) Office of Environmental Health Hazard Assessment (OEHHA) http://oehha.ca.gov/index.html Toxicity Criteria Database http://oehha.ca.gov/tcdb/index.asp Non-cancer health effects Table (RELs) and Cancer Potency Factor (Appendix A and Appendix B) http://www.oehha.ca.gov/air/hot_spots/index.html Chemical Carcinogenesis Research Information System (CCRIS) http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS Developmental and Reproductive Toxicology Database (DART) http://toxnet.nlm.nih.gov/newtoxnet/dart.htm Environment Canada https://www.ec.gc.ca/ European Chemicals Agency http://echa.europa.eu/web/guest Genetic Toxicology Data Bank (GENETOX) http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX Hazardous Substances Data Bank (HSDB) http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm Health Canada First Priority Substances List (PSL1) Assessments http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php Health Canada Second Priority Substances List (PSL2) Assessments http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php	3/24/15	PFOS perfluorooctane sulfonate 1763-23-1

International Agency for Research on Cancer (IARC) Monographs http://monographs.iarc.fr/ENG/Classification/index.php International Programme on Chemical Safety (IPCS) http://www.who.int/ipcs/en/ International Programme on Chemical Safety (IPCS) INCHEM http://www.inchem.org/ International Toxicity Estimates for Risk (ITER) http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?iter National Institute for Occupational Safety and Health (NIOSH) publications database (NIOSHTIC2) http://www2a.cdc.gov/nioshtic-2/ Occupational Safety and Health Administration (OSHA) https://www.osha.gov/ US EPA Acute Exposure Guideline Levels http://www.epa.gov/oppt/aegl/ United State Environmental Protection Agency (US EPA) ChemView http://java.epa.gov/chemview US EPA IRIS http://www.epa.gov/iris/ US EPA Office of Pesticides Chemical Search database http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1 US EPA Office of Water Drinking Water Standards and Health Advisories http://water.epa.gov/drink/standards/hascience.cfm US EPA Provisional Peer Reviewed Toxicity Values (PPRTV) assessment library http://hhpprtv.ornl.gov/quickview/pprtv_papers.php United Stated National Toxicology Program (US NTP) Report on Carcinogens http://ntp.niehs.nih.gov/pubhealth/roc/listings/index.html World Health Organization (WHO) Concise International Chemical Assessment Documents http://www.who.int/ipcs/publications/cicad/en/ WHO Environmental Health Criteria http://www.who.int/ipcs/publications/ehc/en/		

Table A-3. Criteria used to identify references for further consideration or for exclusion

A reference was identified for further consideration if it met one of the following criteria:

- Animal toxicology studies (including rodents, non-human primates, and rabbits)
- Epidemiological studies
- Human exposure
- Mechanistic studies (including studies on absorption, distribution, metabolism, excretion, in vitro studies, in silico studies, genotoxicity)
- Secondary sources of health effects information (i.e., not primary data references such as book chapters, commentaries, editorials, health assessments, review articles)

A reference was excluded if it met at least one of the following criteria:

- Describes analytical methodology (e.g., method development)
- Foreign language reference
- Meeting abstract/poster
- Measurement in consumer products (e.g., packaging) or food for human consumption including drinking water
- Measurement in environmental media (e.g., air, dust, sewage treatment effluent or sludge, soil, water)
- Not enough information to determine relevance (e.g., no abstract and/or readily accessible full text version)
- PFOS is not the test agent
- PFOS used as a chemical reagent in a non-toxicological manner (e.g., use of aqueous firefighting foam)
- Proposed research (e.g., funding application)
- Reference was a duplicate (determined electronically or manually)
- Related to biodegradation, environmental fate or processes, or remediation
- Related to effects or measurement in wildlife (includes crops, livestock, plants)
- Related to chemical or physical properties
- Related to policy (e.g., monitoring or screening programs)
- The abbreviation PFOS returned a non-chemical reference

Table A-4. Backward searches	
Reference used for backward search[1]	Results of backward search[2]
Bach CC, Bech BH, Brix N, Nohr EA, Bonde JP, Henriksen TB. 2015. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: A systematic review. Critical reviews in toxicology 45:53-67.	0 references
USEPA. 2014. Health effects document for perfluorooctane sulfonate (PFOS).	1 reference Haug LS, Thomsen C, Becher G. 2009. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environmental Science & Technology 43:2131-2136.
Chang ET, Adami HO, Boffetta P, Cole P, Starr TB, Mandel JS. 2014. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. Critical reviews in toxicology 44 Suppl 1:1-81	1 reference Bonefeld-Jorgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Kruger T, et al. 2011. Perfluorinated compounds are related to breast cancer risk in greenlandic inuit: A case control study. Environmental Health : A Global Access Science Source 10:88.
Corsini E, Luebke RW, Germolec DR, DeWitt JC. 2014. Perfluorinated compounds: Emerging pops with potential immunotoxicity. Toxicology letters 230:263-270.	0 references
Saikat S, Kreis I, Davies B, Bridgman S, Kamanyire R. 2013. The impact of pfos on health in the general population: A review. Environmental science Processes & impacts 15:329-335.	0 references

Taylor KW, Novak RF, Anderson HA, Birnbaum LS, Blystone C, Devito M, et al. 2013. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: A national toxicology program workshop review. Environmental health perspectives 121:774-783.	0 references
DeWitt JC, Peden-Adams MM, Keller JM, Germolec DR. 2012. Immunotoxicity of perfluorinated compounds: Recent developments. Toxicologic pathology 40:300- 311.	0 references
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Mariussen E. 2012. Neurotoxic effects of perfluoroalkylated compounds: Mechanisms of action and environmental relevance. Archives of toxicology 86:1349-1367.	0 references
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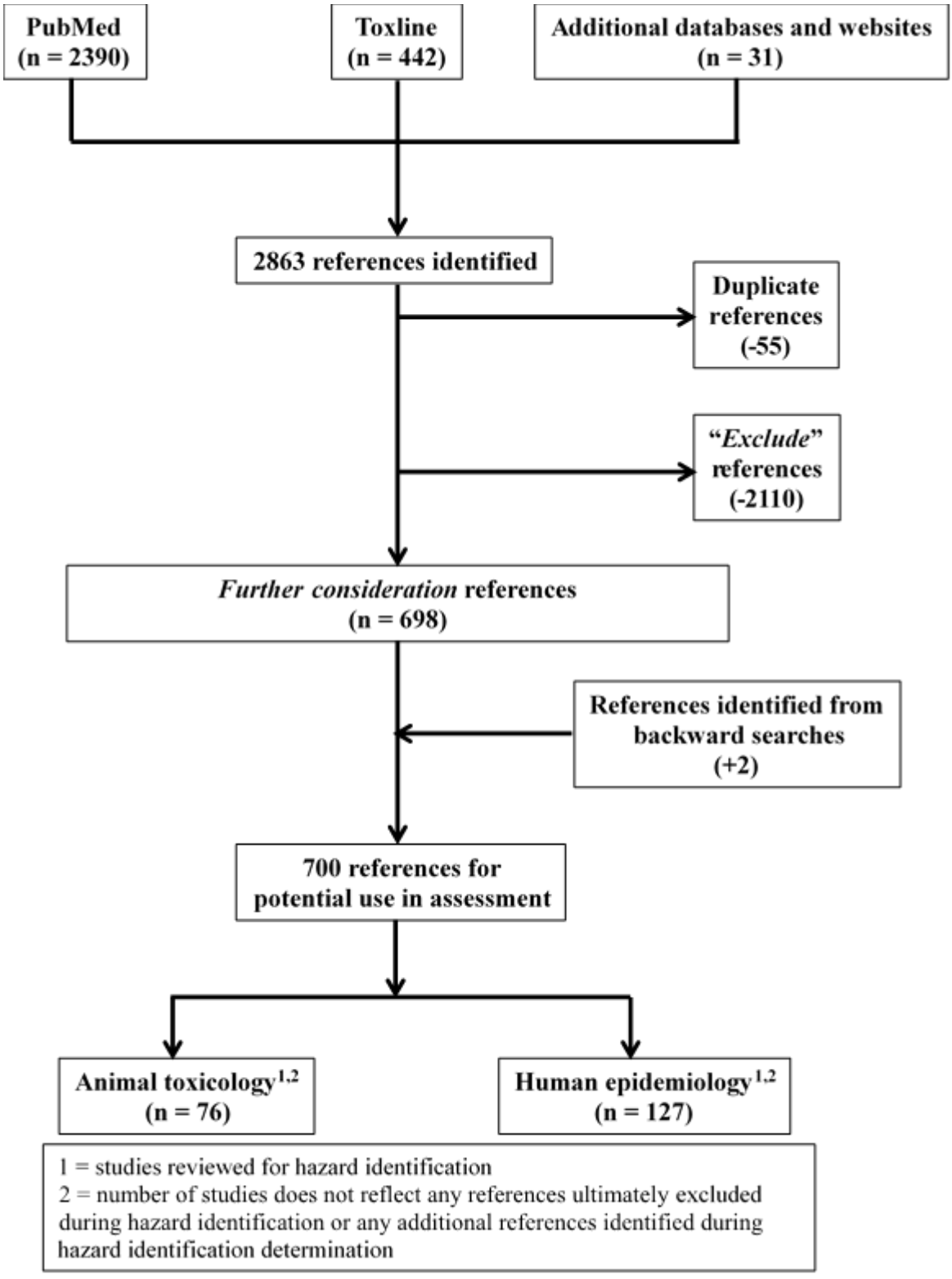


Figure A-1. Graphical representation of literature search

Appendix 2: Comparison of USEPA Office of Water Health Advisory and NJDEP ISGWQC for PFOS

The basis for the USEPA (2016a) Health Advisory and this ISGWQC for PFOS, and other relevant information about these two drinking water values, are compared in the table below. Additional information is provided in the text that follows the table.

Parameter	USEPA Office of Water (OW) Lifetime Health Advisory	NJDEP Health-based concentration/ISGWQC
Drinking Water Concentration	70 ng/L	13 ng/L (rounded to 10 ng/L)
General Statement and Summary	"Protects the most sensitive populations, with a margin of protection from a lifetime of exposure."	"Developed using a risk assessment approach intended to be protective for chronic (lifetime) exposure."
	As discussed in this document, PFOS is associated with several human health effects, including decreased vaccine response and others, within the general population exposure range even without additional exposure from drinking water. The Target Human Serum Level for decreased immune response (decreased plaque forming cell response) in mice (22.5 ng/ml) is only slightly above the exposure range in the general population (95th percentile – 19 ng/ml). Therefore, additional exposure from drinking water may potentially pose some risk of health effects. For this reason, it cannot be definitively concluded that lifetime exposure to these drinking water concentrations is protective of sensitive subpopulations with a margin of exposure. USEPA (2016a) recognizes that human studies provide evidence of associations of several health effects with PFOS. However, USEPA concludes that the human studies do not provide quantitative information on the exposure levels or serum levels associated with these health effects. Therefore, USEPA did not consider the possibility that health effects may result from exposures within the general population range, even in the absence of additional exposure from drinking water. Additionally, USEPA also dismissed the most sensitive toxicological effect in animal studies, decreased plaque forming cell response, from consideration as the basis for risk assessment. See further discussion of these points below.	
Reference Dose (RfD)	20 ng/kg/day (2 x 10^{-5} mg/kg/day)	1.8 ng/kg/day (1.8 x 10^{-6} mg/kg/day)
	Based on decreased body weight in neonatal rats (F_2 generation); selected based on lowest administered dose.	Based on decreased plaque forming cell response in adult male mice; selected based on lowest serum PFOS concentration.

Interspecies conversion	Based on pharmacokinetic modeling used to predict average serum PFOS concentrations.	Based on measured serum PFOS concentrations at end of dosing period.
Estimated lifetime cancer risk at Health Advisory/ ISGWQC	Not assessed by EPA. Estimated as 2×10^{-5} based on the cancer slope factor derived here	Estimated as 3×10^{-6} based on the cancer slope factor derived here.
Relative Source Contribution Factor	20% - to account for non-drinking water exposures.	20% - to account for non-drinking water exposures.
Assumed Drinking Water Consumption	0.054 L/kg/day; 90th percentile for lactating woman	0.029 L/kg/day; Based on default upper percentile adult assumptions: 2 L/day, 70 kg
Increase in serum PFOS concentration predicted from ongoing exposure to USEPA Health Advisory and NJ health-based water concentration	<u>With average water consumption:</u> The USEPA Lifetime Health Advisory is predicted to result in a serum PFOS concentration 3.7 times the U.S. general population median (CDC, 2017) <u>With upper percentile water consumption:</u> The USEPA Lifetime Health Advisory is predicted to result in a serum PFOS concentration 5.8 times the U.S. general population median (CDC, 2017) (Note: These calculations are explained in more detail below)	<u>With average water consumption:</u> The ISGWQC is predicted to result in a serum PFOS concentration 1.5 times the U.S. general population median (CDC, 2017) <u>With upper percentile water consumption:</u> The ISGWQC is predicted to result in a serum PFOS concentration 1.9 times the U.S. general population median (CDC, 2017) (Note: These calculations are explained in more detail below)
Sensitive Subpopulations	Pregnant and lactating women; bottle-fed infants. USEPA does not include women who plan to become pregnant in its definition of sensitive subpopulations, but says that states may choose to expand the sensitive subgroups to include women of childbearing age (ASDWA, 2016). However, the body burden of PFOS remains elevated for many years after exposure ceases. Therefore, if body burden is elevated prior to pregnancy, it will remain elevated during pregnancy and lactation.	As is the case for all ISGWQCs developed by the NJDEP, the ISGWQC for PFOS is intended to be protective of all individuals, including sensitive subpopulations. Sensitive subpopulations for health effects of PFOS include women who plan to become pregnant, pregnant women, lactating women, and breast-fed and bottle-fed infants.

	USEPA (2016a) also calculated a Lifetime Health Advisory value for alternative exposure scenarios for the general population (adults age 21 and older) of 100 ng/L based on standard adult exposure assumptions. USEPA states that the Lifetime Health Advisory of 70 ng/L is protective for effects other than developmental toxicity, such as "liver damage, other developmental effects, and developmental neurotoxicity". It is noted that the news media has reported that the USEPA designation of sensitive subgroups has been misinterpreted by some local authorities to mean that those not in these sensitive subpopulations may continue to drink water exceeding the USEPA Health Advisory.	

Discussion of differences in risk assessment approaches employed here and the the USEPA-OW

Endpoints used as basis for USEPA Office of Water (OW) Health Advisory and this recommeded Health-based ISGWQC

The primary basis for this ISGWQC is an RfD for decreased plaque forming cell response in mice (Dong et al., 2009). We conclude that this immunosuppressive effect in animals is a sensitive and well- established effect of PFOS that is relevant to humans. Based on epidemiologic studies (summarized below), there is evidence that serum PFOS concentrations within the range found in the general population are associated with immunosuppressive effects (i.e., decreased vaccine response).

Although plaque forming cell response as reported by Dong et al. (2009) was the most sensitive endpoint (i.e. occurring with the lowest LOAEL) identified by USEPA for studies of greater than short-term exposure (p. 4-4 of USEPA, 2016b), USEPA did not use this endpoint as the basis of its Health Advisory. Instead, USEPA chose decreased neonatal body weight from the F_2 generation in a two-generation rat study (Luebker et al., 2005a) as the critical endpoint. While this is a valid endpoint for use in human health risk assessment, we conclude that the immunotoxicity endpoint is equally valid and, importantly, more sensitive. A detailed comparison of the LOAELs for the two endpoints is provided below. In light of the weight of evidence for the immunotoxicity of PFOS at low levels of exposure, we conclude that USEPA does not make a strong case for its decision not to choose the animal immune toxicity data for this endpoint as the basis for the PFOS Health Advisory. USEPA provides the following summary statement to justify its decision not to base its Health Advisory on immunotoxicity, and specifically not on the Dong et al. (2009) study:

"*Taken together, the lower antibody titers associated with PFOS levels in humans and the consistent suppression of SRBC [sheep red blood cells] response in animals indicates a concern for adverse effects on the immune system. However, lack of human dosing information and lack of low-dose confirmation of effects in animals for the short-duration study precludes the use of these immunotoxicity data in setting the RfD*."

We agree with USEPA that evidence for the suppression of immune response (SRBC response) in animals is "*consistent*." We also agree with USEPA that the combination of epidemiological (human) and animal data indicates "*a concern for adverse effects*." Therefore, it is not clear what USEPA means by the "*lack of human dosing information*," or "*the lack of low dose confirmation of effects in animals for short duration study*," and why these statements are sufficient to preclude the use of immunotoxicity data in derivation of its Health Advisory.

Several other recent reviews by government and academic scientists have also identified decreased immune response as a sensitive and relevant endpoint for PFOS risk assessment. The National Toxicology Program (NTP, 2016) conducted a systematic review of immunotoxicity of PFOS, based on consideration of human and animal studies, along with mechanistic data. NTP (2016) concludes that exposure to PFOS is presumed to be an immune hazard to humans based on: 1) a high level of evidence that PFOS suppressed the antibody response from animal studies, and 2) a moderate level of evidence from studies in humans. NTP also considered additional, although weaker, evidence from laboratory animal studies suggesting PFOS may suppress infectious disease resistance and NK cell activity in humans. NTP stated that "the bodies of evidence indicating that PFOS suppresses multiple aspects of the immune system add to the overall confidence that PFOS alters immune function in humans."

Additionally, the Minnesota Department of Health Reference Dose (MDH, 2017) and the ATSDR (2018) Minimum Risk Level for PFOS incorporate uncertainty factors for potentially more sensitive immune system toxicity than decreased offspring body weight in Luebker et al. (2005a), the critical effect for the EPA Reference Dose.

Finally, two recent peer reviewed publications have identified immunotoxicity as a sensitive toxicological endpoint for PFOS. Both Lilienthal et al. (2017) and Dong et al. (2017) noted that immune system toxicity is a more sensitive endpoint than the developmental effects used as the basis for the USEPA (2016a) RfD for PFOS. Lilienthal et al. (2017) reviewed recent data on health effects of PFOS in relation to current regulations and guidance values and note that human and animal evidence suggest that low doses of PFOS cause immune system suppression. They further state that decreased immune system response from PFOS (and low-dose developmental effects of PFOA) "likely constitute a sound basis for ongoing and future regulations."

Comparison of LOAELs for decreased plaque forming cells (Dong et al., 2009) and decreased neonatal body weight (Luebker et al., 2005a)

Based on administered dose, the LOAEL for decreased plaque forming cell response used as the critical effect here was 0.083 mg/kg/day (Dong et al., 2009), whereas the LOAEL for decreased neonatal body weight (F_2 generation) used as the critical effect by USEPA was 5-fold higher (0.4 mg/kg/day maternal dose group; Luebker et al., 2005a). Serum PFOS concentrations are more relevant than administered doses for comparison of LOAELs because serum concentrations represent the internal doses that cause toxicological effects. In Dong et al. (2009), terminal sacrifice occurred at the end of the dosing period and therefore, reflects the maximum exposure in the dosed mice. We used serum PFOS levels at terminal sacrifice from Dong et al. (2009) as the dose metric for Reference Dose development. The serum PFOS concentration at the LOAEL for decreased plaque forming cell response was 7,132 ng/ml.

The serum PFOS measurement reflecting the maximum exposure in the neonatal F_2 generation rats from Luebker et al. (2005a) would be the serum concentration in the F_1 dams at or close to parturition of the F_2 pups. However, Luebker et al. (2005a) did not measure maternal F_1 serum PFOS concentrations. Although more uncertain than measured maternal F_1 serum levels would have been, several other measured and modeled serum PFOS provide estimates of the serum PFOS LOAEL for decreased neonatal F_2 body weight from Luebker et al. (2005a).

- Luebker et al. (2005a) measured serum PFOS concentrations in the F_0 dams on day 21 after delivery of the F_1 offspring (i.e. the end of lactation). The serum PFOS concentration in the F_0 dams at the LOAEL (based on decreased neonatal body weight in the F_2 generation) of 0.4 mg/kg/day was **18,900 ng/ml**. This serum concentration is likely lower than that in the F_1 dams at delivery of the F_2 generation at the same dose for two reasons. First, exposure to the F_0 dams began at around 9 weeks of age, while the F_1 dams were exposed *in utero*, through lactation during neonatal life, and via gavage dosing starting at weaning. Secondly, and more importantly, serum levels were measured in the F_0 dams after 21 days of nursing rather than prior to delivery, and a considerable portion of the PFOS body burden in these dams had presumably been excreted in breast milk.

- Luebker et al. (2005b) conducted a one-generation reproductive/developmental in the same strain of rats used in the two-generation study (Luebker et al., 2005a). One of the doses in the one-generation study was the same as the LOAEL for the USEPA RfD from the two-generation study, 0.4 mg/kg/day. In the pharmacokinetic component of the one-generation study, dams were dosed from 42 days prior to cohabitation with males until the end of gestation, and serum PFOS levels were measured on GD 1, 7, 15, and 21. In the 0.4 mg/kg/day dose group, serum PFOS levels on GD 1, 7, and 15 were about **41,000 ng/L** and represent maximum exposure to the developing offspring, while they were lower, **26,200 ng/L**, on GD 21.

(It is noted that the serum PFOS data from the two Luebker et al. [2005a, b] studies are incorrectly presented in the USEPA (2016b) PFOS Health Effects Support Document [Table 4-3]. In Table 4-3, serum PFOS data from GD 21 of the one generation study [Luebker 2005b] are incorrectly shown to be from the end of lactation [PND 21] of the two-generation study [Luebker, 2005a]. It is also incorrectly shown that serum PFOS data are not available from the one generation study, although such data were reported by Luebker et al. [2005b]).

- The USEPA Health Advisory did not use measured serum PFOS concentrations at the LOAEL to derive the Reference Dose for decreased F_2 generation neonatal body weight in Luebker et al. (2005a). Instead, the USEPA Reference Dose is based on pharmacokinetic modeling that predicts the final serum PFOS concentration and final predicted area under the curve (AUC) for serum concentration versus time (Table 4-3,USEPA, 2016b). The average PFOS serum concentration was obtained by dividing the AUC by the study duration. For decreased neonatal body weight in Luebker et al. (2005a), the average serum PFOS concentration at the LOAEL was predicted to be **25,000 ng/ml** (Table 4-6, USEPA, 2016b).

  We note that there are inherent uncertainties in the use of a pharmacokinetic model to predict serum concentrations and the AUC in general. There is also additional uncertainty in the use of this model to predict serum PFOS concentrations for Luebker et al. (2005a) because the model is based on non-pregnant rats, but was used by USEPA to predict serum PFOS concentrations in pregnant rats used in Luebker et al. (2005a).

Notwithstanding the uncertainties discussed above, the measured and modeled serum PFOS concentrations that provide estimates of the LOAEL for decreased neonatal body weight in the F_2 generation (Luebker et al., 2005a) are several-fold higher than the serum concentration at the LOAEL in Dong et al. (2009) of 7,132 ng/L. In summary, decreased plaque forming cell response in Dong et al. (2009) is a more sensitive endpoint than the decreased neonatal body weight in the F_2 generation in Luebker et al. (2005a).

Consideration of data from human epidemiology studies

Both the current derivation and the USEPA Office of Water conducted comprehensive reviews of relevant epidemiology studies investigating possible associations between PFOS exposure and adverse health effects. Both risk assessments used epidemiology data in support of the toxicological endpoints selected as the basis for RfD development. USEPA stated that studies of low birth weight are consistent with the critical endpoint of decreased neonatal weight in rats, we identified studies of vaccine antibody levels that are consistent with the critical endpoint of suppression of cellular immune response as measured by a decrease in plaque forming cell response in mice.

Neither assessment used human epidemiological data as the quantitative basis for derivation of a Reference Dose. USEPA states that, while human studies are useful for hazard identification, they cannot be used quantitatively because the PFOS exposures at which the associations were observed are unknown or highly uncertain. In contrast, we agree that the human data have limitations that preclude their use as the primary basis for risk assessment, but it does not agree with USEPA that the serum PFOS concentrations and PFOS exposures associated with human health effects are highly uncertain or unknown.

USEPA (2016a) provides the following reasons for its conclusions:

- Serum levels may have decreased prior to when the blood sample was taken. Therefore, the effects may have been due to earlier exposures that were higher than indicated by the measured serum PFOS levels.
    - It is unlikely that this is a major source of uncertainty in evaluation of exposure since PFOS serum levels decrease slowly (half-life of several years) and do not fluctuate in the short term. Importantly, the most notable effect associated with human exposure to PFOS is decreased vaccine response in children, which may be associated with prenatal exposure (i.e. maternal serum PFOS levels) or serum PFOS levels in the child at various ages. For effects resulting from exposure at these lifestages, the serum PFOS level was measured at or close to the timepoint at which the effect was initiated. Additionally, if effects were actually due to previous exposures that were higher than those at the time of blood sampling, it would mean that the detrimental effects of PFOS are persistent and do not resolve when exposures decrease, which would increase the level of concern about the effects.

- PFOS measured in serum may result from metabolism of precursors to PFOS rather than direct exposure to PFOS itself.
    - This statement is correct but this does not appear to be a valid reason to dismiss consideration of serum PFOS levels as a measure of PFOS exposure. Effects of PFOS would be the same regardless of whether the source of exposure is PFOS itself or metabolism of precursors to PFOS.

- Co-exposure to other PFCs, even if accounted for as a potential confounding factor in the statistical analysis, increase uncertainty about observed associations of health endpoints with PFOS.
    - However, co-exposure to other chemicals is a general issue for all human studies of exposure to environmental contaminants and does not preclude evaluation of the levels of PFOS exposure

associated with health endpoints.

In considering immunotoxicity in humans, USEPA cites four epidemiological studies that investigated the association of vaccine response with serum PFOS concentration (USEPA, 2016a, b). All of these studies were also reviewed here and discussed in this document. In one study of a population with general population level exposure to PFOS, with all of the children initially vaccinated at 3 months old (Grandjean et al., 2012), PFOS in children's serum measured at 5 years of age (prebooster) was significantly associated with a decrease in their tetanus antibody levels at age 5, but not at age 7 follow-up, following a booster vaccination (28.5% decrease for each doubling of PFOS concentration). PFOS in mothers' serum was significantly associated with a decrease in children's diphtheria antibody levels at age five following a booster vaccination (38.6% decrease for each doubling of PFOS concentration) and child's PFOS serum concentration was significantly associated with decreased response at age 7. Of particular concern, the risk of having diphtheria antibody levels from the initial vaccination that were below the level of clinical protectiveness was significantly associated with both maternal and 5 year-old children's elevated PFOS levels. In another study (Granum et al., 2013) with general population levels of PFOS exposure, mothers' serum PFOS concentration was significantly associated with a decreased level of rubella vaccine in their children. In a third study of general population level PFOS exposure (Stein et al., 2016; NHANES, U.S. population) children's PFOS serum concentration was significantly associated with decreased antibodies to rubella and mumps (13.3 and 5.9% decreases, respectively). PFOS exposure was not associated with decreased immune response to any type of vaccine in only one study (Looker et al., 2014). This study evaluated response to only the influenza vaccine and included adults rather than children. The lack of association of PFOS with influenza vaccine in this study is consistent with the lack of association found in the only other study that evaluated influenza vaccine in children (Granum et al., 2013).

As mentioned above, USEPA notes correctly that similar relationships were found for other PFCs in some of these studies, and that the decrease in immune protectiveness cannot necessarily be attributed to PFOS alone. Nonetheless, the results of these human studies are consistent with the PFOS-specific animal studies of decreased immune response.

Estimation of cancer risk from PFOS in drinking water

Both USEPA and the current derivation characterized PFOS as having "suggestive evidence of carcinogenic potential" under the USEPA's 2005 Guidelines for Carcinogen Risk Assessment. Neither USEPA, nor this assessment used cancer risk as the basis of the drinking water Health Advisory or ISGWQC.

USEPA did not derive a cancer slope factor for PFOS. It stated that, for chemicals categorized as having suggestive evidence of carcinogenic potential, "*a quantitative estimate of risk is generally not performed unless there is a well-conducted study that could serve a useful purpose by providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In the case of PFOS, the existing evidence does not support a strong correlation between the tumor incidence and dose to justify a quantitative assessment."* We agree that the estimated cancer risk for PFOS based on the chronic rat study is too uncertain to use as the basis for a ISGWQC. However, this document developed a cancer slope factor to provide an estimated cancer risk to provide context for the ISGWQC based on a non-cancer endpoint. The cancer slope factor of 8.4×10^{-6} $(ng/kg/day)^{-1}$ derived here is based on the incidence of hepatocellular tumors in female rats the chronic study of Butenhoff et al. (2012).

The estimated lifetime cancer risk at the ISGWQC of 10 ng/L, based on this slope factor, is 3×10^{-6}, which is close to the target risk goal for New Jersey ISGWQCs of 1×10^{-6}. Based on the cancer slope factor derived here

and exposure assumptions, the lifetime cancer risk at USEPA's Health Advisory of 70 ng/L is estimated as 2 x 10^{-5} lifetime cancer risk.

Assumed water consumption rate

The USEPA based its water consumption rate of 0.054 L/kg/day on the 90th percentile for lactating woman. The NJDEP's assumed water consumption rate of 0.029 L/kg/day used default adult exposure assumptions of 2 L/day and a 70 kg body weight, which is intended to represent an upper percentile rate for the general population. Thus, the USEPA consumption rate is 1.9 times larger than that used by the NJDEP. For purposes of comparison, if USEPA had applied the water consumption rate used by NJDEP, the resulting USEPA Health Advisory water concentration would be proportionally larger (1.9 x 70 ng/L = 133 ng/L).

Consideration of increases in serum PFOS levels from exposure to PFOS in drinking water

As noted in the table at the beginning of this Appendix, a clearance factor was used by USEPA to relate PFOS exposures to human PFOS serum levels. This factor can be can be used to predict increases in serum PFOS from ongoing drinking water exposures. The bar graph below (Fig. A-2) shows the predicted increases in serum PFOS levels from ongoing exposure to PFOS in drinking water at the USEPA (2016a) Health Advisory (70 ng/L) and this health-based drinking water concentration (13 ng/L). The predictions shown are based on the recommended mean ingestion rate of 0.016 L/kg/day from the USEPA Exposure Factors Handbook (USEPA, 2011; Table 3-1) and the upper percentile ingestion of 0.029 L/kg/day used here to develop the health-based drinking water concentration.

As part of its toxicokinetic model for PFOS, USEPA (2016b) used the clearance factor (8.1 x 10^{-5} L/kg/day = 8.1 x 10^{-2} ml/kg/day) to convert NOAEL and LOAEL serum levels from laboratory animals to human equivalent doses. The NOAEL and LOAEL serum PFOS levels in these animal studies ranged from 6.26 – 38 µg/ml (6,260 – 38,000 ng/ml) (HEDs; Section 4-14 of USEPA, 2016b). USEPA (2016b, p. 2-23) discussed that this clearance factor relates human PFOS dose to human PFOS serum level, including from drinking water exposure. USEPA (2016c; 2016d) also used the clearance factor for PFOA in the same way as described above forPFOS - i.e. to convert NOAEL and LOAEL serum PFOA levels from animal studies to HEDs in an analogous toxicokinetics model for PFOA.

With respect to PFOA, USEPA (2016e) stated that, "…*the clearance equation cannot justifiably be utilized to predict serum values for humans using a guideline value (70 ppt or 14 ppt) that is well below the range of doses and serum values utilized in the derivation of the [toxicokinetic]model.*" These USEPA conclusions apply equally to the use of the PFOS clearance factor to estimate human serum PFOS concentrations from intake of PFOS in drinking water.

We disagree with the reasoning underlying this statement from USEPA. As discussed in detail in the Toxicokinetics section and Appendix 3 for PFOS (and in DWQI, 2017 for PFOA), the clearance factors for PFOS (and PFOA) were developed from human serum PFOS (or PFOA) data within a range that is more relevant to drinking water exposures than to the much higher range of serum PFOS (or PFOA) levels from animal studies to which it was applied by USEPA (2016e). Furthermore, the PFOS clearance factor is in agreement with estimates from other similarly exposed human populations using both toxicokinetic modeling and direct measurement of exposure media.

Although the ISGWQC is derived on the basis of animal data, as discussed above, there is substantial evidence from epidemiology studies that decreased vaccine response occurs at levels of serum PFOS prevalent in the general population. As shown in Figure A-2 below, exposure to PFOS in drinking water at the USEPA Health Advisory of 70 ng/L is predicted to increase serum PFOS concentrations to the upper end of this range and higher. Therefore, the magnitude of elevations in serum PFOS levels expected from ongoing exposure to PFOS in drinking water at the USEPA Health Advisory level are not desirable and may not be protective of public health.

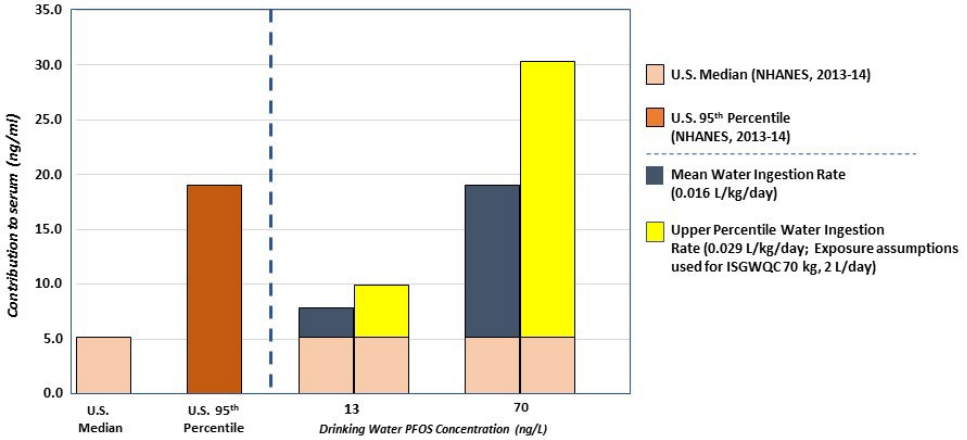
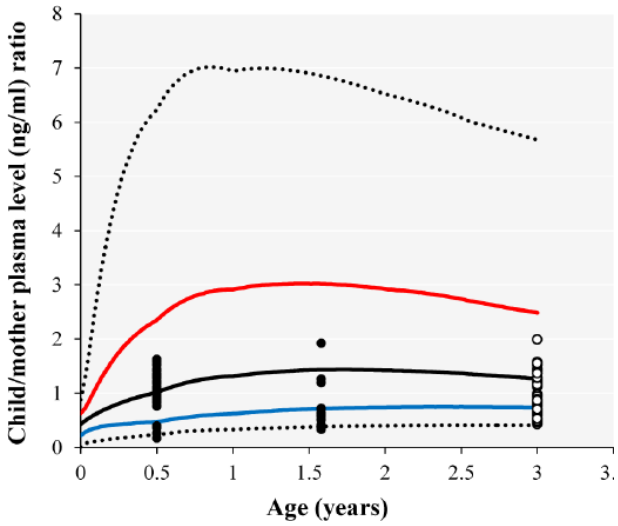


Figure A-2. Median and 95th percentile PFOS serum concentrations in the U.S. population (left of dotted line; from NHANES 2013-2014; CDC, 2017). Increases in the median U.S. serum PFOS concentration (right of dotted line) predicted from mean and upper percentile consumption of drinking water for PFOS concentrations in drinking water at the NJ health-based drinking water concentration (13 ng/L) and the USEPA Health Advisory (70 ng/L) levels.

Finally, as discussed elsewhere in this document, several studies have shown that serum PFOS concentrations in breastfed infants, while lower than maternal levels at birth, increase several fold during the first few months of life to levels which exceed those in the mother (see figure below). Exposures to infants who consume formula prepared with contaminated water are also highest during this time-period, and serum PFOS levels remain elevated for the first several years of life (see figure below). Therefore, increases in serum PFOS levels in infants and children with direct or indirect (via breast milk) exposure to drinking water contaminated with PFOS are expected to be several-fold higher than those shown in the bar graph above.

USEPA recognizes that lactating women and bottle-fed infants are sensitive subpopulations for exposure to PFOS in drinking water. We also conclude that the elevated exposures during infancy and early childhood are of particular concern because sensitive endpoints for health effects, including decreased immune response, may result from shorter term higher exposures early in life. Additionally, we conclude that women who may become pregnant should also be included as sensitive subpopulations, because the body burden of PFOS remains elevated for many years after exposure ceases. Therefore, if serum PFOS levels are elevated when a woman becomes pregnant, they will remain elevated during pregnancy and lactation.



From Verner et al. (2016). Modeling simulation of the ratio of PFOS in blood plasma in breast fed infants/children to plasma concentration in mother. Black line - 50th percentile. Blue line - 5th percentile. Red line - 95th percentile. Dotted lines - minimum and maximum values.

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Appendix 3: Alternate Derivation of the PFOS-Specific Clearance Factor and Basis for USEPA (2016) clearance factor used in ISGWQC development

A chemical-specific clearance factor (CL) of 8.1 x 10^{-5} L/kg/day (8.1 x 10^{-2} ml/kg/day) that relates PFOS serum levels to dose in humans at steady-state was developed by USEPA (2016) and was used in development of the ISGWQC. CL relates administered PFOS dose to serum PFOS level in humans, as follows:

Dose (ng/kg/day) = Serum Level (ng/ml) x CL (ml/kg/day)

The clearance factor was based on the human half-life ($t_{1/2}$) from a study of retired workers (Olsen et al., 2007) and the volume of distribution (V_d) from Thompson et al. (2010a, b) using the equation below:

$CL = V_d \times (\ln 2 / t_{1/2})$

Where:

V_d = 0.23 L/kg
ln 2 = 0.693
$t_{1/2}$ = 5.4 years = 1,971 days

The only direct measure of the human serum $t_{1/2}$ of PFOS is from retired workers who were occupationally (i.e. highly) exposed to PFOS and are older than the general population. It is unknown whether the $t_{1/2}$ of PFOS is age and/or concentration dependent. If that were the case, the estimate of $t_{1/2}$ from a highly exposed older population could overestimate the $t_{1/2}$ in the general population which includes younger individuals and have lower exposure.

Thompson et al. (2010a,b) based the PFOS V_d value on a previously developed V_d for PFOA of 0.17 L/kg that had been calibrated with human data. The PFOA V_d was adjusted by35%, based on the observation of Andersen et al. (2006) that the V_d for PFOS can be 20 to 50% greater than for PFOA in monkeys. It is noted that, although this V_d estimate is supported by the results of Thompson et al. (2010a) and Egeghy and Lorber (2011), the use of the PFOA V_d as a surrogate measure of V_d for PFOS and the adjustment of the PFOA V_d on the basis of a cross-species analogy are sources of uncertainty in its derivation.

Clearance factor developed with alternative approach

CL can also be developed with an alternate derivation that does not require the estimation of V_d or the $t_{1/2}$ from retired workers, using the relationship between the intake dose and the associated serum concentration. This alternate derivation produces an estimate of CL that is in close agreement with the value derived by the USEPA (2016). The alternative derivation is:

As above:

Dose (ng/kg/day) = Serum Level (ng/ml) x CL (ml/kg/day)

Therefore:

CL (ng/kg/day) = Dose (ml/kg/day) / Serum level (ng/ml)

***Dose (ng/kg/day)*:**
Egeghy and Lorber (2011; cited by USEPA (2016) as support for its estimated V_d), estimated the daily average PFOS exposure from all sources in the U.S. population (ng/day) to account for the measured serum PFOS concentration in the U.S. population as reported in the NHANES database. These estimates were based on estimates of PFOS in different media from different sources combined with estimates of media-specific exposure rates of (e.g. food intake, inhalation rate, and house dust ingestion). The estimated the geometric mean value of total PFOS intake for a typical adult (i.e., not exposed to a specific source of contamination) was 160 ng/day.

Assuming the standard risk assessment default for adult body weight of 70 kg, the intake of 160 ng/kg/day is equivalent to a dose of (160 ng/day)/70 kg = **2.3 ng/kg/day**.

Serum concentration (ng/ml):
The estimate of total PFOS exposure in the U.S. adult population developed by Egeghy and Lorber (2011) was based on a large number of studies of PFOS in various media published between 2000 to 2008. Thus, the most appropriate estimate serum PFOS concentration to combine with this estimated daily PFOS intake is the geometric mean serum PFOS concentration in the general adult (i.e, ≥ 20 years old) U.S. population reported by NHANES for that period. NHANES provides data for the period from 1999-2010 mostly in one year in intervals (CDC, 2017).

Based on the NHANES data for adults reported between 2000-2008 (1999-2000, 2003-04, 200506, 2007-08), the average of the geometric mean serum PFOS concentrations is **20.6 ng/ml.** (Note that the NHANES data for this range also includes data for samples collected in 1999).

Clearance factor
From this estimates of daily intake (dose) and geometric mean serum PFOS concentrations given above, CL can be estimated as (2.3 ng/kg/day)/(20.6 ng/ml) = **0.11 ml/kg/day.** This estimate is in close agreement (i.e. 36% higher) with the CL of 0.081 ml/kg/day developed by USEPA (2016).

It is noted that the CL of 0.11 ml/kg/day from the above alternate derivation is uncertain for several reasons. The value used for total intake is based on estimates of PFOS occurrence and exposure rates for different media. The serum PFOS concentration in the U.S. population has been decreasing since at least 1999 (when NHANES began publishing estimates of serum PFOS concentrations in the U.S. population), and there is some uncertainty as to whether NHANES data from 1999-2008 versus 2003-2004 are most appropriate to compare to the total intake estimate of Egeghy and Lorber (2011). Finally, the body weight assumed for this calculation (70 kg) is a default value, and body weight may be correlated with PFOS intake and/or $t_{1/2}$.

Conclusion
The close agreement of the CL of 0.11 ml/kg/day produced by this alternate approach which is independent of estimates of Vd and t1/2 with the USEPA (2016) CL of 0.081 ml/kg/day provides support for use of the USEPA value as a reasonable estimate of the CL for PFOS.

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Egeghy PP, Lorber M. 2011. An assessment of the exposure of Americans to perfluorooctane sulfonate: a comparison of estimated intake with values inferred from NHANES data. J Expo Sci Environ Epidemiol. 2011 Mar-Apr;21(2):150-68.

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Appendix 4: Animal evidence tables

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Abbott et al. (2009a)

Species and strain:
Mice, 129S1/SvImJ wild type (WT) and PPAR alpha knockout (KO)
F0 age not reported

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (potassium salt, >91% pure) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
WT: 0, 4.5, 6.5, 8.5, 10.5 mg/kg/day
KO: 0, 8.5, 10.5 mg/kg/day

See Results column for serum PFOS concentrations at PND 15, only pup data reported herein

Exposure regimen:
GD15 to GD18</td>
<td><u>Internal PFOS concentrations: offspring data</u>

<table>
<tr><td colspan="3">Internal PFOS concentrations in offspring</td></tr>
<tr><td></td><td>Number of pups examined</td><td>Serum PFOS (ng/mL)</td></tr>
<tr><td>WT</td><td></td><td></td></tr>
<tr><td>Control</td><td>8</td><td>7.39±2.92</td></tr>
<tr><td>4.5 mg/kg/day</td><td>6</td><td>24,100±1820</td></tr>
<tr><td>6.5 mg/kg/day</td><td>4</td><td>28,700±2610</td></tr>
<tr><td>8.5 mg/kg/day</td><td>8</td><td>40,700±2680</td></tr>
<tr><td>10.5 mg/kg/day</td><td>6</td><td>41,200±3070</td></tr>
<tr><td>KO</td><td></td><td></td></tr>
<tr><td>Control</td><td>8</td><td>6.88±1.57</td></tr>
<tr><td>8.5 mg/kg/day</td><td>7</td><td>42,800±3600</td></tr>
<tr><td>10.5 mg/kg/day</td><td>12</td><td>52,400±3620</td></tr>
<tr><td colspan="3">Concentrations reported at means ± SEM
Serum PFOS levels determined at PND15 (16 days after last dose)</td></tr>
</table>

<u>Maternal effects</u>
• No statistically significant effect on weight at GD18 and weight gain from GD15 to GD18 in both WT and KO dams
• No statistically significant effect on body weight, liver weight, and relative liver weight on PND15 in both WT and KO dams

<u>Reproductive outcomes</u>
• No statistically significant effect on number of implantation sites, total number of pups at birth (alive and dead), and percent litter loss from implantation to birth in both WT and KO</td>
<td>Major Limitations:
• Serum PFOS measurements at PND15 not informative for endpoints (e.g., maternal weight at GD18) assessed at other time points

Other comments:
• Species and strains appropriate for endpoints assessed
• Sample sizes ranged from generally ≥10 dams for maternal endpoints to ≤10 for some neonatal effects (e.g., body and liver weights)
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous knowledge of potential strain (129S background) sensitivity to perfluorinated chemicals
• Duration of exposure based on previous observations of postnatal death from gestational exposure to PFOS; however, this duration may not identify effects that might arise from exposures occurring earlier in gestation
• Number of doses (i.e., 2) for KO exposures do not allow for determining low-dose effects
• Quantitative data reporting
• Endpoint ascertainment used standardized assessment of</td>
</tr>
</table>

Neonatal effects

- No statistically significant effect on pup birth weight, pup weight on PND15, and weight gain from PND1 to PND15 in both WT and KO
- No statistically significant effect on pup body weight at PND15 in both WT and KO
- Statistically significant ($p<0.01$) trend for increase in absolute liver weight in WT at PND15; no effect on absolute liver weight in KO at PND15
- Statistically significant trend for increase in relative liver weight in WT ($p<0.001$) and KO ($p<0.01$) at PND15
- Statistically significant increase in relative liver weight with 10.5 mg/kg in WT ($p<0.001$) and KO ($p<0.05$) compared to corresponding controls at PND15
- Most postnatal effects occurred by PND2

Percentage postnatal survival on PND15		
	WT	KO
Control	65%±10 (n=16)[a]	84%±9 (n=12)
4.5 mg/kg/day	45%±14[b] (n=8)	NA
6.5 mg/kg/day	55%±6 (n=7)	NA
8.5 mg/kg/day	43%±9[b] (n=20)	56%±12[b] (n=13)
10.5 mg/kg/day	26%±9[b] (n=17)	62%±8[b] (n=14)
a = number (n) of pups surviving at PND15 b = $p<0.001$, compared to corresponding controls NA = not applicable		

Postnatal development

- Delay in both eye opening in WT (PND13) and KO (PND14)

mortality, body and organ weights, and developmental milestone

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Butenhoff et al. (2009)

Species and strain:
Rats, Crl:CD (SD)
Males and females (virgin) mated at ~12 weeks of age

Group size:
4 groups (n = 25 in each)

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 0.3, 1.0 mg/kg/day

Exposure regimen:
GD0 to PND20</td>
<td><u>Maternal effects: body weight</u>
• No statistically significant effect on body weight at GD0, GD20, or PND1 as well as in change in body weight (from GD0 to GD20 and from PND1 to PND21)
• Note: Based on graphically reported data, statistically significant (p<0.05 or p<0.01) reduction in maternal body weight with 1.0 mg/kg/day between PND4 and 21 compared to controls

<table>
<tr><td colspan="5">Maternal body weight at PND21</td></tr>
<tr><td></td><td colspan="4">PFOS (mg/kg/day)</td></tr>
<tr><td></td><td>0</td><td>0.1</td><td>0.3</td><td>1.0</td></tr>
<tr><td>Sample size</td><td>25</td><td>23</td><td>25</td><td>24</td></tr>
<tr><td>Body weight (g)</td><td>365</td><td>365</td><td>363</td><td>351*</td></tr>
<tr><td colspan="5">* p<0.05</td></tr>
</table>

<u>Maternal effects: food consumption</u>
• No statistically significant difference between exposed and controls groups for:
o relative food consumption GD0 to 20
o absolute food consumption PND1 to 21
o relative food consumption PND1 to 21

<table>
<tr><td colspan="5">Maternal absolute food consumption GD0 to 20</td></tr>
<tr><td></td><td colspan="4">PFOS (mg/kg/day)</td></tr>
<tr><td></td><td>0</td><td>0.1</td><td>0.3</td><td>1.0</td></tr>
<tr><td>Sample size</td><td>25</td><td>23</td><td>25</td><td>24</td></tr>
<tr><td>Food consumption (g/rat/d)</td><td>25</td><td>24</td><td>24</td><td>23*</td></tr>
<tr><td colspan="5">* = p<0.05</td></tr>
</table>

<u>Maternal effects: reproductive</u>
• No statistically significant effect on number of litters, length of gestation, implantation sites, and unaccounted sites (potential resorption)</td>
<td>Major Limitations:
• Internal PFOS concentrations not determined
• Lack of histopathology

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size ~25 per dose provided good statistical power
• Oral gavage provided direct maternal exposure to PFOS
• Doses selected based on previous observations of neonatal toxicity but represented a narrow dose range
• Duration of exposure lasted length of gestation
• Number of exposure levels (control plus 3 doses) were standard and allowed for determining any dose-dependent effects
• Qualitative and quantitative data clearly reported
• Endpoint ascertainment used standardized and objective assessment of morphological, observational, and behavioral endpoints</td>
</tr>
</table>

	Maternal effects: internal macroscopic examination
	• No treatment-related findings in dams with failure to deliver or dams necropsied on PND21
	
	Neonatal effects
	• Statistically significant ($p<0.05$) increase in body weight at vaginal patency and body weight at balanopreputial separation with 0.1 mg/kg/day compared to controls
	• No statistically significant differences for delivered litters; pups born per litter; live litter size PND0; % males per litter at birth; % survival PND0 to 4; % survival PND4 to 21; pup weight (male and female separately) at PND1, 21, and 72; age at vaginal patency; and age at balanopreputial separation
	
	Offspring effects: sensory and behavioral outcomes
	• Functional observation battery (observation on PND4, 11, 21, 35, 45, 60)
	  ○ Statistically significant ($p<0.05$) reduction in hind limb grip strength with 1.0 mg/kg/d (males only) on PND21 only; mean value for this group was stated to be within historic control range
	• Locomotor activity (data presented graphically only, cumulative daily counts)
	  ○ Statistically significant ($p<0.05$) increase with 0.3 and 1.0 mg/kg/day (males only) at PND17 compared to concurrent controls
	  ○ Statistically significant ($p<0.05$) increase with 1.0 mg/kg/day (females only) at PND21 compared to concurrent controls
	• Acoustic startle response
	  ○ No statistically significant differences between groups
	• Biel maze swimming
	  ○ No statistically significant differences between groups
	
	Offspring effects: brain morphology (PND21 and 72)
	• No statistically significant dose related effects on brain weight, brain length, and brain width	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Butenhoff et al. (2012)

Species and strain:
Rats, Sprague-Dawley (Crl:CD(SD)ICS)
Males and females
~41 days old at start of treatment

Group size:
For entire exposure duration: 60 to 70/sex/exposure group

For recovery group (20 ppm only): 40/sex

Appears that dose groups had (initially) 60 rats per group excluding those for interim sacrifice

Test article and vehicle:
PFOS (potassium salt, 86.9% pure), acetone vehicle

Route of exposure:
Dietary

Exposure levels:
0, 0.5, 2, 5, 20 ppm

See Results column for serum PFOS concentration</td>
<td><u>Internal PFOS concentration</u>
Note: PFOS liver concentration data determined by authors but are not shown herein

<table>
<tr><td colspan="8">Serum PFOS concentrations (ug/mL)</td></tr>
<tr><td></td><td></td><td colspan="6">Dietary PFOS (ppm)</td></tr>
<tr><td>Week of sampling</td><td>Sex</td><td>0</td><td>0.5</td><td>2</td><td>5</td><td>20</td><td>20 ppm (recovery)</td></tr>
<tr><td>4</td><td>M</td><td>< LOQ</td><td>0.91</td><td>4.33</td><td>7.57</td><td>41.80</td><td>-</td></tr>
<tr><td></td><td>F</td><td>0.026</td><td>1.61</td><td>6.62</td><td>12.60</td><td>54.00</td><td>-</td></tr>
<tr><td>14</td><td>M</td><td>< LOQ</td><td>4.04</td><td>17.10</td><td>43.90</td><td>148.0</td><td>-</td></tr>
<tr><td></td><td>F</td><td>2.67</td><td>6.86</td><td>27.30</td><td>64.40</td><td>223.0</td><td>-</td></tr>
<tr><td>53</td><td>M</td><td>0.025</td><td>-</td><td>-</td><td>-</td><td>146.0 (4)</td><td>-</td></tr>
<tr><td></td><td>F</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>102</td><td>M</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td></td><td>F</td><td>-</td><td>-</td><td>20.20 (9)</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>105</td><td>M</td><td>0.012 (11)</td><td>1.31 (10)</td><td>7.60 (17)</td><td>22.50 (25)</td><td>69.3 (22)</td><td>-</td></tr>
<tr><td></td><td>F</td><td>0.084 (24)</td><td>4.35 (15)</td><td>-</td><td>75 (15)</td><td>233 (25)</td><td>-</td></tr>
<tr><td>106</td><td>M</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2.42 (10)</td></tr>
<tr><td></td><td>F</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>9.51 (17)</td></tr>
<tr><td colspan="8">Values are means (standard deviations not reported herein)
LOQ = limit of quantitation reported to be 0.009 (week 4) or 0.046 ug/mL (week 14)
n=5 unless specified in parenthesis
- = data not available</td></tr>
</table>

<u>Cumulative mortality (through week 105)</u>
• Estimated mortality based on Kaplan-Meier model

Note: For mortality through week 53 (unscheduled deaths): pathological observations consisted of large, mottled, or diffusively dark livers (in 2/3 males and 1/1 females) in 20 ppm group</td>
<td>Major Limitations:
• Data reporting is inadequate
• Incidence of non-neoplastic (and apparently neoplastic effects) are calculated on the basis of the sum of intermediate sacrifices, term sacrifices, and unscheduled mortality. If adverse effects (including tumors) are time dependent and occur with greater frequency with longer durations of exposure, calculation of incidences based on inclusion of examination of intermediate sacrifices and unscheduled mortality will result in an underestimate of the full-term incidence.
• Rats (10/dose group) were interim sacrificed at 52 weeks. Also, 5 rats at 0.5 and 5 ppm diets were sacrificed at weeks 4 and 14. This appears to account for variable numbers (60 or 70) per dose group (i.e., 60 per dose group designated for full term exposure). However, this is not clear.
• Organ weight changes are only provided as</td>
</tr>
</table>

Exposure regimen:
103 to 104 weeks (depending on mortality)

For recovery exposure, 20 ppm diet for 52 weeks followed by control diet until termination at week 104

10 rats/group sacrificed at 52 weeks

10 rats/group (0.5 and 5 ppm groups) sacrificed at weeks 4 and 14

Related studies:
Seacat et al. (2003)

Estimated probability of mortality through 105 weeks in males

	Dietary PFOS (ppm)					
	0	0.5	2	5	20	20 (recovery)
Sample size	70	60	60	60	70	40
Estimated mortality *	0.778	0.800	0.660	0.500	0.565	0.750
p-value	-	0.98	0.18	**0.01**	**0.03**	0.74

* Estimate appears to take interim sacrifices into account based on Kaplan-Meier model
Bold text = statistically significant ($p<0.05$) from controls
After 105 weeks of exposure, appears to be statistically significant (p-trend = 0.0005) decrease across dose groups (excluding 20 ppm recovery groups

Estimated probability of mortality through 105 weeks in females

	Dietary PFOS (ppm)					
	0	0.5	2	5	20	20 (52 weeks recovery
Sample size	70	60	60	60	70	40
Estimated mortality *	0.520	0.700	0.820	0.700	0.498	0.575
p-value	-	0.17	**0.002**	0.23	0.86	0.94

* Estimate appears to take interim sacrifices into account based on Kaplan-Meier model
Bold text = statistically significant ($p<0.05$) from controls

Food consumption

- Overall mean daily food intake increased linearly with PFOS dose (R^2=0.9999 for males and females), statistics not provided

Body weight

- No statistically significant differences in final body weights between exposure groups and controls

Note: statistically significant decrease in interim body weights with 20 ppm
Note: statistically significant decrease in body weights between weeks 3 to 61 with 20 ppm for recovery females, body weights recovered on control diet

comparisons of controls vs. 20 ppm group.

Other comments:

- Species and strain appropriate for endpoints assessed
- Sample size (n) is overall reasonably large, but sample size varies throughout with some sample sizes (e.g., organ weight), marginal. Also, there is variability in n among dose groups whose origin is not clear.
- Dietary exposure allows for PFOS to interact with tissues from the oral cavity to the stomach
- Dose selection based on previous observations of body weight and liver effects in rats (Seacat et al. 2003)
- Chronic duration of exposure
- Number of exposure levels would allow for determining any dose-dependent effects, recovery groups included
- Internal PFOS concentrations determined
- Endpoint ascertainment used standardized assessment of mortality, body and organ weights,

Organ weight

Note: Data in table are from the Supplementary data tables of Butenhoff et al. (2012), which only present data for significant differences between controls and 20 ppm groups

Organ weight and organ weight ratios (to body and brain weights) following 52 weeks of exposure

		Males (n=9)			Females (n=10)		
Organ	Dose group (ppm)	Organ wt (g)	Organ wt/body wt (%)	Organ wt/brain wt (%)	Organ wt (g)	Organ wt/body wt (%)	Organ wt/brain wt (%)
Left adrenal	0 20				0.0501 0.0311		0.0235 0.0141
Right adrenal	0 20						0.0172 0.0144
Brain	0 20					0.5376 0.6752	
Left kidney	0 20					0.3357 0.4149	
Right kidney	0 20					0.3498 0.4193	
Liver	0 20	20.028 26.632	2.811 4.004	8.613 11.366		2.803 4.205	
Spleen	0 20	0.9792 0.8287	0.1382 0.1252	0.4208 0.3529		0.1368 0.1650	
Left thyroid (w parathyroid) *	0 20	0.0246 0.0195		0.0246 0.0083			

Mean weight report (standard deviations not reported herein)
All data presented here are statistically significant differences between controls and 20 ppm at p≤0.05
* Note: No statistically significant differences from controls in right thyroid (with parathyroid) data with 20 ppm for any measure

histopathology, and other endpoints

Note: Due to conflation of interim and term data in outcome reporting both significance and dose-response for term (i.e., chronic) outcomes are not interpretable.

	Clinical chemistry • Note: data presented graphically only Serum ALT (measured at weeks 4, 14, 27, 53 only) • Statistically significant (p≤0.05) increase with 20 ppm (males only) at weeks 14 and 53 compared to controls, apparent borderline statistically significant increase at week 27 Serum AST (measured at weeks 4, 14, 27, 53 only) • Statistically significant (p≤0.05) decrease with 20 ppm (females only) at week 4 compared to controls Serum total cholesterol (measured for all time points) • Statistically significant (p≤0.05) decrease in males with 20 ppm at weeks 14, 27, and 53 (but not at terminal sacrifice) compared to controls • Statistically significant (p≤0.05) decrease in females with ≥2 ppm at week 27, apparent borderline statistical significance at week 53 Serum glucose (measured at weeks 4, 14, 27, 53 only) • Statistically significant (p≤0.05) decrease in males with 20 ppm at weeks 14 and 53 compared to controls • Statistically significant (p≤0.05) decrease in females with ≥2 ppm at week 53 Serum urea nitrogen (measured at weeks 4, 14, 27, 53 only) • Statistically significant (p≤0.05) increased in males with 20 ppm at weeks 14 and 27 or ≥2 ppm at week 53 compared to controls • Statistically significant (p≤0.05) increase in females with 20 ppm at weeks 14 and 27 or ≥5 ppm at week 53 compared to controls Serum creatinine (measured at weeks 4, 14, 27, 53 only) • No statistically significant effects in males • Statistically significant (p≤0.05) increase in females with 2 ppm at week 14 compared to controls	

Urine chemistry

- Statistically significant increase in pH and decrease in sodium ion concentration in males with 2 ppm at week 53 compared to controls
- Statistically significant decrease in potassium ion excretion in males with 0.5 and 5 ppm at week 53 compared to controls

Hematology

- Statistically significant increase in segmented neutrophils in males with 20 ppm at week 14 compared to controls

Microscopic pathology

Non-neoplastic microscopic lesions in livers of male and females (includes interim and terminal sacrifices and unscheduled mortality)

		Dietary PFOS (ppm)						
	sex	0	0.5	2	5	20	20 (recovery)	p-trend
Lymphohistio-cytic infiltrate	F	42/65	42/55	38/55	41/55	56/65 **	32/40	**
Hepatocellular hypertrophy (centrilobular)	M	0/65	2/55	4/55 *	22/55 **	42/65 **	3/40	**
	F	2/65	1/55	4/55	16/55 **	52/65 **	2/40	**
Granular, eosinophilic cytoplasm (centrilobular)	M	0/65	0/55	0/55	0/55	14/65 **	0/40	**
	F	0/65	0/55	0/55	7/55 **	36/65 **	1/40	**
Hepatocellular pigment (centrilobular)	M	0/65	0/55	0/55	0/55	6/65 *	0/40	**
	F	0/65	0/55	0/55	1/55	36/65 **	3/40	**
Individual hepatocyte necrosis	M	5/65	4/55	6/55	13/55	19/55 *	3/40	*
	F	7/65	6/55	6/55	6/55	15/65 *	3/40	*
Hepatocellular vacuoles (midzone/ centrilobular)	M	3/65	3/55	6/55	13/55 **	19/65 **	3/40	**

Cystic degeneration	M F	5/65 0/65	15/55 ** 1/55	19/55 ** 1/55	17/55 ** 2/55	22/65 ** 4/65	15/40 ** 1/40	** *
Degeneration/ Necrosis (centrilobular)	M	1/65	0/55	0.55	1/55	5/65	1/40	*
Periportal hepatocellular hypertrophy	F	12/65	10/55	9/55	4/65	3/65 *	7/40	**
Pigmented macrophage infiltration	F	2/65	3/55	5/55	6/55	23/65 **	7/40 *	**

Note: only statistically significant outcomes shown herein
* p≤0.05, ** p≤0.01

Neoplastic lesions in males and females
(apparently includes interim and terminal sacrifices and unscheduled mortality)

		Dietary PFOS (ppm)						
	sex	0	0.5	2	5	20	20 (recovery)	p-trend
Liver								
Hepatocellular Adenoma	M F	0/60 0/50	3/50 1/50	3/50 1/49	1/50 1/50	7/60 * 5/60 *	0/40 2/40	* *
Hepatocellular adenoma + carcinoma	F	0/60	1/50	1/49	1/50	6/60 *	2/40	**
Thyroid								
Follicular cell adenoma	M	3/60	5/49	4/50	4/49	4/59	9/39 *	

Note: only statistically significant positive outcomes shown herein
* p ≤0.05, ** p≤0.01

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Case et al. (2001)

Note: study authors conducted dose-range finder and developmental toxicity studies. Results from the dose-range finder study are reported herein.

Species and strain:
Rabbits, New Zealand white (Hra: (NZW) SPF)
5 to 6 months of age

Group size:
5/mated females/group

Test article and vehicle:
PFOS (salt not reported, 98.4% pure) in 2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 1.0, 2.5, 5.0, 10, 20 mg/kg/day

Exposure regimen:
GD6 to GD20, animals sacrificed at GD29

Note: study reported to have been conducted according to GLP</td>
<td><u>Maternal toxicity</u>
• Reduced feed consumption, scant feces, and ungroomed hair coats observed with ≥5 mg/kg/day
• Maternal deaths and abortions (see table below) reported to occur between GD17 and GD 26

<table>
<tr><td colspan="5">Endpoints assessed for maternal toxicity</td></tr>
<tr><td></td><td colspan="4">PFOS (mg/kg/day)</td></tr>
<tr><td></td><td>Controls[a]</td><td>5</td><td>10</td><td>20</td></tr>
<tr><td>Body weight loss[b]</td><td>0/5</td><td>3/5</td><td>4/5</td><td>5/5</td></tr>
<tr><td>Deaths</td><td>0/5</td><td>0/5</td><td>0/5</td><td>4/5</td></tr>
<tr><td>Abortions</td><td>0/5</td><td>2/5</td><td>4/5</td><td>1/5</td></tr>
<tr><td>Animals pregnant at GD29</td><td>5/5</td><td>2/3</td><td>0/1</td><td>NA</td></tr>
<tr><td colspan="5">a = observations for 0.1, 1.0, and 2.5 mg/kg/day groups were identical to control observations and are not reported herein
b = >15% less than controls
5 females/group; NA = no animals available to exam</td></tr>
</table>

<u>Fetal toxicity</u>

<table>
<tr><td colspan="4">Endpoints assessed for fetal toxicity (continued in table below)</td></tr>
<tr><td></td><td colspan="3">PFOS (mg/kg/day)</td></tr>
<tr><td></td><td>0 (n=5)[a]</td><td>0.1 (n=5)</td><td>1.0 (n=5)</td></tr>
<tr><td>Corpora lutea</td><td>10.2±1.6</td><td>11.8±2.9</td><td>10.0±0.8</td></tr>
<tr><td>Implantations</td><td>8.8±1.6</td><td>9.5±1.7</td><td>8.5±1.3</td></tr>
<tr><td>Litter size</td><td>8.4±1.1</td><td>9.2±1.5</td><td>8.5±1.3</td></tr>
<tr><td>Resorptions</td><td>0.4±0.5</td><td>0.2±0.5</td><td>0.0±0.0</td></tr>
<tr><td>Fetal weight (g)</td><td>43.8±5.9</td><td>40.8±7.5</td><td>44.0±2.7</td></tr>
<tr><td colspan="4">Mean±SD
a = number of pregnant females in group</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS concentrations not determined
• Results not statistically analyzed

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size limited to 5 females
• Oral gavage provided direct exposure to PFOS
• Doses selected to purposely identify doses to that produce toxicity
• Gestational exposure did not last entire pregnancy
• Number of exposure levels allowed for determining any dose-related effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of mortality, body weights, and reproductive/developmental effects</td>
</tr>
</table>

Endpoints assessed for fetal toxicity (continued from table above)

	PFOS (mg/kg/day)		
	0 (n=5)[a]	2.5 (n=5)	5 (n=2)
Corpora lutea	10.2±1.6	11.0±1.4	10.5±0.7
Implantations	8.8±1.6	8.8±2.0	9.5±0.7
Litter size	8.4±1.1	8.4±1.5	5.5±2.1
Resorptions	0.4±0.5	0.4±0.5	4.0±1.4
Fetal weight (g)	43.8±5.9	38.2±5.6	26.0±5.4

Mean±SD
[a] = number of pregnant females in group

Reference and Study Design	Results	Comment
Case et al. (2001) Note: study authors conducted dose-range finder and developmental toxicity studies. Results from the developmental toxicity study are reported herein. **Species and strain:** Rabbits, New Zealand white (Hra: (NZW) SPF) 5 to 6 months of age **Group size:** 22/mated females/group **Test article and vehicle:** PFOS (salt not reported, 98.4% pure) in 2% Tween 80 **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.1, 1.0, 2.5, 3.75 mg/kg/day **Exposure regimen:** GD7 to GD20, animals sacrificed at GD29 Note: study reported to have been conducted according to GLP	**Maternal toxicity** • No maternal deaths • Statistically significant ($p \leq 0.05$ or $p \leq 0.01$) reductions in body weight gains during exposure (GD6 to GD20) to ≥1 mg/kg/day, non-statistically significant reductions after exposure (GD21 to GD29), 3.75 mg/kg/day data not reported • Reduced body weight gains generally correlated with a reduction in feed consumption **Fetal and developmental toxicity** • One abortion reported with 2.5 mg/kg/day (on GD25) and 10 abortions with 3.75 mg/kg/day (between GD22 and GD28) • Statistically significant ($p \leq 0.05$ or $p \leq 0.01$) reduction in fetal weight with ≥2.5 mg/kg/day • No effect on corpora lutea, implantations, resorptions (early and late), and number of fetuses (alive and dead) • Structural abnormalities included some reversible delays in ossification (sternebrae, hyoid, metacarpal, and pubic bones) with ≥2.5 mg/kg/day	**Major Limitations:** • Internal PFOS concentrations not determined **Other comments:** • Species and strain appropriate for endpoints assessed • Sample size >10 • Oral gavage provided direct exposure to PFOS • Dose selection based on results from a dose-range finder study • Gestational exposure did not last entire pregnancy • Number of exposure levels allowed for determining any dose-related effects • Quantitative data reported • Endpoint ascertainment used standardized assessment of mortality, body weights, and reproductive/developmental effects

Reference and Study Design	Results	Comment
Chang et al. (2009) Note: the results reported by the authors represent thyroid parameters determined as part of a developmental neurotoxicity study with gestational and lactational exposures (Butenhoff et al. 2009). The maternal, neonatal, and developmental neurotoxicity results are reported in a separate table. **Species and strain:** Rats, Sprague-Dawley About 12 weeks old at mating (per Butenhoff et al. 2009) **Group size:** 25 pregnant females/group **Test article and vehicle:** PFOS (potassium salt, 86.9% pure) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.1, 0.3, 1.0 mg/kg/day See **Results** column for PFOS concentrations in specimens from dams and offspring (fetuses and pups)	**Internal PFOS concentration** • Maternal internal PFOS concentrations (i.e., in serum, liver, and brain) correlated with administered dose for GD20, PND4, and PND21 (day of maternal sacrifice) • Maternal liver to serum ratio greater than brain to serum ratio at GD20 (only time point available for ratio determination) • Fetal and pup internal PFOS concentrations (i.e., in serum, liver, and brain) correlated with maternal administered dose for GD20, PND4, PND21, and PND72 • Fetal and pup liver to serum ratio greater than brain to serum ratio at GD20, PND4, PND21, and PND72 • Maternal serum PFOS concentrations less than that of fetuses on GD20 but greater than pup serum PFOS concentrations on PND4 and PND21 • Maternal liver PFOS concentrations greater than that of fetuses on GD20 (no subsequent comparisons possible) • Maternal brain PFOS concentrations less than that of fetuses on GD20 (no subsequent comparisons possible) • Maternal liver and brain samples not collected for PND4 and PND21 analyses **Maternal effects: serum thyroid stimulating hormone (TSH) measurements** • No statistically significant differences between exposure groups at all time points (GD20, PND4, and PND21) **Offspring effects: serum TSH measurements** • No statistically significant differences between exposure groups at all time points (GD20, PND4, and PND21) **Offspring effects: thyroid histology** • No changes observed between 1.0 mg/kg/day group and controls at all time points (GD20, PND4, and PND21) • Thyroids collected for 0.1 and 0.3 mg/kg/day groups but not analyzed microscopically	**Major Limitations:** • Sample size varied by endpoint (e.g., ~10 for thyroid histology, <10 for thyroid proliferation, unclear sample size for TSH measurements) **Other comments:** • Species and strain appropriate for endpoints assessed • Oral gavage provided direct exposure to PFOS • Dose selection aimed to avoid neonatal toxicity based on previous rat studies (per Butenhoff et al. 2009) • Duration of exposure included gestation period through lactation • Number of exposure levels allowed for determining any dose-related effects • Quantitative data reported • Internal PFOS measurements determined • Endpoint ascertainment used standardized assessment for TSH, thyroid morphometry, and thyroid cell proliferation; subjective thyroid histology

Exposure regimen: GD0 to PND20 Dams sacrificed at PND21 F1 weaned at PND21 and sacrifice at PND72 A second group of pregnant females (10/group) were exposed GD0 to GD19 with sacrifice on GD20 **Related studies:** Butenhoff et al. (2009)	**<u>Offspring effects: thyroid morphometry</u>** • Statistically significant ($p<0.05$) increase in thyroid follicular epithelial cell height in males only with 1.0 mg/kg/day at PND21 compared to controls; thyroid follicular epithelial cell height in concurrent male controls noted to be lower compared to female control group at PND21 • No statistically significant differences between exposed and control groups at PND4 • Only control and 1.0 mg/kg/day groups analyzed **<u>Offspring effects: thyroid follicular colloid area</u>** • No statistically significant differences between exposed and control groups at PND4 and PND21 • Only control and 1.0 mg/kg/day groups analyzed **<u>Offspring effects: thyroid proliferation</u>** • Statistically significant ($p<0.05$) increase in thyroid cell proliferation in females only with 1.0 mg/kg/day at GD20 compared to controls; control values noted to have a wide range (4 to 113 cells with positive staning) • Only control and 1.0 mg/kg/day groups analyzed	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Chen et al. (2012a)

Species and strain:
Rats, Sprague-Dawley
Males and females sexually mature, virgin

Group size:
10 dams/exposure group

Test article and vehicle:
PFOS (salt not reported, >98% pure) in 0.05% Tween 80 in deionized water

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 2.0 mg/kg/day
Adjusted daily for body weight changes

See Results column for serum PFOS concentrations

Exposure regimen:
GD1 to GD21

Second set of dams treated as above and survival determined on PND4

At PND0, 2 male and 2 female pups randomly selected from each litter and sacrificed for serum and lung tissue analysis3 males and 3 females per litter maintained to PND21 (weaning) and then sacrificed</td>
<td><u>Internal PFOS concentration</u>
• Note: Lung PFOS concentrations determined for pups on PND0 and PND21 but not reported herein

<table>
<tr><td colspan="3">Serum PFOS levels in pups on PND0 and PND21</td></tr>
<tr><td>Age</td><td>Dose (mg/kg/day)</td><td>Serum concentration (µg/ml)</td></tr>
<tr><td>PND0</td><td>0</td><td>ND</td></tr>
<tr><td></td><td>0.1</td><td>1.7*</td></tr>
<tr><td></td><td>2.0</td><td>47.52**</td></tr>
<tr><td>PND21</td><td>0</td><td>ND</td></tr>
<tr><td></td><td>0.1</td><td>0.41*</td></tr>
<tr><td></td><td>2.0</td><td>4.46**</td></tr>
<tr><td colspan="3">Values are means (standard deviations not reported herein)
ND = not detected (limit of detection not reported)
* p<0.05, ** p<0.01</td></tr>
</table>

<u>Offspring effects: body weight</u>
• Statistically significant (p<0.05) decrease in body weight with 2.0 mg/kg/day for PND0 to 21 compared to controls

<u>Offspring effects: post-natal mortality</u>
• Statistically significant (p<0.01) increase in post-natal mortality with 2.0 mg/kg/day at PND3 compared to controls

<u>Offspring effects: histopathology</u>
• Normal histopathology of pulmonary alveolus in control and 0.1 mg/kg/day (data not shown) groups at PND0 and PND21
• At PND0: marked alveolar hemorrhage, thickened inter-alveolar septa, and focal lung consolidation with 2.0 mg/kg/day
• At PND 21: alveolar hemorrhage, thickened inter-alveolar septa, and inflammatory cell infiltration with 2.0 mg/kg/day</td>
<td>Major Limitations:
• Maternal toxicity not reported
• Sample size not given explicitly, 10 dams/dose group appears to be 10 litters/dose group. Therefore, histopathology sample size appears to be 20/sex/group at PND0 and 60 (30 males, 30 females) at PND21.
• Only qualitative data presented, data presented in figures or micrographs with no tabular data

Other comments:
• Species and strain appropriate for endpoints assessed
• Oral gavage provided direct exposure to PFOS
• Doses selected allowed for the determination of a LOAEL and NOAEL (e.g., for survival and body weight)
• Duration of exposure lasted during entire gestation period
• Two exposure levels may limit ability to demonstrate any dose-related effects
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of mortality, body weight, and lung histopathology

Note: this study also presented data on apoptosis-related endpoints and oxidative stress. These data are not summarized herein.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Dong et al. (2009)

Species and strain:
Mice, C57BL/6
8–10 weeks old

Group size:
10/males/group

Test article and vehicle:
PFOS (potassium salt, >98% pure) in
de-ionized water with
2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
<u>Daily dose</u>: 0, 8.33, 83.33, 416.67, 833.33, 2083.33 ug/kg/day

<u>Targeted total administered dose (TAD)</u>: 0, 0.5, 5, 25, 50, 125 mg/kg

See Results column for serum PFOS concentrations

Exposure regimen:
Once daily for 60 days
Mice sacrificed on day 61 (24 hours after last exposure)</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="2">Serum PFOS concentrations after 60 days of exposure</td></tr>
<tr><td>PFOS (mg/kg TAD)</td><td>Serum PFOS (mg/L)</td></tr>
<tr><td>Control</td><td>0.048±0.014</td></tr>
<tr><td>0.5</td><td>0.674±0.166*</td></tr>
<tr><td>5</td><td>7.132±1.039*</td></tr>
<tr><td>25</td><td>21.638±4.410*</td></tr>
<tr><td>50</td><td>65.426±11.726*</td></tr>
<tr><td>125</td><td>120.670±21.759*</td></tr>
<tr><td colspan="2">For each dose group n = 10
* = p≤0.05, compared to control</td></tr>
</table>

<u>Body weight and food intake</u>
• Statistically significant (p<0.05) reduction in final body weight and body weight change with ≥25 mg/kg TAD compared to controls
• Statistically significant (p<0.05) reduction in food intake with ≥50 mg/kg TAD compared to pre-exposed baseline

<u>Organ weight changes: kidney, liver, spleen, thymus</u>
• Note: organ weights reported by authors as [organ weight (g)/body weight (g)] x 100
• Statistically significant (p≤0.05) reduction in kidney mass with ≥50 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) increase in liver mass with ≥5 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) reduction in spleen and thymus mass with ≥25 mg/kg TAD compared to controls

<u>Changes in serum corticosterone</u>
• Dose-dependent increase in serum corticosterone
• Statistically significant (p≤0.05) increase in serum corticosterone compared to control with TAD of 50 and 125 mg/kg</td>
<td>Major Limitations:
• Only male mice used so response in females not known
• Unclear whether hepatic effects contributed to immune responses, as noted by study authors

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size of 10/group per endpoint
• Oral gavage provided direct exposure to PFOS
• Doses selected based on previous observations of altered immune function in mice
• Subchronic duration of exposure
• Number of exposure levels would allow for determining any dose-dependent effects
• Quantitative data reported
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of endpoints</td>
</tr>
</table>

	Splenic and thymic cellularity • Dose-dependent decrease in cellularity for both the spleen and thymus • Statistically significant (p≤0.05) decreases in cellularity compared to respective controls for both spleen and thymus with TAD of ≥25 mg/kg **Lymphocyte immunophenotypes (splenic and thymic)** • Statistically significant (p≤0.05) decreases in some splenic T cell CD4/CD8 subpopulations with ≥25 mg/kg TAD compared to controls • Statistically significant (p≤0.05) decreases in splenic B cells (B220+) with ≥50 mg/kg TAD compared to controls • Statistically significant (p≤0.05) decreases in some thymic T cell CD4/CD8 subpopulations with ≥25 mg/kg TAD compared to controls **Splenic natural killer (NK) cell activity** • Inverted U-shaped dose-response curve, inflection point = TAD of 5 mg/kg • Statistically significant (p≤0.05, compared to controls) increase with TAD of 5 mg/kg and decrease with TAD of 50 and 125 mg/kg **Splenic lymphocyte proliferation** • Dose-dependent decrease in proliferation index (PI) for both concanavalin A (conA) and lipopolysaccharide (LPS) treated lymphocytes • Statistically significant (p≤0.05) decrease in PI compared to respective controls for both conA and LPS treated cells with TAD of 50 and 125 mg/kg **Antibody plaque forming cell (PFC) response to sheep red blood cells** • Dose-dependent decrease in PFC response • Statistically significant (p≤0.05) decrease in PFC response compared to controls with TAD of 5, 25, 50, and 125 mg/kg	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Dong et al. (2011)

Species and strain:
Mice, C57BL/6
8–10 weeks old

Group size:
12/males/group

Test article and vehicle:
PFOS (potassium salt, >98% pure) in
de-ionized water with
2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
<u>Daily dose</u>: 0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333 mg/kg/day

<u>Targeted total administered dose (TAD)</u>: 0, 0.5, 1, 5, 25, 50 mg/kg

See Results column for serum PFOS concentrations

Exposure regimen:
Once daily for 60 days
Mice sacrificed on day 61 (24 hours after last exposure)</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="2">Serum PFOS concentrations after 60 days of exposure</td></tr>
<tr><td>PFOS (mg/kg TAD)</td><td>Serum PFOS (mg/L)</td></tr>
<tr><td>Control</td><td>0.05±0.01</td></tr>
<tr><td>0.5</td><td>1.07±0.11</td></tr>
<tr><td>1</td><td>2.36±0.47</td></tr>
<tr><td>5</td><td>10.75±0.82*</td></tr>
<tr><td>25</td><td>22.64±2.29*</td></tr>
<tr><td>50</td><td>51.71±3.81*</td></tr>
<tr><td colspan="2">For each dose group n = 6
* = p≤0.05, compared to control</td></tr>
</table>
<u>Body weight and food intake</u>
• Statistically significant (p≤0.05) reduction in body weight change with 50 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) reduction in food intake from day 60 to 61 with 50 mg/kg TAD compared to controls

<u>Organ weight changes: kidney, liver, spleen, thymus</u>
• Note: organ weights reported by authors as [organ weight (g)/body weight (g)] x 100
• No statistically significant changes in kidney mass
• Statistically significant (p≤0.05) increase in liver mass with ≥25 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) decrease in spleen mass with 50 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) decrease in thymus mass with 50 mg/kg TAD compared to controls

<u>Changes in serum corticosterone</u>
• No statistically significant changes observed</td>
<td>Major Limitations:
• Only male mice used so response in females not known
• Sample size of 6/group per endpoint

Other comments:
• Species and strain appropriate for endpoints assessed
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous observations of altered immune function in mice
• Subchronic duration of exposure
• Number of exposure levels would allow for determining any dose-dependent effects
• Quantitative data reported
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of endpoints</td>
</tr>
</table>

	<u>Levels of interferon (IFN)-gamma and interleukin (IL)-4 in splenocytes isolated from exposed mice</u> • Dose-dependent decrease in IFN-gamma levels • Statistically significant ($p \leq 0.05$) decrease in IFN-gamma compared to control with TAD of 50 mg/kg • Dose-dependent increase in IL-4 levels • Statistically significant ($p \leq 0.05$) increase in IL-4 compared to control with TAD 5, 25, and 50 mg/kg **<u>Number of T-cells secreting IL-2^+ and IL-10^+ from splenocytes isolated from exposed mice</u>** • Dose-dependent decrease in number of IL-2^+-secreting cells • Statistically significant ($p \leq 0.05$) decrease in number of IL-2^+-secreting cells compared to control with TAD 50 mg/kg • Dose-dependent increase in number of IL-10^+-secreting cells • Statistically significant ($p \leq 0.05$) increase in number of IL-10^+-secreting cells compared to control with TAD 50 mg/kg **<u>Immunoglobulin levels in serum</u>** • Statistically significant ($p \leq 0.05$) reduction in IgM levels with ≥5 mg/kg TAD compared to controls • Statistically significant ($p \leq 0.05$) increases in IgG, IgG1, and IgE levels with 50 mg/kg TAD compared to controls • No statistically significant change on IgG2a levels **<u>Delayed-type hypersensitivity text</u>** • No statistically significant change on footpad thickness	

Reference and Study Design	Results	Comment
Dong et al. (2012b) **Species and strain:** Mice, C57BL/6 Males only 8–10 weeks old **Group size:** 12/group **Test article and vehicle:** PFOS (potassium salt, >98% purity) in de-ionized water with 2% Tween-80 **Route of exposure:** Oral gavage **Exposure levels:** Daily dose: 0, 0.0167, 0.0833, 0.833 mg/kg/day Total administered dose (TAD): 0, 1, 5, 50 mg/kg See **Results** column for serum PFOS concentrations **Exposure regimen:** Once daily for 60 days Sacrifice on day 61	**Internal PFOS concentration** Serum PFOS concentrations after 60 days of exposure | PFOS (mg/kg TAD) | Sample size | Serum PFOS (mg/L) | |---|---|---| | 0 | 12 | 0.04 | | 1 | 12 | 4.35* | | 5 | 12 | 8.21* | | 50 | 12 | 59.74* | Values are means (standard errors not reported herein) * = p≤0.05 compared to controls **Body weight and food intake** Change in body weight and food intake after 60 days of exposure | PFOS (mg/kg TAD) | Change in body weight over 60 d (g) | Food intake on day 60 | |---|---|---| | 0 | 4.49 | 4.22 | | 1 | 4.16 | 4.94 | | 5 | 3.78 | 3.90 | | 50 | -1.34* | 2.24* | Values are means (standard errors not reported herein) For each dose group n = 12 * = p≤0.05 compared to controls **Organ weights** Relative organ weight after 60 days of exposure | PFOS (mg/kg TAD) | Spleen | Thymus | Kidney | Liver | |---|---|---|---|---| | 0 | 0.53 | 0.32 | 1.52 | 4.87 | | 1 | 0.50 | 0.31 | 1.58 | 5.09 | | 5 | 0.47 | 0.27 | 1.54 | 5.51* | | 50 | 0.31* | 0.22* | 1.41 | 9.03* | Values are means (standard errors not reported herein) For each dose group n = 12; * = p≤0.05 compared to controls Note: relative organ weight determined by: [organ weight (g)/body weight (g)] x 100	**Major Limitations:** • Only males used • Subchronic exposure **Other comments:** • Species and strain appropriate for endpoints assessed • Sample size of 12/group per endpoint • Oral gavage provided direct exposure to PFOS • Doses selected yielded clear NOAEL and LOAEL • Number of exposure levels would allow for determining any dose-dependent effects • Quantitative data reported • Internal PFOS concentrations determined • Endpoint ascertainment used standardized assessment for body weight and organ weights Note: This study also provides data on mechanistic outcomes that are not reported herein.

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Dong et al. (2012a)

Species and strain:
Mice, C57BL/6

8–10 weeks old

Group size:

6/males/group (for each of 2 studies, see Exposure regimen below)

Test article and vehicle:
PFOS (potassium salt, >98% pure) in de-ionized water with 2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:

Daily dose: 0, 0.0083, 0.0167, 0.0833, 0.4167, 0.8333, 2.0833 mg/kg/day
Targeted total administered dose (TAD): 0, 0.5, 1, 5, 25, 50, 125 mg/kg

See Results column for serum PFOS concentrations

Exposure regimen:

Exposed for 60 consecutive days, on day 61 sacrificed directly following exposure or exposed to lipopolysaccharide (LPS) and then sacrificed 2 hours later</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="2">Serum PFOS concentrations after 60 days of exposure</td></tr>
<tr><td>PFOS (mg/kg TAD)</td><td>Serum PFOS (mg/L)</td></tr>
<tr><td>Control</td><td>0.04±0.01</td></tr>
<tr><td>0.5</td><td>0.58±0.19*</td></tr>
<tr><td>1</td><td>4.35±0.63*</td></tr>
<tr><td>5</td><td>8.21±1.15*</td></tr>
<tr><td>25</td><td>24.53±5.56*</td></tr>
<tr><td>50</td><td>59.74±12.16*</td></tr>
<tr><td>125</td><td>114.19±23.72*</td></tr>
<tr><td colspan="2">For each dose group n = 6
* = p≤0.05, compared to control</td></tr>
</table>

<u>Body weight and food intake</u>
• Statistically significant (p≤0.05) reduction in final body weight with ≥25 mg/kg TAD compared to controls
• Reduced food intake in the last day of exposure with ≥25 mg/kg TAD compared to controls (note: statistical significance not reported)

<u>Organ weight changes: kidney, liver, spleen, thymus</u>
• Note: organ weights reported by authors as [organ weight (g)/body weight (g)] x 100
• Statistically significant (p≤0.05) reduction in kidney mass with ≥50 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) increase in liver mass with ≥5 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) reduction in spleen mass with ≥25 mg/kg TAD compared to controls
• Statistically significant (p≤0.05) reduction in thymus mass with ≥25 mg/kg TAD compared to controls</td>
<td>Major Limitations:
• Only male mice used so response in females not known
• Sample size of 6/group per endpoint

Other comments:
• Species and strain appropriate for endpoints assessed
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous observations of altered immune function in mice
• Subchronic duration of exposure
• Number of exposure levels would allow for determining any dose-dependent effects
• Quantitative data reported
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of endpoints</td>
</tr>
</table>

	Macrophage numbers in the spleen and peritoneal cavity • Statistically significant (p≤0.05) reduction in splenic cellularity (i.e., total cell population in spleen) with ≥25 mg/kg TAD compare to controls • Non-statistically significant reductions in the numbers of splenic macrophages • Statistically significant (p≤0.05) increase in percentage of splenic macrophages with ≥50 mg/kg TAD compare to controls, authors noted that this increase was due to reductions in splenic cellularity • Statistically significant (p≤0.05) reduction in peritoneal cavity cellularity with 125 mg/kg TAD compared to controls • Non-statistically significant reductions in number of peritoneal cavity macrophages • Statistically significant (p≤0.05) increase in percentage of peritoneal cavity macrophages with ≥1 mg/kg TAD compared to controls **Cytokine production following *in vivo* LPS stimulation** • Note: following LPS stimulation, cells were isolated from peritoneal cavity or spleen for *ex vivo* measurement of cytokines • Statistically significant (p≤0.05) increases in TNF-alpha (≥25 mg/kg TAD), IL-1beta (≥50 mg/kg TAD), and IL-6 (125 mg/kg TAD) in cells from the peritoneal cavity compared to controls • Statistically significant (p≤0.05) increases in TNF-alpha (≥50 mg/kg TAD), IL-1beta (≥50 mg/kg TAD), and IL-6 (125 mg/kg TAD) in cells from the spleen compared to controls **Serum cytokines** • Note: following LPS stimulation, serum was collected for *ex vivo* measurement of cytokines • Without LPS stimulation: statistically significant (p≤0.05) increase in IL-1beta and IL-6 (≥50 mg/kg TAD) compared to controls, non-statistically significant increase in TNF-alpha • With LPS stimulation: statistically significant (p≤0.05) increase in TNF-alpha (125 mg/kg TAD), IL-1beta (≥50 mg/kg TAD), and IL-6 (125 mg/kg TAD)	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Era et al. (2009)

Species and strain:
Mice, ICR
Mature females mated with a male

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (potassium salt, >98% pure) in 0.5% Tween-20

Route of exposure:
Oral gavage

Exposure levels:
Experiment 1: 0, 9, 13, 20, 30 mg/kg/day

Experiment 2: 20 or 50 mg/kg/day

Note: different set of dams apparently used for each experiment

See Results column for serum and amniotic fluid PFOS concentrations

Exposure regimen:
Experiment 1: GD1 to GD17</td>
<td><u>Internal PFOS concentrations at GD17 (Experiment 1)</u>
• Note: serum and amniotic PFOS concentration data presented only graphically
• Dam serum PFOS concentration increased with dose up to the administered dose of 30 mg/kg (measured to be 162.3±25 µg/ml)
• Fetal serum PFOS concentration similar to dam serum PFOS concentration until the administered dose of 20 mg/kg, the fetal concentration then declined
• Amniotic PFOS concentration about one-sixth of the fetal serum PFOS concentration

<u>Fetal effects: cleft palate at GD17 (Experiment 1)</u>
• Note: statistical significance not reported; data for all doses presented graphically but in text for only ≥13 mg/kg/day
• Incidence of cleft palate for 13, 20, and 30 mg/kg/day groups were 7.3%, 78.3%, and 93.8%, respectively; incidence of cleft palate in control group appeared to be ~0% as estimated by visual inspection of graphical data
• Authors reported ED50 = 17.7 mg/kg/day or a fetal serum PFOS concentration of 121 µg/ml

<u>Maternal effects (Experiment 2)</u>

<table>
<tr><td colspan="5">Maternal effects at term</td></tr>
<tr><td></td><td colspan="4">Maternal Dosing Period</td></tr>
<tr><td></td><td colspan="2">GD1–17</td><td colspan="2">GD11–15</td></tr>
<tr><td></td><td>0 mg/kg/d</td><td>20 mg/kg/d</td><td>0 mg/kg/d</td><td>50 mg/kg/d</td></tr>
<tr><td>Number dams examined</td><td>6</td><td>9</td><td>5</td><td>7</td></tr>
<tr><td>Body weight (g)</td><td>71.3</td><td>56.7*</td><td>68.4</td><td>65.6</td></tr>
<tr><td>Body weight gain (g)</td><td>36.6</td><td>23.8*</td><td>34.8</td><td>33.1</td></tr>
<tr><td>Liver weight (g)</td><td>2.9</td><td>5.0*</td><td>2.6</td><td>5.0**</td></tr>
<tr><td>Relative liver weight (%)</td><td>4.1</td><td>8.8*</td><td>3.8</td><td>7.7**</td></tr>
</table>
</td>
<td>Major Limitations:
• Data reporting incomplete for cleft palate (control and low dose not reported; statistical significance not reported for full dose range in GD1–17; number of fetuses examined in each dose group for full dose range at GD17 not given; number of litters represented not reported for GD1–17 vs. GD11–15 comparison)

Other comments:
• Strain of mouse not very common and appropriateness for endpoints assessed is unclear
• Overall sample size is moderate; for full dose range study (GD17) it appears that 3 litters were examined per dose group, but number of fetuses not given; for maternal endpoints, n = 5–9, for fetal endpoints (GD1–17 vs. 11–15) n = 67–103, number of litters = 5–7.
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous observations of fetal defects in mice; however, dose range is narrow; from graphical incidence data, not clear if NOAEL was achieved
• For maternal endpoints, dosing period of ≤17 days is short; for fetal developmental, exposure encompassed most of gestation</td>
</tr>
</table>

Experiment 2: GD1 to GD17 (20 mg/kg/day) or GD11 to GD15 (50 mg/kg/day)	Body weight minus liver weight at GD18 (g)	68.4	51.7**	65.8	60.6
	Implantation sites/litter	16.5	15.9	14.2	15.6
	Number of prenatal losses/litter	1.8 (11.1%)	1.9 (11.8%)	0.6 (4.2%)	1.3 (8.3%)
	Values are means (standard deviations not reported herein) Values in parentheses are prenatal loss percentage per litter = mean of ((number of implantation sites – number of fetuses)/ number of implantation sites) in each dam, corresponding confidence intervals not reported herein * p<0.05; **p<0.01				

Fetal effects: GD1–17 vs. GD11–15 (Experiment 2)

Fetal effects at term				
	Maternal dosing period			
	GD1–17		GD11–15	
	0 mg/kg/d	20 mg/kg/d	0 mg/kg/d	50 mg/kg/d
Total number of fetuses	88	112	68	100
Number of live fetuses examined	82	103	67	99
Fetuses/litter	14.7	14.0	13.6	14.3
Number of cleft palate	0	92 (89.3%)**	0	6 (6.1%)*
Body weight (g)	1.69	1.27**	1.66	1.45**
Liver weight (mg)	126.7	110.5**	125.0	124.5
Relative liver weight (%)	7.5	8.7**	7.5	8.5**
Brain weight (mg)	84.4	75.9**	85.6	80.7**
Implantation sites/litter	16.5	15.9	14.2	15.6
Relative brain weight (%)	5.0	6.1**	5.2	5.7**
Values are means (standard deviations not reported herein) Values in parentheses are percentage of live fetuses with cleft palate (corresponding confidence intervals not reported herein) * p<0.05; **p<0.01				

- Number of exposure levels would allow for determining any dose-dependent effects, but dose response above threshold is very steep and dose range does not provide detail on this portion of range
- Internal PFOS concentrations determined, but only reported graphically
- Endpoint ascertainment used standardized assessment of morphology, body weight, and organ weights

Note: this study included mechanistic data from ex-vivo tissue and histology studies that are not reported herein

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Fuentes et al. (2006)

Species and strain:
Mice, Charles River CD1
Adult females mated with adult males

Group size:
Maternal = 10/group (except 1.5 mg/kg/d where 11/group)
Litters = 9–10/group
Fetuses = 67–71/group

Test article and vehicle:
PFOS (potassium salt, purity not reported) in 0.5% Tween-20

Route of exposure:
Oral gavage

Exposure levels:
0, 1.5, 3, 6 mg/kg/day

Exposure regimen:
GD6 to GD18

All animals sacrificed on GD18</td>
<td><u>Maternal effects</u>
• No statistically significant effects on:
- maternal body weight at GD18 and body weight gain
- maternal food consumption
- gravid uterine weight
- kidney weight
- relative kidney weight
- maternal thyroid hormones or corticosterone
<table>
<tr><td colspan="5">Maternal effects at GD18</td></tr>
<tr><td></td><td colspan="4">Dose (mg/kg/day) for GD1–18</td></tr>
<tr><td></td><td>0
(vehicle control)</td><td>1.5</td><td>3</td><td>6</td></tr>
<tr><td>Liver wt (g)</td><td>2.3</td><td>2.5</td><td>2.8*</td><td>3.1*</td></tr>
<tr><td>Relative liver wt (%)</td><td>4.3</td><td>4.4</td><td>5.0</td><td>5.8*</td></tr>
<tr><td colspan="5">Values are means (standard error of the mean not reported herein).
* p<0.05 compared to control</td></tr>
</table>
<u>Fetal effects: reproductive performance</u>
• No statistically significant effects on:
- implants per litter
- live fetuses per litter
- dead fetuses per litter
- litters with dead fetuses
- early resorptions per litter
- late resorptions per litter
- post-implantation loss
- mean fetal weight
- fetal sex ratio
<u>Fetal effects: developmental effects</u>
• No statistically significant effects on:
- number of litters examined skeletally
- assymetrical sternebrae
- diminished ossification of caudal vertebrae
- supernumerary ribs
- total of litters with skeletal defects
• Statistically significant (p<0.05) decrease in diminished ossification (calcaneous) with 3 mg/kg/day, but not at other doses (including 6 mg/kg/day)</td>
<td>Major Limitations:
• Internal PFOS concentration not determined
• PFOS purity not reported

Other comments:
• Species and strains appropriate for endpoints assessed
• Sample size 10–11/group (maternal effects) and 9–10/group (fetal effects)
• Oral gavage provided direct exposure to PFOS
• Doses selected based on previous observations in rats and mice; concentration range produced LOAEL and NOAEL for maternal liver weight, but no other observed effects
• Exposure lasted most of gestation (for fetal effects); maternal effects, exposure was short-term
• Number of exposure levels allow for determining any dose-dependent effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of maternal and fetal endpoints

Note: This study also examined outcomes associated with the combination of maternal PFOS dosing and maternal stress due to restraint. Restraint-related data are not reported herein.</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Grasty al. (2003)

Species and strain:
Rats, Sprague-Dawley
F0 age not reported

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (potassium salt, purity not reported) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
Four-day regimen: 0, 25 mg/kg
Two-day regimen: 0, 25, 50 mg/kg

For four-day regimen, maternal serum PFOS levels determined 24 hours after final exposure and on GD21, data not reported herein

Exposure regimen:
Four-day regimen: GD2 to GD5, GD6 to GD9, GD10 to GD13, GD14 to GD17, GD17 to GD20; after fourth day of dosing pregnancies were carried out to full term
Two-day regimen: GD19 to GD20</td>
<td><u>Four-day regimen: maternal effects</u>
• Statistically significant (p<0.05) decrease in weight gain during dosing in all treatment groups compared to controls, weight loss noted following exposure on GD2 to GD5 and GD6 to GD9
• Reduced food and water consumption by treated animals during and immediately following exposure (data not shown), consumption exceeded control levels several days after the end of exposure
<u>Four-day regimen: pup effects</u>
• Decreased pup survival for all treatment groups, controls near 100% survival
• Survival decreased as treatment occurred later in gestation
• Deaths primarily occurred during PND1
• Following exposure during GD17 to GD20: pups born pale and rigid, mortality near 100% within 24 hours
• No statistically significant effect on live litter size
• Statistically significant (p<0.05) decrease in pup weight for GD2 to GD5, GD6 to GD9, and GD10 to GD14 groups, compared to controls
<u>Two-day regimen: maternal and pup effects</u>
• Statistically significant (p<0.05) lower weight gain in treated dams groups compared to controls

Effects on pups at PND0
<table><tr><th></th><th>Number of pups</th><th>Live litter size</th><th>% survival</th><th>Pup weight (g)</th></tr><tr><td>0 mg/kg</td><td>26</td><td>13.6±0.5[a]</td><td>100[a]</td><td>6.6±0.1[a]</td></tr><tr><td>25 mg/kg</td><td>21</td><td>11.9±0.5[b]</td><td>94[a]</td><td>5.9±0.1[b]</td></tr><tr><td>50 mg/kg</td><td>27</td><td>11.1±0.8[b]</td><td>29[b]</td><td>5.4±0.2[b]</td></tr></table>Data are mean±SE
Groups not sharing a common letter have statistically significant difference (p<0.05)</td>
<td>Major Limitations:
• No serum PFOS measurement for pups
• PFOS purity not reported

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size generally ≥10 litters
• Oral gavage provided direct exposure to PFOS
• Doses selected meant to induce neonatal mortality
• Duration of exposure limited to specific gestational periods
• Number of doses selected (i.e., 1 or 2) limited the ability to determine dose-related effects
• Data generally quantitative, qualitative information on food and water consumption reported
• Endpoint ascertainment used standardized assessment of body weight and mortality; lung examination relied on subjective assessment of histology</td>
</tr>
</table>

	• Pups in 50 mg/kg group were moribund with troubled breathing after birth, only 3% survived by PND5 • Pups in 25 mg/kg group varied in physical appearance (e.g., size and color) at birth, 66% survived by PND5 • Pup weight remained lower ($p<0.05$) in 25 mg/kg group compared to control through PND5; pup weight for 50 mg/kg group not included due to only 1 litter surviving past PND0 • Decreased lung expansion in pups from treated dams compared to prenatal controls • Difference in lung histology (i.e., thinning of epithelial walls) between pups from treated dams and control pups	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Grasty et al. (2005)

Species and strain:
Rats, Sprague-Dawley
F0 age not reported

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (potassium salt, 91% pure) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
0, 25, 50 mg/kg/day

Exposure regimen:
GD19 to GD20

Rescue studies conducted with co-exposure to either dexamethasone (Dex) or retinyl palmitate (RP) on GD19 to either GD20 or GD21

Related studies:
Grasty et al. (2003)</td>
<td><u>Maternal and developmental toxicity</u>
• Not determined by authors during this exposure
• Authors referred to earlier work (Grasty et al. [2003]) for effects resulting from an identical exposure regimen
• Suppressed maternal weight gain compared controls
• Statistically significant decreases in live litter size and pup birth weight compared to controls
• Increased neonatal mortality compared to controls

<u>Lung histology</u>
• No differences in alveolar wall thickness between treated and control animals at GD21 with microscopic examination
• Morphological resemblance between GD21 controls and PND0 treated groups: 17% and 50% of 25 and 50 mg/kg/day groups, respectively, determined to be affected by treatment
<table>
<tr><td colspan="4">Morphometric analysis of neonatal lung tissue</td></tr>
<tr><td>PFOS (mg/kg/day)</td><td>Solid tissue proportion</td><td>Small airway proportion</td><td>Solid tissue: small airway ratio</td></tr>
<tr><td>0</td><td>0.34±0.02</td><td>0.61±0.02</td><td>0.57±0.05</td></tr>
<tr><td>25</td><td>0.43±0.03</td><td>0.47±0.02[a]</td><td>0.93±0.09[a]</td></tr>
<tr><td>50</td><td>0.45±0.02[a]</td><td>0.50±0.02[a]</td><td>0.94±0.09[a]</td></tr>
<tr><td colspan="4">For all groups, lungs from 12 pups (2 per litter) were examined
Data are mean±SEM
a = p<0.05, compared to controls</td></tr>
</table>
<u>Rescue studies</u>
• No statistically significant increase in neonatal survival from co-exposure to PFOS and Dex or RP</td>
<td>Major Limitations:
• Serum PFOS concentrations not reported

Other comments:
• Species and strain appropriate for endpoints assessed
• Small sample size for some endpoints (e.g., ≤10 pups for lung histopathology)
• Oral gavage provided direct exposure to PFOS
• Doses selected on previous observations of neonatal mortality
• Duration of exposure limited to specific gestational period
• Number of doses selected do not allow for determining low dose effects
• Quantitative data generally reported
• Endpoint ascertainment used standardized assessment of mortality; lung assessed by quantitative morphometric analyses

• Study also assessed mechanistic endpoints (e.g., phospholipid profile, RNA microarray) that are not reported herein</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Kawamoto et al. (2011)

Species and strain:
Rats, Wistar
4 weeks old

Group size:
5 or 6/males/group

Test article and vehicle:
PFOS (potassium salt, purity not reported) in aqueous solution mixed with powdered diet

Route of exposure:
Dietary

Exposure levels:
0, 2, 8, 32, 128 ppm

See Results column for serum, brain, kidney, and liver PFOS concentrations

Exposure regimen:
7 days a week for 13 weeks
Rats sacrificed after 13 weeks of exposure

Rats also exposed biweekly to ultrasonic stimulus (47 kHz, 10 sec at 30 cm)

Related studies:
Sato et al. 2009</td>
<td><u>Internal PFOS concentrations</u>

<table>
<tr><th colspan="3">PFOS concentrations (mg/kg) after 13 weeks of exposure</th></tr>
<tr><td>Dose group</td><td>Serum</td><td>Brain</td></tr>
<tr><td>0 ppm</td><td>NR</td><td>NR</td></tr>
<tr><td>2 ppm</td><td>9.50±0.68</td><td>1.91±0.37</td></tr>
<tr><td>8 ppm</td><td>44.1±5.60</td><td>6.91±1.38</td></tr>
<tr><td>32 ppm</td><td>177±20.0</td><td>22.3±114</td></tr>
<tr><td>128 ppm</td><td>432±75.3</td><td>105±19.8</td></tr>
<tr><td>Dose group</td><td>Liver</td><td>Kidney</td></tr>
<tr><td>0 ppm</td><td>NR</td><td>NR</td></tr>
<tr><td>2 ppm</td><td>59.7±8.96</td><td>14.8±4.60</td></tr>
<tr><td>8 ppm</td><td>135±42.7</td><td>36.0±11.2</td></tr>
<tr><td>32 ppm</td><td>647±113</td><td>188±46.8</td></tr>
<tr><td>128 ppm</td><td>1180±156</td><td>628±169</td></tr>
<tr><td colspan="3">n = 5; NR = not reported</td></tr>
</table>

• Tissue PFOS concentrations relative to serum PFOS: brain, 0.13 to 0.24; liver, 2.7 to 6.3; and kidney, 0.82 to 1.6

<u>General effects: food consumption and body weight</u>
• Statistically significant ($p<0.05$) decrease in food consumption with ≥32 ppm compared to control
• Statistically significant ($p<0.05$ or $p<0.01$) decrease in body weight with ≥32 ppm compared to control

<u>Organ weights (at end of study): brain, kidney, liver</u>
• Statistically significant ($p<0.05$) increase in relative brain weight with ≥32 ppm
• No statistically significant effect on kidney weight
• Statistically significant ($p<0.05$ or $p<0.01$) increase in absolute (with 128 ppm) and relative (with ≥32 ppm) liver weights</td>
<td>Major Limitations:
• Serum and tissues PFOS concentrations not reported in control animals
• Only males used
• Biological significance of ultrasonic-induced convulsions not clear

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size was at least 5 rats per endpoint
• Dietary exposure allows for PFOS to interact with tissues from the oral cavity to the stomach
• Doses selected span over 50-fold increase between lowest and highest dose
• Subchronic duration of exposure appropriate
• Number of exposure levels allow for determining any dose-related effects
• Generally quantitative data reported, qualitative (textual) reporting for some endpoints (behavioral abnormalities)
• Internal PFOS concentrations determined in multiple tissues
• Endpoint ascertainment used standardized assessment of body and organ weights, histopathology, and neurological testing</td>
</tr>
</table>

	Neurotoxicity: convulsions after biweekly ultrasonic stimulus • No observations of convulsions in 2, 8, and 32 ppm groups • In 128 ppm group, convulsions observed in 5/6 animals at week 6; recovery observed in all animals except in 1 that was found dead next morning, ultrasonic stimulus ceased thereafter **Neurotoxicity: behavioral abnormalities** • Textual reporting of data only • No observed behavioral abnormalities (e.g., startle response, touch response, pain response, righting reflex, visual placing, abdominal tone, and limb tone) **Neurotoxicity: histopathology and ultrastructure** • No histopathological changes observed in neuronal or glial cells of the cerebrum and cerebellum (textual reporting of data only) • No ultrastructural changes observed in the neurons in the cortex and hippocampus as well as the neurons and granules cells in the cerebellum	

Reference and Study Design	Results	Comment

Keil et al. (2008)
 	
Species and strain:
Mice, B6C3F1 obtained from breeding C57BL/6N females with C3H/HeJ males
 	
Group size:
Varied by endpoint
 	
Test article and vehicle:
PFOS (potassium salt, 91% pure) in distilled water with 0.5% Tween-20
 	
Route of exposure:
Oral gavage
 	
Exposure levels:
0, 0.1, 1.0, 5.0 mg/kg/day
 	
Exposure regimen:
GD 1 to GD17
 	
Pups sacrificed at 4 and 8 weeks of age
	Maternal effects: body weight
	• No significant weight loss in pregnant dams (data not shown by authors)
	
	Offspring effects: body weight
	• No statistically significant differences between exposure groups and controls at 4 weeks (6/sex/group) and 8 weeks (5–6/sex/group) of age
	
	Offspring effects: organ weight
	• Note: weights normalized to body weight [(organ weight/body weight) x 100]
	• At 4 weeks of age (6/sex/group):
	  ○ Females: statistically significant ($p \leq 0.05$ compared to controls) decrease in liver weight (0.1 mg/kg/day only) and in kidney weight (5 mg/kg/day); no effect on spleen and thymus weights
	  ○ Males: statistically significant ($p \leq 0.05$ compared to controls) increase in liver weight (5 mg/kg/day); no effect on kidney, spleen, and thymus weights
	• At 8 weeks of age (5–7/sex/group):
	  ○ Females and males: no effect on kidney, liver, spleen, and thymus
	
	Offspring effects: spleen and thymus cellularity
	• No statistically significant differences between exposure and control groups for females and males at 4 weeks (6/sex/group) and 8 weeks (5–7/sex/group except 0.1 mg/kg/day where 2–3/sex/group) of age
	
	Offspring effects: natural killer cell function
	• At 4 weeks of age (genders combined for analysis, 12/group):
	  ○ No statistically significance differences between exposure and controls groups
	• At 8 weeks of age (genders analyzed separately, 6/sex/group unless noted otherwise):
		Major Limitations:
		• Internal PFOS levels not determined
		• Interpretation of immunotoxicity with respect to significance of adversity is not clear
		• Quantitative data reported for immunotoxicity but individual litter data not reported for non-immunotoxicity endpoints (e.g., body weight, organ weights)
		
		Other comments:
		• Species and strain appear to be appropriate for endpoints assessed
		• Sample size for most endpoints was 5–7 animals/group, may have reduced power to detect changes or dose-response
		• Oral gavage provides direct exposure to PFOS
		• Dose selection based on previous observations in rodents, dose range was adequate to detect LOAEL and NOAEL for some endpoints
		• Duration of exposure covered gestational period
		• Number of exposure levels allowed for determining and dose-dependent effects
		• Endpoint ascertainment used standardized methods for endpoints assessed
		
		Note: peritoneal macrophage nitric oxide was also assessed, but is not

	○ Females (3/group with 0.1 mg/kg/day): statistically significant (p<0.05) decrease (35.1%) with 5.0 mg/kg/day compared to controls ○ Males (2/group with 0.1 mg/kg/day): statistically significant (p<0.05) decrease with 1.0 mg/kg/day (42.5%) and 5.0 mg/kg/day (32.1%) compared to controls **Offspring effects: specific IgM response to sheep red blood cell (SRBC) immunization** • Note: analysis only performed at 8 weeks of age at 6/sex/group • Females: no statistically significant differences between exposure and controls groups • Males: statistically significant (p<0.05) decrease (53%) with 5.0 mg/kg/day compared to controls **Offspring effects: lymphocyte immunophenotypes (subpopulations)** • Note: CD3+, CD4+, CD8+, DP (CD4+/CD8+), DN (CD4-/CD8-), B220+ assessed • At 4 weeks of age (6/sex/group): ○ Female: statistically significant (p≤0.05) decrease (21%) in splenic B220 cells with 5.0 mg/kg/day compared to controls, no statistically significant differences between exposure and control groups for other splenic subpopulations ○ Male: no statistically significant differences between exposure and controls groups for any splenic subpopulation ○ For both males and females: no statistically significant differences between exposure and controls groups for thymic subpopulations • At 8 weeks of age (6/sex/group): ○ Female: no statistically significant differences between exposure and controls groups for thymic and splenic subpopulations ○ Male: statistically significant (p≤0.05) reduction in thymic CD3+ (23%) and CD4+ (29%) cells with 5.0 mg/kg/day compared to controls, no statistically significant differences between exposure and controls groups for other thymic or any splenic subpopulations	summarized herein as this is an intermediate rather than apical endpoint

Reference and Study Design	Results	Comment
Lau et al. (2003) Note: authors assessed endpoints within 3 general outcomes, herein broadly defined as: reproductive/developmental effects (e.g., birth outcomes, age at eye opening and puberty), effects dues to cross-fostering, and neurodevelopmental effects (e.g., choline acetyltransferase activity, T-maze). Of these, neurodevelopmental effects are reported in a separate table. Study authors also conducted exposures using mice. These mice data are presented in a separate table. **Species and strain:** Rats, Sprague-Dawley F0 age not reported **Group size:** Varied by endpoint **Test article and vehicle:** PFOS (potassium salt, 91% pure) in 0.5% Tween 20 **Route of exposure:** Oral gavage	**Postnatal effects: mortality** • Statistically significant ($p<0.05$) reduction in postnatal survival with ≥2 mg/kg • 100% of pups in 10 mg/kg group died ~60 minutes following birth • 95% of pups in 5 mg/kg group died within 24 hours of birth • 50% of pups in 3 mg/kg group survived **Postnatal effects: reproductive/developmental milestones** • Statistically significant ($p<0.05$) delay in eye opening by ~1 day with ≥2 mg/kg, control group eye opening between PND14 and PND15 • No effect on vaginal opening, onset and profiles of the estrous cycle, and preputial separation **Postnatal effects from cross-fostering: mortality** • Cross-fostering pups from 5 mg/kg group with control dams did not improve postnatal survival • All control pups cross-fostered with PFOS-exposed dams survived duration of observation (3 days)	**Major Limitations:** • Internal PFOS concentrations not determined **Other comments:** • Species and strain appropriate for endpoints assessed • For most endpoints, sample size was ≥10 rats • Oral gavage provided direct exposure to PFOS • Doses selected allowed for overt toxicity at highest dose • Duration of exposure lasted length of gestation • Number of exposure levels allowed for determining any dose-dependent effects • While generally quantitative, data not reported for some endpoints • Endpoint ascertainment used standardized assessment of mortality and reproductive/developmental endpoints

Exposure levels: 0, 1, 2, 3, 5, 10 mg/kg/day Note: internal PFOS concentrations not determined from rats assessed for developmental and cross-fostering effects **Exposure regimen:** GD2 to GD21 Note: newborns from control and 5 mg/kg groups participated in a 3-day cross-fostering experiment: 1) control pups with their dams; 2) PFOS-exposed pups with their dams; 3) PFOS-exposed pups with control dams; and 4) control pups with PFOS-exposed dams **Related studies:** Thibodeaux et al. (2003)		

Reference and Study Design	Results	Comment
Lau et al. (2003) Note: authors assessed endpoints within 3 general outcomes, herein broadly defined as: reproductive/developmental effects (e.g., birth outcomes, age at eye opening and puberty), effects dues to cross-fostering, and neurodevelopmental effects (e.g., thyroid hormones, T-maze). Neurodevelopmental effects are reported herein. Study authors also conducted exposures using mice. These mice data are presented in a separate table. **Species and strain:** Rats, Sprague-Dawley F0 age not reported **Group size:** 17 to 28 dams/group **Test article and vehicle:** PFOS (potassium salt, 91% pure) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 2, 3, 5 mg/kg/day	**Internal PFOS concentrations in neonatal rats** • At PND0, serum PFOS concentrations were proportional to administered dose, but not in a linear relationship • At PND5, serum PFOS levels in each surviving group were lower than on PND0 • At PND0, liver PFOS concentrations were proportional to administered dose and similar to serum PFOS concentrations **Postnatal effects: body weight and liver weight** • Body weights were lower with ≥ 2 mg/kg compared to controls, statistically significant ($p<0.05$) results typically within first week of postnatal life • Absolute liver weights comparable between controls and exposed groups • Relative liver weights increased with ≥1 mg/kg compared to controls, statistically significant ($p<0.05$) results typically within first 3 weeks of postnatal life **Postnatal effects: thyroid hormones** • Serum levels of total thyroxine and free thyroxine were decreased compared to controls • Decrease in serum free thyoxine persisted through end of experiment (PND35) • No significant effects on serum triiodothyronine or thyroid stimulating hormone compared to controls **Postnatal effects: learning behavior** • No significant difference between exposed (3 mg/kg) and control groups for T-maze test	**Major Limitations:** • Measurements for internal PFOS concentrations limited to PND1 to PND5 for serum and PND0 for liver • Thyroid hormone measurements may be subject to negative bias based on analytical method used **Other comments:** • Species and strain appropriate for endpoints assessed • For most endpoints, sample size was ≥10 rats, for T-maze and thyroid hormones sample size was <10 rats • Oral gavage provided direct exposure to PFOS • Doses selected allowed for overt toxicity at highest dose as well as survival throughout duration of experiment in lower doses • Duration of exposure lasted length of gestation • Number of exposure levels allowed for determining any dose-dependent effects • Quantitative data reported • Endpoint ascertainment used standardized assessment of body and organ weights

See **Results** column for serum and liver PFOS concentrations for neonatal rats **Exposure regimen:** GD2 to GD21 Postnatal observations performed through PND35, weaning at PND21 **Related studies:** Thibodeaux et al. (2003)		

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lau et al. (2003)

Note: authors conducted two separate mouse studies, each employing the same exposure conditions but assessing different endpoints. Mice from an initial exposure were assessed for mortality, body weight, and eye opening. Mice from a separate exposure were assessed for liver weight and serum thyroid hormone.

Study authors also conducted exposures using rats. These rat data are presented in a separate table.

Species and strain:
Mice, CD-1
F0 age not reported

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (potassium salt, 91% pure) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
0, 1, 5, 10, 15, 20 mg/kg</td>
<td><u>Postnatal effects: mortality</u>
• Dose-dependent reduction in postnatal survival
• Majority of pups in 15 and 20 mg/kg groups did not survive past 24 hours post birth
• Survival in 1 and 5 mg/kg groups similar to that of controls
• LD50 estimated to be 10 mg/kg

<u>Postnatal effects: body weight and liver weight</u>
• Postnatal body weight generally comparable between exposed and controls groups, trend (p<0.05 vs control) toward growth deficit observed with 10 mg/kg
• Absolute and relative liver weights increased in exposed groups compared to controls throughout observation period (until PND35), statistically significant (p<0.05) results typically with ≥5 mg/kg

<u>Postnatal effects: thyroid hormone</u>
• Only total serum thyroxine levels reported for mice
• Levels in exposed and control groups generally comparable except for 5 and 10 mg/kg groups which tended to be lower than controls

<u>Postnatal effects: reproductive/developmental milestones</u>

<table>
<tr><td colspan="2">Postnatal observations after PFOS exposure</td></tr>
<tr><td>PFOS (mg/kg/day)</td><td>Age at eye opening (PND)</td></tr>
<tr><td>0</td><td>14.8±0.1</td></tr>
<tr><td>1</td><td>15.1±0.1</td></tr>
<tr><td>5</td><td>15.5±0.1</td></tr>
<tr><td>10</td><td>15.6±0.1</td></tr>
<tr><td colspan="2">mean±SE
Number of mice examined not reported
Statistically significant (p<0.0001) treatment effect</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS concentrations not determined
• Thyroid hormone measurements may be subject to negative bias based on analytical method used

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample sizes ranged from ≥20 mice for body and liver weights to <10 for serum thyroid hormone measurements
• Oral gavage provided direct exposure to PFOS
• Doses selected allowed for overt toxicity at highest dose as well as survival throughout duration of experiment in lower doses
• Duration of exposure lasted length of gestation
• Number of exposure levels allowed for determining any dose-dependent effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of mortality, body and organ weights, and reproductive/developmental milestone</td>
</tr>
</table>

Exposure regimen: GD1 to GD17 Postnatal observations performed through PND35, weaning at PND21 **Related studies:** Thibodeaux et al. (2003)		

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lee et al. (2015)

Species and strain:
Mice, CD-1
Time-mated, entered study at GD10

Group size:
10 pregnant mice/group

Test article and vehicle:
PFOS (potassium salt, purity not reported) in 0.5% Tween

Route of exposure:
Oral gavage

Exposure levels:
0, 0.5, 2.0, 8.0 mg/kg/day

Exposure regimen:
GD11 to GD16

Pregnant dams sacrificed on GD17 and fetuses and placentas were harvested</td>
<td><u>Maternal effects: body weight</u>
• No statistically significant difference in body weight gain between any group during GD10–13
• Statistically significant (p<0.05 or p<0.001 according to Kruskal-Wallis group test) differences in body weight gain among four groups during GD14–17
• At GD17, mean maternal body weights of control, 0.5, 2.0, and 8.0 mg/kg/day groups were 61.44, 60.03, 57.68, and 48.32g, respectively

<u>Fetal effects: developmental and placental parameters</u>

Fetal effects at GD17
<table><tr><td></td><td colspan="4">Dose (mg/kg/day)</td></tr><tr><td></td><td>0</td><td>0.5</td><td>2.0</td><td>8.0</td></tr><tr><td>Number of pregnant dams</td><td>10</td><td>10</td><td>10</td><td>10</td></tr><tr><td>Placental weight (mg)</td><td>185.63</td><td>177.32*</td><td>163.22*</td><td>151.54*</td></tr><tr><td>Fetal weight (g)</td><td>1.72</td><td>1.54</td><td>1.30*</td><td>1.12*</td></tr><tr><td>Placental capacity[a]</td><td>9.30</td><td>8.68*</td><td>7.96*</td><td>7.39*</td></tr><tr><td>Number of implantations[b]</td><td>13.45</td><td>13.20</td><td>13.68</td><td>13.71</td></tr><tr><td>Number of resorptions and dead fetuses</td><td>0.57</td><td>1.62*</td><td>4.84*</td><td>7.58*</td></tr><tr><td>Number of live fetuses</td><td>12.88</td><td>11.58</td><td>8.84*</td><td>6.13*</td></tr><tr><td>Post-implantation loss[c]</td><td>4.24%</td><td>12.27%</td><td>35.38%</td><td>55.29%</td></tr></table>Values are means (standard deviations not reported herein)
Note: Fetal analyses utilized litters as units of analysis
* p<0.01 compared to controls
a = ratio of fetal weight/placental weight
b = implantation occurred prior to PFOS dosing
c = [(total implantations – live implantations)/total implantations] x 100</td>
<td>Major Limitations:
• No data on purity of PFOS
• Internal PFOS concentrations not determined

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample sized generally 10/group
• Oral gavage provided direct exposure to PFOS
• Doses selected based on previous observations of development toxicity in mice; as the lowest dose is a LOAEL for most endpoints, dose range does not permit a NOAEL
• Duration of exposure lasted most of gestation
• Number of exposure levels allowed for determining any dose-dependent effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of most endpoints, determining placental area of injury partially unclear

Note: This research included measurement of non-apical (molecular and mechanistic) endpoints that are not summarized herein.</td>
</tr>
</table>

	Placental necrosis at GD17 Dose (mg/kg) | Area of injury[a] Control | 0% 0.5 | 12.7% 2.0 | 26.3% 8.0 | 42.4% a = approximately defined as ratio of placental area with injury to total placental area Note: for each group, three placental sections from five different animals (15 sections/group)	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Long et al. (2013)

Species and strain:
Mice, C57BL6
8 weeks old, males and females

Group size:
15/group (gender distribution not reported)

Test article and vehicle:
PFOS (salt not reported, purity not reported) in normal saline

Route of exposure:
Oral (presumed by gavage)

Exposure levels:
0, 0.43, 2.15, 10.75 mg/kg

Exposure regimen:
Once daily for 3 months

Endpoints assessed after the 3-month exposure</td>
<td><u>Neurotoxicity: spatial learning</u>

<table>
<tr><td colspan="5">Escape latency on day 3</td></tr>
<tr><td></td><td colspan="4">Dose (mg/kg/day)</td></tr>
<tr><td></td><td>control</td><td>0.43</td><td>2.15</td><td>10.75</td></tr>
<tr><td>Escape latency (seconds)</td><td>32.5</td><td>NR</td><td>56.75*</td><td>61.5**</td></tr>
<tr><td colspan="5">Values are means (standard deviation not reported herein) for four trials
* = p<0.05 compared to controls; ** = p<0.01 compared to controls
NR = numerical data not reported, but no statistically significant difference compared to control
Note: no statistically significant difference between genders
Note: mice with poor swimming velocity (<5 cm/s for >50% of swim time) excluded from analysis (number of mice not provided)</td></tr>
</table>

<u>Neurotoxicity: spatial memory</u>

<table>
<tr><td colspan="5">Time spent in target quadrant on day 4</td></tr>
<tr><td></td><td colspan="4">Dose (mg/kg/day)</td></tr>
<tr><td></td><td>control</td><td>0.43</td><td>2.15</td><td>10.75</td></tr>
<tr><td>Percent time in target quadrant</td><td>~43%</td><td>~35%</td><td>~25%*</td><td>~20%**</td></tr>
<tr><td colspan="5">Note: percent values not provided by study authors, values in above table are estimated from Figure 1b of the Long et al study
* = p<0.05 compared to controls; ** = p<0.01 compared to controls
Note: no statistically significant differences between genders
Note: mice with poor swimming velocity (<5 cm/s for >50% of swim time) excluded from analysis (number of mice not provided)</td></tr>
</table>
</td>
<td>Major Limitations:
• PFOS purity not reported
• Internal PFOS concentration not determined
• Missing quantitative data (i.e., lowest dose for escape latency on day 3)
• No specific information given on the number of poor swimmers that were excluded from analyses

Other comments:
• Species and strain appropriate for endpoints assessed
• Oral exposure provided direct exposure to PFOS
• Doses selected represent a reasonable range (factor of 25) and encompass NOAEL, LOAEL, and high dose
• Subchronic duration of exposure
• Number of exposure levels allowed for determining any dose-dependent effects
• Endpoint ascertainment used standardized assessment of spatial learning and memory

Note: this study also provided mechanistic data that is not reported herein</td>
</tr>
</table>

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Luebker et al. (2005a)

Note: study authors conducted two-generation and cross-foster studies. Of the F0, F1, and F2 results from the two-generation study, only the F0 results are reported herein. F1 and F2 results and the results from the cross-foster study are reported in separate tables.

Species and strain:
Rats, Crl:CD® (SD)IGS BR VAF®
F0 male and females were 62 days old at receipt followed by 14-day acclimation period prior to exposure

Group size:
35/sex/group (for exposure), group size then varied by endpoint

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in 2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 0.4, 1.6, 3.2 mg/kg/day</td>
<td><u>Internal PFOS concentrations for F0 rats</u>

<table>
<tr><td colspan="5">Internal PFOS concentrations for F0 males and females</td></tr>
<tr><td></td><td colspan="2">F0 females
Internal PFOS at LD21</td><td colspan="2">F0 males
Internal PFOS after 42 to 56 days of exposure</td></tr>
<tr><td>Dose group (mg/kg/day)</td><td>Serum (ug/mL)</td><td>Liver (ug/g)</td><td>Serum (ug/mL)</td><td>Liver (ug/g)</td></tr>
<tr><td>Control</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>0.1</td><td>5.28±0.358</td><td>14.8±1.71</td><td>10.5±0.946</td><td>84.9±6.28</td></tr>
<tr><td>0.4</td><td>18.9±1.30</td><td>58±6.73</td><td>45.4±5.49</td><td>176±23.4</td></tr>
<tr><td>1.6</td><td>82±17.5</td><td>184±88.3</td><td>152±7.91</td><td>323±36.2</td></tr>
<tr><td>3.2</td><td>NR</td><td>NR</td><td>273±49.8</td><td>1360±40.7</td></tr>
<tr><td colspan="5">mean±SD; NR = not reported</td></tr>
</table>
<u>F0 male effects: mortality, clinical signs, body weight, food consumption</u>
• No deaths or treatment-related clinical signs observed
• Non-statistically significant (p≤0.05) reduction in body weight with 0.4 mg/kg/day at various times between the first and terminal days of the study
• Statistically significant (p≤0.05) reduction in body weight with 1.6 mg/kg/day after the mating/cohabitation period compared to controls
• Statistically significant (p≤0.01) reduction in body weight with 3.2 mg/kg/day prior to (day of study 36) mating/cohabitation through termination compared to controls

<table>
<tr><td colspan="2">Overall body weight gain (day 0 to termination) in F0 males</td></tr>
<tr><td>Dose group (mg/kg/day)</td><td>Overall body weight gain (g)</td></tr>
<tr><td>0</td><td>153.6±41.5</td></tr>
<tr><td>0.1</td><td>149.2±34.5</td></tr>
<tr><td>0.4</td><td>132.8±34.0[a]</td></tr>
<tr><td>1.6</td><td>121.9±30.2[a]</td></tr>
<tr><td>3.2</td><td>91.0±29.9[a]</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS measurements determined after some effects were initially observed (e.g., F0 female reproductive effects at birth and F0 female internal PFOS measurements at LD21)
• Control values for internal PFOS measurements not reported

Other comments:
• Species and strain appropriate for endpoints assessed
• Most F0 endpoints had n>20, but GD10 observations had n≤10
• Oral gavage provided direct exposure to PFOS
• Dose selection presumptively based on observations of rat neonatal mortality in previous studies
• Duration of F0 exposures (i.e., ≥42 days) were subchronic (i.e., >30 days)
• Number of exposure levels allowed for determining any dose-related effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of mortality, body weight, food consumption, fertility indices, and reproductive effects</td>
</tr>
</table>

See **Results** column for serum and liver PFOS concentrations for F0 males and females **Exposure regimen:** F0 males: dosed once daily during the 42 day pre-mating period and then once daily during the mating/cohabitation period (with a maximum of 14 days of mating), F0 males then sacrificed 1 week after mating/cohabitation F0 females: dosed once daily during the 42 day pre-mating period, then once daily during the mating/cohabitation period, then either until GD9 (for caesarean group, sacrifice at GD10) or lactation day (LD)20 (natural delivery group, sacrifice at LD21). F1 weaning reported to be LD21 or LD22. **Related studies:** Luebker et al. (2005b)	• Prior to mating/cohabitation, statistically significant reductions in absolute (g/day) and relative (g/kg/day) feed consumption with 1.6 mg/kg/day (p≤0.05) and 3.2 mg/kg/day (p≤0.01) • After mating/cohabitation, statistically significant reduction in absolute feed consumption with 0.4 mg/kg/day (p≤0.05) and >1.6 mg/kg/day (p≤0.01), statistically significant reduction (p≤0.01) in relative feed consumption with 3.2 mg/kg/day **F0 female effects: mortality, clinical signs, body weight, food consumption** • No deaths observed • Localized areas of partial alopecia with >0.4 mg/kg/day • Statistically significant (p≤0.05) reduction in body weight with 1.6 mg/kg/day during periods within gestation and lactation compared to control • Statistically significant (p≤0.01) reduction in body weight with 3.2 mg/kg/day during all pre-mating, mating/cohabitation, and lactation periods	

Overall body weight gain in F0 females

	Overall body weight gain (g)		
Dose group (mg/kg/day)	Pre-mating	Gestation	Lactation
0	37.1±15.8	125.1±15.9	32.8±19.7
0.1	36.0±10.5	123.8±13.3	27.8±12.3
0.4	34.5±12.9	121.9±20.2	33.8±17.8
1.6	25.0±11.9[a]	123.1±18.3	32.0±14.6
3.2	5.4±10.2[a]	108.0±10.6[a]	NR

mean±SD, NR = not reported
a = p≤0.01 compared to controls

	• Prior to mating/cohabitation, statistically significant (p≤0.01) reduction in absolute and relative feed consumption with 3.2 mg/kg/day compared to controls	

- During gestation, statistically significant (p≤0.01) reduction in absolute feed consumption with 3.2 mg/kg/day compared to controls
- During lactation, statistically significant (p≤0.01) reduction in absolute and relative feed consumption with 1.6 mg/kg/day compared to controls, 3.2 mg/kg/day data not reported

F0 male and female effects: fertility indices

Fertility indices[a] in F0 males and females		
Dose group (mg/kg/day)	Male	Female
Control	94.3%	94.3%
0.1	91.4%	91.4%
0.4	81.8%	82.4%
1.6	85.3%	85.3%
3.2	87.5%	85.7%
a = defined as number of pregnancies per number of rats that mated		

F0 female effects: general reproductive effects

- Comparable values between control and exposed groups for: estrous cycle, number of pregnancies per number of matings, number of days to inseminate, and number of matings during the first week of cohabitation

F0 female effects at GD10 (caesarean-section group): reproductive effects

- No effect on litter averages for corpora lutea, implantations, and viable embryos

F0 female effects for natural birth group: reproductive effects

- No effect on reproductive endpoints with exposure to 0.1 mg/kg/day or 0.4 mg/kg/day, observations with exposure to 1.6 mg/kg/day and 3.2 mg/kg/day reported in table below

Reproductive effects in F0 females following natural birth			
	PFOS (mg/kg/day)		
	Control	1.6	3.2
Rats assigned to natural delivery	25	24	25
Delivered litters (%)	23 (100.0)	20 (100.0)	21 (100.0)
Duration of gestation[a] (mean±SD)	22.7±0.4	22.4±0.5	22.2±0.4[c]
Implantation sites per delivered litter (mean±SD)	14.9±1.9	14.8±1.7	12.5±1.4[c]
Dams with stillborn pups (%)	5 (21.7)	4 (20.0)	15 (71.4)[c]
Gestation index[b] (%)	23/23 (100.0)	20/20 (100.0)	20/21 (95.2)
Dams with all pups dying postpartum days 1 to 4 (%)	0[d] (0.0)	2 (10.0)	20 (100.0)[c]
[a] = defined as time in days elapsed between confirmed mating (day 0) and the time in days the first pup was delivered [b] = number of rats with live offspring/number of pregnant rats [c] = p≤0.01 compared to control [d] = historical control incidence also 0			

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Luebker et al. (2005a)

Note: study authors conducted two-generation and cross-foster studies. Of the F0, F1, and F2 results from the two-generation study, only the F1 results are reported herein. F0 and F2 results and the results from the cross-foster study are reported in separate tables.

Species and strain:
Rats, Crl:CD® (SD)IGS BR VAF®
F0 male and females were 62 days old at receipt followed by 14-day acclimation period prior to exposure

Group size:
35/sex/group (for F0 exposure), group size then varied by endpoint

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in 2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 0.4, 1.6, 3.2 mg/kg/day

See Results column for liver PFOS concentrations for F1 pup</td>
<td><u>Internal PFOS concentration for F1 rats</u>

<table>
<tr><td colspan="2">Internal PFOS concentrations for F1 at LD21</td></tr>
<tr><td>Maternal dose group (mg/kg/day)</td><td>Liver (ug/g)</td></tr>
<tr><td>Control</td><td>NR</td></tr>
<tr><td>0.1</td><td>6.19±0.879</td></tr>
<tr><td>0.4</td><td>57.6±6.72</td></tr>
<tr><td>1.6</td><td>70.4±14.5</td></tr>
<tr><td colspan="2">mean±SD; NR = not reported
Note: all F1 pups in 3.2 mg/kg/day group dead by LD21</td></tr>
</table>

<u>F1 effects prior to weaning: mortality</u>
<table>
<tr><td colspan="4">F1 survival at birth</td></tr>
<tr><td></td><td colspan="3">Maternal (F0) dose (mg/kg/day)</td></tr>
<tr><td></td><td>Control</td><td>1.6</td><td>3.2</td></tr>
<tr><td>Delivered litters with ≥1 liveborn pup</td><td>23</td><td>20</td><td>20</td></tr>
<tr><td>Total pups delivered</td><td>323</td><td>260</td><td>200</td></tr>
<tr><td>Liveborn (mean±SD)</td><td>13.6±2.3[a]</td><td>12.7±2.6</td><td>7.8±4.0[b]</td></tr>
<tr><td>Stillborn/litter (mean±SD)</td><td>0.3±0.7</td><td>0.3±0.6</td><td>2.2±2.3[b]</td></tr>
<tr><td colspan="4">Note: data for 0.1 mg/kg/day and 0.4 mg/kg/day groups not reported herein but were comparable to control values
a = historical range of liveborn pups was reported to be 12.2 to 15.5
b = p≤0.01 compared to controls</td></tr>
</table>

• With maternal dose of 3.2 mg/kg/day, 45.5% and 100% F1 pup mortality by end of LD1 and LD4, respectively (p≤0.01 compared to control for both time points)
• With maternal dose of 1.6 mg/kg/day, 10.6% and 26.0% F1 pup mortality by end of LD1 and between LD2 to LD4,</td>
<td>Major Limitations:
• Internal PFOS measurements determined after some effects were initially observed (e.g., F1 pup effects at birth and F1 pup internal PFOS measurements at LD21)
• Control values for internal PFOS measurements not reported

Other comments:
• Species and strain appropriate for endpoints assessed
• Most F0 endpoint had n>20
• Oral gavage provided direct exposure to PFOS
• Dose selection (for F0 parents and in utero for F1) presumptively based on observations of rat neonatal mortality in previous studies, F1 gavage exposures based on surviving dose groups
• F1 exposure duration included gestation and lactation periods as well as for >70 days post-weaning
• Due to mortality and effects at 2 highest doses, observations post-weaning limited to 2 dose groups
• Generally quantitative but some qualitative reporting (e.g., F1 reproductive effects)
• Endpoint ascertainment used standardized assessment of mortality, body weight, food consumption, developmental milestones, reproductive toxicity, and neurotoxicity</td>
</tr>
</table>

Exposure regimen: F1 started gavage exposure on lactation day (LD)22 at same dose level as F0 parent. Around PND90, exposure continued as F1 rats were mated/cohabitated (for a maximum of 14 days). F1 males were sacrificed after mating/cohabitation, between 100 and 112 days of age. F1 females were exposed through gestation and LD20 (sacrifice on LD21 along with F2 pup). Note: F0 dams of F1 had been exposed during pre-conception, gestation, and lactation periods (weaning at LD21/LD22). **Related studies:** Luebker et al. (2005b)	respectively (p≤0.05 compared to control for LD2 to LD4 observation) • With maternal doses ≤0.4 mg/kg/day, >98% pup survived to LD4 • Of F1 pups found dead or moribund: no clear cause of death, no signs of respiratory distress, no milk in stomachs of 75% of necropsied pups from 1.6 mg/kg/day and 3.2 mg/kg/day groups Note: due to 100% mortality of F1 pups in 3.2 mg/kg/day group after LD2, there was no further evaluation of pups in this group **F1 effects prior to weaning: body weight change** • Statistically significant (p≤0.01) reduction in pup weight per litter at LD1 with 1.6 mg/kg/day and 3.2 mg/kg/day compared to controls, the reduction (p≤0.01) in the 1.6 mg/kg/day group continued until LD21 • Statistically significant (p≤0.01) reduction in pup weight gain per litter with 1.6 mg/kg/day compared to controls, this effect was observed at the end of LD4 through the end of LD21 **F1 effects prior to weaning: developmental milestone** • For 1.6 mg/kg/day maternal dose group, F1 pups had statistically significant delays compared to controls for mean number of days for: 50% of pups to attain pinna unfolding (1.6 days, p<0.01); eye opening (1.4 days, p<0.01); surface righting (2.2 days, p<0.05); and air righting (2.0 days, p<0.01) • For 0.4 mg/kg/day maternal dose group, F1 pups had statistically significant delay compared to controls for eye opening (0.6 day, p<0.01) • At weaning, pupil constriction normal in all F1 pups Note: F1 pups in the 1.6 mg/kg/day maternal dose group were observed to be in poor clinical condition and not evaluated past weaning (LD21)	

F1 effects post weaning (during oral gavage): mortality, clinical signs

- For 0.1 mg/kg/day and 0.4 mg/kg/day groups, no deaths or clinical signs observed

F1 effects post weaning (during oral gavage): body weight, feed consumption

- Body weights and body weight gains in exposed groups similar to controls for both males and females
- Absolute and relative feed consumption values in exposed groups similar to controls for both males and females

F1 effects post weaning: sexual maturation

Sexual maturation in F1 males and females		
	Days postpartum	
Dose group (mg/kg/day)	Preputial separation for males	Vaginal patency for females
Control	45.0±2.1	31.1±1.8
0.1	45.7±2.3	31.1±2.0
0.4	45.1±1.8	30.5±1.4
Mean±SD		

F1 effects post weaning: neurotoxicity

- No difference between exposed groups and controls for passive avoidance and water maze performance (learning, short-term retention, long-term memory)

F1 effects post weaning: reproductive

- No effect on reproductive performance or natural delivery parameters: duration of gestation, number of implantations, and number of live pups

Reference and Study Design	Results	Comment
Luebker et al. (2005a) Note: study authors conducted two-generation and cross-foster studies. Of the F0, F1, and F2 results from the two-generation study, only the F2 results are reported herein. F0 and F1 results and the results from the cross-foster study are reported in separate tables. **Species and strain:** Rats, Crl:CD® (SD)IGS BR VAF® F1 male and females were ~90 days old at mating/cohabitation **Group size:** Not reported **Test article and vehicle:** PFOS (potassium salt, 86.9% pure) in 2% Tween 80 **Route of exposure:** Oral gavage (of F1) **Exposure levels:** 0, 0.1, 0.4 mg/kg/day **Exposure regimen:** F1 dams of F2 had been exposed during F1 gestation and lactation periods (F1 weaning at LD21/LD22), from post-weaning through mating/cohabitation, and	**F2 effects: mortality** • Pup mortality similar between control and exposed groups throughout the lactation period **F2 effects: body weight change** • For 0.4 mg/kg/day maternal dose group, transient reduction ($p \leq 0.05$) in body weight and body weight gain • On LD21, body weight parameters of exposed groups decreased but not statistically different from controls	**Major Limitations:** • Internal PFOS concentration not determined for F2 **Other comments:** • Species and strain appropriate for endpoints assessed • Sample size not reported • Oral gavage provided direct exposure to PFOS • Dose selection based on F1 neonatal effects • Duration of exposure included gestation and lactation periods • Two exposure levels may limit ability to demonstrate dose-related effects • Quantitative and qualitative (e.g., mortality) data reported • Endpoint ascertainment used standardized assessment of mortality and body weight

then through F2 gestation until F2 reached LD21 (sacrifice on LD21 for F2 pups and F1 dams). **Related studies:** Luebker et al. (2005b)		

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Luebker et al. (2005a)

Note: study authors conducted two-generation and cross-foster studies. Only the cross-foster results are reported herein. Two-generation (i.e., F0, F1, and F2) results are reported in separate tables.

Species and strain:
Rats, Crl:CD® (SD)IGS BR VAF®
Females were 66 days of age at receipt followed by an acclimation period prior to exposure

Group size:
33 controls females, 27 exposed females

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in 2% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
0, 1.6 mg/kg/day

Exposure regimen:
F0 females exposed for 42 days then mated/cohabitated with an untreated male. F0 females further exposed for a maximum</td>
<td><u>Internal PFOS concentrations</u>
• For treated dams on LD14: serum PFOS concentrations (n=2 dams) reported to be 97.5 and 218 ug/mL, PFOS concentrations in whole milk samples (n=2 dams nursing own pups) reported to be 100 and 13.7 ug/mL
• For pups from treated dam: serum PFOS concentration reported to be 89.3 ug/mL (n=1 pooled litter from dam with 97.5 ug/mL serum PFOS concentration)

Serum PFOS concentrations for F0 and F1 participating in cross-foster study at LD21
<table>
<tr><td></td><td colspan="2">Mean PFOS serum concentration (ug/mL)</td></tr>
<tr><td></td><td>Pups (pooled by litter)</td><td>Dams</td></tr>
<tr><td>CL/CD</td><td><0.05[a] (6)</td><td><0.05[b] (12)</td></tr>
<tr><td>CL/TD</td><td>22.4±17.5[c] (6)</td><td>83.0±27.6 (13)</td></tr>
<tr><td>TL/CD</td><td>53.9±5.0 (6)</td><td>2.02±1.58[d] (13)</td></tr>
<tr><td>TL/TD</td><td>89.7±7.1 (6)</td><td>89.0±28.0 (12)</td></tr>
</table>
mean±SD
a = values below the limit of quantitation (LOQ) were assigned the LOQ value (i.e., 0.05 ug/mL)
b = all values were <LOQ except for one value at 0.0507 ug/mL
c = Two of six values were <LOQ but were assigned LOQ value for calculating mean and SD
d = Two of thirteen values were <LOQ but were assigned LOQ value for calculating mean and SD
Note: number in parenthesis is number of samples

<u>F0 female effects: body weight</u>
• Statistically significant (p value not reported) reductions in body weight with 1.6 mg/kg/day compared to control during latter portion of mating/cohabitation (i.e., day 36 onward)
• Statistically significant (p value not reported) reductions in body weight with 1.6 mg/kg/day (CL/TD and TL/TD) compared to controls (CL/CD) during LD4 through LD14</td>
<td>Major Limitations:
• Only 1 dose tested

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size generally ≥10
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous observations of neonatal mortality
• Duration of exposure included gestation and lactation periods
• Quantitative data generally reported but p values not reported for some endpoints (e.g., F0 reproductive effects)
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of mortality, body weight, food consumption, reproductive effects, and liver ultrastructural effects (i.e., peroxisome number); subjective assessment of lung ultrastructural effects and liver glycogen</td>
</tr>
</table>

of 6 days during gestation and through lactation day (LD)21

Upon birth, litters were cross-fostered with other dams to create the following groups:
CL/CD=control litters fostered by control dams (12 litters)
CL/TD=control litters fostered by treated dams (13 litters)
TL/CD= treated litters fostered by control dams (13 litters)
TL/TD=treated litters fostered by treated dams (12 litters)

Cross-fostering dams sacrificed on LD22, cross-fostered pups sacrificed on LD21

F0 dams and F1 pups not participating in cross-fostering sacrificed on LD14 (PFOS measurements)

Related studies:
Luebker et al. (2005b)

F0 female effects: feed consumption

- Statistically significant reduction in absolute (g/day) feed consumption with 1.6 mg/kg/day compared to controls during premating ($p \leq 0.05$) and gestation ($p \leq 0.01$), no statistically significant effect for relative (g/kg/day) feed consumption
- Statistically significant reduction ($p \leq 0.05$ or $p \leq 0.01$) in absolute and relative feed consumption with 1.6 mg/kg/day (CL/TD and TL/TD groups) compared to control (CL/CD) during LD1 to LD14
- Statistically significant reduction ($p \leq 0.01$) in absolute feed consumption for dams in TL/CD group compared to controls (CL/CD) during LD1 to LD14, no statistically significant effect for relative feed consumption

F0 effects: reproductive effects

- No effects on mating or fertility

Reproductive effects in F0 females		
	Control	1.6 mg/kg/day
Length of gestation (days)	22.4	22.0
Implantation sites per litter	17.7	16.0
Total litter size	16.4	15.1
Live litter size	16.2	14.9
Note: reductions compared to controls listed in this table were reported to be statistically significant but no p value(s) reported		

F1 effects: mortality

- No deaths at end of postpartum day 1
- Most neonatal deaths occurred by postpartum day 4

F1 mortality observations				
	CL/CD	CL/TD	TL/CD	TL/TD
Litters assigned to cross-fostering	13	12	12	13
Pup cross-fostered per litter (mean±SD)	15.9±2.1	16.4±1.6	15.1±1.7	14.8±1.9
Pup mortality between postpartum days 2 and 4	3/191 (1.6)	2/181 (1.1)	15/166 (9.0)	34/177 (19.2)[a]
Viability index[b]	188/191 (98.4)	179/181 (98.9)	151/166 (91.0)	143/177 (80.8)[a]

a = p≤0.01
b = defined as number of live pups on postpartum day 4 (pre-culling)/number of liveborn pups on postpartum day 1
Note: number in parenthesis is percentage

F1 effect: body weight

- Statistically significant (p≤0.05 or p≤0.01) reductions in body weight and body weight change in pups born to or fostered by treated dams (i.e., CL/TD, TL/CD, TL/TD), effect in TL/CD and TL/TD occurred from LD1 through LD21

F1 effect: ultrastructural examination of lung and liver

- Note: tissues from treated pups (i.e., born to treated dams) collected from pups found dead, tissues from control pups collected 1 to 3 hours after birth
- Statistically significant (p<0.0001) increase in mean number of peroxisomes per hepatocyte in liver tissue of treated pups (n=4, 16.1±1.5) compared to control (n=5, 7.0±1.9); glycogen stores appeared larger in treated pups; no apparent difference in cellular membranes or mitochondria between treated and control pups
- Apparent increase in number of type II pneumocytes and lamellar bodies in lungs of treated pups; no difference between treated and control groups regarding the presence of lamellar material (surfactant) within alveolar lumina

Reference and Study Design	Results	Comment
Luebker et al. (2005b) Note: study authors conducted dose-response and pharmacokinetic studies. Only the dose-response results are reported herein. Results from the pharmacokinetic study are reported in a separate table. **Species and strain:** Rats, Crl:CD® (SD)IGS VAF/Plus® F0 females were 71 to 72 days old at receipt followed by a 7 to 9 day acclimation period prior to exposure; age of F0 breeder males (same strain as females) not reported **Group size:** 20 dams/natural delivery group 8 dams/caesarean group **Test article and vehicle:** PFOS (potassium salt, 86.9% pure) in 0.5% Tween 80 **Route of exposure:** Oral gavage **Exposure levels:** 0, 0.4, 0.8, 1.0, 1.2, 1.6, 2.0 mg/kg/day (natural delivery group) 0, 1.6, 2.0 mg/kg/day (caesarean group)	**Internal PFOS concentrations** • Paired maternal and pup serum PFOS concentrations on LD5 increased proportional to maternal dose, concentrations comparable between dams and pups within the same dose group • Paired maternal and pup liver PFOS concentrations on LD5 increased proportional to maternal dose, concentrations in pup livers were about 50 to 250% higher than in the livers of paired dams **F0 female effects (natural delivery group): mortality, necropsy observations** • No deaths were attributed to test agent or vehicle • Necropsy observations (thoracic, abdominal, and pelvic viscera) were not considered related to the test agent **F0 female effects (natural delivery group): body weight** • Statistically significant (p values not reported) reduction in body weight with 1.6 mg/kg/day and 2.0 mg/kg/day compared to controls during gestation and lactation (for 2.0 mg/kg/day only) • Statistically significant ($p \leq 0.05$ or $p \leq 0.01$, compared to controls) reduction in body weight gain during pre-mating (2.0 mg/kg/day only) and lactation (with doses ≥0.8 mg/kg/day) • No apparent differences in body weight change during gestation **F0 female effects (natural delivery group): feed consumption** • General trend of decreased absolute and relative (mean feed consumption/kg of body weight) feed consumption with increasing dose during periods of pre-mating, gestation, and lactation • Statistically significant results observed during some periods **F0 female effects (natural delivery group): liver weight** • Statistically significant (p value not reported, compared to controls) increase in relative liver weight by 10%, 17%, and 12% with 0.8, 1.2, and 2.0 mg/kg/day, respectively	**Major Limitations:** • Limited sample size (<10) or no samples available for some thyroid hormone measurements • Quantitative data for internal PFOS measurements for control animals not reported **Other comments:** • Species and strain appropriate for endpoints assessed • Oral gavage provided direct exposure to PFOS • Dose selection based on previous observations of neonatal effects • Duration of F0 exposures (i.e., ≥42 days) were subchronic (i.e., >30 days), F1 exposures lasted most of gestation period • Six doses used to determine dose-response curve (for dose-response study), only two doses used in caesarean group • Quantitative data reported • Internal PFOS measurements determined • Endpoint ascertainment used standardized assessment of mortality, body weight, food consumption, liver weight, reproductive and fetal effects, biochemical parameters (in serum, liver, milk), and histopathology. Multiple approaches used to measure serum thyroid hormones to avoid potential of a negative bias.

See **Results** column for liver and serum PFOS concentrations for F0 and F1

Exposure regimen:
F0 females: dosed once daily for 42 days prior to mating/cohabitation, then once daily during mating/cohabitation (with a maximum of 14 days of mating), then either until gestation day (GD)20 (for caesarean group, pup and dam sacrifice on GD21) or lactation day (LD)4 (natural delivery group, pup and dam sacrifice on LD5).

F0 males: no exposure

Related studies:
Luebker et al. (2005a)

F0 female effects (natural delivery group): reproductive effects

- Comparable observations between control and exposed groups for fertility index (number of dams pregnant/number of dams mated), average number of implantation sites, gestation index (number of dams with live offspring/number of pregnant dams), and number of liveborn pups
- Statistically significant ($p \leq 0.05$ or $p \leq 0.01$, compared to controls) differences reported for:
  - Gestation length, decreased with ≥0.8 mg/kg/day
  - Dams with stillborn pups, increased with 0.4 mg/kg/day
  - Dams with stillborn pups, decreased with ≥1.0 mg/kg/day
  - Dams with all pups dying between postpartum days 1 and 5, increased with 2.0 mg/kg/day
  - Viability index (number of live pups on postpartum day 5/number of live births), decreased with ≥1.6 mg/kg/day

F0 female effects (caesarean group): reproductive and fetal effects

- No statistically significant effects for litter averages for corpora lutea, implantations, viable fetuses, and dead fetuses; no effect on percent live male fetuses and pooled fetal body weight
- All fetuses were alive and normal placentas observed

F0 female effects at GD21 (caesarean group)

	Dose group (mg/kg/day)		
	Control	1.6	2.0
Dams with any resorptions (%)	8 (100.0)	6 (75.0)	3 (37.5)[a]
Percent dead or resorbed concepti/litter	9.1±6.4	8.0±5.0	2.4±3.4[b]
Early resorptions/litter	1.4±1.1	0.9±1.0	0.4±0.5[b]

a = $p \leq 0.01$
b = $p \leq 0.05$

	F1 effects (natural delivery): body weight • Statistically significant ($p<0.05$ or $p<0.01$) reduction in pup body weight (average per litter) at birth and LD5 with ≥0.4 mg/kg/day compared to controls • Statistically significant ($p<0.05$ or $p<0.01$) reduction in pup weight gain from birth to LD5 with ≥0.4 mg/kg/day compared to controls **F1 effects (natural delivery): mortality** • Dose-dependent increase in pup mortality through LD5, with statistically significant ($p<0.01$) increase in mortality with ≥1.6 mg/kg/day compared to controls **F0 female effects (caesarean group): serum and liver biochemical parameters** • No statistically significant difference compared to controls in serum biochemical parameters: total cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TRIG), glucose (GLUC), and mevalonic acid lactone (MAL) • Statistically significant reduction in liver CHOL with 1.6 mg/kg/day ($p\leq0.05$) and 2.0 mg/kg/day ($p\leq0.01$) compared to controls • No statistically significant difference in liver TRIG compared to controls **Fetal effects (caesarean group): serum and liver biochemical parameters** • Statistically significant ($p\leq0.05$) increase in serum CHOL with ≥1.6 mg/kg/day compared to controls • Statistically significant ($p\leq0.01$) increase in serum LDL with ≥1.6 mg/kg/day compared to controls • No statistically significant differences compared to controls for the serum biochemical parameters: HDL, TRIG, GLUC, and MAL • No statistically significant differences compared to controls for liver biochemical parameters: CHOL and TRIG	

	F0 female effects (natural delivery group): serum, milk, and liver biochemical parameters • Statistically significant (p≤0.01) reduction in serum CHOL with ≥0.4 mg/kg/day compared to controls • Statistically significant reduction in serum TRIG with 1.6 mg/kg/day (p≤0.05) and 2.0 mg/kg/day (p≤0.01) compared to controls • Statistically significant (p≤0.01) increase in serum GLUC with 2.0 mg/kg/day compared to controls • No statistically significant differences compared to controls for the serum biochemical parameters: LDL, HDL, and MAL • No statistically significant difference compared to controls for milk CHOL • Statistically significant (p≤0.01) increase in liver TRIG with ≥1.6 mg/kg/day compared to controls • No statistically significant difference compared to controls for liver CHOL and malic enzyme activity **F1 effects (natural delivery group): serum and liver biochemical parameters** • Statistically significant (p≤0.05) reduction in serum MAL; however, n=2 and both samples were below limit of quantitation • No statistically significant differences compared to controls for the serum biochemical parameters: CHOL, LDL, HDL, TRIG, and GLUC • Statistically significant (p≤0.05 or p≤0.01) reductions compared to controls in liver TRIG for males (with ≥1.0 mg/kg/day) and females (with ≥1.0 mg/kg/day but not 2.0 mg/kg/day) • No statistically significant differences compared to controls for liver CHOL in males and females • No statistically significant difference compared to controls for liver glycogen content and malic enzyme activity	

	F0 female effects (natural delivery group): thyroid hormone measurements
	• Statistically significant ($p<0.01$) reduction in total thyroxin (TT4) with ≥0.4 mg/kg/day compared to controls when measured by analog radioimmunoassay (RIA) approach
	• Statistically significant ($p<0.01$) reduction in total triiodothyronine (TT3) with ≥1.2 mg/kg/day compared to controls when measured by analog RIA approach
	• No statistically significant effect on thyroid stimulating hormone (TSH) when measured by analog RIA approach
	• No statistically significant effect on free thyroxin (FT4) when measured by equilibrium dialysis RIA approach
	
	F1 effects (natural delivery group): thyroid hormone measurements
	• Measurements using the analog RIA approach
	  ○ Non-statistically significant reductions in TT3 with ≥0.8 mg/kg/day
	  ○ Statistically significant ($p\leq0.01$, compared to control) reduction in TT4 with ≥0.4 mg/kg/day, non-detectable levels with 0.4 mg/kg/day and 0.8 mg/kg/day and no samples available for 2.0 mg/kg/day
	  ○ Statistically significant ($p\leq0.05$, compared to control) increase in TSH with 1.6 mg/kg/day, increased TSH levels at 1.0 mg/kg/day and 2.0 mg/kg/day but n=1 for each group, no sample available for 0.4 mg/kg/day and 0.8 mg/kg/day groups
	• Measurement using the analogy chemiluminometric approach
	  ○ Non-statistically significant reductions in TT3 and TT4 with 0.4, 0.8, and 1.0 mg/kg/day, no samples for ≥1.2 mg/kg/day
	• Measurements using equilibrium dialysis RIA approach
	  ○ Comparable levels of FT3 between controls and 0.4, 0.8, and 1.0 mg/kg/day groups, no samples for ≥1.2 mg/kg/day
	  ○ Non-statistically significant reduction in FT4 with 0.4 mg/kg/day, no samples for ≥0.8 mg/kg/day	

	F1 effects (natural delivery group): histopathology of heart and thyroid • No microscopic changes observed with 2.0 mg/kg/day compared to controls, based on data from 1 male and 1 female	

Reference and Study Design	Results	Comment

Luebker et al. (2005b)
 	
Note: study authors conducted dose-response and pharmacokinetic studies. Only the pharmacokinetic study results are reported herein. Results from the dose-response study are reported in a separate table.
 	
Species and strain:
Rats, Crl:CD® (SD)IGS VAF/Plus®
F0 females were ≥60 days old at receipt; age of F0 breeder males (same strain as females) not reported
 	
Group size:
16 dams/group
 	
Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in 0.5% Tween 80
 	
Route of exposure:
Oral gavage
 	
Exposure levels:
0, 0.1, 0.4, 1.6, 3.2 mg/kg/day
 	
See **Results** column for PFOS concentrations in specimens from F0 and F1
	Internal PFOS concentrations
	• Dam PFOS concentrations
	  ○ Serum: linearly proportional to dose after 42 days of dosing, concentrations and linearity remained similar through GD15, concentrations declined (<50%) on GD21 with decrease in 1.6 mg/kg/day group not as severe
	  ○ Liver: concentrations were linearly proportional to dose at GD21, no liver concentrations determined prior to GD21
	  ○ Urine: concentrations were linearly proportional to dose and were similar in urine collected prior to cohabitation and after GD7; concentrations remained roughly similar through GD21 with ≤0.4 mg/kg/day but fluctuated with ≥1.6 mg/kg/day
	  ○ Feces: concentrations were linearly proportional to dose and remained consistent at all time points
	• Paired maternal and pup serum PFOS concentrations on GD21 increased proportional to maternal dose, concentrations in pup serum were 40 to 50% greater than in the serum of paired dams, expect in the 3.2 mg/kg/day group where serum concentrations were about equal
	• Paired maternal and pup liver PFOS concentrations on GD21 increased proportional to maternal dose, concentrations in pup liver were about one-half that in the liver of the paired dams
	
	F0 effects (GD15 and GD21 groups) : mortality, clinical and necropsy observations
	• No deaths attributed to test agent
	• Clinical observations were not considered related to the test agent
	• No gross lesions found by necropsy (thoracic, abdominal, and pelvic viscera)
		Major Limitations:
		• No quantitative reporting of control values for internal PFOS concentrations
		• Internal PFOS measurements limited to GD21 for F1
		
		Other comments:
		• Species and strain appropriate for endpoints assessed
		• Sample sizes (n=8 to 16) for dam endpoints varied
		• Oral gavage provided direct exposure to PFOS
		• Dose selection based on previous observations of neonatal effects
		• Duration of F0 exposures (i.e., ≥42 days) were subchronic (i.e., >30 days), F1 exposures lasted most of gestation period
		• Number of exposure levels allowed for determining any dose-related effects
		• Quantitative data reported but some qualitative reporting of data (e.g., litter parameters)
		• Endpoint ascertainment used standardized assessment of mortality, clinical and necropsy observations, body weight, food consumption, reproductive effects, and fetal effects

Exposure regimen: F0 females: dosed once daily for 42 days prior to mating/cohabitation then through gestation day (GD)14 or GD20. Some dams (8/dose group) sacrificed and caesarean sectioned on GD15 (GD15 group). The remaining dams (8/dose group) sacrificed and caesarean sectioned on GD21 (GD21 group). F0 males: no exposure **Related studies:** Luebker et al. (2005a)	**F0 effects (GD15 and GD21 groups): body weight** • At end of pre-mating/pre-cohabitation period, body weights were 98.0, 96.3, 93.6, and 85.3% of controls for the 0.1, 0.4, 1.6, and 3.2 mg/kg/day groups, respectively • During pre-mating/pre-cohabitation period, body weight gains were 88.8, 80.8, 66.3, and 17.4% of controls for the 0.1, 0.4, 1.6, and 3.2 mg/kg/day groups, respectively • During GD0 to GD7, reduced body weight gains with ≥0.4 mg/kg/day **F0 effects: feed consumption** • During pre-mating/pre-cohabitation period and first week of gestation, reduced absolute (g/day) and relative (g/kg/day) feed consumption with ≥0.4 mg/kg/day • After first week of gestation until the end of dosing, reduced absolute feed consumption with ≥0.4 mg/kg/day in the GD15 group or with 3.2 mg/kg/day in the GD21 group **F0 and F1 effects: reproductive and fetal effects** • GD15 group: no effect on caesarean section or litter parameters • For GD21 group: reductions in litter averages for implantations, litter sizes, and live fetuses (values for these endpoints were below historical ranges observed by laboratory conducting the study); 2 rats in 3.2 mg/kg/day group delivered on GD21 prior to scheduled caesarean section; reduced fetal body weight with 3.2 mg/kg/day, no observed fetal gross external alterations	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Lv et al. (2013)

Species and strain:
Rats, SPF Wistar
F0 age not reported

Group size:
10 pregnant females/group (for exposure), group size then varied by endpoint

Test article and vehicle:
PFOS (potassium salt, >98% purity) in 0.5% Tween 20

Route of exposure:
Oral (presumably gavage)

Exposure levels:
0, 0.5, 1.5 mg/kg/day

See Results column for serum and liver PFOS concentrations at PND0 and PND21

Exposure regimen:
GD0 to PND21 (weaning)

Pups sacrificed 19 weeks after weaning</td>
<td>Note: maternal effects not reported

<u>Internal PFOS concentrations: PND0 and PND21</u>

<table>
<tr><td colspan="4">Internal PFOS concentrations in offspring of exposed rats</td></tr>
<tr><td></td><td></td><td colspan="2">PFOS</td></tr>
<tr><td>Age</td><td>Treatment (mg/kg/day)</td><td>Serum (ug/mL)</td><td>Liver (ug/g)</td></tr>
<tr><td>PND0</td><td>Control</td><td>ND[a]</td><td>ND[a]</td></tr>
<tr><td></td><td>0.5</td><td>3.98±0.80[b]</td><td>10.49±0.80[b]</td></tr>
<tr><td></td><td>1.5</td><td>36.25±4.26[b]</td><td>114.93±6.14[b]</td></tr>
<tr><td>PND21</td><td>Control</td><td>ND[a]</td><td>ND[a]</td></tr>
<tr><td></td><td>0.5</td><td>11.00±1.35[b]</td><td>42.22±2.55[b]</td></tr>
<tr><td></td><td>1.5</td><td>71.35±3.27[b]</td><td>139.68±4.38[b]</td></tr>
<tr><td colspan="4">mean±SEM; n=6 rats per group, PND0 samples pooled by litter
a = lower limit of detection
b= p<0.05</td></tr>
</table>

<u>Neonatal effects: survival and body weight</u>
• No neonatal deaths at birth, all neonates appeared active
• Survival rates through lactation period were comparable between groups: control, 98.7%; 0.5 mg/kg, 98.8%; and 1.5 mg/kg, 98.8%
• General decrease in body weight in exposed groups compared to control (see below for PND0 and PND21 data, body weights for other PNDs not reported herein)

<table>
<tr><td colspan="4">Neonatal body weights at birth and weaning (combined males and females)</td></tr>
<tr><td></td><td></td><td colspan="2">PFOS</td></tr>
<tr><td>Body weight (g)</td><td>Control</td><td>0.5 mg/kg</td><td>1.5 mg/kg</td></tr>
<tr><td>PND0</td><td>6.7±0.4</td><td>5.9±0.4</td><td>5.7±0.1[a]</td></tr>
<tr><td>PND21</td><td>41.8±0.9</td><td>39.2±0.3[a]</td><td>38.5±0.8[a]</td></tr>
<tr><td colspan="4">mean±SEM, n=6 per group
a = p<0.05 compared to control</td></tr>
</table>
</td>
<td>Major Limitations:
• Maternal effects not reported
• Only 2 dose levels

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size generally ≥25 F1 rats per group but <10 for internal PFOS measurements and some lipid metabolism endpoints
• Oral gavage provided direct exposure to PFOS
• Authors noted that PFOS doses used in study were 2 to 3 orders of magnitude higher than concentrations observed in the general population
• Duration of exposure included entire gestational period through weaning
• Generally quantitative data were reported, but some data not reported (e.g., fasting serum cholesterol)
• Exposure characterized by internal PFOS concentrations (e.g., serum and liver)
• Endpoint ascertainment used standardized assessment of body weight, survival, and glucose and lipid metabolism</td>
</tr>
</table>

	• Body weights in exposed males and females generally similar to controls from 9 weeks to 18 weeks after weaning **F1 effects: glucose metabolism** • At 10 weeks after weaning, statistically significant ($p<0.05$) increase in area under the curve (AUC) value for the oral glucose tolerance test (OGTT) with 1.5 mg/kg compared to controls • At 15 weeks after weaning, statistically significant ($p<0.05$) increase in AUC value for OGTT with 0.5 mg/kg compared to controls, non-statistically significant decrease observed for 1.5 mg/kg • No effect on fasting serum glucose and glycosylated serum protein levels **F1 effects at 18 weeks after weaning: hormone levels** • Statistically significant ($p<0.01$) increase in fasting serum insulin with 1.5 mg/kg compared to controls • Statistically significant ($p<0.05$) increase in insulin resistance index with 1.5 mg/kg compared to controls • Statistically significant ($p<0.05$) increase in serum leptin with 1.5 mg/kg compared to controls, non-statistically significant increase with 0.5 mg/kg • Statistically significant decrease in serum adiponectin with 0.5 mg/kg ($p<0.05$) and 1.5 mg/kg ($p<0.01$) compared to controls **F1 effects at 19 weeks after weaning: lipid metabolism** • Statistically significant ($p<0.01$) increase in liver fat accumulation (hepatic steatosis, as measured by oil red O staining) with 1.5 mg/kg compared to controls • Statistically significant ($p<0.05$) increase in liver triglyceride content with 1.5 mg/kg compared to controls • No effect on fasting serum triglyceride and serum cholesterol levels • Statistically significant ($p<0.01$) increase in gonadal fat pad weight with ≥0.5 mg/kg compared to controls, no increase in adipocyte size with exposure	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Ngo et al. (2014)

Unless stated otherwise, results reported herein are for those endpoints where wild-type (WT) and Min/+ mice were assessed together and for maternal effects Results for WT mice and Min/+ mice are reported in separate tables.

Species and strain:
Mice, C57BL/6J
F0 females 6-7 weeks at mating

F1 resulted from mating C57BL/6J-Apc^{+/+} females with C57BL/6J-Ap ^{Min/+} males; offspring genotype identified by polymerase chain reaction for Apc gene

Group size:
Varied when reported; 10 to 24 dams/group; 3 to 27 pups/group

Test article and vehicle:
PFOS (potassium salt, ≥98% pure) in water

Route of exposure:
Oral gavage

Exposure levels:
Two experimental blocks (e.g., exposures) needed to produce enough offspring for statistical analyses</td>
<td><u>Background levels of PFOS in water and feed</u>
• Both PFOS and PFOA were detected at pg/l levels in tap water and vehicle water and at pg/g levels in breeding and maintenance feed
• Potential for up to 30% decrease in dosing solution concentration as determined by a separate stability experiment

<table>
<tr><td colspan="4">Serum PFOS levels (ng/ml) in exposed dams and pups</td></tr>
<tr><td></td><td>Dams GD18[a]</td><td>Dams after weaning</td><td>Pups after weaning</td></tr>
<tr><td colspan="4">Experimental block 1[b,c]</td></tr>
<tr><td>Water (vehicle)</td><td>0/0[d]</td><td>0/0</td><td>0/0</td></tr>
<tr><td>0.1 mg/kg</td><td>1334/1237 (23/25)[e]</td><td>476/544 (7.7/7.2)</td><td>377/298 (3.1)</td></tr>
<tr><td>3.0 mg/kg</td><td>36646/44634</td><td>17227/22249</td><td>NA</td></tr>
<tr><td colspan="4">Experimental block 2[f,g]</td></tr>
<tr><td>Water (vehicle)</td><td>NA</td><td>0/0</td><td>NA</td></tr>
<tr><td>0.01 mg/kg</td><td>131</td><td>66/37 (23)</td><td>20/39</td></tr>
<tr><td>0.1 mg/kg</td><td>NA</td><td>710/496</td><td>NA</td></tr>
<tr><td colspan="4">a = Pregnant dams sacrificed at GD18 (24 hours after last exposure)
b = Dams sacrificed 2 days after weaning on PND21 (PND23)
c = pups sacrificed 4 to 6 days after weaning
d = samples taken from one or two mice (sample 1/sample 2)
e = values in parentheses are PFOA contamination
f = Dams sacrificed 1 to 3 days after weaning on PND25 (PND26 to 28)
g = pups sacrificed 1 day after weaning
NA = not analyzed</td></tr>
</table>

<u>Duration of exposure and time to conception</u>
• Duration of exposure varied from 14 to 17 total days during gestation</td>
<td>Major Limitations:
• Data reporting sometimes combined WT and Min/+ data, which did not allow for determining how genotype affected the endpoint observation
• Internal PFOS concentrations determined but used small sample size (n=2) and at time points earlier than some of the endpoint observations
• PFOS degradation observed
• Potential PFOA contamination in some exposure groups

Other comments:
• Species and background strain (C57BL/6J) appropriate for endpoints assessed
• Sample size varied by endpoint and not always reported
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous perinatal observations in mice
• Duration of exposure included gestational period
• Only 2 exposure levels assessed, may not clarify shape of dose-response curve
• Endpoint ascertainment used standardized assessment of endpoints</td>
</tr>
</table>

Experimental block 1: 0, 0.1, 3.0 mg/kg
Experimental block 2: 0, 0.01, 0.1 mg/kg

See **Results** column for serum PFOS concentrations

Exposure regimen:
GD1 to GD17
GD1 set as day after female and male co-habitation; actual duration of exposure determined based on actual day of birth and counting 21 days backwards

Weaning occurred at PND21 and 25 for experimental block 1 and experimental block 2, respectively

WT and Min/+ offspring were terminated at 20 and 11 weeks, respectively

Study also treated and assessed a separate group of mice exposed to PFOA, data not reported herein

- No statistical difference between treatment groups for mean number of days to conception

Maternal effects

- No overt toxicity observed during GD1 to GD17

Reproductive effects

- No statistically significant differences in incidence of pregnancy between treatment groups and experimental blocks
- No overt toxicity observed for pups surviving past weaning

Experimental block 1: reproductive observations			
	Water	0.1 mg/kg	3.0 mg/kg
# of dams exposed	20	21	21
# of dams pregnant (%)	15 (75)	13 (62)	14 (67)
# of successful births	12	7	5
# of litters that died perinatally	1	4	7
# of litters that died around weaning	0	3	1
# of surviving litters	12	4	4
# of surviving pups	70[a]	18[a]	20
Mean # surviving pups/litter	6.0	5.0	5.0
a = does not include 2 pups/group sacrificed after weaning for PFOS analysis			

Experimental block 2: reproductive observations			
	Water	0.01 mg/kg	0.1 mg/kg
# of dams exposed	10	23	24
# of dams pregnant (%)	7 (70)	16 (70)	15 (63)
# of successful births	4	9	9
# of litters that died perinatally	3	6	6
# of litters that died around weaning	0	1	0
# of surviving litters	4	8	9

# of surviving pups	15	40[a]	41	
Mean # surviving pups/litter	3.8	5.3	4.6	
a = does not include 2 pups/group sacrificed after weaning for PFOS analysis				

Experimental block 1 and 2: reproductive observations				
	Water	0.01 mg/kg	0.1 mg/kg	3.0 mg/kg
# of surviving litters	16	8	13	4
# of surviving pups	85[a]	40[a]	59[a]	20
Mean # surviving pups/litter	5.4	5.3	4.7	5.0
a = does not include 2 pups/group sacrificed after weaning for PFOS analysis				

Feed intake

- Data presented graphically (as g feed/g body weight/day)
- No statistically significant differences in feed intake between any of the exposure groups at either week 6 or week 10
- Statistically significant differences were observed for comparisons between genders and time periods (not reported herein)

Body weight development

- Maternal data presented graphically (as area under the curve [AUC] in arbitrary units) for dams weighed on GD1 to GD18
- No statistically significant difference in maternal AUC between exposure groups
- Pup data for both genotypes presented graphically for pups weighed between PND3 to weaning (PND21 to PND25)
- No statistically significant differences in pup AUC between any exposure group and water group
- Statistically significant (P=0.023) decreased pup AUC for 3.0 mg/kg group compared to the 0.1 mg/kg group

	Blood glucose levels • Statistically significant (P=0.016) increase in blood glucose levels when comparing all pups in the 0.01 mg/kg group to all pups in the 0.1 mg/kg group • Statistically significant (P=0.033) increase in blood glucose levels when comparing all male pups in the 0.01 mg/kg group to all male pups in the 0.1 mg/kg group	

Reference and Study Design	Results	Comment
Ngo et al. (2014) Unless stated otherwise, results reported herein are for those endpoints where only wild-type (WT) mice were assessed. Results for Min/+ mice are reported in a separate table. **Species and strain:** Mice, C57BL/6J F0 females 6-7 weeks at mating F1 resulted from mating C57BL/6J-*Apc*$^{+/+}$ females with C57BL/6J-*Ap* $^{Min/+}$ males; WT genotype identified by polymerase chain reaction for *Apc* gene **Group size:** Varied when reported **Test article and vehicle:** PFOS (potassium salt, ≥98% pure) in water **Route of exposure:** Oral gavage **Exposure levels:** Two experimental blocks (e.g., exposures) needed to produce enough offspring for statistical analyses Experimental block 1: 0, 0.1, 3.0 mg/kg	**Feed intake** • No statistically significant differences in feed intake between any of the exposure groups at week 20 **Body weight development** • Pup data presented graphically (as area under the curve [AUC] in arbitrary units) for pups weighed between week 3 and week 11 • No statistically significant difference in pup AUC between exposure groups • Pup data presented graphically for pups weighed between week 12 and week 20 • No statistically significant difference in pup AUC between exposure groups **Terminal body mass index (BMI)** • Data not shown • No statistically significant differences in pup BMI between exposure groups **Blood glucose levels** • Data presented graphically • Statistically significant (P=0.029) increase in blood glucose levels at 20 weeks when comparing all pups in the 0.01 mg/kg group to all pups in the 0.1 mg/kg group • No statistically significant differences between exposure groups and water group • All blood glucose levels were within the normal range (>3.3 to <13.3 mmol/l) **Terminal absolute and relative liver and spleen weights (at week 20)** • Data presented numerically • No statistically significant difference in absolute or relative liver weights between exposure groups and water group	**Major Limitations:** • Internal PFOS concentrations determined but used small sample size (n=2) and at time points earlier than some of the endpoint observations • PFOS degradation observed • Potential PFOA contamination in some exposure groups **Other comments:** • Species and background strain (C57BL/6J) appropriate for endpoints assessed • Sample size varied by endpoint and not always reported • Oral gavage provided direct exposure to PFOS • Dose selection based on previous perinatal observations in mice • Duration of exposure included gestational period • Only 2 exposure levels assessed, may not clarify shape of dose-response curve • Quantitative data provided but not all data reported (e.g., terminal BMI) • Endpoint ascertainment used standardized assessment of endpoints

Experimental block 2: 0, 0.01, 0.1 mg/kg For serum PFOS concentrations, see **Results** column of Ngo et al. (2014) table for maternal and wild-type and Min/+ results **Exposure regimen:** GD1 to GD17 GD1 set as day after female and male co-habitation; actual duration of exposure determined based on actual day of birth and counting 21 days backwards Study also treated and assessed a separate group of mice exposed to PFOA, data not reported herein	• No statistically significant difference in absolute or relative spleen weights between exposure groups and water group • Statistically significant ($p<0.05$) increase in relative spleen weights in water group and 0.1 mg/kg group females compared to corresponding males	

Reference and Study Design	Results	Comment
Ngo et al. (2014) Unless stated otherwise, results reported herein are for those endpoints where only Min/+ mice were assessed. Results for wild-type (WT) mice are reported in a separate table. **Species and strain:** Mice, C57BL/6J F0 females 6-7 weeks at mating F1 resulted from mating C57BL/6J-*Apc*$^{+/+}$ females with C57BL/6J-*Ap* $^{Min/+}$ males; WT genotype identified by polymerase chain reaction for *Apc* gene **Group size:** Varied when reported **Test article and vehicle:** PFOS (potassium salt, ≥98% pure) in water **Route of exposure:** Oral gavage **Exposure levels:** Two experimental blocks (e.g., exposures) needed to produce enough offspring for statistical analyses Experimental block 1: 0, 0.1, 3.0 mg/kg	**Body weight development** • Pup data presented graphically (as area under the curve [AUC] in arbitrary units) for pups weighed between week 3 and week 11 • No statistically significant difference in pup AUC between exposure groups **Terminal body mass index (BMI)** • Data not shown • No statistically significant differences in pup BMI between exposure groups **Blood glucose levels** • Data presented graphically • No statistically significant differences between exposure groups and water group • All blood glucose levels were within the normal range (>3.3 to <13.3 mmol/l), except one male (13.6 mmol/l) at 6 weeks in the 0.01 mg/kg group **Terminal absolute and relative liver and spleen weights (at week 11)** • Data presented numerically • No statistically significant difference in absolute or relative liver weights between exposure groups and water group • No statistically significant difference in absolute or relative spleen weights between exposure groups and water group **Intestinal tumors** • Tumor number, diameter, and localization data presented graphically • Small intestinal tumors observed in all mice, with the majority being located in the middle and distal parts of the small intestine • No statistically significant difference in the number of small intestinal tumors between exposure groups and water group	**Major Limitations:** • Internal PFOS concentrations determined but used small sample size (n=2) and at time points earlier than some of the endpoint observations • PFOS degradation observed • Potential PFOA contamination in some exposure groups **Other comments:** • Species and background strain (C57BL/6J) appropriate for endpoints assessed; however, direct relevance to general human population of observations in mutant mice unclear • Sample size varied by endpoint and not always reported • Oral gavage provided direct exposure to PFOS • Dose selection based on previous perinatal observations in mice • Duration of exposure included gestational period • Only 2 exposure levels assessed, may not clarify shape of dose-response curve • Quantitative data provided but not all data reported (e.g., terminal BMI) • Endpoint ascertainment used standardized assessment of endpoints

Experimental block 2: 0, 0.01, 0.1 mg/kg For serum PFOS concentrations, see **Results** column of Ngo et al. (2014) table for maternal and wild-type and Min/+ results **Exposure regimen:** GD1 to GD17 GD1 set as day after female and male co-habitation; actual duration of exposure determined based on actual day of birth and counting 21 days backwards Study also treated and assessed a separate group of mice exposed to PFOA, data not reported herein	• No linear increase in small intestinal tumor number with increasing exposure dose • Statistically significant ($p<0.05$) increase in small intestinal tumor size in 0.01 and 3.0 mg/kg females compared to water group • Statistically significant ($p<0.05$) increase in small intestinal tumor size in 3.0 mg/kg females compared to 0.1 mg/kg females • No statistically significant effects on small intestinal tumor size in males • Statistically significant increase in number of colonic tumors in water group ($P=0.002$) and 0.01 mg/kg group ($P=0.007$) males compared to corresponding females • No statistically significant differences in number of colonic tumors between exposed groups and water group	

Reference and Study Design	Results	Comment
Rosen et al. (2009) **Species and strain:** Mice, CD1 F0 age not reported **Group size:** 5 dams/group 2 pups/litter for liver and lung histology **Test article and vehicle:** PFOS (potassium salt) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 5, 10 mg/kg/day **Exposure regimen:** GD1 to GD17 Dams and fetuses sacrificed at term	**Maternal effects** • No observable effect on body weight or general appearance **Fetal effects** • No effects on litter size (data not reported) • Liver: eosoinphilic granules suggesting peroxisome proliferation observed in 5 and 10 mg/kg groups • Lung: no apparent effects with exposure, as determined by light microscopy	**Major Limitations:** • Limited observations (n=2) for fetal histology • No internal PFOS concentrations determined **Other comments:** • Species and strain appropriate for endpoints assessed • Oral gavage provided direct exposure to PFOS • Doses selected based on previous pre- and post-natal observations in rodents • Exposure occurred during gestational period • Only 2 exposure levels assessed, may not clarify shape of dose-response curve • Only qualitative data reported • Endpoint ascertainment used standardized assessment of endpoints, subjective histopathology observations

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Seacat et al. (2002)

Species and strain:
Monkeys, cynomolgus
Young-adult to adult males and females, acclimated 57 days prior to exposure

Group size:
6/sex/group, expect for 0.03 mg/kg/day group where 4/sex

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in lactose

Route of exposure:
Intragastric intubation of a capsule

Exposure levels:
Nominal doses: 0, 0.03, 0.15, 0.75 mg/kg/day
Cumulative doses: 0, 4.6, 22.9, 114.7 mg/kg

See Results column for liver and serum PFOS concentrations

Exposure regimen:
26 weeks

Sacrifice on days 184 and 185 for most animals

Recovery group (2/sex/group in control, 0.15, and 0.75</td>
<td><u>Internal PFOS concentrations</u>

Internal PFOS concentrations in males and females after 183 days of exposure
<table>
<tr><td></td><td colspan="2">Male</td><td colspan="2">Female</td></tr>
<tr><td>Daily dose mg/kg/day</td><td>Serum (ppm)</td><td>Liver (ppm)</td><td>Serum (ppm)</td><td>Liver (ppm)</td></tr>
<tr><td>0</td><td>0.05±0.01</td><td>0.12±0.03</td><td>0.05±0.02</td><td>0.11±0.03</td></tr>
<tr><td>0.03</td><td>15.8±1.4[a]</td><td>17.3±4.7[a]</td><td>13.2±1.4[a]</td><td>22.8±2.1[a]</td></tr>
<tr><td>0.15</td><td>82.6±25.2[a]</td><td>58.8±19.5[a]</td><td>66.8±10.8[a]</td><td>69.5±14.9[a]</td></tr>
<tr><td>0.75</td><td>173±37[a]</td><td>395±24[a]</td><td>171±22[a]</td><td>273±14[a]</td></tr>
<tr><td colspan="5">Mean±SD
a = p≤0.05 compared to controls</td></tr>
</table>

Percent of cumulative PFOS that was given during 183 days of treatment present in the liver ranged from 4.4±1.6% to 8.7±1.0% with no apparent correlation to dose or gender

<u>Mortality during exposure</u>

One male death on day 155 with 0.75 mg/kg/day likely due to severe acute recurrence of pulmonary inflammation, monkey had elevated serum creatinine phosphokinase and lost 13% of initial body weight
One male sacrificed due to moribund condition on day 179 with 0.75 mg/kg/day likely due to hyperkalemia, monkey had numerous elevations in serum clinical chemistry and gained 14% of initial body weight

<u>Body weight after 183 days of exposure</u>

No statistically significant differences in body weight between controls and exposed groups
Statistically significant (p≤0.05) reduction in body weight change (from day 0 to sacrifice) in males and females with 0.75 mg/kg/day compared to controls

</td>
<td>Major Limitations:

Sample sizes generally 2 to 6 monkeys per group but with increased frequency of endpoint measurements (i.e., during the course of exposure)

Other comments:

Species and strain appropriate for endpoints assessed
Oral intubation provided direct exposure to PFOS
Doses selected based on previous observations in monkeys
Duration of exposures were subchronic
Number of exposure levels allowed for determining any dose-related effects
Quantitative data reported but some qualitative reporting of data (e.g., pathology)
Internal PFOS measurements
Endpoint ascertainment used standardized assessment of mortality, body and organ weights, hematological and clinical parameters, urinalyses, hormones, cell proliferation, and microscopy. More than one technique used to assess serum thyroid hormone (e.g., free T4)

</td>
</tr>
</table>

mg/kg/day groups) were monitored for 1 year following exposure then sacrificed Note: most aspects of study reported to have been conducted according to GLP	**Liver weight after 183 days of exposure** • Statistically significant (p≤0.05) increase in absolute liver weights in females with 0.75 mg/kg/day compared to controls • Statistically significant (p≤0.05) increase in relative (to body weight) liver weights in males and females with 0.75 mg/kg/day compared to controls • Statistically significant (p≤0.05) increase in relative (to brain) liver weights in females with 0.75 mg/kg/day compared to controls **Organ weights (non-liver) after 183 days of exposure** • Statistically significant (p≤0.05) increase in relative (to body weight) left adrenal gland weights in males with 0.75 mg/kg/day compared to controls • No statistically significant changes in absolute or relative (to body weight or to brain weight) organ weights with 0.3 mg/kg/day or 0.15 mg/kg/day Note: authors obtained organ weights for 9 different organs **Hematological parameters** • Statistically significant (p<0.05) reduction in hemoglobin in males with 0.75 mg/kg/day compared to controls at end of exposure, values were considered within normal range • No statistically significant changes (compared to controls) in other male parameters at the end of exposure • No statistically significant changes were consistently observed in females during or at the end of exposure Note: authors obtained measurements for 15 parameters **Clinical chemistry parameters** • Statistically significant (p<0.05) reductions in serum total cholesterol in males and females with 0.75 mg/kg/day compared to controls from 91 days of exposure to the end of exposure, male levels significantly (p=0.013) lower than females after 183 days of exposure • Statistically significant (p<0.05) reductions in high-density lipoprotein (HDL) cholesterol in males (with 0.03 and 0.75 mg/kg/day) and females (with 0.15 and 0.75 mg/kg/day)	

	compared to controls at 153 and 182 days of exposure, authors did not measure HDL prior to day 153 • Statistically significant ($p<0.05$) reduction in serum bilirubin in males with 0.75 mg/kg/day compared to controls at 91, 153, and 182 days of exposure, no statistically significant effect in females • Statistically significant ($p<0.05$) increase in serum bile acids in males with 0.75 mg/kg/day compared to controls at 182 days of exposure, no statistically significant effect in females • Authors noted high background (i.e., prior to exposure) levels of creatine phosphokinase in males and females, measurements during the course of exposure generally significantly lower • No statistically significant effects noted for sorbitol dehydrogenase, transaminases, or alkaline phosphatase as well as other clinical chemistry parameters Note: authors obtained measurements for >20 parameters **Urinalyses** • No statistically significant changes expect on day 62 where females (0.75 mg/kg/day) had lower pH than controls Note: authors obtained measurements for >10 parameters **Thyroid hormones** • Thyroid stimulating hormone (TSH): increased (by about twice control values) at day 182 and day 184 (by two techniques) in males and females with 0.75 mg/kg/day, statistically significant ($p\leq0.05$ compare to control) with some measurements • Total thyroxine (T4): no consistent changes in terms of dose response or duration of exposure in males and females, day 184 measurements comparable between two different techniques • Total triiodothyronine (T3): decreased at day 182 and day 184 (by two techniques) in males and females with ≥0.15 mg/kg/day, statistically significant ($p\leq0.05$ compare to control) with some measurements	

	• Free T4: no change at day 184 (only day of measurement) in males and females, values obtained by equilibrium dialysis technique slightly higher than standard approach • Free T3: statistically significant (p≤0.05) decrease at day 184 (only day measured and by only one technique) in males and females with 0.75 mg/kg/day **Hormone analysis** • Statistically significant (p≤0.05) reduction in estradiol at day 182 in males with 0.75 mg/kg/day compared to controls, reduction confirmed with analysis on day 184 (data not reported) • Non-statistically significant reduction in estradiol at day 182 in females with ≥0.15 mg/kg/day • No statistically significant changes in testosterone at day 182 in males and females **Cell proliferation** • No statistically significant effects in the liver, pancreas, and testes at day 182 **Anatomic pathology, histopathology, and electron microscopy** • Anatomic pathology: no significant changes in tissues (liver, thymus, and spinal cord) and doses (0.03 and 0.15 mg/kg/day) analyzed • Histopathology: centrilobular vacuoluation, hypertrophy, and mild bile stasis in some livers from 0.75 mg/kg/day group • Electron microscopy: accumulation of lipid droplets (2 of 2 males, 2 of 4 females) and increased glycogen content (1 of 2 males, 2 of 4 females) in livers from 0.75 mg/kg/day group Note: authors obtained >30 different tissues for histopathological evaluation **1-year recovery group; internal PFOS concentration** • Rate of elimination from serum varied between groups at beginning of recovery then similar slopes in elimination curves near end of recovery	

	• Similar rate of serum PFOS decrease between males and females during recovery phase • Liver PFOS concentrations after 1-year recovery averaged 19±8% of concentrations measured at end of exposure **1-year recovery group: clinical chemistry parameters** • Serum total cholesterol returned to pre-treatment values in males and females within 36 days after exposure ended • HDL cholesterol returned to control values in males and females within 61 days after exposure ended **1-year recovery group: thyroid hormones** • Values for total T3 returned to normal between 33 and 61 days after exposure ended **1-year recovery group: hormone analysis** • Estradiol levels in males returned to control values after 63 days after exposure ended **1-year recovery group: histopathology and electron microscopy** • Histopathology: complete recovery observed in liver tissues collected 7 months after exposure ended, hepatocellular hypertrophy and vacuolation not observed after 1 year of recovery • Electron microscopy: complete recovery observed in liver tissues collected 7 months after exposure ended; liver samples collected 1 year after exposure ended were considered ultrastructurally normal	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Seacat et al. (2003)

Note: the results reported by the authors represent data from 4- and 14-week interim sacrifices of a 2-year bioassay (Butenhoff et al. 2012). Only 14-week sacrifice results are reported herein. Data from the 4-week sacrifice are not summarized in a table but are discussed in text.

Species and strain:
Rats, Crl:CD® (SD) IGS BR
About 41 days old at start of study

Group size:
5/sex/dose for 14-week sacrifice

Test article and vehicle:
PFOS (potassium salt, 86.9% pure) in acetone

Route of exposure:
Dietary

Exposure levels:
Nominal doses: 0, 0.5, 2.0, 5.0, 20 ppm

See Results column for liver and serum PFOS concentrations

Exposure regimen:
14 weeks</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="5">Internal PFOS concentration in males and females after 14 weeks of exposure</td></tr>
<tr><td></td><td colspan="2">Male</td><td colspan="2">Female</td></tr>
<tr><td>Dietary dose (ppm)</td><td>Serum (ug/mL)</td><td>Liver (ug/g)</td><td>Serum (ug/mL)</td><td>Liver (ug/g)</td></tr>
<tr><td>0</td><td><LOQ[a]</td><td>0.46±0.06</td><td>2.67±4.58</td><td>12.0±22.4</td></tr>
<tr><td>0.5</td><td>4.04±0.80</td><td>23.8±3.5</td><td>6.96±0.99[b]</td><td>19.2±3.8</td></tr>
<tr><td>2</td><td>17.1±1.22</td><td>74.0±6.2</td><td>27.3±2.3</td><td>69.2±3.5</td></tr>
<tr><td>5</td><td>43.9±4.9</td><td>358±26</td><td>64.4±5.5</td><td>370±22</td></tr>
<tr><td>20</td><td>148±14</td><td>568±107</td><td>223±22</td><td>635±49</td></tr>
<tr><td colspan="5">Mean±SD, n=5 unless specified
a = limit of quantitation (LOQ)=0.046 ug/mL
b = n=4</td></tr>
</table>

<u>Body weight</u>
• No statistically significant decreases in body weight in males and females

<u>Food consumption</u>
• Statistically significant (p<0.05) decrease in food consumption (presumably in males and females) with 20 ppm
• No effect on food efficiency (g weight gain/g food consumed)

<u>Liver weight</u>
• Statistically significant (p<0.05) increase in absolute liver weight in males only with 20 ppm
• Statistically significant (p<0.05) increase in relative (to body weight) liver weight in males and females with 20 ppm

<u>Hematology</u>
• Statistically significant (p<0.05) increase in the absolute count of segmented neutrophils in males only with 20 ppm
Note: authors performed 8 different hematological evaluations</td>
<td>Major Limitations:
• Sample size ≤5 rats per endpoint

Other comments:
• Species and strain appropriate for endpoints assessed
• Dietary exposure more closely mimics potential human exposure
• Dose selection based on previous observations of body weight and liver effects in rats
• Duration of exposures were subchronic
• Number of exposure levels allowed for determining any dose-related effects
• Quantitative data reported but some qualitative reporting of data (e.g., pathology, urinalysis)
• Internal PFOS measurements determined
• Endpoint ascertainment used standardized assessment of body and organ weights, food consumption, hematological and clinical chemistry parameters, urinalyses, microscopy, and cell proliferation</td>
</tr>
</table>

Related studies: Butenhoff et al. (2012)	**Urinalysis** • No toxicological important changes were observed (data not reported) Note: authors obtained measurements for >10 parameters **Clinical chemistry** • Statistically significant ($p<0.05$) decrease in serum cholesterol in males only with 20 ppm • Statistically significant ($p<0.05$) increase in alanine aminotransferase in males only with 20 ppm • Statistically significant ($p<0.05$) increase in urea nitrogen in males and females with 20 ppm Note: authors obtained measurements for >15 parameters **Histopathology** • Histopathological changes observed in the livers of males (≥5 ppm) and females (20 ppm) included centrilobular hepatocyte hypertrophy and midzonal to centrilobular vacuolation, incidence and severity generally greater in 20 ppm males Note: authors obtain 10 different tissues for microscopic analysis **Cell proliferation** • No increase in hepatocellular proliferation index	

Reference and Study Design	Results	Comment
Thibodeaux et al. (2003) Study authors also conducted exposures using mice. These mouse data are presented in a separate table. **Species and strain:** Rats, Sprague-Dawley F0 age not reported **Group size:** Varied by endpoint **Test article and vehicle:** PFOS (potassium salt, 91% pure) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 2, 3, 5, 10 mg/kg/day **Exposure regimen:** GD2 to GD20 Maternal and fetal sacrifices on GD21 A separate group of non-pregnant adult female rats was exposed to 3 or 5 mg/kg for 20 days **Related studies:** Lau et al. (2003)	**Internal PFOS concentrations: maternal and fetal** • Negligible PFOS levels in maternal and fetal control samples • Maternal serum PFOS initially increased monotonically with administered dose during pregnancy but fell after GD14 • Maternal serum PFOS at term (GD21) increased linearly with administered dose • Maternal liver PFOS at term increased linearly with administered dose • Maternal liver PFOS was approximately four times greater than corresponding serum samples • Fetal liver PFOS increased with administered dose and was approximately half the levels as in maternal counterparts **Maternal effects: weight gain and food and water consumption** • Statistically significant ($p<0.0001$) reduction in weight gain with ≥2 mg/kg, in dose-dependent manner • Initial observations of statistically significant ($p<0.001$) reductions in weight gain started on GD7, GD5, and GD3 for the 3 mg/kg, 5 mg/kg, and 10 mg/kg groups, respectively • No weight gain in 10 mg/kg group until last week of pregnancy • Statistically significant reduction in food ($p<0.0001$) and water ($p<0.05$) consumption with 5 mg/kg and 10 mg/kg **Maternal effects: liver weight** • No effect on absolute liver weight • Statistically significant ($p<0.05$) increase in relative liver weight with 10 mg/kg **Maternal effects: serum chemistry** • Statistically significant ($p<0.05$) reductions in cholesterol and triglycerides with 10 mg/kg • No effect on bile acid, bilirubin, glucose, and sorbitol dehydrogenase	**Major Limitations:** • Thyroid hormone measurements may be subject to negative bias based on analytical method used **Other comments:** • Species and strain appropriate for endpoints assessed • Most endpoints had ≥9 rats/groups • Oral gavage provided direct exposure to PFOS • Doses selected apparently based on previous perinatal effects in laboratory animals • Duration of exposure included gestational period • Number of exposure levels would allow for determining dose-related effects • Quantitative data reported • Internal PFOS concentrations determined

	Maternal effects: serum hormones • No effect on corticosterone and prolactin **Maternal effects: thyroid hormones (data presented graphically)** • Statistically significant reductions in total and free thyroxine ($p<0.0001$) and triiodothyronine ($p<0.002$) • No effect on thyroid-stimulating hormone • Similar effects observed in non-pregnant adult female rats exposed to PFOS **Fetal effects: liver weight** • No effect on absolute and relative liver weight **Fetal effects: reproductive and developmental indices** • No effect on number of implantation sites and percentage of live fetuses • Statistically significant ($p<0.05$) reduction in body weight with 10 mg/kg • Statistically significant ($p<0.05$) increases in cleft palate, sternal defects, anasarca, enlarged right atrium, and ventricular septal defects, generally with 10 mg/kg	

Reference and Study Design	Results	Comment
Thibodeaux et al. (2003) Study authors also conducted exposures using rats. These rat data are presented in a separate table. **Species and strain:** Mice, CD-1 F0 age not reported **Group size:** Varied by endpoint **Test article and vehicle:** PFOS (potassium salt, 91% pure) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 1, 5, 10, 15, 20 mg/kg/day **Exposure regimen:** GD1 to GD17 Sacrifices on GD6, GD12, and GD18 **Related studies:** Lau et al. (2003)	**Internal PFOS concentrations: maternal** • Negligible PFOS levels in maternal control samples • Maternal serum PFOS at term (GD21) increased linearly with administered dose • Maternal liver PFOS at term increased linearly with administered dose but reached saturation between 15 and 20 mg/kg • Maternal liver PFOS was approximately four times greater than corresponding serum samples • Internal fetal PFOS concentrations not determined **Maternal effects: weight gain and food and water consumption** • Statistically significant ($p<0.05$) reduction in weight gain with 20 mg/kg during late gestation • No effect on food consumption but statistically significant ($p<0.05$) effect for water consumption **Maternal effects: liver weight** • Statistically significant ($p<0.05$) increases in absolute and relative liver weights with ≥5 mg/kg **Maternal effects: serum chemistry** • Statistically significant ($p<0.05$) decrease in triglycerides, in a dose-dependent manner • No effect on cholesterol and sorbitol dehydrogenase **Maternal effects: thyroid hormones** • Only data for total serum thyroxine reported • Statistically significant ($p<0.05$) decrease in thyroxine with 20 mg/kg at GD6, levels returned to control levels by last week of pregnancy **Fetal effects: liver weight** • Statistically significant ($p<0.05$) increase in absolute and relative liver weights with 20 mg/kg	**Major Limitations:** • Thyroid hormone measurements may be subject to negative bias based on analytical method used • Internal PFOS concentrations determined for dams but not for fetal tissue **Other comments:** • Species and strain appropriate for endpoints assessed • Most endpoints had ≥10 rats/groups • Oral gavage provided direct exposure to PFOS • Doses selected apparently based on previous perinatal effects in laboratory animals • Duration of exposure included gestational period • Number of exposure levels would allow for determining dose-related effects • Quantitative data reported

	Fetal effects: reproductive and developmental indices • No effect on the number of implantation sites • Statistically significant ($p<0.05$) decrease in percentage of live fetuses with 20 mg/kg • Statistically significant ($p<0.05$) reductions in body weight with 10 mg/kg and 15 mg/kg • Statistically significant ($p<0.05$) increases in cleft palate, sternal defects, enlarged right atrium, and ventricular septal defects, generally at ≥15 mg/kg	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wan et al. (2010)

Species and strain:
Rats, Sprague-Dawley
Age not reported
Mated females

Group size:
10 dams/ group

Test article and vehicle:
PFOS (salt not reported, >98% pure) in 0.05% Tween 80

Route of exposure:
Oral gavage

Exposure levels:
0, 0.1, 0.6, 2.0 mg/kg/day

See Results column for serum and liver PFOS concentrations in offspring

Exposure regimen:
GD2 to GD21

6 pups/litter selected on PND4 were maintained to sacrifice on PND21</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="3">Serum and liver PFOS concentrations in pups at PND21</td></tr>
<tr><td>Maternal dosing (mg/kg/day)</td><td>PFOS in serum (ug/mL)</td><td>PFOS in liver (ug/g)</td></tr>
<tr><td>0</td><td>ND</td><td>ND</td></tr>
<tr><td>0.1</td><td>0.37±0.12</td><td>1.43±0.59</td></tr>
<tr><td>0.6</td><td>1.86±0.35</td><td>7.68±1.62</td></tr>
<tr><td>2.0</td><td>4.26±1.73</td><td>20.52±4.59</td></tr>
<tr><td colspan="3">ND = value below the limit of detection (limit not reported by study authors)
Note: data are mean of 6 litters/group</td></tr>
</table>

<u>Maternal effects: body weight</u>
• Statistically significant reduction in maternal body weight with 2.0 mg/kg/day at GD21 compared to controls
• No statistically significant reductions observed during other gestational time points

<u>Offspring effects: reproductive and developmental</u>

<table>
<tr><td colspan="3">Pups delivered and mortality at PND3</td></tr>
<tr><td>Maternal dosing (mg/kg/day)</td><td>Delivered pups</td><td>Mortality (%)</td></tr>
<tr><td>0</td><td>13.5±1.3</td><td>3.6±0.1</td></tr>
<tr><td>0.1</td><td>13.6±2.3</td><td>3.2±0.1</td></tr>
<tr><td>0.6</td><td>12.7±2.1</td><td>3.5±0.1</td></tr>
<tr><td>2.0</td><td>11.0±2.5*</td><td>22.9±0.1*</td></tr>
<tr><td colspan="3">* = p<0.05 compared to control
Note: data are mean of 10 litters/group</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS concentrations only reported for PND21, corresponding internal PFOS concentrations at PND3 (i.e., time point assessed for pup mortality) either not reported or not determined

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size 6 or 10 litters/group
• Oral gavage provided direct exposure to PFOS
• Doses selected yielded clear LOAEL and NOAEL, doses also produced rat serum PFOS concentrations similar to human serum PFOS concentrations in occupational exposed workers (as reported by the study authors)
• Duration of exposure lasted through the majority of gestational period, lactational exposure (through PND21) from residual exposure PFOS in dams
• Number of exposure levels would allow for determining any dose-dependent effects
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of pup mortality, body weight, and liver weight

Note: this study presented additional mechanistic data (e.g., DNA</td>
</tr>
</table>

Offspring effects: body and liver weights

Pup body and liver weights at PND21			
Maternal dosing (mg/kg/day)	Body weight (g)	Liver weight (g)	Relative liver weight
0	52.8±3.4	2.13±0.19	0.040±0.002
0.1	53.5±3.7	2.18±0.18	0.040±0.002
0.6	50.4±3.4	2.10±0.18	0.041±0.003
2.0	45.3±3.8*	2.12±0.18	0.046±0.001*
* = p<0.05 compared to control Note: data are mean of 6 litters/group			

Offspring effects: liver histopathology

- No significant differences in pathology between exposure and controls groups (e.g., no cytoplasmic vacuolation or hepatocyte hypertrophy)

methylation) that are not presented herein

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wan et al. (2014)

Species and strain:
Mice, CD-1
F0 females: 6 to 8 weeks old

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (salt not reported, 98% pure) in 0.05% DMSO and corn oil

Route of exposure:
Oral gavage

Exposure levels:
0, 0.3, 3 mg/kg

See Results column for serum and liver PFOS concentrations at PND21 and PND63

Exposure regimen:
GD3 to PND21 (weaning)

Note: All F0 dams and some F1 pups (2 per dam) sacrificed at PND21; remaining F1 pups allowed access to either a standard diet (STD) or high-fat diet (HFD) until sacrifice at PND63</td>
<td><u>Internal PFOS concentrations: PND21 and PND63</u>

<table>
<tr><td colspan="3">Internal PFOS concentrations for dams (F0) at PND21</td></tr>
<tr><td>PFOS</td><td>Serum PFOS (ug/mL)</td><td>Liver PFOS (ug/g)</td></tr>
<tr><td>Control</td><td>0.25±0.11</td><td>0.15±0.11</td></tr>
<tr><td>0.3 mg/kg</td><td>15.33±4.62</td><td>49.09±9.88</td></tr>
<tr><td>3 mg/kg</td><td>131.72±30.71</td><td>338.87±100.71</td></tr>
<tr><td colspan="3">mean±SD; n=4 per group</td></tr>
</table>

<table>
<tr><td colspan="3">Internal PFOS concentrations for pups (F1) at PND21</td></tr>
<tr><td>PFOS</td><td>Serum PFOS (ug/mL)</td><td>Liver PFOS (ug/g)</td></tr>
<tr><td>Control</td><td>M: 0
F: 0</td><td>M: 0
F: 0</td></tr>
<tr><td>0.3 mg/kg</td><td>M: 12.73±1.96
F: 11.35±1.08</td><td>M: 20.14±4.06
F: 17.96±6.38</td></tr>
<tr><td>3 mg/kg</td><td>M: 98.74±4.58[a]
F: 87.23±4.28</td><td>M: 242.98±55.62
F: 178.44±79.03</td></tr>
<tr><td colspan="3">mean±SD; n=4 per group
a = p<0.05
F = females; M = males</td></tr>
</table>

<table>
<tr><td colspan="5">Serum PFOS concentrations (ug/mL) in F1 adults at PND63</td></tr>
<tr><td></td><td colspan="2">Males</td><td colspan="2">Female</td></tr>
<tr><td>PFOS</td><td>STD</td><td>HFD</td><td>STD</td><td>HFD</td></tr>
<tr><td>Control</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.3 mg/kg</td><td>0.30±0.06</td><td>1.20±0.29[a]</td><td>0.51±0.11</td><td>1.50±0.27[a]</td></tr>
<tr><td>3 mg/kg</td><td>3.36±1.07</td><td>5.38±0.30[a]</td><td>3.40±1.08</td><td>5.76±1.24[a]</td></tr>
<tr><td colspan="5">mean±SD; n=4 per group
a = p<0.05 compared between STD and HFD within the same gender
HFD = high-fat diet; STD = standard diet</td></tr>
</table>
</td>
<td>Major Limitations:
• Only 2 dose levels used

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample sized generally ≥6 dams or F1 mice
• Oral gavage provided direct exposure to PFOS
• Dose selection approximated human occupational exposure levels
• Duration of exposure lasted gestational period to weaning
• Quantitative data reported
• Exposure characterized by internal PFOS concentrations (e.g., serum and liver)
• Endpoint ascertainment used standardized assessment of body and liver weights and glucose metabolism</td>
</tr>
</table>

	Liver PFOS concentrations (ug/g) in F1 adults at PND63 mean±SD; n=4 per group a = p<0.05 compared between STD and HFD within the same gender HFD = high-fat diet; STD = standard diet	

	Males		Female	
PFOS	STD	HFD	STD	HFD
Control	0	0	0	0
0.3 mg/kg	3.97±0.50	5.43±0.98[a]	3.34±0.50	4.27±1.75[a]
3 mg/kg	12.30±1.59	24.54±1.06[a]	13.77±4.05	21.34±3.36[a]

Maternal (F0) effects at PND21: body and liver weights

- No effect on body weight
- Statistically significant ($p<0.05$) increase in relative liver weight with 3 mg/kg
- No effect on absolute liver weight

Maternal (F0) effects at PND21: glucose metabolism

- Increased serum fasting glucose and fasting insulin with increasing dose but no statistical significance
- Statistically significant ($p<0.02$) increase in homeostatic model assessment for insulin resistance (HOMA-IR) index with ≥0.3 mg/kg compared to control

F1 effects at PND21: body and liver weights

- No difference in body weights between exposure groups as measured from PND1 to PND21
- Statistically significant ($p<0.05$) increase in relative liver weight with 3 mg/kg in males and females compared to control
- Statistically significant ($p<0.05$) increase in absolute liver weight with 3 mg/kg in males compared to controls, increased absolute liver weights in females but no statistically significance

	F1 effects at PND21: glucose metabolism • No effect on fasting serum glucose in males and females • Statistically significant ($p<0.05$) increase in fast serum insulin with ≥0.3 mg/kg in males compared to controls, no effect in females • No effect on HOMA-IR in males and females **F1 effects at PND63 (STD): body and liver weights** • No effect on body weights (measured between PND21 and PND63) between exposed and control groups in both males and females • Statistically significant ($p<0.05$) increase in absolute liver weight with 3 mg/kg compared to controls (in males only) • Statistically significant ($p<0.05$) increase in relative liver weight with ≥0.3 mg/kg compared to controls (in males only) **F1 effects at PND63 (STD): glucose metabolism** • Statistically significant ($p<0.05$) increase in fasting serum glucose with ≥0.3 mg/kg compared to controls in both males and females • Statistically significant ($p<0.05$) increase in fasting serum insulin with 3 mg/kg compared to controls in both males and females • No significant effect on oral glucose tolerance test (OGTT) between control and exposed groups • Statistically significant ($p<0.01$) increase in HOMA-IR with 3 mg/kg compared to controls in both males and females **F1 effects at PND63 (HFD): body and liver weights** • No effect on body weights (measured between PND21 and PND63) between exposed and control groups in both males and females • Statistically significant ($p<0.05$) increase in absolute and relative liver weights with 3 mg/kg compared to controls in males only	

	F1 effects at PND63 (HFD): glucose metabolism • Statistically significant ($p<0.05$) increase in fasting serum glucose in males (3 mg/kg) and females (≥0.3 mg/kg) compared to controls • Statistically significant ($p<0.05$) increase in fasting serum insulin with 3 mg/kg compared to controls in males and females • Statistically significant ($p<0.02$) increase in blood glucose area under the curve (OGGT) with 3 mg/kg compared to controls in both males and females • Statistically significant ($p<0.01$) increase in HOMA-IR with 3 mg/kg compared to controls in both males and female **F1 effects at PND63 comparing STD and HFD groups: liver weights** • Statistically significant ($p<0.05$) increase in relative liver weight with 3 mg/kg for HFD group compared to STD group in males only **F1 effects at PND63 comparing STD and HFD groups: glucose metabolism** • Statistically significant ($p<0.05$) increase in fasting serum glucose with 3 mg/kg for HFD group compared to STD group in males only • Statistically significant ($p<0.05$) increase in fasting serum insulin with 3 mg/kg for HFD group compared to STD group in females only • Statistically significant ($p<0.01$) increase in HOMA-IR with 0.3 mg/kg for HFD group compared to STD group in males and females	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wang et al. (2011c)

Species and strain:
Rats, Wistar
F0 age not reported

Group size:
Varied
4 to 9 dams/group
5 to 8/female pups/group
5 to 8/male pups/group

Test article and vehicle:
PFOS (potassium salt, >98% pure) in 2% Tween 20

Route of exposure:
Dietary

Exposure levels:
0, 3.2, 32 mg/kg feed

See Results column for serum and brain PFOS concentrations

Exposure regimen:
GD1 to PND14
Rats sacrificed on PNDs 1, 7, and 14

This study also exposed rats to 2,2’,4,4’-tetrabromodiphenyl ether (BDE-47) alone and in combination with PFOS. Results reported herein are for PFOS only exposures.</td>
<td><u>Internal PFOS concentrations</u>

<table>
<tr><td colspan="4">Serum and cortex PFOS concentrations in dams</td></tr>
<tr><td>PFOS (mg/kg feed)</td><td>Serum PFOS (ug/ml)</td><td>Cortex PFOS (ug/g tissue)</td><td>Cortex/serum ratio</td></tr>
<tr><td>Dams PND1</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td><LLOQ[a] (3)</td><td><LLOQ[b] (3)</td><td>NA</td></tr>
<tr><td>3.2</td><td>2.29±0.15 (4)</td><td>---</td><td>---</td></tr>
<tr><td>32</td><td>16.9±0.43 (3)</td><td>0.76±0.05 (3)</td><td>0.046±0.002[c]</td></tr>
<tr><td>Dams PND7</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td><LLOQ (3)</td><td><LLOQ (3)</td><td>NA</td></tr>
<tr><td>3.2</td><td>4.16±0.04 (3)</td><td>---</td><td>---</td></tr>
<tr><td>32</td><td>27.3±0.43 (4)</td><td>1.33±0.03 (4)</td><td>0.050±0.002[c]</td></tr>
<tr><td>Dams PND14</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td><LLOQ (3)</td><td><LLOQ (3)</td><td>NA</td></tr>
<tr><td>3.2</td><td>3.15±0.21 (6)</td><td>---</td><td>---</td></tr>
<tr><td>32</td><td>28.7±1.44 (6)</td><td>1.04±0.02 (6)</td><td>0.035±0.003[c]</td></tr>
<tr><td colspan="4">Concentrations reported as Mean±SE
Number in parentheses is sample size
a = lower limit of quantitation (LLOQ) for serum PFOS is 0.010ug/ml
b = LLOQ for brain PFOS is 0.025 ug/g
c = p<0.05 cortex/serum ratio for PFOS in neonate compared to dam
NA = not applicable as ratio could not be calculated as PFOS concentrations were below the LLOQ
--- = no samples available</td></tr>
</table>
</td>
<td>Major Limitations:
• Sample size reported to be <10 but not reported for any given endpoint

Other comments:
• Species and strain appropriate for endpoints assessed
• Oral gavage provided direct exposure to PFOS
• Dose selection based on previous observations of thyroid hormone effects
• Exposure lasted through gestation
• Only 2 exposure levels assessed, may not clarify shape of dose-response curve
• Quantitative data reported, clinical signs assessed not reported
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of endpoints</td>
</tr>
</table>

Serum and cortex PFOS concentrations in pups			
PFOS (mg/kg feed)	Serum PFOS (ug/ml)	Cortex PFOS (ug/g tissue)	Cortex/serum ratio
Pups PND1			
0	<LLOQ[a] (3)	<LLOQ[c] (3)	NA
3.2	5.85±0.33 (7)	2.05±0.13 (7)	0.36±0.07
32	32.9±0.81 (6)	11.5±0.82 (6)	0.37±0.05
Pups PND7			
0	<LLOQ (3)	<LLOQ (3)	NA
3.2	3.65±0.23 (6)	1.52±0.10 (6)	0.42±0.01
32	21.3±1.06 (5)	6.79±0.48 (5)	0.32±0.03
Pups PND14			
0	<LLOQ (3)	<LLOQ (3)	NA
3.2	4.89±0.29 (5)	1.45±0.06 (5)	0.30±0.01
32	25.2±1.27 (6)	4.92±0.29 (6)	0.20±0.04
Concentrations reported as Mean±SE Number in parentheses is sample size a = lower limit of quantitation (LLOQ) for serum PFOS is 0.010ug/ml b = LLOQ for brain PFOS is 0.025 ug/g NA = not applicable as ratio could not be calculated as PFOS concentrations were below the LLOQ --- = no samples available			

Maternal effects: general observations

- No signs of general toxicity during daily observations
- Dam food intake similar between groups for GD1 to GD21

Reproductive and offspring endpoints

- Statistically significant ($p<0.05$) decreased pup body weight at PNDs1, 7, and 14 in 32 mg/kg feed group compared to controls
- Pups appeared pale and delicate in 32 mg/kg feed group

Reproductive and offspring effects			
PFOS (mg/kg feed)	Pregnancy length (days)	Litter size	Mortality on PND1 (%)
0	22	8 to 14	0 to 25
3.2	22	8 to 14	0 to 20
32	22	6 to 14	0 to 29

	Maternal effects: serum levels of total triiodothyronine (TT3) and total thyroxine (TT4) • Statistically significant ($p<0.05$) decrease in maternal TT3 levels at PND1 with 32 mg/kg compared to controls; data incomplete for PNDs7 and 14 • Statistically significant ($p<0.05$) decrease in maternal TT4 at PND1 (≥3.2 mg/kg) and PND7 (only 3.2 mg/kg data reported) compared to controls, no control values reported at PND14 **Offspring effects: serum levels of TT3 and TT4** • Statistically significant ($p<0.05$) decrease in TT3 levels at PND14 with 32 mg/kg compared to controls, no effects at PNDs1 and 7 • Statistically significant ($p<0.05$) decreases in TT4 levels at PND1 with 32 mg/kg and at PNDs7 and 14 with ≥3.2 mg/kg compared to controls	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Wang et al. (2015)

Species and strain:
Rats, Wistar
Age not reported
Pregnant females

Group size:
Varied by endpoint

Test article and vehicle:
PFOS (salt not reported, ≥97% pure) in 2% Tween 20 (this stock solution was diluted 500-fold with sterile tap water for exposure)

Route of exposure:
Drinking water (ad libitum)

Exposure levels:
0, 5, 15 mg/L

See Results column for maternal serum and offspring hippocampus PFOS concentrations

Exposure regimen:
Dams exposed GD1 to weaning (PND not specified), offspring were then exposed from weaning to PND35

On PND1, control and exposure groups were</td>
<td><u>Internal PFOS concentrations</u>

<table>
<tr><td colspan="4">Maternal serum PFOS concentrations (ug/mL)</td></tr>
<tr><td></td><td colspan="3">PFOS dose (mg/L)</td></tr>
<tr><td></td><td>0</td><td>5</td><td>15</td></tr>
<tr><td>PND7</td><td>ND</td><td>25.7±0.8**</td><td>99.3±2.0**</td></tr>
<tr><td>PND35</td><td>ND</td><td>64.3±9.5**</td><td>207.7±10.5**</td></tr>
<tr><td colspan="4">For each dose group, n = 3
* = p<0.05, ** = p<0.01
ND = not detectable</td></tr>
</table>

<table>
<tr><td colspan="8">PFOS concentrations (ug/g) in hippocampus of litters</td></tr>
<tr><td></td><td colspan="7">Groups</td></tr>
<tr><td></td><td>CC</td><td>TT5</td><td>TT15</td><td>TC5</td><td>TC15</td><td>CT5</td><td>CT15</td></tr>
<tr><td>PND1</td><td>ND</td><td>123.3**</td><td>373.4**</td><td>----</td><td>----</td><td>----</td><td>----</td></tr>
<tr><td>PND7</td><td>ND</td><td>11.4**</td><td>32.30**</td><td>4.6**##</td><td>10.8**##</td><td>1.0</td><td>3.5**</td></tr>
<tr><td>PND35</td><td>ND</td><td>6.7**</td><td>14.66**</td><td>0.3#</td><td>0.3##</td><td>1.9**</td><td>5.7**</td></tr>
<tr><td colspan="8">Values are means (standard errors not reported herein)
For each dose group, n = 3
Compared to control (CC): * = p<0.05; ** = p<0.01
Compared to CT of same PFOS dose: # = p<0.05, ## = p<0.01
ND = not detectable
---- = group did not exist at time of sampling</td></tr>
</table>

<u>Reproductive/developmental effects</u>

<table>
<tr><td colspan="4">Litter parameters</td></tr>
<tr><td></td><td colspan="3">PFOS dose (mgL)</td></tr>
<tr><td></td><td>0</td><td>5</td><td>15</td></tr>
<tr><td>Number of pups born per litter</td><td>10.50±0.55</td><td>11.59±0.80</td><td>10.26±0.8</td></tr>
<tr><td>Number of pup surviving to PND1</td><td>10.36±0.52</td><td>11.24±0.74</td><td>8.74±0.81</td></tr>
<tr><td>Birth to PND1 survival (% per litter)</td><td>99±1.0</td><td>97±1.0</td><td>87±6.0**</td></tr>
<tr><td colspan="4">Mean±SE
* = p<0.05, ** = p<0.01</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS concentration in offspring determined only for PND35 and not for time points where effects were observed (e.g., decrease in time spent in target quadrant with TT15 on PND42)
• Maternal toxicity not reported

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample sizes ≤10
• Drinking water exposure allows for PFOS to interact with tissues from the oral cavity to the stomach
• Doses selected based on acute toxicity tests (LD50 determinations) in rats, as stated by the study authors
• Duration of exposure lastrd from the beginning of gestation until PND35
• Two exposure levels may limit ability to demonstrate any dose-related effects, NOAEL not identified (for escape latency)
• Quantitative data reported
• Endpoint ascertainment used standardized assessment of reproductive/developmental and neurological endpoints</td>
</tr>
</table>

cross-fostered to produce the following groups:

- CC = no prenatal and no postnatal exposure
- TT5 or TT15 = prenatal and postnatal exposure to 5 or 15 mg/L, respectively
- CT5 or CT15 = only postnatal exposure to 5 or 15 mg/L, respectively
- TC5 or TC15 = only prenatal exposure to 5 or 15 mg/L, respectively

Some pups sacrificed on PND7 and PND35, other pups tested for spatial learning and memory ability starting on PND35

Neurotoxicity (offspring): visual and motor functions

- No statistically significant differences in swimming speeds and time to reach the visible platform between exposure groups and controls

Neurotoxicity (offspring): learning ability

Escape latency (time to hidden platform) in offspring							
Test day	PND35	PND36	PND37	PND38	PND39	PND40	PND41
Sample size	8	6	10	10	10	9	10
CC	77.27	41.48	23.76	17.76	23.64	16.59	17.60
TT5	80.10	49.21	19.72	22.49	21.96	15.14	15.44
TT15	85.88	58.49	44.13**	29.75*	26.19	22.74	23.78
TC5	80.02	51.38	35.4	38.82*	27.24*	20.41	23.65
TC15	91.47	65.66*	49.41**	35.69*	41.50**	29.61**	31.01*
CT5	83.92	48.45	39.99*	28.14*	24.17	25.36	22.67
CT15	80.08	57.80	35.57	28.63*	24.15	20.53	21.29
Values are means reported in seconds (standard errors not reported herein) * = p<0.05, compared to controls (CC); ** = p<0.01, compared to controls (CC)							

Escape distance (distance swum before reaching submerged platform) in offspring	
Training day	Observations for escape distance[a]
1	• No statistically significant differences between exposed groups and control
2	• No statistically significant differences between exposed groups and control
3	• Statistically significant (p<0.05) increase with TT15, TC5, TC15, and CT5 compared to control
4	• Statistically significant (p<0.05) increase with TC5 and TC15 compared to control
5	• Statistically significant (p<0.01) increase with TC15 compared to control
6	• Statistically significant (p<0.05) increase with TC15 compared to control
7	• Statistically significant (p<0.05) increase with TC5 and TC15 compared to control
Note: Training day 1 was PND35 a = data by study authors were only provided in a figure	

Note: this study also presented data on mechanistic and neurochemical effects of PFOS. Those data are not reported herein.

	<u>Neurotoxicity (offspring): memory ability</u> • Note: probe test conducted on PND42 (i.e., 24 hours after the last hidden platform test) • Statistically significant ($p<0.05$) decrease in time spent in target quadrant with TT15 compared to controls • Statistically significant ($p<0.05$) decrease in number of platform crossings with TT15 compared to controls	

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Yahia et al. (2008)

Species and strain:
Mice, ICR
F0: 7 weeks

Group size:
5 dams/group

Test article and vehicle:
PFOS (potassium salt, 98% pure) in 0.5% Tween 20

Route of exposure:
Oral gavage

Exposure levels:
0, 1, 10, 20 mg/kg/day (only two highest doses for histopathology study)

Exposure regimen:
Prenatal study: GD0 to GD17, sacrifice on GD18
Postnatal study: GD0 to GD18, sacrifice following natural birth

Histopathology study: GD0 to GD17 or GD18, sacrifice prior to or after birth</td>
<td><u>Maternal effects</u>
• No maternal deaths
• Statistically significant (p<0.05 or p<0.01) decrease in weight gain from GD11 until end of gestation with 20 mg/kg
• Statistically significant (p<0.05) decrease in daily feed consumption from GD14 onward with 20 mg/kg
• Increased daily water consumption with 20 mg/kg (intermittent statistical significance [p<0.05] from GD11 onward)
• Dose-dependent increase in liver weight (statistically significant [p<0.01] with 10 and 20 mg/kg) with hypertrophy at highest dose
• No effect on organ weight for kidneys, lungs, and brain

<u>Prenatal effects</u>
• Bilateral swelling in back of neck in all fetuses with 20 mg/kg and in some fetuses (incidence not reported) with 10 mg/kg
<table>
<tr><td colspan="5">Fetal observations following PFOS exposure</td></tr>
<tr><td></td><td>Control</td><td>1 mg/kg</td><td>10 mg/kg</td><td>20 mg/kg</td></tr>
<tr><td># of dams</td><td>5</td><td>5</td><td>5</td><td>5</td></tr>
<tr><td>Total # of fetuses</td><td>80</td><td>76</td><td>79</td><td>71</td></tr>
<tr><td>% live fetuses</td><td>98.75±1.25</td><td>98.88±1.12</td><td>96.85±1.97</td><td>90.06±3.02*</td></tr>
<tr><td>% resorbed fetuses</td><td>1.25±1.25</td><td>1.11±1.11</td><td>3.15±1.97</td><td>5.36±2.63</td></tr>
<tr><td>% dead fetuses</td><td>0</td><td>0</td><td>0</td><td>4.58±3.25</td></tr>
<tr><td>Fetal body weight (g)</td><td>1.49±0.01</td><td>1.46±0.01</td><td>1.41±0.01**</td><td>1.10±0.02**</td></tr>
<tr><td colspan="5">* = p<0.05, compared to control; ** = p<0.01, compared to control</td></tr>
</table>
</td>
<td>Major Limitations:
• Internal PFOS concentrations not determined
• Sex of offspring not reported

Other comments:
• Strain of mouse not very common and appropriateness for endpoints assessed is unclear
• Sample size generally ≥10 dams or pups
• Oral gavage provided direct exposure to PFOS
• Dose selection allowed for overt toxicity at highest dose
• Duration of exposure lasted gestational period
• Generally 3 doses assessed per endpoint, expect 1 dose for histopathology
• Generally quantitative data but some qualitative (textual) reporting of data
• Endpoint ascertainment used standardized assessment of mortality, body and organ weights, reproductive/developmental endpoints, and histology

• Note: biological significance of intracranial blood vessel dilation not clear.</td>
</tr>
</table>

Fetal observations following PFOS exposure				
	Control	1 mg/kg	10 mg/kg	20 mg/kg
# fetuses examined	60	44	68	60
% cleft palate	0	1.96±1.96	26.36±8.27**	98.56±1.44**
% sternal defects	0	15.77±0.99**	52.44±2.79**	100**
% delayed ossification of phalanges	0	1.96±1.96	4.34±1.80	57.23±9.60**
% delayed eruption of incisors	3.25±1.89	6.90±0.53	22.12±2.68	36.10±4.64**
% extra ribs	27.81±13.35	13.01±6.59	36.11±11.85	32.08±8.04
% wavy ribs	0	0	7.31±0.34*	84.09±2.56**
% tail abnormalities	4.41±4.41	18.38±8.73	23.05±3.25	65.00±6.71**
% curved fetus	3.55±2.11	4.94±2.47	33.38±8.47**	68.47±1.30**
% spina bifida occulta	0	1.96±1.96	23.13±3.94**	100**
* = p<0.05, compared to control; ** = p<0.01, compared to control				

Postnatal effects

- Neonates (100%) in 20 mg/kg group born pale, weak, and inactive; died immediately after or within hours after birth
- Neonates (45%) in 10 mg/g group born pale and inactive; died within 24 hours after birth
- Bilateral firm swelling in back of neck in all neonates of 20 mg/kg group and in some (incidence not reported) of 10 mg/kg group
- Histological examination of pup lungs showed atelectasis-like histology in all pups (n=5) in 20 mg/kg group and in some (incidence not reported) pups in 10 mg/kg group; 1 mg/kg and control pups had intact lung structure

Neonatal observations following PFOS exposure				
	Control	1 mg/kg	10 mg/kg	20 mg/kg
# of dams	5	5	5	5
# of pups	53	59	49	40
Neonatal body weight (g)	1.51±0.02	1.55±0.02	1.41±0.01**	1.08±0.01**
% survival rats at PDN4	98.18±1.82	100	55.20±18.98*	0**
* = p<0.05, compared to control; ** = p<0.01, compared to control PND = postnatal day				

Histopathology of fetal (20 mg/kg) and neonatal (10 mg/kg) heads and lungs

- Normal lung structure in all (n=15) fetal lungs
- All fetal heads (n=15) showed mild to severe intracranial dilatation of blood vessels with no inflammatory or hemorrhagic reactions
- Lung atelectasis (slight) in 27% of pups accompanied with moderate to severe intracranial blood vessel dilatation
- Brain blood vessel dilatation (moderate to severe) in 87% of pups

Reference and Study Design	Results	Comment
Ye et al. (2012) **Species and strain:** Rats, Sprague-Dawley F0 age not reported **Group size:** 10 dams/group **Test article and vehicle:** PFOS (salt and purity not reported) in 0.5% Tween 20 **Route of exposure:** Oral gavage **Exposure levels:** 0, 5, 20 mg/kg **Exposure regimen:** GD12 to GD18 Pregnant dams sacrificed on GD18.5	**Maternal effects** • No dams died from exposure **Fetal effects** • No histological differences observed in lungs between exposure groups Note: body weights of dams and fetus were recorded but not reported by authors	**Major Limitations:** • Qualitative data reported; dam and fetal birth weights not reported • No internal PFOS concentrations determined, purity of PFOS not reported **Other comments:** • Species and strain appropriate for endpoints assessed • Sample size 10 dams/group but number of fetuses used endpoint observation (lung pathology) not reported • Oral gavage provided direct exposure to PFOS • High dose used apparently based on previous observations of neonatal mortality in rats • Exposure occurred during a part of gestational period • Only 2 exposure levels assessed, may not clarify shape of dose-response curve • Endpoint ascertainment used standardized assessment of endpoints, subjective histopathology observations

<table>
<tr><th>Reference and Study Design</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Yu et al. (2009a)

Species and strain:
Rats, Sprague-Dawley
Males only
Age not reported

Group size:
8–10/group

Test article and vehicle:
PFOS (potassium salt, >98% pure) in drinking water

Route of exposure:
Drinking water (ad libitum)

Exposure levels:
0, 1.7, 5.0, 15.0 mg/L

See Results column for serum PFOS concentrations

Exposure regimen:
91 days</td>
<td><u>Internal PFOS concentration</u>

<table>
<tr><td colspan="2">Serum PFOS concentrations after 91 days of exposure</td></tr>
<tr><td>Exposure dose (mg/L)</td><td>Serum PFOS (mg/L)</td></tr>
<tr><td>0</td><td><LOQ</td></tr>
<tr><td>1.7</td><td>5.0±0.3</td></tr>
<tr><td>5.0</td><td>33.6±2.1</td></tr>
<tr><td>15.0</td><td>88.2±4.2</td></tr>
<tr><td colspan="2">For each dose group, n = 7–8/group
Limit of quantitation (LOQ) was 0.5 ug/L</td></tr>
</table>

<u>Body weight</u>

<table>
<tr><td colspan="2">Body weight after 91 days of exposure</td></tr>
<tr><td>Exposure dose (mg/L)</td><td>Body weight (g)</td></tr>
<tr><td>0</td><td>397±29.3</td></tr>
<tr><td>1.7</td><td>406±40.3</td></tr>
<tr><td>5.0</td><td>434±19.2</td></tr>
<tr><td>15.0</td><td>385±26.7</td></tr>
<tr><td colspan="2">For each dose group, n = 8–10/group</td></tr>
</table>

<u>Organ weights: liver and thyroid</u>

<table>
<tr><td colspan="5">Organ weights after 91 days of exposure</td></tr>
<tr><td></td><td colspan="2">Liver</td><td colspan="2">Thyroid</td></tr>
<tr><td>Exposure dose (mg/L)</td><td>Absolute (g)</td><td>Relative[a]</td><td>Absolute (mg)</td><td>Relative[a] (x10^3)</td></tr>
<tr><td>0</td><td>13.7±1.1</td><td>0.035±0.002</td><td>27.4±3.2</td><td>0.068±0.004</td></tr>
<tr><td>1.7</td><td>15.1±1.5</td><td>0.037±0.001</td><td>23.6±2.0</td><td>0.060±0.005</td></tr>
<tr><td>5.0</td><td>17.9±1.0*</td><td>0.041±0.001**</td><td>26.7±1.9</td><td>0.061±0.002</td></tr>
<tr><td>15.0</td><td>19.8±1.5**</td><td>0.052±0.002**</td><td>25.9±2.6</td><td>0.067±0.004</td></tr>
<tr><td colspan="5">For each dose group, n = 8–10/group
a = organ weight to body weight ratio
* = p<0.05 compared to control, ** = p<0.01 compared to control</td></tr>
</table>
</td>
<td>Major Limitations:
• Only males used, females may be more sensitive
• Exact sample size per dose group not provided

Other comments:
• Species and strain appropriate for endpoints assessed
• Sample size ≤10/group
• Drinking water exposure allows for PFOS to interact with tissues from the oral cavity to the stomach
• Doses selected cover ~1 order of magnitude and produce rat serum PFOS concentrations that are greater than human PFOS serum concentrations from occupational and non-occupational exposures, as reported by the study authors
• Subchronic duration of exposure
• Number of exposure levels would allow for determining any dose-dependent effects
• Quantitative data reported
• Internal PFOS concentrations determined
• Endpoint ascertainment used standardized assessment of body and organ weights; based on authors' description of methods, unclear whether free T4 measurements were potentially subject to negative bias due to analytical method used</td>
</tr>
</table>

Thyroid hormones

Thyroid hormone levels after 91 days of exposure

Exposure dose (mg/L)	Total T3 (ug/L)	Total T4 (ug/L)	Free T4 (pmol/L)	TSH (IU/L)
0	0.29±0.04	40.9±1.8	19.0±1.3	0.72±0.30
1.7	0.48±0.08*	23.9±1.3**	16.7±1.4	0.67±0.27
5.0	0.23±0.05	16.4±5.4**	12.6±1.5*	1.12±0.34
15.0	0.23±0.03	8.5±1.6**	17.3±1.1	1.62±0.67

For each dose group, n = 5–6/group
Note: thyroid hormones measured by radioimmunoassay
T3 = triiodothyronine
T4 = thyroxine
TSH = thyrotropin
* = $p<0.05$ compared to control, ** = $p<0.01$ compared to control

Note: This paper also includes mechanistic data not reported herein.

Appendix 5: Animal tabular review tables

Reference and Study Design	Results	Comment
Author Asakawa et al. (2007) **Species, strain, age of animals:** Mice, ddy, M, 8-9 wks old Rats, Wistar, M, 8-10 wks old **Group size:** N = 3-7 **Test article and vehicle:** PFOS, in artificial cerebrospinal fluid w 1% DMSO **Route of exposure:** Intracerebrovemtricular injection **Exposure levels:** Vehicle, 30, 100, 300 µg/kg **Exposure regimen:** Single dose	**Endpoint 1** Inhibition of feeding **NOAEL** 30 µg/kg **LOAEL** 100 µg/kg **Endpoint 2** Gastro-duodenal motility **NOAEL** --- **LOAEL** 300 µg/kg (single dose level) **Endpoint 3** Rate of gastric emptying **NOAEL** 100 µg/kg **LOAEL** 300 µg/kg	Study also contains information on gene expression, and hypothalamus cellular function. Unusual route-of-exposure

Reference and Study Design	Results	Comment
Author Austin et al. (2003) **Species, strain, age of animals:** Rats, S-D, adult, F **Group size:** N = 8 for each dose group **Test article and vehicle:** K-PFOS in DMSO **Route of exposure:** Intarperitoneal injection **Exposure levels:** Vehicle, 1, 10 mg/kg Serum conc (mean) = ND, 10,480, 45,446 ng/ml **Exposure regimen:** [day/week, duration] Daily for 14 d **Other information** PFOS measured in various tissue in addition to serum Monoamines measured in hypothalmus	**Endpoint 1** Body wt **NOAEL** 1 mg/kg **LOAEL** 10 mg/kg ↓ (for d 11-14) **Endpoint 2** Food intake **NOAEL** 1 mg/kg **LOAEL** 10 mg/kg ↓ (for d 5-14) **Endpoint 3** Estrous cycling (percent animals w regular cycles) **NOAEL** 1 mg/kg (also irregular cycle and ↑ persistent diestrus vs. no observed in controls) **LOAEL** 10 mg/kg ↓ % normal (also irregular cycle and ↑ persistent diestrus vs. no observed in controls) **Endpoint 4** Serum leptin **NOAEL** 1 mg/kg **LOAEL** 10 mg/kg ↓	

Reference and Study Design	Results	Comment
Author Bijland et al. (2011) **Species, strain, age of animals:** E3LCEPT mice, M, **Group size:** N = 5-8 (depending on experiment) **Test article and vehicle:** K-PFOS in food **Route of exposure:** Diet (western-type) **Exposure levels:** ~3 mg/kg/d (single dose) <u>Serum conc</u> 4 wks – 85.6, 95.3 μg/ml 6 wks – 124.7 μg/ml **Exposure regimen:** 4-6 wks	**<u>Endpoint 1</u>** Body wt **NOAEL** 3 mg/kg/d **LOAEL** --- **<u>Endpoint 2</u>** Food intake **NOAEL** 3 mg/kg/d **LOAEL** --- **<u>Endpoint 3</u>** Triglycerides, plasma (4 wks) **NOAEL** --- **LOAEL** 3 mg/kg/d ↓ **<u>Endpoint 4</u>** Total cholesterol, plasma **NOAEL** --- **LOAEL** 3 mg/kg/d ↓	Also addresses non-apical endpoints that may be useful for mechanistic understanding

	Endpoint 5 VLD-cholesterol, plasma **NOAEL** **---** **LOAEL** 3 mg/kg/d ↓ **Endpoint 6** HD-cholesterol, plasma **NOAEL** --- **LOAEL** 3 mg/kg/d ↓ **Endpoint 7** Liver wt **NOAEL** --- **LOAEL** 3 mg/kg/d ↑ **Endpoint 8** Liver triglyceride content **NOAEL** --- **LOAEL** 3 mg/kg/d ↑	

Reference and Study Design	Results	Comment
Author Bjork et al. (2008) **Species, strain, age of animals:** Rats, S-D **Group size:** Dams/fetuses N =5-6 (litters constituted single unit) **Test article and vehicle:** PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 3 mg/kg **Exposure regimen:** Dams dosed daily GD-2 - 20 **Other information** Dams weighed and sacrificed d-21 Fetuses extracted	**Endpoint 1** Maternal body wt **NOAEL** 3 mg/kg **LOAEL** --- **Endpoint 2** Maternal liver wt **NOAEL** 3 mg/kg **LOAEL** --- **Endpoint 3** Fetal liver wt **NOAEL** 3 mg/kg **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Chang et al. (2008) **Species, strain, age of animals:** Rats, S-D, M & F, 8-10 wks old **Group size:** 5-15/group **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 15 mg/kg Serum conc 61.58 μg/ml (at 24 hr) **Exposure regimen:** Single dose (sacrifice at various time pts ≤ 24 post dosing) **Other information** This study presents data on malic enzyme mRNA transcripts and activity (not summarize here)	**Endpoint 1** Total serum T4 **NOAEL** --- **LOAEL** 15 mg/kg ↓ **Endpoint 2** Total T3 **NOAEL** --- **LOAEL** 15 mg/kg (at 24 hr) ↓ **Endpoint 3** rT3 **NOAEL** --- **LOAEL** 15 mg/kg (at 24 hr) ↓ **Endpoint 4** Free T4 **NOAEL** 15 mg/kg (at 24 hr) **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Cui et al. (2009) **Species, strain, age of animals:** Rats, S-D, M, ~2 mos. old **Group size:** N = 10/group **Test article and vehicle:** PFOS in Mili-Q water **Route of exposure:** gavage **Exposure levels:** 0, 5, 20 mg/kg/d Blood conc at 28 d 5 mg/kg/d → 72,0 μg/g 20 mg/kg/d → not avialable **Exposure regimen:** Daily for 28 days **Other information** Paper also presents data for tissue distribution	**Endpoint 1** Behavioral abnormalities **NOAEL** --- **LOAEL** 5 mg/kg/d **Endpoint 2** lethality **NOAEL** ? unclear **LOAEL** ? unclear Complete lethality by 26 days for 20 mg/kg/d **Endpoint 3** Body wt **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 4** Food consumption **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓	All 10 rats at 20 mg/kg/d died before 28 d For spleen and brain histopath results, unclear which pathology was observed at the 5 mg/kg/d dose compared to observations at 20 mg/kg/d

	Endpoint 5 Rel. liver wt **NOAEL** --- **LOAEL** 5 mg/kg/d ↑ **Endpoint 6** Rel kidney wt **NOAEL** --- **LOAEL** 5 mg/kg/d ↑ **Endpoint 7** Rel gonadal wt **NOAEL** --- **LOAEL** 5 mg/kg/d ↑ **Endpoint 8** Liver histopathology **NOAEL** --- **LOAEL** 5 mg/kg/d (Cytoplasmic vacuolization, focal/flakelike necrosis) **Endpoint 9** Lung histopathology **NOAEL** ---	

	LOAEL 5 mg/kg/d Pulmonary congestion, focal/diffuse thickening of epithelial walls **<u>Endpoint 10</u>** Kidney histopathology **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d Turbidness/tumefaction in epithelium of proximal convoluted tubules, congestion in renal cortex/medulla, enhanced cytoplasmic acidophelia **<u>Endpoint 11</u>** Spleen histopathology **NOAEL** --- **LOAEL** 5 mg/kg/d (?) Congestion, mild dilation of splenic antrum **<u>Endpoint 12</u>** Brain histopathology **NOAEL** --- **LOAEL** 5 mg/kg/d (?) Focal hyperplasia of gliocytes, dilation/congestion in inferior caval veins of cerebral arachnoid matter, slight focal hemorrhaging	

Reference and Study Design	Results	Comment
Author Curran et al. (2008) **Species, strain, age of animals:** Rats, S-D, 35-37 day old, M, F **Group size:**] 11-15/sex/group **Test article and vehicle:** K-PFOS in feed **Route of exposure:** diet **Exposure levels:** 2, 20, 50, 100 mg/kg feed Intake M – 0, 0.14, 1.33, 3.21, 6.34 mg/kg/d F – 0, 0.15, 1.43, 3.73, 7.58 mg/kg/d Serum conc (μg/g) M – 0.47, 0.95, 13.45, 20.93, 29.88 F – 0.95, 1.50 15.40, 31.93, 43.20 **Exposure regimen:** 28 d **Other information** Study also contains data on RBC deformability and liver fatty acid profiles	**Endpoint 1** Body wt **NOAEL** 20 mg/kg feed **LOAEL** 50 mg/kg feed ↓ (males) 100 mg/kg feed ↓ (females, day 15) **Endpoint 2** Rel organ wts (rel to bw) **NOAEL** Brain – 20 mg/kg feed Liver – 2 M, - F Kidney – 50 M, 20 F Adrenal – 100 Heart – 100 Thyroid – 50 M, F **LOAEL** Brain – 50 mg/kg feed M,F ↑ Liver – 20 M, 2 F ↑ Kidney – 100 M, 50 F ↑ Adrenal - Heart - Thyroid – 100 M, F ↑ **Endpoint 3** Liver pathology **NOAEL** 20 mg/kg feed **LOAEL** 50 mg/kg feed Hepatocyte hypertrophy (M only)	

	Endpoint 4 Blood cell pathology **NOAEL** 100 mg/kg feed - M 50 - F **LOAEL** 100 mg/kg feed – F only RBC, hematocrit, Hb conc ↓ **Endpoint 5** Clinical Chem **NOAEL** 20 mg/kg feed – M, F **LOAEL** 50 mg/kg feed Amylase – F ↑ Bicarbonate – F ↓ Conjug bilirubin - F ↑ Cholesterol - M. F ↓ Lipase – M ↓ Urea – F ↓ (50 but not 100) **Endpoint 6** Thyroid hormones **NOAEL** T3 – 50 mg/kg feed – M, 20 mg/kg feed – F T4 – 2 mg/kg feed – M, F **LOAEL** T3 – 50 mg/kg feed – F, 100 mg/kg feed – M T4 – 20 mg/kg feed – M, F	

Reference and Study Design	Results	Comment
Author Elcombe et al. (2012a) **Species, strain, age of animals:** Rats, S-D, M, 6-7 wks old (at start) **Group size:**] As indicated by endpoint **Test article and vehicle:** K-PFOS **Route of exposure:** diet **Exposure levels:** 0, 20, 100 ppm in diet -, 1.27, 5.62 mg/kg/d Serum conc (μg/ml): ND, 94, 411 **Exposure regimen:** Diet for 28 d ***** **Other information** ***** This study also exposed rats for 1 and 7 days and sacrificed rats on 2, 8, and 29 d. Only 28 d exposures w 29 d sacrifices are reported here.	**Endpoint 1** Body wt (control – n = 30 20 ppm – n = 30; 100 ppm – n = 9) **NOAEL** 20 ppm feed **LOAEL** 100 ppm feed ↓ **Endpoint 2** Food consumption (n = 4-5) **NOAEL** 20 ppm feed **LOAEL** --- **Endpoint 3** Rel liver wt **NOAEL** --- **LOAEL** 20 ppm feed ↑ **Endpoint 4** Plasma liver enzymes (ALT, AST) (n = 9-10) **NOAEL** 20 ppm feed **LOAEL** ---	Stat sig not provided for liver histopathology results.

	Endpoint 5 Plasma cholesterol (n = 9-10) **NOAEL** --- **LOAEL** 20 ppm feed ↓ **Endpoint 6** Plasma triglycerides (n = 9-10) **NOAEL** 20 ppm feed **LOAEL** 100 ppm ↓ **Endpoint 7** Plasma glucose (n = 9-10) **NOAEL** 20 ppm feed **LOAEL** 100 ppm ↓ **Endpoint 8** Liver histopathology (n = 10) **NOAEL** --- **LOAEL** 20 ppm feed Hypertrophy ↑	

Reference and Study Design	Results	Comment
Author Elcombe et al. (2012b) **Species, strain, age of animals:** Rats, S-D, M, 6-7 wks old **Group size:** 40/group **Test article and vehicle:** K-PFOS **Route of exposure:** diet **Exposure levels:** 0, 20, 100 ppm in feed <u>Serum conc</u> (recovery d 1) 39.49 (20 ppm), 140.40 μg/ml (100 ppm), **Exposure regimen:** Diet for 7 d Followed by 1, 28, 56, 84 d of recovery **Other information** Study also presents data on liver biochemical assays related to proliferation and metabolism (not summarized here) **Related studies:** Elcombe et al. (2012a)	**<u>Endpoint 1</u>** Body wt **NOAEL** --- **LOAEL** 20 ppm in feed ↓ (sig on recovery d 21 and 28 only) **<u>Endpoint 2</u>** Food consumption **NOAEL** 100 ppm in feed **LOAEL** --- **<u>Endpoint 3</u>** Rel liver wt **NOAEL** --- **LOAEL** 20 ppm in diet (recovery d 1) ↑ (Also on recovery d 84) **<u>Endpoint 4</u>** Plasma liver enzymes **NOAEL** AST – 100 ppm in feed ALT – no NOAEL **LOAEL** (recovery d 1) AST – no LOAEL ALT – 20 ppm in feed ↓	Note that ↑ liver wt was observed on d 84 of recovery (although not ond 28, 56) PFOS serum conc in control serum not provided

	Endpoint 5 Plasma cholesterol **NOAEL** --- **LOAEL** 20 ppm in feed (recovery d 1) ↓ (also recovery d 28 and recovery d 84 for 100 ppm) **Endpoint 6** Plasma triglycerides **NOAEL** 20 ppm in feed **LOAEL** 100 ppm in feed (recovery d 1) ↓ **Endpoint 7** glucose **NOAEL** 20 ppm in feed **LOAEL** 100 ppm in feed (recovery d 56 only) ↑ **Endpoint 8** Liver histopathology **NOAEL**--- **LOAEL** 20 ppm in feed (hepatocellular hypertrophy – recovery d 1: grade 1; grades 1 & 2 for 100 ppm) ↑ incidence through recovery d 84	

	Endpoint 9 Thyroid histopathology **NOAEL** 100 ppm in feed **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Fair et al. (2011) **Species, strain, age of animals:** Mice, B3C6F1, F, 7-8 wks old **Group size:** N = 5/group **Test article and vehicle:** K-PFOS in Milli-Q water, 0.5% Tween-20 **Route of exposure:** Gavage **Exposure levels:** (as $PFOS^-$) Administered 0, 3.31, 16.6, 33.1, 166 μg/kg/d Total av dose 0, 0.1, 0.5, 1, 5 mg/kg Serum conc ND, ND, 1.16, 2.15, 12.47 μg/ml **Exposure regimen:** Daily, 28 d	**Endpoint 1** Body wt **NOAEL** 166 μg/kg/d **LOAEL** --- **Endpoint 2** Uterine rel wt **NOAEL** 33.1 μg/kg/d **LOAEL** 166 μg/kg/d ↓ Sig for trend **Endpoint 3** histopathology **NOAEL** 166 μg/kg/d (spleen, lung, thymus, liver, adrenals, uterus, kidney) **LOAEL** **---** **Endpoint 4** Glucose, serum **NOAEL** 166 mg/kg/d (1.3 x ↑ but not sig) **LOAEL** ---	Small N

	Endpoint 5 cholesterol **NOAEL** 166 mg/kg/d (27% ↓ but not sig) **LOAEL** --- **Endpoint 6** Thyroid hormones (T3, T4) **NOAEL** 166 mg/kg/d **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Fuentes et al. (2007b) **Species, strain, age of animals:** Mice, CD-1, F, adult **Group size:** N = 8-10/dose/treatment group **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** Gavage (maternal) **Exposure levels:** 0, 6 mg/kg/d w and w/out stress by constraint **Exposure regimen:** GD 12-18	**Endpoint 1** Maternal food/water consumption **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 2** Length of gestation **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 3** Live pups **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 4** Time to physical maturation **NOAEL** --- **LOAEL** 6 mg/kg/d For M testes descent only ↑	

	<u>Endpoint 5</u> Neuromotor development **NOAEL** **---** **LOAEL** 6 mg/kg/d (tail pull resistance - PND 10, 11 (not 12) ↓ Vertical climb, forelimb grip – PND 11 (not 10, 12) ↓ **<u>Endpoint 6</u>** Habituation (open field) **NOAEL** 6 mg/kg/d **LOAEL** **---** **<u>Endpoint 7</u>** Coordination/balance (rotorod) **NOAEL** 6 mg/kg/d **LOAEL** **---**	

Reference and Study Design	Results	Comment
Author Fuentes et al. (2007c) **Species, strain, age of animals:** Mice, CD-1, F, adult **Group size:** N = 8-10 **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 6 mg/kg/d (maternal) **Exposure regimen:** GD 12-18 **Other information** Evaluation of offspring 3 mos post-natal Additional data reported on corticosterone levels **Related studies:** Appears to be continuation of Fuentes et al. (2007a)	**Endpoint 1** Open field activity (rearing, distance traveled) **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 2** Water maze **NOAEL** --- **LOAEL** 6 mg/kg/d (F only – acquisition phase d 3, 4) ↑ distance traveled	Maternal toxicity not determined

Reference and Study Design	Results	Comment
Author Fuentes et al. (2007a) **Species, strain, age of animals:** Mice, CD-1, 3 mos old, M **Group size:** 10/group **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 3, 6 mg/kg/d **Exposure regimen:** Daily for 4 wks	**Endpoint 1** Functional observation battery (CNS activity, neuromuscular function, autonomic function, sensorimotor reactivity) **NOAEL** 6 mg/kg/d (sig ↑ ease of removal for 3, but not 6 mg/kg/d) **LOAEL** --- **Endpoint 2** Open field **NOAEL** --- **LOAEL** 3 mg/kg/d (time spent in center middle 5 min of 15 min total – only)	

Reference and Study Design	Results	Comment
Author Guruge et al. (2009) **Species, strain, age of animals:** Mice B6C3F1, F, 6-7 wks (at PFOS exposure) **Group size:** PFOS-only exposure (sacrifice at 21 d) N = 3 PFOS + virus N = 23-25 **Test article and vehicle:** K-PFOS in Milli-Q water and 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 5, 25 μg/kg/d **Exposure regimen:** Daily for 21 d (21 d prior to influenza A infection) Virus incubated 20 d post-infection	**Endpoint 1** Body wt (PFOS-only) **NOAEL** 25 μg/kg/d **LOAEL** --- **Endpoint 2** Liver wt **NOAEL** 25 μg/kg/d **LOAEL** --- **Endpoint 3** Other organ wts (rel to bw) (spleen, thymus, kidney, lung) **NOAEL** 25 μg/kg/d **LOAEL** **---** **Endpoint 4** Body wt following PFOS + virus infection **NOAEL** --- **LOAEL** 5 μg/kg/d ↓	* Authors report no sig diff (i.e., ↓) in survival between controls and 5 μg/kg/d group. However, graphic shows clear diff.

	<u>Endpoint 5</u> Virus resistance (survival w PFOS + infection – control = infection, but no PFOS) **NOAEL** 5 μg/kg/d ***** **LOAEL** 25 μg/kg/d	

Reference and Study Design	Results	Comment
Author Johansson et al. (2008) **Species, strain, age of animals:** Mice, NMRI, M offspring at 10 d **Group size:** 10/group ***** **Test article and vehicle:** K-PFOS in mixture of egg lecithin and peanut oil **Route of exposure:** gavage **Exposure levels:** 0, 0.75, 11.3 mg/kg **Exposure regimen:** Single dose Testing at 2 and/or 4 mos	**Endpoint 1** Body wt **NOAEL** 11.3 mg/kg **LOAEL** --- **Endpoint 2** Spontaneous behaviour **NOAEL** 0.75 mg/kg **LOAEL** 11.3 mg/kg (locomotion, rearing, total activity – 2 and 4 mos) ↓ **Endpoint 3** habituation **NOAEL** 0.75 mg/kg **LOAEL** 11.3 mg/kg **Endpoint 4** Activity w nicotine challenge **NOAEL** 0.75 mg/kg **LOAEL** 11.3 mg/kg (locomotion, rearing, total activity) ↓	* N = 10/group reported for one behavioral test, but group size does not appear to be given for other tests

	<u>Endpoint 5</u> Performance in elevated plus maze **NOAEL** 11.3 mg/kg/d **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Kim et al. (2011) **Species, strain, age of animals:** Rats, S-D, M, F, 5 wk old **Group size:** 12 M, 12 F/group **Test article and vehicle:** K-PFOS in DMSO diluted w saline **Route of exposure:** Gavage **Exposure levels:** 0, 1.25, 5, 10 mg/kg/d **Exposure regimen:** Daily for 28 d	**Endpoint 1** Body wt **NOAEL** 5 mg/kg/d – F 10 mg/kg/d – M **LOAEL** 10 mg/kg/d – F only ↓ **Endpoint 2** Serum liver enzymes **NOAEL** 5 mg/kg/d **LOAEL** 10 mg/kg/d (AST M only ↑) **Endpoint 3** Serum lipids **NOAEL** 5 mg/kg/d **LOAEL** 10 mg/kg/d (triglycerides, M only ↓) **Endpoint 4** Hematology **NOAEL** 10 mg/kg/d **LOAEL** ---	Stat sig not given for histopathology endpoints

	<u>Endpoint 5</u> Liver wt (rel to bw) **NOAEL** 5 mg/kg/d **LOAEL** 10 mg/kg/d – M and F ↑ **<u>Endpoint 6</u>** Liver histopathology **NOAEL** 1.25 mg/kg/d **LOAEL** 5 mg/kg/d (“fatty change” M only; Hypertrophy and cellular swelling in F only – LOAEL = 10 mg/kg/d)	

Reference and Study Design	Results	Comment
Author Lefebvre et al. (2008) **Species, strain, age of animals:** Rats, S-D, adult, M and F **Group size:** 15 M, 15 F/dose group **Test article and vehicle:** K-PFOS in feed **Route of exposure:** dietary **Exposure levels:** diet 0, 2, 20, 50, 100 mg/kg/feed Intake M - 0, 0.14, 1.33, 3.21, 6.34 mg/kg/d F – 0, 0.15, 1.43, 3.73, 7.58 mg/kg/d Serum conc. 0.47 (control), 0.95, 13.45, 20.93, 29.88 μg/g **Exposure regimen:** 28 d **Other information** This study also presented information (not summarized here) on sub-clinical immunological parameters	**Endpoint 1** Body wt **NOAEL** 20 mg/kg feed - M, F **LOAEL** 50 mg/kg feed – M,F ↓ **Endpoint 2** Rel liver wt **NOAEL** --- F 2 mg/kg feed – M **LOAEL** 2 mg/kg feed – F ↑ 20 mg/kg feed – M ↑ **Endpoint 3** Rel spleen wt **NOAEL** 50 mg/kg feed – F 100 mg/kg feed – M **LOAEL** 100 mg/kg feed – F ↑ **Endpoint 4** Rel thymus wt **NOAEL** 100 mg/kg feed – M, F **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Lopez-Doval et al. (2014) **Species, strain, age of animals:** Rats, S-D, adult, M, **Group size:** 5/group **Test article and vehicle:** K-PFOS in 2.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 0.5, 1.0, 3.0, 6.0 mg/kg/d **Exposure regimen:** Daily for 28 d	**Endpoint 1** Organ wts (rel to bw) (hypothalamus, pituitary, testes) **NOAEL** 6.0 mg/kg/d **LOAEL** --- **Endpoint 2** Serum LH **NOAEL** --- **LOAEL** 0.5 mg/kg/d ↓ **Endpoint 3** Serum FSH **NOAEL** --- **LOAEL** 0.5 mg/kg/d ↑ **Endpoint 4** Serum testosterone **NOAEL** --- **LOAEL** 0.5 mg/kg/d ↓	

	Endpoint 5 Histopathology – hypothalamic neurons **NOAEL** 1.0 mg/kg/d **LOAEL** 3.0 mg/kg/d (reduced size, basophilia of nuclei and cytoplasm) **Endpoint 6** Histopathology – pituitary gonadotrophic cells **NOAEL** --- **LOAEL** 0.5 mg/kg/d (ultrastructural changes) **Endpoint 7** Histopathology - testes **NOAEL** 0.5 mg/kg/d **LOAEL** 1.0 mg/kg/d (interstitial edema, degeneration of sperm heads)	

Reference and Study Design	Results	Comment
Author Martin et al. (2007) **Species, strain, age of animals:** Rats, S-D (CrtcCD(SD)IGS BR), M, 10 wks old **Group size:** 5/group **Test article and vehicle:** K-PFOS **Route of exposure:** gavage **Exposure levels:** 0, 10 mg/kg/d Serum conc 87.7 μg/ml (d-3) **Exposure regimen:** 5 d **Other information** This study also presented data on gene expression (not summarized here)	**Endpoint 1** Body wt **NOAEL** 10 mg/kg/d **LOAEL** --- **Endpoint 2** Rel liver wt **NOAEL** --- **LOAEL** 10 mg/kg/d ↑ **Endpoint 3** Liver histopathology **NOAEL** --- **LOAEL** 10 mg/kg/d (hepatocyte eosinophilia, hepatocyte hypertrophy, non-zonal microvesicular lipid) **Endpoint 4** Serum cholesterol **NOAEL** --- **LOAEL** 10 mg/kg/d ↓	

	Endpoint 5 Serum testosterone **NOAEL** 10 mg/kg/d **LOAEL** --- **Endpoint 6** Total T4 **NOAEL** --- **LOAEL** 10 mg/kg/d ↓ **Endpoint 7** Free T4 **NOAEL** --- **LOAEL** 10 mg/kg/d ↓ **Endpoint 8** Total T3 **NOAEL** --- **LOAEL** 10 mg/kg/d ↓	

Reference and Study Design	Results	Comment
Author Mollenhauer et al. (2011) **Species, strain, age of animals:** Mice, B6C3F1, adult, F **Group size:** 5/group **Test article and vehicle:** K-PFOS in Milli-Q water w 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 0.0331, 0.0993, 9.93 mg/kg/d Total admin dose 0, 1, 3, 300 mg/kg **Exposure regimen:** Daily for 28 d	**Endpoint 1** Body wt **NOAEL** 3 mg/kg/d **LOAEL** 300 mg/kg/d ↓	

Reference and Study Design	Results	Comment
Author Onishchenko et al. (2011) **Species, strain, age of animals:** Mice, C56BL/6/Bkl, adult **Group size:** maternal control, n = 10 PFOS, n = 6 Offspring Control, exposed – n = 8 (1-2 per litter) **Test article and vehicle:** K-PFOS in 95% ethanol **Route of exposure:** Food **Exposure levels:** 0.3 mg/kg/d Offspring brain – 3.1 μg/g Offspring liver – 11.8 μg/g **Exposure regimen:** Maternal GD 1 – delivery	**Endpoint 1** Maternal wt gain **NOAEL** 0.3 mg/kg/d **LOAEL** --- **Endpoint 2** Litter size, sex ratio **NOAEL** 0.3 mg/kg/d **LOAEL** --- **Endpoint 3** Offspring body wt **NOAEL** 0.3 mg/kg/d **LOAEL** --- **Endpoint 4** Offspring brain wt **NOAEL** 0.3 mg/kg/d **LOAEL** ---	

	Endpoint 5 Offspring liver wt **NOAEL** 0.3 mg/kg/d **LOAEL** **---** **Endpoint 6** Locomotor activity **NOAEL** --- **LOAEL** 0.3 mg/kg/d (M only) ↓ **Endpoint 7** Circadian activity **NOAEL** --- **LOAEL** 0.3 mg/kg/d Novel environment (M only) ↓ **Endpoint 8** Elevated plus maze **NOAEL** --- **LOAEL** 0.3 mg/kg/d (various parameters) M only	

	<u>Endpoint 9</u> Muscle strength (hanging wire test) **NOAEL** --- **LOAEL** 0.3 mg/kg/d (M only) ↓ fall latency **<u>Endpoint 10</u>** Motor coordination (accel. rotorod test) **NOAEL** --- **LOAEL** 0.3 mg/kg/d (M and F, but only on some trials)	

Reference and Study Design	Results	Comment
Author Peden-Adams et al. (2008) **Species, strain, age of animals:** Mice, B6C3F1, adult, M, F **Group size:** 5/group (for antigen challenge, 10/group) **Test article and vehicle:** K-PFOS in Milli-Q water w 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** Dose (as $PFOS^-$) 0, 0.166, 1.66, 3.31, 16.6, 33.1, 166 μg/kg/d Total admin dose 0, 0.005, 0.05, 0.1, 0.5, 5 mg/kg Serum conc (ng/g) M – 12.1 (control), 17.8, 91.5, 131, -, -, - * F – 16.8 (control), 88.1, -, 123, 666, -, - * **Exposure regimen:** Daily for 28 d (for antigen challenge – daily for 21 d) **Other information** Study also reports lymphocyte proliferation response, and lymphocyte phenotypes (not summarized here)	**Endpoint 1** Body wt **NOAEL** 166 μg/kg/d **LOAEL** --- **Endpoint 2** Organ wts (rel to bw) **NOAEL** 166 μg/kg/d (spleen, thymus, liver, kidney) **LOAEL** --- **Endpoint 3** Spleen cellularity/cell viability **NOAEL** 166 μg/kg/d **LOAEL** --- **Endpoint 4** Thymus cellularity/cell viability **NOAEL** 166 μg/kg/d **LOAEL** ---	* PFOS serum concentrations indicated by '–' were not reported by authors

	<u>Endpoint 5</u> IgM antigen challenge **NOAEL** M - 0.0166 μg/kg/d F – 3.31 μg/kg/d **LOAEL** M – 1.66 μg/kg/d ↓ F - 16.6 μg/kg/d ↓	

Reference and Study Design	Results	Comment
Author Pereiro et al. (2014) **Species, strain, age of animals:** Rats, S-D, M, adult **Group size:** 10/group **Test article and vehicle:** K-PFOS in 2.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 0.5, 1.0, 3.0, 6.0 mg/kg/d **Exposure regimen:** Daily for 28 d **Other information** Study presents data of effects on corticosterone and ACTH, NOS gene expression and SOD activity (not summarized here)	**Endpoint 1** Rel wt hypothalamus, pituitary **NOAEL** 6.0 mg/kg/d **LOAEL** --- **Endpoint 2** Rel wt adrenal gland **NOAEL** --- **LOAEL** 0.5 mg/kg/d ↓ (although adrenal wt was sig ↓ compared to controls at all doses, adrenal wt ↑ w ↑ dose) **Endpoint 3** Histopathology of fasciculata zona cells of adrenal cortex **NOAEL** 6.0 mg/kg/d ?? * **LOAEL** ---	* Authors report that fasculata zona cells of adrenal cortex did not appear to have "important" morphological or ultrastructural alterations, but then describe the appearance of these cells as "activated" with the presence of liposomes in the cytoplasm.

Reference and Study Design	Results	Comment
Author Qazi et al. (2009b) **Species, strain, age of animals:** Mice, C57BL/6(H-2^b), M, 6-8 wks old Mice, PPARα-null 129/Sv And corresponding wild-type (WT), age? **Group size:** 4/group **Test article and vehicle:** Tetrabutylammonium-PFOS in acetone and mixed w feed **Route of exposure:** diet **Exposure levels:** 0, 0.001%, 0.005%, 0.02% in feed Serum conc (C57BL mice) 0.0287 (control), 50.8, 96.7, 340 μg/ml **Exposure regimen:** 10 d	**Endpoint 1** Body wt (C57BL) **NOAEL** 0.005% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 2** Food consumption (C57BL) **NOAEL** 0.005% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 3** Rel liver wt (C57BL) **NOAEL** --- **LOAEL** 0.001% in feed ↑ **Endpoint 4** Rel thymus wt (C57BL) **NOAEL** 0.005% in feed **LOAEL** 0.02% in feed ↓	* For studies w PPARα-null/WT mice, only 0, 0.005% and 0.02% concentrations in food were used (no 0.001% exposure group)

	Endpoint 5 Rel spleen wt (C57BL) **NOAEL** 0.005% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 6** Epididymal fat wt **NOAEL** 0.005% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 7 *** Abs liver wt (PPARα-null, WT) **NOAEL** PPARα-null – no NOAEL WT – no NOAEL **LOAEL** PPARα-null – 0.005% in feed ↑ WT – 0.005% in feed ↑ **Endpoint 8** Abs thymus wt (PPARα-null, WT) **NOAEL** PPARα-null – 0.005% in feed WT – 0.005% in feed	

	LOAEL PPARα-null – 0.02% in feed ↓ WT – 0.02% in feed ↓ **<u>Endpoint 9</u>** Abs spleen wt (PPARα-null, WT) **NOAEL** PPARα-null – 0.005% in feed WT – 0.005% in feed **LOAEL** PPARα-null – 0.02% in feed ↓ WT – 0.02% in feed ↓ **<u>Endpoint 10</u>** Abs epididymal fat wt (PPARα-null, WT) **NOAEL** PPARα-null – 0.02% in feed WT – 0.005% in feed **LOAEL** PPARα-null – no LOAEL WT – 0.02% in feed	

Reference and Study Design	Results	Comment
Author Qazi et al. (2009a) **Species, strain, age of animals:** Mice, C56BL/6 (H-2^b), M, 6-8 wks old **Group size:** 4/group **Test article and vehicle:** Tetraammonium-PFOS in acetone added to feed **Route of exposure:** diet **Exposure levels:** 0, 0.001%, 0.02% in feed\ Total intake for 0.02% ~6 mg Serum conc by ref to Qazi et al. 2009b **Exposure regimen:** 10 d **Related studies:** Study also presents data on populations of macrophages in different organs/tissues; inflammatory response of macrophages, and *in vivo* cytokine response (not summarized here)	**Endpoint 1** Liver wt **NOAEL** 0.001% **LOAEL** 0.02% in feed ↑ **Endpoint 2** Thymus wt (absolute) **NOAEL** 0.001% **LOAEL** 0.02% in feed ↓ **Endpoint 3** Body wt (0.02% only) **NOAEL** --- **LOAEL** 0.02% ↓ **Endpoint 4** Spleen wt (absolute) **NOAEL** 0.001% **LOAEL** 0.02% ↓	

	Endpoint 5 Epididymal fat wt **NOAEL** 0.001% **LOAEL** 0.02% ↓ **Endpoint 6** Food consumption (0.02% only) **NOAEL** --- **LOAEL** 0.02% ↓ **Endpoint 7** Total WBC count **NOAEL** 0.001% **LOAEL** 0.02% ↓ (sig for lymphocytes, but not for neutrophils)	

Reference and Study Design	Results	Comment
Author Qazi et al. (2010b) **Species, strain, age of animals:** Mice, C57BL6(H-2^b), M, 6-8 wks **Group size:** 4/group **Test article and vehicle:** Tetraammonium-PFOS in water mixed w feed **Route of exposure:** diet **Exposure levels:** 0, 0.005% in feed Serum conc 0.052 (control), 125.8 μg/ml **Exposure regimen:** Diet for 10 d **Other information** Study presents effects on functional properties of isolated B and T cells, hepatic levels of cytokines, and hepatic levels of erythropoietin (not summarized here)	**Endpoint 1** Body wt **NOAEL** 0.005% **LOAEL** --- **Endpoint 2** Food intake **NOAEL** 0.005% **LOAEL** - **Endpoint 3** Rel liver wt **NOAEL** --- **LOAEL** 0.005% ↑ **Endpoint 4** Rel spleen, rel thymus wt, rel epididymal fat pad wt **NOAEL** 0.005% **LOAEL** ---	

	Endpoint 5 Serum liver enzymes **NOAEL** 0.005% (ALT, AST) **LOAEL** 0.005% - ALP ↑ **Endpoint 6** Serum cholesterol (total) **NOAEL** --- **LOAEL** 0.005% ↓ **Endpoint 7** Serum triglycerides **NOAEL** 0.005% **LOAEL** --- **Endpoint 8** Hematological parameters (hematocrit, Hb) **NOAEL** 0.005% **LOAEL** ---	

	Endpoint 9 Liver histopathology **NOAEL** --- **LOAEL** 0.005% (hypertrophy of parenchymal cells, cytoplasmic acidophilic granules)	

Reference and Study Design	Results	Comment
Author Qazi et al. (2010a) **Species, strain, age of animals:** Mice, B6C3F1(H-$2^{b/k}$), M, 7-8 wks old **Group size:** 5/group **Test article and vehicle:** Tetraethylammonium-PFOS **Route of exposure:** diet **Exposure levels:** administered 1.56 μg/kg feed Intake ~250 μg/kg/d Total admin dose ~ 7mg/kg Serum conc Control – 0.0409 μg/ml Exposed – 11.6 μg/ml **Exposure regimen:** Diet for 28 d **Other information** Study presents data on effects on sub-populations of thymic cells (not summarized here)	**Endpoint 1** Body wt **NOAEL** --- **LOAEL** 250 μg/kg/d ↓ **Endpoint 2** Food consumption **NOAEL** 250 μg/kg/d ↑ **LOAEL** **---** **Endpoint 3** Liver wt (rel to bw) **NOAEL** --- **LOAEL** 250 μg/kg/d ↑ **Endpoint 4** Thymus wt, spleen wt (rel to bw) **NOAEL** 250 μg/kg/d **LOAEL** ---	PFOS concentration in diet is reported prior to drying of feed.

	<u>Endpoint 5</u> Specific antigen response **NOAEL** 250 μg/kg/d **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Qazi et al. (2012) **Species, strain, age of animals:** Mice, C57BL/6 (H-2^b), M, 6-8 wks old **Group size:** 4/group **Test article and vehicle:** Tetraammonium-PFOS in water and mixed w feed **Route of exposure:** diet **Exposure levels:** 0, 0.001%, 0.002%, 0.02% in feed **Exposure regimen:** 10 d **Other information** This study also presents data on the effect of PFOS exposure on the populations of B-lymphoid and myeloid cells in bone marrow (not summarized here)	**Endpoint 1** Body wt **NOAEL** 0.002% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 2** Food consumption **NOAEL** 0.002% in feed **LOAEL** 0.02% in feed ↓ **Endpoint 3** Rel liver wt **NOAEL** --- **LOAEL** 0.001% ↑ **Endpoint 4** Rel thymus wt **NOAEL** 0.002% **LOAEL** 0.02% ↓	35% diet restriction resulted in comparable ↓ in body wt, thymus wt, spleen wt, and wt of epididymal fat, but did not affect bone marrow cell number. However, note that for 0.02% PFOS in feed the reduction in food consumption was 24% (not 35%).

	Endpoint 5 Rel spleen wt **NOAEL** 0.002% **LOAEL** 0.02% ↓ **Endpoint 6** Rel epididymal fat **NOAEL** 0.002% **LOAEL** 0.02% ↓ **Endpoint 7** Cellularity of thymus, cellularity of spleen **NOAEL** 0.002% **LOAEL** 0.02% ↓ **Endpoint 8** Cell content of bone marrow **NOAEL** 0.002% **LOAEL** 0.02% ↓	

Reference and Study Design	Results	Comment
Author Qazi et al. (2013) **Species, strain, age of animals:** Mice, C57BL/6 (H-2b), M, 6-8 wks **Group size:** 6-8/group **Test article and vehicle:** Tetraammonium-PFOS in feed **Route of exposure:** diet **Exposure levels:** 0.004% in feed – 10 d exposure 0.0001% in feed – 28 d expousre 10 d exposure - 6 mg/kg/d 28 d exposure – 0.144 mg/kg/d **Exposure regimen:** Dietary, 10 and 28 d **Related studies:** Study also presents data on liver effects of PFOS in conjunction w ConA-induced hepatitis (not summarized here)	**Endpoint 1** Body wt **NOAEL** 6 mg/kg/d – 10 d 0.144 mg/kg/d – 28 d **LOAEL** --- **Endpoint 2** Spleen, thymus, epididymal fat pad (absolute) **NOAEL** 6 mg/kg/d – 10 d 0.144 mg/kg/d – 28 d **LOAEL** --- **Endpoint 3** Liver wt (rel to bw) **NOAEL** 0.144 mg/kg/d – 28 d **LOAEL** 6 mg/kg/d – 10 d ↑ **Endpoint 4** Serum enzymes – AST, ALT **NOAEL** 6 mg/kg/d – 10 d 0.144 mg/kg/d – 28 d **LOAEL** **---**	PFOS concentration in feed measured prior to drying of feed

Reference and Study Design	Results	Comment
Author Qiu et al. (2013) **Species, strain, age of animals:** Mice, ICR, 8 wks old **Group size:** 20/group **Test article and vehicle:** PFOS (salt not reported) in corn oil **Route of exposure:** gavage **Exposure levels:** 0, 0.25, 2.5, 25, 50 mg/kg/d **Exposure regimen:** 28 days **Other information** Serum and testes levels of PFOS reported	**Endpoint 1** Sperm count **NOAEL** 0.25 mg/kg/d **LOAEL** 2.5 mg/kg/d ↓ **Endpoint 2** Testicular histopathology (light microscopy of seminiferous tubules) **NOAEL** 0.25 mg/kg/d **LOAEL** 2.5 mg/kg/d ↑ (Sertoli cell vacuolization, derangement of cell layers) **Endpoint 3** Testicular histopathology (electron microscopy of seminiferous epithelia) **NOAEL** 0.25 mg/kg/d **LOAEL** 2.5 mg/kg/d ↑ (Sertoli cell vacuolization)	

Reference and Study Design	Results	Comment
Author Ribes et al. (2010) **Species, strain, age of animals:** Mice, CD-1, adult, F **Group size:** maternal N = 5/group Offspring N = 10 M,F/treatment group (1-2/ litter) **Test article and vehicle:** 0.5% in Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 6 mg/kg/d **Exposure regimen:** GD 12-18 **Other information** Study also includes measurement of corticosterone in serum **Related studies:** Design and open-filed portion appear to be close to or identical to Fuentes et al. 2007b)	**Endpoint 1** Body wt (offspring) **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 2** Maternal care **NOAEL** 6 mg/kg/d **LOAEL** --- **Endpoint 3** Open field activity **NOAEL** 6 mg/kg/d **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Rogers et al. (2014) **Species, strain, age of animals:** Rats, S-D pregnant **Group size:** Maternal, n = 21 (control and treatment) Offspring, n = 21 litters/group (for bw) 1-2/litter for BP **Test article and vehicle:** In 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 18.75 mg/kg/d **Exposure regimen:** GD 2-6 **Other information** Fostering on unexposed dams	**Endpoint 1** Maternal wt gain **NOAEL** --- **LOAEL** 18.75 mg/kg/d ↓ **Endpoint 2** Birth wt **NOAEL** --- **LOAEL** 18.75 mg/kg/d (F only) **Endpoint 3** Wt gain (offspring) **NOAEL** 18.75 mg/kg/d **LOAEL** --- **Endpoint 4** Systolic blood pressure (offspring) **NOAEL** --- **LOAEL** 18.75 mg/kg/d ↑ (M at 7, 52 wks; F at 37, 65 wks – not 7 wks)	

	<u>Endpoint 5</u> Nephron endowment (offspring) (at 22 d, M only) **NOAEL** --- **LOAEL** 18.75 mg/kg/d ↓	

Reference and Study Design	Results	Comment
Author Rosen et al. (2010) **Species, strain, age of animals:** Mice, wild type-129S1/Svdm, PPARα-null 129S4/Sv]ae-Ppara^{tm1Gomz}/, M, 6-9 mos old **Group size:** 5/group **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 3, 10 mg/kg/d **Exposure regimen:** 7 d **Other information** This study also presents data on gene profiling for WT and null mice (not summarized here)	**Endpoint 1** Rel liver wt **NOAEL** 3 mg/kg/d (WT and null) **LOAEL** 10 mg/kg/d (WT and null) ↑ **Endpoint 2** Liver histopathology **NOAEL** 3 mg/kg/d **LOAEL** 10 mg/kg/d (WT and null) (vacuole formation)	

Reference and Study Design	Results	Comment
Author Ryu et al. (2014) **Species, strain, age of animals:** Mice, Balb/c, pregnant **Group size:** 4-5 M, 4-5 F per group **Test article and vehicle:** In food **Route of exposure:** dietary **Exposure levels:** 4 mg/kg in food Maternal ~0.016-0.024 mg/d/animal Offspring No serum data (PFOA data only) **Exposure regimen:** Maternal - GD 2-lactation Offspring – weaning-12 wks (dietary)	**Endpoint 1** Body wt gain (offspring, 12 wks) **NOAEL** --- **LOAEL** 4 mg/kg feed ↑ **Endpoint 2** Liver enlargement (rel liver weight, offspring) **NOAEL** --- **LOAEL** 4 mg/kg feed ↑ **Endpoint 3** Airway hyperresponsiveness (offspring) **NOAEL** 4 mg/kg feed **LOAEL** --- **Endpoint 4** Airway sensitivity (methacholine challenge in offspring) **NOAEL** --- **LOAEL** 4 mg/kg feed	

	Endpoint 5 Airway allergic hyperresponsiveness (offspring) **NOAEL** 4 mg/kg feed **LOAEL** --- **Endpoint 6** Lung inflammation (offspring) **NOAEL** 4 mg/kg feed **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Sato et al. (2009) **Species, strain, age of animals:** Rats, Wistar, M, 6 to 7 weeks old Mice, ICR, M, 6 to 7 weeks old **Group size:** Neurobehavioral observations = 2 to 3/group (rats and mice) Histopathology = 3/group (rats only) **Test article and vehicle:** PFOS (potassium salt, ≥98% pure) in 2% carboxymethyl cellulose **Route of exposure:** Oral gavage **Exposure levels:** 0, 125, 250, 500 mg/kg Brain, kidney, liver, and serum PFOS concentrations determined 24 hrs after exposure for rats only (not reported herein) **Exposure regimen:** Single exposure **Other information** Neurobehavioral observations made following a daily exposure to ultrasonic stimulus	**Endpoint 1** Body wt (rats and mice) **NOAEL** 125 mg/kg **LOAEL** 250 mg/kg ↓ **Endpoint 2** Brain histopathology (neuronal or glial cells of cerebrum and the cerebellum) Note: no exposure to ultrasonic stimulus **NOAEL** 500 mg/kg **LOAEL** --- **Endpoint 3** Neurobehavioral observation (e.g., excited locomotion, convulsion) **NOAEL** Rats: 125 mg/kg Mice: - **LOAEL** Rats: 250 mg/kg Mice: 125 mg/kg ↑ locomotion	

Reference and Study Design	Results	Comment
Author Wan et al. (2012) **Species, strain, age of animals:** Mice, CD-1, M, 6-8 wks old **Group size:** "≥ 4/group" **Test article and vehicle:** PFOS (salt?) in < 0.4% DMSO and corn oil **Route of exposure:** gavage **Exposure levels:** 0, 1, 5, 10 mg/kg/d **Exposure regimen:** Daily for 21 d (also, 3, 7, 14 d) **Other information** Study data reported at d-3, 7, 14 as well as 21. Only d-21 data are summarized here.	**Endpoint 1** Body wt **NOAEL** 5 mg/kg/d **LOAEL** 10 mg/kg/d ↓ **Endpoint 2** Liver wt **NOAEL** --- **LOAEL** 1 mg/kg/d ↑ **Endpoint 3** Liver size (length) **NOAEL** 1 mg/kg/d **LOAEL** 5 mg/kg/d ↑ **Endpoint 4** Liver triglycerides **NOAEL** 1 mg/kg/d **LOAEL** 5 mg/kg/d	

Reference and Study Design	Results	Comment
Author Wang et al. (2011a) **Species, strain, age of animals:** Mice, BALB/c, M, F, 5-6 wks old (after adaptation period) **Group size:** 8 M, 8F/group Normal diet and high-fat diet groups **Test article and vehicle:** PFOS (salt?) in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 5, 20 mg/kg/d **Exposure regimen:** Daily for 2 wks	**Endpoint 1** Body wt **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓ **Endpoint 2** Food intake **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓ **Endpoint 3** Rel Liver wt **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↑ Fat diet – 20 mg/kg/d ↑	* “fat index” is not defined. Unclear what organ(s) this applies to. For 20 mg/kg/d exposure (normal and fat diet) this is reported as 0. The meaning of this is unclear. Summary effects for this endpoint are as per the text of the paper rather than the tabular results from the table. ** Text notes subtle histopathology changes in thymus at 5 mg/kg/d in regular diet. No data are reported for 5 mg/kg/d for high fat diet.

	<u>Endpoint 4</u> "fat index" ***** **NOAEL** Reg diet – 5 mg/kg/d Fat diet - no NOAEL **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 5 mg/kg/d ↓ **<u>Endpoint 5</u>** Rel. thymus wt **NOAEL** Reg diet – 5 mg/kg/d Fat diet – no NOAEL (M) (for F, NOAEL is 5 mg/kg/d) **LOAEL** Reg diet – 20 mg/kg/d (F) ↓ Fat diet – 5 mg/kg/d (M) ↓ **<u>Endpoint 6</u>** Rel spleen wt **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓	

	<u>Endpoint 7</u> Thymus histopathology ****** **NOAEL** Reg diet – no NOAEL Fat diet - ? ****** **LOAEL** (vasodilation, congestion) Reg diet – 5 mg/kg/d Fat diet - ? ****** **<u>Endpoint 8</u>** Spleen histopathology (dilation of splenic sinus) **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d	

Reference and Study Design	Results	Comment
Author Wang et al. (2014a) **Species, strain, age of animals:** Mice, BALB/c, M, 4-5 wks old **Group size:** 8/group **Test article and vehicle:** PFOS in 0.5% Tween-20 **Route of exposure:** gavage **Exposure levels:** 0, 5, 20 mg/kg/d **Exposure regimen:** Daily for 14 d Mice received either regular or high fat diets	**Endpoint 1** Body wt **NOAEL** Reg diet – 5 mg/kg/d Fat diet – no NOAEL **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 5 mg/kg/d ↓ **Endpoint 2** Food consumption **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓ **Endpoint 3** Rel liver wt **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL** Reg diet – 5 mg/kg/d ↑ Fat diet – 5 mg/kg/d ↑	* "Fat content" is not defined in the paper. This appears to be different from "liver fat content," that is addressed separately. ** Liver pathology was more severe at each dose group for the high fat diet

	<u>Endpoint 4</u> Rel fat content ***** **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓ **<u>Endpoint 5</u>** Liver fat content **NOAEL** Reg diet – no NOAEL Fat diet – 20 mg/kg/d **LOAEL** Reg diet – 5 mg/kg/d ↑ Fat diet – no LOAEL **<u>Endpoint 6</u>** Liver glycogen content **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL** Reg diet – 5 mg/kg/d ↓ Fat diet – 5 mg/kg/d ↓	

	<u>Endpoint 7</u> Liver histopathology **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL **** (hydropic degeneration and vacuolation) Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **<u>Endpoint 8</u>** Serum glucose **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓ **<u>Endpoint 9</u>** Serum triglycerides **NOAEL** Reg diet – 5 mg/kg/d Fat diet – 5 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 20 mg/kg/d ↓	

	<u>Endpoint 10</u> Serum HDL cholesterol **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL** Reg diet – 5 mg/kg/d ↓ Fat diet – 5 mg/kg/d ↓ **<u>Endpoint 11</u>** Serum albumin **NOAEL** Reg diet – no NOAEL Fat diet – no NOAEL **LOAEL** Reg diet – 5 mg/kg/d ↑ Fat diet – 5 mg/kg/d ↑ **<u>Endpoint 12</u>** Serum cholesterol **NOAEL** Reg diet - 5 mg/kg/d Fat diet – no NOAEL **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – 5 mg/kg/d ↓	

	<u>Endpoint 13</u> Serum LDL cholesterol **NOAEL** Reg diet - 5 mg/kg/d Fat diet – 20 mg/kg/d **LOAEL** Reg diet – 20 mg/kg/d ↓ Fat diet – no LOAEL	

Reference and Study Design	Results	Comment
Author Yu et al. (2009b) **Species, strain, age of animals:** Rats, Wistar, adult, F **Group size:** Dams - N = 20 (control, exposed) Pups – 5 M, 5 F per treatment group **Test article and vehicle:** K-PFOS in 0.5% Tween-20 **Route of exposure:** dietary **Exposure levels:** 3.2 mg/kg feed Serum conc. (range over time) - gest exp only M = 3.78-0.41 μg/ml F = 3.78-1.02 - lact exp only M = 1.22-6.64 F = 1.22-7.04 - gest + lact exp M = 10.6 F = 11.5 **Exposure regimen:** Exposure from diet from GD 0 – PND 0-35 Full cross-fostering design (pups cross-fostered w exposed dams received PFOS diet post-weaning)	**Endpoint 1** Body wt (pups) **NOAEL** 3.2 mg/kg feed **LOAEL** --- **Endpoint 2** Rel. liver wt **NOAEL** --- **LOAEL** 3.2 mg/kg feed ↑ **Endpoint 3** Total T3 **NOAEL** 3.2 mg/kg feed (all exposure groups) **LOAEL** --- **Endpoint 4** Total T4 **NOAEL** --- **LOAEL** 3.2 mg/kg feed ↓ (gest, lact, gest + lact)	Maternal toxicity determined in a separate, preliminary experiment

	<u>Endpoint 5</u> Reverse T3 **NOAEL** 3.2 mg/kg feed **LOAEL** ---	

Reference and Study Design	Results	Comment
Author Zheng et al. (2009) **Species, strain, age of animals:** Mice, C57BL/6, M, 8-10 wks old **Group size:** 12/group **Test article and vehicle:** K-PFOS in deionized water and 2% Tween-80 **Route of exposure:** gavage **Exposure levels:** 0, 5, 20, 40 mg/kg/d Serum conc ND (control), 110.46, 280.65, 338.01 μg/ml **Exposure regimen:** 7 d **Other information** This study also presents data on serum corticosterone, lymphocyte immunophenotypes, NK cell function (not summarized here)	**Endpoint 1** Body wt **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 2** Food intake **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 3** Rel spleen wt **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 4** Rel thymus wt **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓	

	Endpoint 5 Rel liver wt **NOAEL** --- **LOAEL** 5 mg/kg/d ↑ **Endpoint 6** Spleen/thymus cellularity **NOAEL** 5 mg/kg/d (for both organs) **LOAEL** 20 mg/kg/d (for both organs) ↓ **Endpoint 7** Lymphocyte proliferation and plaque formation (in response to antigen challenge) **NOAEL** --- **LOAEL** 5 mg/kg/d ↓	

Reference and Study Design	Results	Comment
Author Zheng et al. (2011) **Species, strain, age of animals:** Mice, C57BL/6, M 8-10 wks old **Group size:** 12/group **Test article and vehicle:** K-PFOS in deionized water and 2% Tween-80 **Route of exposure:** gavage **Exposure levels:** 0, 5, 20 mg/kg/d Serum conc ND (control), 97.25, 250.34 μg/ml **Exposure regimen:** 7 d **Other information** This study presents data on serum corticosterone levels, interleukin levels, cytokines (not summarized here)	**Endpoint 1** Body wt **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 2** Food intake **NOAEL** 5 mg/kg/d **LOAEL** 20 mg/kg/d ↓ **Endpoint 3** Rel spleen, rel thymus wt **NOAEL** 5 mg/kg/d (for both organs) **LOAEL** 20 mg/kg/d (for both organs) ↓ **Endpoint 4** Rel liver wt **NOAEL** --- **LOAEL** 5 mg/kg/d ↑	

	<u>Endpoint 5</u> Serum IgM **NOAEL** --- **LOAEL** 5 mg/kg/d ↓ **<u>Endpoint 6</u>** Serum IgG **NOAEL** --- **LOAEL** 5 mg/kg/d ↑ (not sig diff from control for 20 mg/kg/d)	

Appendix 6: Epidemiology evidence tables

Reference and Study Design	Exposure Measures	Results	Comment
Study: **Alexander and Olsen (2007)** "Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Ann Epidemiol. 2007 Jun;17(6):471-8 **Study Design:** Information on cases (current and deceased) of bladder cancer among current and former employees. Combinatio of self-reporting (with physician follow-up) and death certificate data. Follow-up 1970-2002 **Location:** Decatur, AL **Population:** Same population as Alexander et al. (2003) – workers in 3M Decatur facility. ≥365 cumulative days of employment prior to 1998. 1,400/2083 current employees responded, plus death certificate data on 185/188 decedents.	**Exposure Assessment:** Same as in Alexander et al. (2003). Assignment of exposure by job title based on limited biomonitoring of serum PFOS in Olsen (2003b) **Population-Level Exposure:** - Non-expousre – 0.11-0.29 µg/ml - Low– 0.39-0.89 µg/ml - High – 1.30-1.97 µg/ml Cumulative exposure estimated on basis of summation of weighted assigned to job titles on basis of exposure potential: - Non = 1 - Low = 3 - High = 10	**Stat Method:** SIRs calculated based on exposure categories; and by weighted cumulative exposures Rate ratios calculated based on Non-exposure category as internal referent and SIRs based on US pop. Incidence data **Outcome:** Confirmed bladder cancer cases **Major Findings:** Cases were more likely to have smoked regularly compared to non-cases (83% vs. 56%). However, similar to national smoking rates 11 total cases of bladder cancer observed 8.6 expected (SIR = 1.28; CI = 0.64-2.29; **not sig**) - 2 (18%) of cases were "Non-exposed" - 9 (82%) of cases worked in L or H exposure job. 6 of these for ≥1 yr - 3 (27%) worked in H exposure job ≥1 yr SIRs = 1.12-2.26 for the exposure groups (highest SIR for L exp group)	**Major Limitations:** Exposure classification based on correspondence of job category to exposure levels (serum PFOS). However, correspondence was based on a sample of 186 = 12% of the number of respondants. Variability for some job categories was high including some with high PFOS exposure (95% UCI/geom.mean ≈ 3) (Olsen et al. 2003b)). "No-exposure" category is 5.5 times the median serum PFOS reported by NHANES = 0.02 ppm (Fourth National Report on Human Exposure to Environmental Chemicals; http://www.cdc.gov/exposurereport/pdf/fourthreport.pdf) Thus, use of "no-exposure" category as referent will bias against finding significantly elevated risk ratios based on No-exposures as internal referenants. **Other comments:** This study was straightforward in terms of case definition and ascertainment, However, exposure assessment is subject to uncertainty due to small biomonitoring sample size, significant variability of serum PFOS within exposure categories and sig background exposure in "No-exposure" referants. Lack of clear evidence of elevated bladder cancer as a function of exposure. However, consistently elevated (but not sig) risk for exposed workers.

Reference and Study Design	Exposure Measures	Results	Comment
73.9% response relative to eligible (43,739 person-yrs of follow-up) **Related Studies:** Alexander et al. (2003)		Highest SIR for cumulative exp = 2.72 for 5-10 yrs exposure in H exp job (CI = 0.55-73.95; **not sig**) Rate ratios for cumulative exp for 5-10 yrs and >10 yrs exposure = 1.92 and 1.52 (**not sig**) (based on internal referent grouo) Sensitivity analysis for inclusion of non-respondants assuming doubling of expected bladder cancer rate. Overall SIRs not sig.	

Reference and Study Design	Exposure Measures	Results	Comment
Study: *Alexander et al. (2003)* ***Study Design:*** *Mortality study linking employment records with cause of death-specific vital records search. Comparison to sister plant with no specific PFC exposure and to AL state and local counties mortality* ***Location:*** *3M plant, Decatur, AL* ***Population:*** *All employees working ≥365 days by end of 1997 with a verified death certificate* *M = 83% (84% of H exposure)* ***Related Studies:*** *Olsen et al.(2003a)* *Olsen et al. (2003b)* *Olsen et al.(2004)* *Grice et al. (2007)* *Alexander et al. (2007)* *Olsen et al. (2012)*	***Exposure Assessment:*** *Assignment of exposure by job title based on limited biomonitoring of serum PFOS in Olsen (2003b)* ***Population-Level Exposure:*** *Exposure Category* - *Ever-H – n = 982 (47%)* - *Ever-L, but Never-H – n = 298 (14%)* - *Ever No/minimal exposure – n = 812 (39%)*	***Stat Method:*** *Calculation of SMR adjusted for age, gender and calendar period.* ***Outcome:*** *All-cause and specific cause mortality* ***Major Findings:*** *All-cause mortality* - *Total - SMR = 0.63* - *Ever H – SMR = 0.69* - *Ever L, but never H – SMR = 0.64* - *Ever No/minimal – SMR = 0.60* - *<1.0 for ≥ 1 yr H or Ever L* *All cancer mortality* - *Total – SMR = 0.72* - *Ever H – SMR = 0.84* - *Ever L, but never H – SMR = 0.52* - *Ever No/minimal – SMR = 0.73* - *SMR <1.0 for ≥ 1 yr H or Ever L* *Liver cancer* *SMR = 1.61 (2 obs. vs. 1.24 expected) – not stat. sig.* *Bladder cancer* *SMR = 4.81 (border line stat. sig – lower CI = 0.99) 3 obs. vs. 0.62 expected. All M, all worked H exposure job for ≥ 5 yr. SMR for ≥5 yrs = 25.5 (3 obs. vs. 0.12 expected)*	***Major Limitations:*** *Significant co-exposure to PFOA.* *Exposure classification based on correspondence of job category to exposure levels (serum PFOS). However, correspondence was based on a sample of 186 = 13% of the number of questionnaire respondents. Variability for some job categories was high including some with high PFOS exposure (95% UCI/geom.mean ≈ 3) (Olsen et al. 2003b)).* *Observation of high SMR for bladder cancer rests on only 3 observations.* *Mortality as an endpoint does not address the full potential range of adverse outcomes.* ***Other comments:*** *The cause-of-mortality data collection and ascertainment were well conducted and appear to be reasonably comprehensive. The exposure assignment was based on a relatively small sample and could not control for confounding by (e.g.) smoking.*

Study:	**Exposure Assessment:**	**Stat Method:**	**Major Limitations:**
Andersen et al. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. Am J Epidemiol. 172(11):1230-7.. Erratum in: Am J Epidemiol. 2011 Jun 15;173(12):1475. **Study Design:** Danish National Birth Cohort Blood sample collected during regular antenatal care visit during 1st trimester. Telephone interviews - preg. wks 16 and 30 and 6 and 18 mos postnatal Self-reported data on maternal pregnancy wt. and ht. → BMI Birthweight and gestational age from Danish Nat'l Birth Reg. Child wt and length obtained from mothers based on recorded information in child's data book entered by physician and kept by mother **Location:** Denmark	Maternal Plasma PFOS and PFOA by HPLC-MS **Population-Level Exposure:** <u>PFOS</u> (ng/ml) median = 33.4 IQR = 17.2 Range = 6.4-106.7 <u>PFOA</u> (ng/ml) Med. = 5.21 IQR = 3.06 Range = 0.5-21.9	Multiple linear regression of wt, length and BMI (as z-scores) against PFOS (and PFOA) Co-variates – maternal age; parity; pregnancy BMI; smoking during pregnancy; SES; geststional wk at blood samples; duration of breastfeeding; child's exact age at measurements; wt, length, BMI at 5 mos (for models at 12 mos). Child's sex, in stratified analyses. Exclusion of one hig-value outlier for PFOA **Outcome:** Children's wt, length and BMI as function of PFOS (PFOA) and co-variates **Major Findings:** <u>All Children</u> PFOS Sig. inverse assoc. with wt (adjusted, but not crude model *****) Sig. inverse assoc. BMI at 12 mos.(adjusted and crude models *****) PFOA Sig. inverse assoc with birth wt. (crude and adjusted models) ***** crude model – adjusted for child's exact age at measurement only Adjusted model – as detailed above	Significant co-exposure to PFOA. Although outcomes associated with PFOS and PFOA did not completely overlap (little effect of PFOA at 12 mos), interactions between PFOS and PFOA were not investigated. Maternal self-reporting of wt and length data. However, data were generated by physicians and provided to mothers using a formal and common format. Fetal exposure estimated from maternal blood sample from first trimester. Variability in maternal fetal transfer and changes in maternal exposure after 1st trimester introduce some uncertainty in the exposure assessment. However, resulting exposure misclassification would tend to bias outcomes away from observing relationships between plasma PFOS and infant measures of growth. **Other comments:** This was a well designed and conducted longitudinal cohort study using well supported and standardized databases and a reasonable surrogate of fetal gestational exposure (1st trimester maternal plasma PFOS and PFOA). Co-exposure to PFOA prevents clear conclusions about the independent influence of PFOS.

Population: 1,400 mothers with 1st trimester blood samples, and 4 telephone interviews 1,147 w weight and height data children at 5 mos.; 1,076 w wt and ht data at 12 mos. **1010 with data at both time points** **Related Studies:** Fei et al. (2008) Fei et al. (2007) Andersen et al. (2013)		****** crude model – adjusted for gestational age (quadratic and linear terms) Adjusted model – as detailed above <u>Boys only</u> PFOS Sig. inverse assoc w wt at 12 mos (adjusted model only) Sig inverse assoc w BMI at 12 mos (crude and adjusted models) PFOA Sig. inverse assoc w birth wt (crude and adjusted models Sig inverse assoc w wt at 5 mos (adjusted model only) Sig inverse assoc w BMI at 5 mos (adjusted model only) Sig inverse assoc w BMI at 12 mos (crude model only) <u>Girls only</u> PFOS Sig. inverse assoc w birth wt (crude and adjusted models) PFOA Sig inverse assoc w birth wt (crude model only) <u>Breastfeeding</u> Duration of breastfeeding as a co-variate did not produce sig changes in βs for wt or BMI. Thus, effects at 12 mos do not appear to be due to continued exposure through breast milk	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Andersen et al. (2013) Andersen CS, Fei C, Gamborg M, Nohr EA, Sørensen TI, Olsen J. Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. Am J Epidemiol. 2013 Sep 15;178(6):921-7. **Study Design:** Danish National Birth Cohort 1996-2002 Blood sample collected during regular antenatal care visit during 1st trimester. Telephone interviews - preg. wks 16 and 30 and 6 and 18 mos postnatal **Mailed questionnaire during month child turned 7 years old** Self-reported data on height weight, waist cirmcumference - 33% obtained by school physician, public health nurse, or personal physician - 67% obtained by another person (usually parents) Birthweight and gestational age from Danish Nat'l Birth Reg.	**Exposure Assessment:** Maternal plasma PFOS and PFOA by HPLC-MS Apparently utilized 1st trimester blood sample data from Andersen et al. (2010) **Population-Level Exposure:** <u>PFOS</u> (ng/ml) median = 33.8 IQR = 17.6 Range = 6.4-106.7 <u>PFOA</u> (ng/ml) Med. = 5.25 IQR = 2.99 Range = 0.5-21.9	**Stat Method:** Multiple linear regression of BMI, waist circum and risk of overweight (as z-scores) against PFOS (and PFOA) as continuous or categorical variables Lowest quartile of PFOS (PFOA) used as reference group for categorical variables Analyses stratified by sex <u>Covariates</u> Maternal age Parity Maternal pregnancy BMI Smoking during pregnancy SES Preg wk at blood draw Gestational wt gain Child's brith wt Duration of breastfeeding Child's wt at 5 and 13 mos **Outcome:** Children's BMI, waist circum. and risk of overweight at 7 yrs Overweight defined at 7 yrs from Int'l Obesity Taks Force <u>cutpoints</u> Boys = 17.92 kg/m^2 Girls = 17.75 kg/m^2	**Major Limitations:** Relatively low (~58%) retention of original cohort from Anderson et al. (2010). Possible self-selection bias. Sig co-exposure to PFOA BMI and waist circumference measurements taken by different sources (some medical personnel, some parents) Population exposure to PFOS appears high relative to US population (although direct comparison is difficult) – Med PFOS = 33.8 – based on 4th annual NHANES for 12-19 yr old, this is equivalent to bet 75th and 90th percentiles. Therefore, comparison of upper quartiles to lowest quartiles may underestimate changes relative to background exposure. Does not appear that regression analyses controlled for PFOA in analysis of PFOS **Other comments:** The major weakness in this study is the co-exposure to PFOA and apparent failure to control analysis of PFOS for PFOA. In addition, measurements by parents were not standardized leading to potential for error (but not necessarily bias) in endpoint determination

Location: Denmark **Population:** 1,400 mothers with 1st blood sample, and 4 telephone interviews from Andersen et al (2010) eligible for this 7 yr follow-up if provided information on - Height and wt (n = 811) Or - Waist circumference (n = 804) ~58% recruitment of original cohort **Related Studies:** Fei et al. (2008) Fei et al. (2007) Andersen et al. (2010)		**Major Findings:** No differences with original cohort for PFOS (PFOA), maternal age, preg BMI, preg wt gain, or child's growth measures. However, sig. differences with original cohort Original cohort mothers "slightly" older, higher preg BMI, and higher birth wt **No sig effect of PFOS (PFOA) on BMI or waist circumference for boys or girls**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Apelberg et al.(2007) Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007 Nov;115(11):1670-6. **Study Design:** Cross-sectional, All singleton, live births at Johns Hopkins U. Hospital bet 11/26/2004 and 3/16/2005 Major congenital abnormalities excluded Cord blood collected Maternal characteristics and infant anthropometric data obtained from hospital medical records Birth wt, length, head circum., Ponderal index (birth wt/length^3 x 100) **Location:** Baltimore, MD	**Exposure Assessment:** PFOS, PFOA and other PFCs by HPLC-MS LOD for PFOS and PFOA = 0.2 ng/ml **Population-Level Exposure:** PFOS detected in >99% of samples (PFOA in 100%) PFOS median conc = 5 ng/mL [range, < LOD (0.2) to 34.8 ng/mL] PFOA median conc = 1.6 ng/mL (range, 0.3 to 7.1 ng/mL)	**Stat Method:** Univariagte and multivariate linear regression analysis of assoc. of PFOS and PFOA on: gestational age; birthwt; length, head circumference; ponderal index Conc's below LOD set to LOD for regression analysis <u>Co-variates</u> For gestational age – smoking status, age, race, prepregnancy BMI, previous preterm birth, diabetes,hypertension. For birthweight and birth size – smoking status, age, gestational age, race, prepregnancy BMI, net weight gain during pregnancy (weight gain minus birth weight), height, parity, infant sex, diabetes, hypertension Investigated interaction term between PFOS (PFOA) and birth mode (vaginal and Caesarian) Analysis w and w/out controlling for total lipids, total cholesterol, triglycerides For subjects (<4%) with missing data on preg wt., height or wt gain, median values were imputed	**Major Limitations:** 50% of births meeting other inclusion criteria did not have a cord blood sample or had too small a blood sample volume and were, therefore, excluded from the study. Births without useable blood samples had lower gestational age and birth wt.(sig?). This could bias findings of study against finding assoc. with these outcomes. Sig co-exposure to PFOA with similar associations. Unclear whether PFOS results reflect control for PFOA. **Other comments:** This is a cross-sectional study. However, direct contact with mothers allowed control of key co-variates including smoking (based on cotinine concentration). The main weaknesses of this study are: 1. the co-exposure to PFOA and lack of clarity as to statistical control for PFOA in effects associated with PFOS 2. Loss of 50% of subjects from full cohort and differences between full cohort and lost subjects in outcome variables

Population: n = 293 **Related Studies:**		**Outcome:** **Major Findings:** Assoc. of PFOS with anthropometric measures <u>Birthweight</u> – Stat sig decrease in birthwt only with model adjusted for gestational age (but not other co-variates) <u>Head circumference</u> – Stat sig decrease for full adjusted model and for gestational age adjust only Inclusion of (sig) interaction term with mode of delivery (vaginal/Cesarean) limited assoc to vaginal births <u>Ponderal Index</u> – Stat sign decrease for univariate, gestational age adjust only, and fully adjusted models Note: PFOA showed essentially the same relationships with approx. the same coefficients. <u>Total serum cholesterol, total lipids, triglycerides</u> - No sig assoc with PFOS (PFOA)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Audet-Delage (2013) Audet-Delage Y1, Ouellet N, Dallaire R, Dewailly E, Ayotte P. Persistent organic pollutants and transthyretin-bound thyroxin in plasma of Inuit women of childbearing age. Environ Sci Technol. 2013 Nov 19;47(22):13086-92. doi: 10.1021/es4027634. Epub 2013 Nov 11. **Study Design:** Archived plasma samples from 2004 study Regression of T4-TTR (transthyretin-bound T4) levels against PFOS (and OH-PCBs and chlorophenols) (Note: transthyretin is one of the T4 transport protein in plasma) **Location:** Nunavik, Quebec **Population:** Inuit women previously participating in 2004 cross-sectional study 18-39 yrs old Restrictions – pregnant, use of thyroid medication	**Exposure Assessment:** PFOS by LC-MS/MS (OH-PCBs and chlorophenols by GC-MS) LOD = 0.10 ng/ml Plasma conc of contaminants <LOD reported as LOD/2 (Note; LODs not reported) T4-TTR measured by polyacrylamide gel electrophoresis **Population-Level Exposure:** PFOS detected in 100% of samples Geom mean = 10.92 ng/ml 95% CI = 9.84-12.13 ng/ml Range = 2.30-97.00 ng/ml OH-PCB conc geom mean = 0.11-0.02 ng/ml (for 10 congeners) Pentachlorophenol geom mean = 0.80 ng/ml Tetrachlorophenol geom mean = 0.21 ng/ml PFOS plasma conc in this population is in the range of US adult pop based on 4th NHANES Biomonitoring Report	**Stat Method:** Multiple linear regression models created separately for PFOS, OH-PCBs and chlorophenols <u>Co-variates</u> Total T4, Total thyroid binding globin (TBG), Total TTR, Plasma lipids Age, BMI, smoking status, alcohol, total marine food (g/d), education level **Outcome:** T4-TTR **Major Findings:** PFOS not a sig determinant of T4-TTR in regression model (likewise PCB-OH, and chlorophenols)	**Major Limitations:** T4-TTR levels in this population were lower than expected based on other populations. Although it does not appear that PFOS (or PCB-OH, or chlorophenols) influenced these levels, there are other contaminants not measured in this study that could have competed with TTR for T4 binding. In the absence of these competitors, PFOS might have significantly competed with TTR for T4 binding. **Other comments:** This is a well conducted study with good control for known co-variates and a reasonable sample size. The exposure of this population to other POPs at high in the Arctic environment could have confounded assessment of the ability of PFOS to bind T4. However, overall the study did not indicate decreased T4 due to PFOS.

N = 120 - randomly selected from eligible pop. **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Bloom et al. (2010) Bloom MS1, Kannan K, Spliethoff HM, Tao L, Aldous KM, Vena JE. Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol Behav. 2010 Feb 9;99(2):240-5. doi: 10.1016/j.physbeh.2009.02.005. Epub 2009 Feb 10. **Study Design:** Nested cross-sectional study "Hypothesis screening" investigating associations between 8 PFCs (incl. PFOS) and TSH and free T4 (FT4) in sub-population from NY State Angler's Cohort Study cohort Blood sample and survey questionnaire (sportfish, game, lifestyle, demographics, medical conditions) completed 1995-1997. **Location:** NY State **Population:** 31 of 38 cohort members previously selected on the basis of high level sportfish consumption	**Exposure Assessment:** Analysis of TSH and FT4 from archived serum samples in 2003 by immunoassay Analysis of PFC from archived serum samples in 2006 PFOS PFDA PFNA PFOA PFHpA PFUmDA PFHxS PFOSA Analysis by Electrospray tandem MS (ESj-MS/MS) LOD for PFOS = 2.00 ng/ml (LOD for other PFC were ≤LOD for PFOS by ≥10x) **Population-Level Exposure:** PFOS geom mean = 19.57 (7.25-76.88) ng/ml 83% of total PFCs PFOS serum concentration consistent with NHANES levels from 4th National Report on Human Exposure to Environmental Chemicals PFOS sig correlated with PFDA (r = 0.7); PFNA (0.53).	**Stat Method:** Multiple linear regression for total PFCs and individual PFCs <u>Covariates</u> Included if p<0.1 in bivariate analysis Variables examined for potential inclusion in models: Age, BMI, gender, smoking, self-reported sportfish consumption **Outcome:** Assoc of PFOS (and other PFCs) with TSH and FT4 **Major Findings:** **Neither TSH, or FT4 associated with PFOS (or other PFCs) in multiple linear regression**	**Major Limitations:** Authors suggest that pop size would need to be increased 9x and 3x in order to achieve 80% power to detect sig associations for TSH and FT4 (respectively) at observed effect size. Thus, study appears to be underpowered. Due to small n, study did not conduct simultaneous regression modeling of all measured PFCs. Thus, PFOS analysis did not control for pos or neg effects of other PFCs on PFOS assoc with TSH or FT4. **Other comments:** Study was well conducted, but was limited by small sample size

N = 31 (4 F) Mean age = 39 (31-45) yrs No history of thyroid or goiter problems **Related Studies:**	Non-sig assoc with PFOA (r = 0.35)		

Reference and Study Design	Exposure Measures	Results	Comment
Study: Bonefeld-Jorgensen et al. (2011) Bonefeld-Jorgensen EC1, Long M, Bossi R, Ayotte P, Asmund G, Krüger T, Ghisari M, Mulvad G, Kern P, Nzulumiki P, Dewailly E. Environ Health. 2011 Oct 6;10:88. doi: 10.1186/1476-069X-10-88. Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. **Study Design:** Case-control Cases – 80% of **breast cancer** cases in Greenland 2000-2003 Controls – from study of POP exposure and Artic Monitoring and Assessment Prgm (AMAP) Age, district-matched to cases Blood samples on diagnosis (cases) or on enrollment (controls) Analysis blind to disease status Plasma fatty acids Serum cotinine Serum 17β-estradiol Measurement of ER, AR, and AhR transactivaties	**Exposure Assessment:** PFOS extraction by ion pairing Analysis by LC-MS-MS w electrospray ionization LOD = 0.1-0.4 ng/ml **Population-Level Exposure:** PFOS median conc - cases = 45.6 ng/ml - controls = 21.9 ng/ml (NOTE: PFOS concs ~ 2.5 -5 x current US F (NHANES 4th Rpt)	**Stat Method:** PFOS and other vars ln-transformed OR from unconditional logistic regression <u>Co-variates considered</u> - age - BMI - no.full term pregnancies - breastfeeding - menopausal status - serum cotinine Included in model if $\Delta\beta > 15\%$ **Outcome:** OR for breast cancer as function of unit increase in PFOS **Major Findings:** (adj model) **OR for breast cancer per unit PFOS sig > 1.0** (OR = 1.03, p = 0.05) (OR for unadj analysis not sig >1.0)	**Major Limitations:** Small n for cases (9 for PFOS OR analysis) PFOS analysis not adj for PFOA or other PFCs **Other comments:** Case-control study Small N Sig, but small effect (However, see Ghisari et al. follow-up study) Relatively high exposure

Location: Greenland **Population:** Greenland Inuit F Full N: Cases – n = 31 Controls – n = 115 N for PFOS OR analyses: <u>Unadj analysis</u> Cases = 31 Controls = 98 **<u>Adj analysis</u>** **Cases= 9** **Controls = 69** **Related Studies:** Ghisari et al. (2014)			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Caserta et al. (2013) Caserta D, Ciardo F, Bordi G, Guerranti C, Fanello E, Perra G, Borghini F, La Rocca C, Tait S, Bergamasco B, Stecca L, Marci R, Lo Monte G, Soave I, Focardi S, Mantovani A, Moscarini M Correlation of endocrine disrupting chemicals serum levels and white blood cells gene expression of nuclear receptors in a population of infertile women.. Int J Endocrinol. 2013;2013:510703. doi: 10.1155/2013/510703. Epub 2013 Apr 21. **Study Design:** Lifestyle questionnaire Exclusions: - smoking - vegetarian diet - occup exposure to EDCs - BMI > 30 - inflammatory/infectious disease - diagnosis of M infertility factor Blood sample - for infertile, collection before hormone treatment Nuclear receptor gene expression determined on peripheral blood mononuclear cells (PBMNCs)	**Exposure Assessment:** Liquid-liquid separation HPLC w electrospray ionization-MS PFOS LOD = 0.4 ng/ml **Population-Level Exposure:** % > LOD - infertile = 32.4 - fertile = 18.2	**Stat Method:** Comparison of normally distrib variables compared w t-test, non-normally distrib var by Mann-Whitney U test. Chi-sq and Fisher for comparison of rates and proportions **Outcome:** Assoc PFOS w fertility status **Major Findings:** **No sig diff** in % PFOS detects between fertile and infertile women **Outcome:** Assoc PFOS w nuclear receptors **Major Findings:** <u>Infertile</u> PFOS **sig corr** w AR (r = 0.236) (androgen receptor) and PXR (r = 0.239) (not w ERα, ERβ, AHR PPARγ) <u>Fertile</u> PFOS not sig corr w any nuclear receptor	**Major Limitations:** Low level of PFOS detects (LOD mod high) Comparison of PFOS conc by fetility status based on prop <> LOD rather than continuous data **Other comments:** Small prop PFOS detects

Location: Rome, Ferrara, Sora; Italy **Population:** Infertile **n = 111** F, 18-40 Enrolled in IVF clinics Recruited 6/09-4/10 Fertile **n = 44** F 18-40 Spontaneous preg in prev year Regular menstrual cycle Stopped breastfeeding ≥ 6 mos prev **Related Studies:**			

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>
Study:

Chan et al. (2011)

Chan E, Burstyn I, Cherry N, Bamforth F, Matrin JW.
Perfluorintated acids and hypothyroxinemia in pregnant women.
Environ Res. 2011 May; 111(4): 559-64
doi: 10.1016/j.envres.2011.01.011.
Epub 2011 Feb 9.

Study Design:

Matched case-control.

<u>Cases</u>- Normal TSH, no hyperthyroidism, free T4 in lowest 10th percentile of samples
N=96

<u>Controls</u>- Normal TSH, free T4 in 50th-90th percentile of samples
N=175

<u>Matching-</u> Cases matched to 1-3 controls each based on: Referring physician; maternal age (+/-3 yrs)

Location:
Edmonton, Alberta, Canada

Population:
Pregnant women providing second trimester blood samples in conjunction
</td>
<td>
Exposure Assessment:

Serum TSH and free T4 by chemoluminescent immunoassay – “standard laboratory procedure”

CV for TSH at lowest conc.=10%, CV at greater values=2.7%

CV for free T4=3-4%

PFOS, PFOA, and PFHxS by HPLC- triples quadripole MS LOD (for ea.) =0.25 ng/ml

PFC measurement precision demonstrated in QC analyses

Population-Level Exposure:

Geom. Mean (nmol/L)
<table>
<tr><th></th><th>PFOS</th><th>PFOA</th><th>PFHxS</th></tr>
<tr><td>cases</td><td>14.15</td><td>3.10</td><td>2.86</td></tr>
<tr><td>controls</td><td>15.18</td><td>3.32</td><td>2.59</td></tr>
</table>
(PFOS conc in ng/ml=
Cases-7.08
Controls-7.50)
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Stat Method:

PFC conc <LOD enetered as ½ LOD

OR by conditional logistic regression

Co-variates- maternal age, maternal weight, gestational age at blood draw (dichotomized), race (Caucasian/ other)

Outcome:
TSH, free T4

Major Findings:

For PFOS independently (in model without other PFCs), OR <1.0

For model with all PFCs, OR for PFOS <1.0 (OR for PFHxS adj OR=1.27, but not stat sig)

For sum of PFCs, OR <1.0
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Major Limitations:

N for cases and controls is modest.

Women self-selected for the trisomy/Down’s/spina bifida screening and therefore, cohort is not necessarily representative of al pregnancies.

Other comments:

This was a well-controlled study with minimal opportunity for uncontrolled confounding. However, the small N and non-randomness of the sample reduce the generalizability of the findings.
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</table>

with trisomy 18//Down's syndrome/spina bifida screening (Dec. 2005- June 2006). Women ≥ 18 yrs old, singleton delivery > 22 wks N for total samples= 974 **Related Studies:**			

Reference and Study Design:	Exposure Measures:	Results:	Comments:
Study: Château-Degat et al. (2010) Château-Degat ML1, Pereg D, Dallaire R, Ayotte P, Dery S, Dewailly E. Effects of perfluorooctanesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec). Environ Res. 2010 Oct;110(7):710-7. doi: 10.1016/j.envres.2010.07.003. Epub 2010 Aug 8. **Study Design:** Cross-sectional study based on large-scale community stratified health study (2004) Investigation of association between PFOS and plasma lipid levels Blood samples collected in conjunction with large-scale community health study Questionnaires (self-administered and interview) on socio-demographic, environmental, dietary, lifestyle factors **Location:** Nunavik Inuit.	**Exposure Assessment:** Fasting HDL-C, LDL-C, triglycerides (TG) and glucose determined in plasma samples by autoanalyzer PFOS extracted by alkaline ion-pairing extraction. Quantification by HPLC-quadrapole-MS ^{13}C4-PFOS internal std. Recovery = 87% LOD = 0.1 ng/ml LOQ = 0.3 ng/ml Intra, and inter assay CVs = 4%, 6% **Population-Level Exposure:** PFOS (geom mean) = 18.5 ng/ml (95% CI = 17.8-19/5)	**Stat Method:** Assoc. of lipids and PFOS investigated with multiple linear regression Confounders considered: age; gender; self-identified smoking; fasting glycaemia; fasting insulinaemia; circulating DHA + EPA; lipid lowering drugs; BMI Interaction between PFOS and gender investigated Co-factors included in model if inclusion resulted in >10% change in dependent variable **Outcome:** Assoc. of lipid parameters with plasma PFOS **Major Findings:** Interaction term sig for PFOS-gender for PFOS-HDL and PFOS-triglycerides. These outcomes were stratified by gender <u>Adjusted models</u> HDL (good cholesterol) sig. positively assoc w. PFOS (M and F) TC/HDL sig negatively assoc w PFOS	**Major Limitations:** PFOS w/in range of age comparable US pop according to CDC-NHANES **Other PFCs not reported. Cannot determine confounding by exposure to other PFCs** **Results are opposite from most reported associations in US pop (i.e., PFOS → ↓HDL, ↑ TG** PUFA (DHA + EPA) exposure very high in this pop. Authors hypothesize that high PUFA intake could confound effects of PFOS (despite inclusion of PUFA in models as statistically appropriate) **Other comments:** Except for the failure to investigate potential confounding by other PFCs, this study was well controlled with a reasonably sixed N. Although cross-sectional, long PFOS serum half-life and likely consistency of diet suggests that observations are generalizable in this pop.

Population: Participants in community-based stratified randomized household sampling. Exclusion criteria: Pregnancy, non-Inuit, not fasted for 8-hrs N = 723 Mean age = 36.9 yrs F = 55% Mean BMI = 27.2 kg/m^2 **Related Studies:** Dallaire et al. (2009)		TG sig (p = 0.040 negatively assoc w PFOS for F only (M neg., but not sig)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Chen et al. (2013) Chen MH, Ha EH, Liao HF, Jeng SF, Su YN, Wen TW, Lien GW, Chen CY, Hsieh WS, Chen PC. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology. 2013 Nov;24(6):800-8. doi: 10.1097/EDE.0b013e3182a6dd46. **Study Design:** Longitudinal birth cohort Investigation of assoc between cord plasma PFCs and neurodevelopment in 2-yr olds "Comprehensive Developmental Inventory for Infants and Toddlers" Domains – cognitive; language; motor, social; self-help Tests administered by "specially trained physical therapists" **Location:** Taiwan **Population:** Children at 2 yrs old from birth cohort assembled 2004-2005	**Exposure Assessment:** PFOS and PFOA measured in cord plasma by UPL-triple quadrupole MS LOQ = 0.22 ng/ml PFOS, 1.58 ng/ml PFOA **Population-Level Exposure:** PFOS detection = 100% PFOA detection = 82% Mean conc (sd) PFOS = 7.0 (5.8) ng/ml PFOA = 2.5 (2.6) ng/ml	**Stat Method:** Co-factors/confounders HOME scale (support available for children at home) Cord blood cotinine Sex Gestational age Maternal education (≤ > 12 yr)Family income (dichotomized) Breastfeeding (never/ever) Postnatal ETS Linear and logistic regression PFOS, PFOA as continuous and categorical variables **Outcome:** Whole test and sub-test outcomes of Comprehensive Developmental Inventory for Infants and Toddlers **Major Findings:** (adjusted model) PFOS ↑ in PFOS equal to inter-quartile range of cord plasma conc → stat sig ↓ in whole test score ↑ in PFOS equal to inter-quart range → stat sig ↓ in gross motor test component All other components assoc w non-sig decrease for inter-quart ↑ in PFOS	**Major Limitations:** No indication of inter-tester QA determinations. Number of testers not specified. Testers were "physical therapists." Not clear if this is a mis-translation. However, not clear that physical therapists are appropriate for this testing. Does not appear that PFOS models were adjusted for PFOA conc. **Other comments:** Study was well controlled with reasonable N. However, lack of information about testers, testers qualifications, number of testers, and inter-tester variability results in uncertainties. Failure to adjust PFOS models for other PFCs (although PFOA, alone, not assoc with outcomes)

Initial cohort n = 402. After exclusion for incomplete information and loss to follow-up, **n = 239 mother-child pairs** Av. Materinal age = 32 yrs First birth for 40% of mothers Education >12 yrs over-represented in study pop. compared to full cohort **Related Studies:** Chen et al. (2012b)		For categorical analysis, test score for gross motor for highest quartile PFOS conc stat sig. ↓ compared to lowest quartile PFOS OR for lowest 10% of performance for gross-motor component w inter-quart ↑ in PFOS = 2.4 (95% CI = 1.3-4.2) For boys only, OR = 4.2 (1.7-10.8) <u>PFOA</u> No sig effects on test outcomes	

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
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Study:

Christensen et al. (2011)

Christensen KY, Maisonet M, Rubin C, Holmes A, Calafat AM, Kato K, Flanders WD, Heron J, McGeehin MA, Marcus M Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort.. Environ Int. 2011 Jan;37(1):129-35. doi: 10.1016/j.envint.2010.08.007. Epub 2010 Sep 16.

Study Design:

Prospective case-control nested within ALSPAC (Avon Longitudinal Study of Parents and Children)

"Self"-reporting (by mothers?) of menarche status and age at first menarche

Maternal serum samples collected "during pregnancy." If multiple samples, earliest preg sample was chosen.

Investigation of OR for early menarche (cases) with maternal prenatal PFCs

Location:

Avon, UK

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Exposure Assessment:

Analyte	LOD (ng/ml)
PFOS	0.2
PFOA	0.1
PFOSA	0.1
Et-PFOSA-AcOH	0.2
Me-PFOSA-AcOH	0.2
PFHxS	0.1
PFNA	0.1
PFDeA	0.2

Analysis by CDC – on-line solid phase extraction coupled to isotope dilution HPLC-tandem MS

For analytes in >30% of samples, < LOD → LOD/2
For analystes in < 30% of samples, < LOD entered as missing

Population-Level Exposure:

Analyte	Median (ng/ml)
PFOS	19.8
PFOA	3.7
PFOSA	0.2
Et-PFOSA-AcOH	0.6
Me-PFOSA-AcOH	0.4
PFHxS	1.6
PFNA	0.6
PFDeA	-

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Stat Method:

<u>Confounders investigated</u>
Maternal pre-preg BMI
Maternal age at delivery
Maternal age at own menarche
Maternal education
Child's ethnicity (white/non-white)
Child's birth order
SES/class

Outcome:

OR for assoc PFOS with ↓ age at menarche.

Major Findings:

OR for PFOS < 1.0 for continuous and binary analysis - non-adj and adjusted models.

No OR sig > 1.0 for any PFCs.

Non-sig ↓ ORs for PFOS

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Major Limitations:

Modest n's

Sig PFOA exposure

PFOS exposure is consistent with US exposure in NHANES 4th Report

Analysis based on single serum sample (however, relatively long half life).

Because preg period sampling dates varied, later samples, maternal-fetal transport could reduce measured maternal serum levels leading to underestimating fetal exposure

Other comments:

The study was generally well conducted and well controlled. However, concerns about exposure misclassification based on preg sampling time (see above), and small N, make lack of assoc uncertain.

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Population: From original cohort of 14,610 → singleton F → ≥ 1 maternal prenatal serum sample → ≥2 puberty stage questionnaires (one, post-menarche) → report of age at menarche →analyzable samples Menarche < 11.5 yrs = cases (n = 218) Menarche > 11.5 yrs = controls Random sample → n = 230 N's based on calc to achieve 80% power to detect OR ≥ 1l7 w control/cases n = 225 **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Dallaire et al. (2009) Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. Environ Health Perspect. 2009 Sep;117(9):1380-6. doi: 10.1289/ehp.0900633. Epub 2009 May 12. **Study Design:** Investgation of assoc of plasma polyhalogenated cmpds (incl. PFOS) and thyroid function in adult pop. of Nunavik, Quebec Based on large-scale cross-sectional health community stratified random study (2004) among permanent Inuit residents ≥ 18 yrs old **Location:** Nunavik, Quebec, Canada **Population:** Adult Inuit ≥ 18 yr Exclusions – pregnant; thyroid medication N = 621 Age - 36.8 ± 13.9, range = 18–73	**Exposure Assessment:** PFOS in plasma by LC/MS-MS LOD = 0.1 ng/ml (suppl. material.) TSH, freeT4, total T3, thyroid binding globin (TBG) by radioimmunoassay. **Population-Level Exposure:** PFOS detected in 100% of samples PFOS geom mean = 18.28 ng/ml	**Stat Method:** Multiple linear regression 5 participants with extreme TSH excluded Interaction terms for sex not sig. M and F combined in analyses. Co-variates with $p \leq 0.1$ considered - Sex; menopause; age, BMI; Se; smoking (no. cigarettes); alcohol freq; fish consumption; marine mammal consumption; education; thyroid altering medication, plasma lipids Included in PFOS model if inclusion altered PFOS β by > 10% Included co-variates age, sex, BMI, plasma lipids, smoking, education PCB-153, and BDE-47 examined in model w PFOs **Outcome:** Assoc PFOS w THS, free T4, total T3, TBG **Major Findings:** PFOS correlated w PCBs and metabolites (r = 0.47-0.55) Other org chlor r = 0.36-0.51 BDE-153 r = 0.23 (adj models)	**Major Limitations:** Plasma conc other PFC (esp. PFOA) not determined PFOS in range of US pop (NHANES) Cross-sectional **Other comments:** The study was reasonably conducted. However, lack of controlling for other PFCs creates uncertainties as to the specificity of results to PFOS

Related Studies: Chateau-Degat et al. (2010)		PFOS Sig assoc w ↓ TSH Sig assoc w ↑ free T4 Sig assoc w ↓ total T3 Sig assoc w ↓ TBG For TSH, and free T4, β for adj model for PFOS was largest of all contaminants. And second largest for TBG.	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Dalsager et al., 2016 Dalsager, L., N. Christensen, S. Husby, H. Kyhl, F. Nielsen, A. Host, P. Grandjean and T. K. Jensen (2016). "Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort." Environ Int **96**: 58-64. **Study Design:** Prospective birth cohort **Location:** Odense, Denmark **Population:** Odense Child Cohort – an ongoing prospective study on children's health where PFASs measured in 649 pregnancy women recruited from 2010-2012. Of these women, n=359 were included in this study (200 selected randomly and 449 based on availability of information). **Outcome Assessment:** Mothers reported on symptoms of infection in their child (aged 1 to 3.3 years old) every two weeks for a one-year period. Collected data: days without symptoms, fever, stuffed or runny nose, cough, wheezy or whistling breathing, eye inflammation, ear pain, discharge from ear, feeling unwell,	**Exposure Assessment:** Maternal serum PFASs concentrations before gestational week 16 (in utero) **Population-Level Exposure:** Median (ng/ml) 8.07 PFOA: 1.68 Tertile concentration (ng/mL) Low (0-6.93) Medium (6.94-10.18) High (10.19-25.10)	**Stat Method:** Associations were estimated using logistic regression and negative binomial regression model. All models were adjusted for maternal age, education level, parity and child age. Outcomes were analyzed as dichotomous (above or below the median) and ordinal data. PFAS concentrations were log-transformed and divided into tertiles. A test for linear trend across the exposure groups was conducted. Potential covariates and confounders considered include maternal age, educational level, and parity and adjusted for childhood age. Also maternal smoking, child sex, day-care attendance, and exclusive breastfeeding. Bonferroni adjustment also considered. **Outcome:** Symptoms of infection **Major Findings:** **Fever:** *Proportion* T2 v. T1 OR=1.14 (0.81, 2.44) **T3 v. T1 OR=2.35 (1.34, 4.11)** ***Findings were not significant following Bonferroni adjustment** *Number* T2 v. T1 OR=1.23 (0.93, 1.63) **T3 v. T1 OR=1.65 (1.24, 2.18)**	**Major Limitations:** Did not control for other co-occurring environmental contaminants as potential confounders. Moderate sample size. **Other comments:** Strong outcome and exposure assessment.

diarrhea, blood in stool,and vomiting. Number of days throughout the year were summarized to calculate mean for the year		***P for Trend < 0.001** **IRR=1.06 (1.03, 1.09)** **Cough:** *Proportion* T2 v. T1 OR=1.16 (0.67, 2.01) T3 v. T1 OR=1.03 (0.59, 1.79) *Number* T2 v. T1 OR=1.03 (0.80, 1.34) T3 v. T1 OR=0.88 (0.67, 1.15) **Nasal Discharge:** *Proportion* T2 v. T1 OR=1.11 (0.65, 1.93) T3 v. T1 OR=1.07 (0.62, 1.85) *Number* T2 v. T1 OR=1.22 (0.93, 1.61) T3 v. T1 OR=1.02 (0.76, 1.35) **Diarrhea:** *Proportion* T2 v. T1 OR=0.89 (0.51, 1.56) T3 v. T1 OR=1.04 (0.59, 1.82) *Number* T2 v. T1 OR=1.41 (0.79, 2.51) T3 v. T1 OR=1.19 (0.67, 2.12) **Vomiting:** *Proportion* T2 v. T1 OR=1.47 (0.86, 2.54) T3 v. T1 OR=0.78 (0.45, 1.35) *Number* T2 v. T1 OR=1.18 (0.80, 1.74) T3 v. T1 OR=0.87 (0.58, 1.31) Co-occurrence of fever & coughing and fever & nasal discharge – IRR appear to increase with increasing tertile but no statistically significant associations.	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Darrow et al. (2013) Darrow LA, Stein CR, Steenland K. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environ Health Perspect. 2013 Oct;121(10):1207-13. doi: 10.1289/ehp.1206372. Epub 2013 Jul 8. **Study Design:** Prospective study Assoc of birth outcomes w PFOS serum conc in blood samples collected from mothers at enrollment in C8 Health Project (2005-6) Birth outcome ascertained by interview Births 2005-2010 Live birth data obtained from birth records - Preterm - Low birth wt - Birth wt (continuous variable) of full-term infants **Location:** Mid-Ohio Valley	**Exposure Assessment:** Solid-phase extraction Reverse-phase HPLC-MS Inter- and intra-lab CV for PFOS = 0.1 LOD (PFOS) = 0.5 ng/ml Sample < LOD = 0.25 ng/ml **Population-Level Exposure:** <u>Geom mean (SD) (ng/ml)</u> PFOS = 13.1 (1.9) PFOA = 16.2 (2.8) <u>95th percentile (ng/ml)</u> PFOS = 31.8 PFOA = 114.1 Corr PFOS and PFOA - r = 0.3	**Stat Method:** Analyses conducted w and w/out participants with blood samples collected pre-conception. Binary outcomes by logistic regression Continuous outcomes by linear regression Also, by quintiles (compared to lowest quintile). Lowest quintile PFOS ≈ 10th percentile US pop (NHANES) <u>Co-variates</u> Parity, smoking status, maternal age, yrs education, BMI, non-pregnancy diabetes, PFOS and PFOA modeled separately and (in sens. Analyses) together **Outcome:** <u>Assoc. PFOS (and PFOA) with:</u> - Preterm birth - Preg induced hypertension (PIH) (maternal) - Low birth wt - Birth wt in full-term infants (continuous)	**Major Limitations:** ~100% of births ≤ 3 yrs from serum collection. Despite rel. long half-life and environmental exposure, this creates uncertainty as to gestational PFOS exposure 26% of births prior to serum sample Geom mean PFOS exposure ~32% lower than US female pop (NHANES) Sig PFOA co-exposure, esp in upper percentiles. However, co-exposure controlled for in sensitivity analyses Authors raise theoretical concern re. reverse causality for PIH (i.e., pre-disposition to PIH may affect PK of PFC excretion). However, PFOS and PFOA can also be causal for PIH through kidney and liver toxicity. **Other comments:** This was a well conducted study, w a relatively large N. For analyses excluding post-partum blood samples, this was a prospective study. The analyses were well controlled and sensitivity analyses addressed potential study weaknesses.

Population: Pop living near Dupont Washington Works Births to participants in C8 Community Follow-Up study after Jan. 1, 2005 - Enrollment in C8 2005-2006, - completion of demographic health questionnaire, - provided blood sample, - participated in ≥ 1 follow-up Interview 2008-2011, - ≥ 1 live birth 2005-2010 - Singleton births - White mothers - Maternal age at birth ≤ 45 yrs N = 1,630 ~26% of births were in 2005, but prior to C8 enrollment ~52% of PFOS samples collected prior to conception **Related Studies:**		**Major Findings:** Pretern - No sig assoc w PFOS (also not sig with PFOS and PFOA in same model) **PIH - ↑ PFOS (and PFOA) sig assoc w ↑ incidence PIH (higher β and OR when analysis restricted to post-partum blood samples). Also sig w PFOA in same model** Low birth wt - No sig assoc w PFOS **Continuous birth wt in full term - ↑ PFOS (but not PFOA) sig assoc w ↓ birth wt (first preg. post-sample only). Also sig for trend (but not monotonic) across quintiles**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Darrow et al. (2014) Darrow LA1, Howards PP, Winquist A, Steenland K. Epidemiology. 2014 Jul;25(4):505-12. doi: 10.1097/EDE.0000000000000103. PFOA and PFOS serum levels and miscarriage risk. **Study Design:** Nested cohort (C8 study), prospective pregnancy outcome Not preg at enrollment (exclusion) Blood sample at enrollment, interview reporting ≥ 1 pregnancy conceived after blood sample Ending (successfully or unsuccessfully) prior to follow-up interview Follow-up interview – reproductive history 40% online 60% by telephone Gestational age from OH birth records Miscarriage = ges age ≤ 20 wks Stillbirth = > 20 wks	**Exposure Assessment:** PFOS LOD = 0.5 ng/ml < LOD (n = 7) = LOD/2 **Population-Level Exposure:** Mean PFOS = 16.9 ng/ml (sd = 9.7 ng/ml) Geom mean PFOS = 14.3 ng/ml (sd = 1.9 ng/ml)	**Stat Method:** Logistic regression w generalized estimating equations Log-PFOS as continuous measure and quintiles Covariates (a priori) - maternal race - pre-preg BMI - education - diabetes - maternal age at conception - smoking at conception - time between serum measurement and conception **Outcome:** OR for miscarriage rel to serum PFOS Full analysis (miscarriages = 304; live births = 1,438) **Major Findings:** **OR not sig > 1.0** for continuous analysis or for any quintile However, continuous analysis borderline sig OR = 1.21 (0.94-1.55) **Outcome:** OR for miscarriage rel to serum PFOS Restricted to first preg (miscarriages = 213; live births = 1,129)	**Major Limitations:** **Other comments:** Large overall N (moderate number of cases Prospective study design Good analytical reliability Multiple sensitivity analyses Results are ambiguous and difficult to interprt

Location: OH, WV **Population:** C8 study cohort F ≥ 20 yrs old - Live births, n = 1,134 (incl 11 stillbirths) - miscarriage, n = 304 **Related Studies:**		**Major Findings:** **OR sig > 1.0** For continuous analysis (OR = 1.34 (1.02-1.76) And for Q2-Q5 (but response not monotonic) **Outcome:** OR for miscarriage rel to serum PFOS Restricted to first preg and excluding recent preg (≤ 40 wks before last interview) (miscarriages = 190; live births = 1,105) (Note: recent preg exclusion corrects bias of miscarriages but not live births reported) **Major Findings:** **OR not sig > 1.0** For continuous analysis Or for any quintile except Q3 **Outcome:** Condition at enrollment: Gravity = 0; parity = 0; or parity >0 **Major Findings:** **OR not sig >1.0** For continuous analysis Or for any quintile except Q3	

Reference and Study Design	Exposure Measures	Results	Comment
Study: de Cock et al. (2014a) de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M. Int J Environ Res Public Health. 2014 Jul 10;11(7):7001-21. doi: 10.3390/ijerph110707001. First year growth in relation to prenatal exposure to endocrine disruptors - a Dutch prospective cohort study. **Study Design:** Recruited 1/2011-1/2013 Preg F recruited through midwife clinics Recruitment at 1st ante-natal visit (10-12 wks of preg) Exclusions - twins - major congenital abnormalities Cord blood, breast milk (at mean 6.3 wks post-natal) collected Growth during first yr obtained from regional youth health authority (pop has regularly scheduled visits – aver = 6 visits) Parental anthropometry from midwives	**Exposure Assessment:** Plasma Isotope dilution, on-line trapping column-LC-triple quadrupole MS CV = 16-17% (internal? External repeats?) PFOS (cord plasma) LOQ 0.04-1.4 ng/ml **Population-Level Exposure:** Mean cord plasma PFOS = 1.6 ng/ml (NOTE: PFOS conc appears low compared to US pop (NHANES 4th Rpt), but pop data on cord plasma not available)	**Stat Method:** Mixed models PFOS as quartiles Exposure quartile, timing of anthropomorphic meas, sex, as fixed effects in model, random effect added for subject <u>Co-variates</u> - Maternal/paternal BMI - gest age - parity - alcohol - smoking - education - duration breast feeding Co-variates added to model if $\Delta\beta > 10\%$ **Outcome:** BMI **Major Findings:** PFOS **not sig assoc** w BMI **Sig interaction** w time (post-natal) and w sex **Outcome:** Weight	**Major Limitations:** Small n Low PFOS expsoure **Other comments:** Small n Low PFOS exposure Incomplete statistical reporting (βs not given)

Questionnaire on parental health, lifestyle, prev preg Follow-up visits to child health centers at 1, 2, 4, 6, 9, 11 mos. after birth **Location:** Zwolle, The Netherlands **Population:** LINC cohort (maternal-child) 89 mother child pairs from general regional pop M = 56 F = 33 **N for PFOS = 61** **Related Studies:**		**Major Findings:** PFOS **not sig assoc** w weight **Sig interaction** w time (post-natal) and w sex **Outcome:** Height **Major Findings:** PFOS **not sig assoc** w height **Sig interaction** w time (post-natal) and w sex **Outcome:** Head circum **Major Findings:** PFOS **not sig assoc** w head circum **Sig interaction** w time (post-natal) and w sex	

Reference and Study Design	Exposure Measures	Results	Comment
Study: de Cock et al. (2014b) de Cock M1, de Boer MR, Lamoree M, Legler J, van de Bor M. Environ Health. 2014 Dec 10;13:106. doi: 10.1186/1476-069X-13-106. Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study. **Study Design:** Prospective birth cohort Recruited 1/2011-1/2013 Preg F recruited through midwife clinics Recruitment at 1st ante-natal visit (10-12 wks of preg) Exclusions - twins - major congenital abnormalities Cord blood, breast milk (at mean 6.3 wks post-natal) collected T4 from heel-prick blood sample collected between postnatal days 4-7 Parental anthropometry from midwives	**Exposure Assessment:** Plasma Isotope dilution, on-line trapping column-LC-triple quadrupole MS CV = 16-17% (internal? External repeats?) PFOS (cord plasma) LOQ 0.04-1.4 ng/ml No PFOS samples < LOQ **Population-Level Exposure:** Mean and median PFOS cord serum conc = 1.6 ng/ml (range 0.57-3.2 ng/ml)	**Stat Method:** <u>Co-variates investigated</u> - Thyroid related health issues - thyroid related meds during preg - birth wt - maternal/paternal wt at10-12 wks preg - maternal/paternal length at 10-12 wks preg) - maternal wt at 36 wks preg (gest wt gain) - caesarian delivery (Y/N) - maternal birth date - parity - 1st trimmest maternal smoking - 1st trimester alcohol Linear regression Stratified by sex Analysis by quartiles Sensitivity analyses (for maternal factors) by exclusion of - gest wt gain - birth wt **Outcome:** T4 (from heel-prick on filter paper) **Major Findings:** (full adj model) **T4 not sig assoc w PFOS** for either M or F	**Major Limitations:** Low PFOS exposure level Small N No controlling of PFOS analyses for PFOA **Other comments:** Well controlled Low LOQ for PFOS Low power given small sample size and low PFOS exposure

Questionnaire on parental health, lifestyle, prev preg **Location:** Zwolle, The Netherlands **Population:** LINC cohort (maternal-child) infants 62 M 62 F PFOS N = 64 **Related Studies:**		(for M, PFOS Q2 and Q3 sig neg assoc w T4 in crude model and for Q2 in partial adj model. No sig assoc in F)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Donauer et al. (2015) Donauer S, Chen A, Xu Y, Calafat AM, Sjodin A, Yolton K J Pediatr. 2015 Mar;166(3):736-42. doi: 10.1016/j.jpeds.2014.11.021. Epub 2014 Dec 16. Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior. **Study Design:** Prospective birth-cohort Neonatal Intensive Care Unit Network Neurobehavioral Scale administered during home visits (13 dimensions) Maternal serum collection at 16 wks gestation (85% of mothers), or 26 wks gest (10% mothers), delivery (5%) **Location:** Cincinnati, OH **Population:** Mother-child participants in Health Outcomes and Measurements of the Environment (HOME) Study Recruited 3/03-1/06	**Exposure Assessment:** PFOS analytical methodology per CDC analysis **Population-Level Exposure:** PFOS geom mean conc = 13.25 ng/ml (NOTE: PFOS conc ~1.7 times current US F, but consistent with US F for 2003-6 (NHANES 4th Rpt))	**Stat Method:** PFOS conc log-transformed Multiple linear regression of endpoints on maternal serum PFOF for all individual NNNS endpoints except: - hypotonicity (logistic regression - assymetric reflexes (Poisson regression) NNNS composite endpoints (high arousal/difficult or hypotonic vs. social/easygoing) by logisitic regression <u>Co-variates investigated</u> - maternal age - race - income - marital status - maternal depression - BMI at 13-19 wks gest - alcohol during preg - marijuana during preg - cotinine - infant monthly wt change (birth-5 wks) - maternal BPb during preg (max of 16, 26 wks, delivery) - gestational age < 37 wks Co-variates retained if Δ in β PFOS w removal > 10% Multivariate models constructed for NNNS outcomes w bivariate $p < 0.15$	**Major Limitations:** Range of maternal sampling periods for PFOS PFOS analysis not controlled for PFOA **Other comments:** Moderate N Good analytical methodology Issues w comparability of PFOS exposure measurements across time

N = 349 infants M = 164 F = 185 **Related Studies:**		**Outcome:** NNNS outcomes **Major Findings:** **PFOS not sig assoc** w NNNS for: Attention Self-regulation Quality of movement Arousal Excitability Special handling required Lethargy Non-optimal reflexes Asymmetric reflexes Hypotonicity Stress abstinence (borderline sig)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Dong et al.(2013) Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, Jin YH, Hsieh WS, Lee YL, Chen PC. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect. 2013 Apr;121(4):507-13, 513e1-8. doi: 10.1289/ehp.1205351. Epub 2013 Jan 7. **Study Design:** Case-control study of assoc of asthma w PFOS exposure 8-hr fasting urine and serum samples **Location:** Taiwan **Population:** 10-15 yr old children diagnosed w asthma by physician 1 yr prior to entry into study (2009-2010) Controls (non-asthmatic) selected from 7 public schools w various SES, and geographic/climate	**Exposure Assessment:** <u>Outcomes</u> Venous blood Absolute eosinophil count (AEC) x 10^6 by automatic analyzer Eosinophil cationic protein (ECP) µg/L by ELISA IgE (IU/ml) by Pharmacia UniCap assay test Asthma control test (ACT) questionnaire for asthma symptoms in prev 4 wks and asthma severity questionnaire administered to cases <u>PFC exposure</u> PFC from serum by HPLC-QQQ-MS/MS PFOS LOQ = 0.03 ng/ml **Population-Level Exposure:** PFOS ≥ 97% detect <u>PFOS (ng/ml)</u> mean = 33.4 controls; 45.5 cases median = 28.9 controls; 33.9 cases <u>PFOA (ng/ml)</u> Mean = 1.0 controls; 1.5 cases	**Stat Method:** PFC < LOQ = LOQ/√2 OR for asthma by logistic regression A priori model adj for age and sex Other confounders considered: Parental education BMI ETS Month of survey Factor included if inclusion changed PFC effect by ≥ 10% Multiple gen linear regression for IgE, AEC, ECP by PFC quartile **Outcome:** Assoc PFOS w asthma and immune markers **Major Findings:** <u>Asthma</u> OR for PFOS sig for all quartiles (compared to lowest) OR 4^{th} quartile = 2.63 Also sig for (pos) trend ORs also sig for most other PFCs	**Major Limitations:** PFOS conc is higher (median ≈ 75^{th} percentile of US 12-19 yrs old (NHANES) PFTA conc is comparable to PFOS. Overall p-value sig for controls > cases. However, mean and median conc differ as to cases or controls higher **Authors state that because of intercorrelations among PFCs contribution of individual PFCs cannot be determined (i.e., other PFCs were not controlled for in PFOS model)** **Other comments:** The study was reasonably well designed and conducted. The N was modest. However, the failure and/or inability to statistically isolate PFOS (or other PFCs) does not permit ascertainment of a specific PFOS effect.

locations in Taiwan. Same age group as cases. No family or personal asthma history Cases = 225 Controls = 231 **Related Studies:**	 <u>PFTA (ng/ml)</u> Mean = 29.9 controls; 54.6 cases Median = 5.2 controls; 4.1 cases <u>PFDoA (ng/ml)</u> Mean = 4.5 controls; 3.8 cases Note: all other PFCs < PFDoA	<u>IgE</u> No sig diff among quartiles of any PFC for controls For cases, PFOS 4th quart sig > 1st (ref) quartile Sig for (pos) trend Also sig for upper quartiles and trend for other PFCs (PFOA, PFDA, PFNA) <u>AEC</u> No sig diff among quartiles of any PFC for controls For PFOS, not sig for any individual quartile, but sig for (pos) trend <u>ECP</u> No sig diff among quartiles of any PFC for controls For PFOS, 4th quart sig > 1st quart. Sig for trend Upper quartiles and trend also sig for several other PFCs	

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<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
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<td>Study:

Eriksen et al. (2009)

Eriksen KT, Sørensen M, McLaughlin JK, Lipworth L, Tjønneland A, Overvad K, Raaschou-Nielsen O. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. J Natl Cancer Inst. 2009 Apr 15;101(8):605-9. doi: 10.1093/jnci/djp041. Epub 2009 Apr 7.

Study Design:

Prospective cohort enrolled 12/93-5/97. Age 50-65 yrs. No prev cancer diagnosis
Total cohort n = 57,051

Nested case-control w/in cohort

Questionnaire at enrollment

Location:

Denmark

Population:

Danish cancer and pathology reg’s used to identify spec cancers diagnosed 0-12 (median = 7) years) post-enrollment</td>
<td>Exposure Assessment:

Plasma samples at recruitment

PFOS and PFOA analysis by HPLC-MS

LOQ (apparently for all PFCs) = 1 ng/ml

Non-detects as LOQ/√2

Mean CV for PFOS (50 samples) = 1.8%

Population-Level Exposure:

PFOS (ng/ml)
<table>
<tr><td></td><td>M</td><td>F</td></tr>
<tr><td>cases</td><td>35.1</td><td>32.1</td></tr>
<tr><td>controls</td><td>35.0</td><td>29.3</td></tr>
</table>
PFOA conc ≈ 20% of PFOS conc

PFOS correlated w PFOA, r = 0.7</td>
<td>Stat Method:

Confounders investigated:

<u>Prostate cancer</u>
Yrs school
BMI
Fat intake
Fruit and veg intake

<u>Bladder cancer</u>
Smoking (status, duration, intensity)
Yrs of school
Specific occupation exposures

<u>Pancreatic cancer</u>
Smoking (status, duration, intensity)
Fat intake
Fruit and veg intake

<u>Liver cancer</u>
Smoking (status, duration, intensity)
Yrs of school
Alcohol intake
Specific occupation exposures

Quartiles of PFC exposure defined on basis of separate distributions for each cancer

Linear assoc of PFOS conc and each cancer by linear spline to yield incidence rate per 10 ng/ml ↑ in PFOS

Analysis for total pop and stratified by sex</td>
<td>Major Limitations:

Plasma sample represent exposure ≤ 12 yrs prior to diagnosis. Potential for exposure misclassification

PFOS exposure higher than US adult pop (~ 75th percentile) (NHANES)

Other comments:

This is a high quality study with a reasonable n and relevant exposure levels. The potential for exposure misclassification due to temporal offset of sampling and diagnosis is the main caveat.</td>
</tr>
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<table>
<tr>
<td>Prostate (n = 713)
Bladder (n = 332)
Pancreatic (n = 128)
liver (n = 67)

Control group 680 M, 92 F (~ ratio among cases) randomly selected from same cohort

Related Studies:

Eriksen et al. (2013) (non-cancer)</td>
<td></td>
<td>Outcome:

Incident rate ratio (IRR) for each cancer by PFOS (and PFOA) plasma conc

Major Findings:

No sig ↑ IRR for PFOS (or PFOA) for any cancer at any quartile. No sig trend for any cancer (crude or adj models)

No sig influence of sex

<u>For prostate</u>

<table>
<tr><td>quartile</td><td>IRR</td><td>95% CI</td></tr>
<tr><td>1</td><td>1.00 (ref.)</td><td></td></tr>
<tr><td>2</td><td>1.35</td><td>0.97-1.87</td></tr>
<tr><td>3</td><td>1.31</td><td>0.94-1.82</td></tr>
<tr><td>4</td><td>1.38</td><td>0.99-1.93</td></tr>
</table>
Given lack of trend authors suggest either a low threshold for (modest) ↑ risk, or chance</td>
<td></td>
</tr>
</table>

Reference and Study Design	Exposure Measures	Results	Comment
Study: Eriksen et al. (2013) Eriksen KT, Raaschou-Nielsen O, McLaughlin JK, Lipworth L, Tjønneland A, Overvad K, Sørensen M. Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PLoS One. 2013;8(2):e56969. doi: 10.1371/journal.pone.0056969. Epub 2013 Feb 18. **Study Design:** Danish Diet, Cancer, and Health study. Prospective cohort enrolled 12/93-5/97. Age 50-65 yrs. No prev cancer diagnosis Total cohort n = 57,053 M = 27,178 F = 29,875 Nested cross-sectional case-control w/in cohort Questionnaire at enrollment Blood for PFOS and cholesterol samples taken at enrollment Analysis of assoc bet PFOS (PFOA) and cholesterol levels	**Exposure Assessment:** <u>PFOS</u> Plasma samples at recruitment PFOS and PFOA analysis by HPLC-MS LOQ (apparently for all PFCs) = 1 ng/ml Non-detects as LOQ/√2 Mean CV for PFOS (50 samples) = 1.8% <u>Cholesterol</u> Determination by reflectance photometer reading of test strips (range 100-500 mg/dL) **Population-Level Exposure:** Mean PFOS = 36.1 ng/ml Mean PFOA = 7.1 ng/ml M > F (mean Δ = 6.1 ng/ml)	**Stat Method:** Generalized linear analysis Linearity verified graphically by linear splines PFOS (PFOA) as continuous variables and as octiles (100 in ea). <u>Co-variates</u> Age Sex Yrs school BMI Smoking Alcohol Phys activity (hrs/wk) Egg intake Animal fat intake **Outcome:** Cholesterol **Major Findings:** (fully adj model) For total pop, ↑ PFOS sig → ↑ cholesterol Stratified by sex, assoc sig only for F (and β ~ 3 x for M) Cholesterol ↑ ~ 4 mg/dL (1.7% of total mean conc) for each interquartile range of PFOS	**Major Limitations:** Study pop highly skewed to M (due to previous use of cohort as controls for cancer incidence study (Eriksen et al. (2009)) PFOS exposure > US adult pop (~75th percentile) Unclear if regression for PFOS controlled for PFOA Total cholesterol, not LDL measured Although sig, overall effect of PFOS on cholesterol is small **Other comments:** This is a generally well-conducted study with a reasonable N. However, it is hampered somewhat by lack of clarity as to possible contribution of PFOA to PFOS assoc

Location: Denmark **Population:** Danish (middle-aged), native born Control pop from Eriksen et al. (2009). Excluded under medication for high cholesterol, and no cholesterol blood data N = 754 M = 663 F = 90 **Related Studies:** Eriksen et al. (2009) (cancer)		diabetes increased β for assoc PFOS w cholesterol BMI had no effect on PFOS-cholesterol assoc	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei and Olsen (2011) Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Fei C, Olsen J. Environ Health Perspect. 2011 Apr;119(4):573-8. doi: 10.1289/ehp.1002026. Epub 2010 Nov 9. **Study Design:** Assoc between pre-natal PFOS exposure (maternal) and behavioral, social and motor dev. of children at 7 yrs Danish National Birth Cohort. Maternal PFOS exposure in plasma Blood draw pre-preg Parental interview w questionnaires when child was 7 yrs based on assessment in prev 6 mos - Strength & Difficulties Questionnaire (SDQ) - (behavioral problems) - Dev Coordination Disorder Questionnaire (DCDQ) For SDQ, scores > highest 10% defined as high behavior score	**Exposure Assessment:** ((Note: The following information is from Fei (2007), which used the same population and blood samples. The current publication provides less detail) Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml Sample < LOQ as LOQ/2 **Population-Level Exposure:** Median PFOS = 34.4 ng/ml (IQR = 26.6 -44.5) (Median PFOA = 5.4 ng/ml PFOS-PFOA correlated - r_s = 0.70	**Stat Method:** Logistic reg using dichotomous outcomes for “high” DSQ and “low” DCDQ scores Also ordinal linear regression for DSQ and DCDQ scores as categorical variables (3-6 categories depending on spec subscales) PFOS plasma conc in quartiles <u>Potential confounders investigated</u>: Parity Maternal age Pre-preg BMI Preg smoking Preg alcohol Maternal SES Sex of child Parental behavior problems score Breastfeeding Birth yr Household density Gestational age at blood draw Co-variates retained in model if changed PFOS estimates by ≥ 5% **Outcome:** High DSQ scores (i.e., elevated behavioral difficulties scores) **Major Findings:** No sig or consistent assoc w PFOS	**Major Limitations:** Does not appear that PFOS analyses were controlled for PFOA (However, high corr. between PFOS and PFOA may have precluded including both in same model) Although the overall N was mod high, the top j10% of (SDQ) and bottom (DCDQ) scores defining the high category for dichotomous analysis had rel small n’s for each subscore category (n = 15-36). Thus, power may have been low No clear indication of accuracy of parental scoring (no gold std applied to assess reliability of scoring) **Other comments:** Study design was reasonable, but (see above) uncertainties in high/low n’s and reliability of parental scoring.

For DCDQ, scores in < lowest 10% defined as potential dev coordination disorder **Location:** Denmark **Population:** Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected for follow-up study at 7 yrs (children) → **n = 787 for SDQ** and **n = 537 for DCDQ** **Related Studies:** Fei et al. (2007, 2008, 2009, 2010a, 2010b)		**Outcome:** Low DCDQ scores (i.e., low dev coordination ability) **Major Findings:** No sig or consistent assoc w PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei (2007) Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Fei C, McLaughlin JK, Tarone RE, Olsen J. Environ Health Perspect. 2007 Nov;115(11):1677-82. **Study Design:** Nested cross-sectional study (birth outcomes w single 1st trimester blood sample) Maternal preg assoc between PFOS (PFOA) and birth wt, length of gestation from Danish Nat'l birth cohort Interviews at ges. wks 12 and 30, and post natal mos. 6 and 18 Food freq questionnaire at ges wk 25 Blood drawn 1st and 2nd trimester Cord blood sample at birth Birth wt and gestational age from Danish Nat'l Hospital Discharge Reg. **Location:** Denmark	**Exposure Assessment:** Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml Sample < LOQ as LOQ/2 **Population-Level Exposure:** No overall mean PFOS reported Maternal mean for F = 35.3 ng/ml Maternal mean for M = 35.2 ng/ml PFOs and PFOA correlated (r = 0.87)	**Stat Method:** Stat analyses based on 1st maternal blood sample Multiple linear reg for continuous birth wt OR by logistic regression for low birth wt; small for gest age (SGA); and preterm birth PFOS (PFOA) as continuous and categorical variables (< 25th percentile as ref group) Log-transf and non-transf PFOS conc investigated in models <u>Co-variates investigated in models</u> Maternal age Parity SES Pre-preg BMI Smoking during preg Infant sex Gest wk of blood drawing Models also stratified by Parity, pre-preg BMI and pre-term/term/post-term birth **Outcome:** Birth wt	**Major Limitations:** PFOS exposure > 75th percentile US F >20 yrs old (NHANES 4th Biomonit Rpt) Does not appear that PFOS models were adjusted for PFOA Only 1st trimester maternal blood sample used in stat analyses, but 2nd trimester sample differed (↓ mean) analyses could have differed with the later exposure metric **Other comments:** The study had thorough statistical analysis. However, the n was small and the later of the two blood samples was not analyzed in the models

Population: Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected → 200/1,102 w 2nd blood sample randomly selected → **50**/146 w cord blood sample randomly selected **(i.e., N = 50)** **Related Studies:** Fei et al. (2008, 2009, 2010a, b; Fei and Olsen 2011)		**Major Findings:** For continuous variable No sig assoc of PFOS with birth wt For OR for low birth wt (< 2,500 g) - ORs for all quartiles elevated but – - No quartile OR sig - Trend not sig For OR SGA (< 10th perc of corresponding gest age - No elevated ORs for any quartile - No sig ORs - Trend not sig **Outcome:** Length of gestation **Major Findings:** For continuous var No sig assoc of PFOS w length of gestation For OR for pe-term birth - ORs for all quartiles elevated but – - Only OR for 3rd quart sig - Trend not sig	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei et al. (2008) Fei C, McLaughlin JK, Tarone RE, Olsen J. Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. Am J Epidemiol. 2008 Jul 1;168(1):66-72. doi: 10.1093/aje/kwn095. Epub 2008 May 5. **Study Design:** Nested cross-sectional study (birth outcomes w single 1st trimester blood sample) Maternal preg assoc between PFOS (PFOA) and birth wt, length of gestation from Danish Nat'l birth cohort Interviews at ges. wks 12 and 30, and post natal mos. 6 and 18 Food freq questionnaire at ges wk 25 Blood drawn ges wk 4-14 (median = 8 wks) Birth wt and gestational age from Danish Nat'l Hospital Discharge Reg. **Location:** Denmark	**Exposure Assessment:** ((Note: *The following information is from Fei (2007), which used the same population and blood samples. The current publication provides less detail)* Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml Sample < LOQ as LOQ/2 Plasma preparation not available for 12 samples. Sampled as whole blood and concentrations x 2 to estimate plasma conc. **Population-Level Exposure:** Mean PFOS = 35.3 ng/ml Mean PFOA = 5.6 ng/ml	**Stat Method:** PFOS (PFOA) as continuous and categorical (quartile) variables (< 25th percentile as ref group) Investigated as log-transformed and unstransformed variables Placental wt, birth length, head circum., abdominal circum., ponderal index (kg/m3) as continuous variables Coveriates investigated Ges. age Infant sex Parity SES Pre-preg BMI Smoking in preg Ges wk of blood draw Alcohol Diet (fish, protein, fat, carbohydrates, energy) Maternal preg wt gain Maternal hypertension Maternal diabetes Mode of delivery Co-variates retained in model if changed parameter (presumably PFOS, PFOA) by ≥ 5% Gest age at birth as linear and quadratic term PFOS-PFOA interaction terms with outcome variables investigated and	**Major Limitations:** PFOS exposure > 75th percentile US F >20 yrs old (NHANES 4th Biomonit Rpt) Does not appear that PFOS analysis were controlled for PFOA concentration **Other comments:** Other than apparent failure to control for PFOA in PFOS analyses, this study was well designed and appropriately analyzed with a large N

Population: Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected **Related Studies:** Fei et al. (2007, 2009, 2010a, b, 2011)		**Outcome:** (Results for adj models unless indicated) Placental wt **Major Findings:** For categorical analysis Inconsistent β across quartiles no quartile sig For continuous analysis Neg β **No sig assoc w PFOS** **Outcome:** Birth wt **Major Findings:** For categorical analysis Inconsistent β across quartiles no quartile sig For continuous analysis Neg β **No sig assoc w PFOS** **Outcome:** Head circum	

		Major Findings: For categorical analysis Inconsistent β across quartiles no quartile sig For continuous analysis Neg β **No sig assoc w PFOS** **Outcome:** Abdominal circum **Major Findings:** For categorical analysis Inconsistent β across quartiles no quartile sig For continuous analysis Neg β Sig in for crude β (unadjusted model) In adjust model, **no sig assoc w PFOS**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei et al (2009) Fei C, McLaughlin JK, Lipworth L, Olsen J. Maternal levels of perfluorinated chemicals and subfecundity. Hum Reprod. 2009 May;24(5):1200-5. doi: 10.1093/humrep/den490. Epub 2009 Jan 28. **Study Design:** Nested case-control study (birth outcomes w single 1st trimester blood sample) Maternal preg assoc between PFOS (PFOA) and birth wt, length of gestation from Danish Nat'l birth cohort Interviews at ges. wks 12 and 30, and post natal mos. 6 and 18 Time-to-pregnancy (TTP) determination based self-reporting in 1st interview Food freq questionnaire at ges wk 25 Blood drawn ges wk 4-14 (median = 8 wks) Birth wt and gestational age from Danish Nat'l Hospital Discharge Reg.	**Exposure Assessment:** ((Note: *Parts of the following information are from Fei et al. (2007), which used the same population and blood samples. The current publication provides less detail)* Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml **Population-Level Exposure:** All PFOS samples > LOQ Median PFOS = 33.7 ng/ml (IQR = 26.6-43.5 ng/ml) (Median PFOA = 5.3 (IQR = 4.0-7.0 ng/ml)	**Stat Method:** PFOS (PFOA) as continuous and categorical (quartile) variables (< 25th percentile as ref group) OR for infertility by logistic regression for elevated PFOS compared to lowest quartile Fecundity OR (FOR) by Cox model modify for discrete time data (FOR = odds of successful conception at a given PFOS quartile) in a given month given non-conception in prev month Potential confounders investigated: Maternal age at delivery Parity Pre-preg BMI History of miscarriage Abdominal disease Maternal SES Pre-preg alcohol Paternal age Paternal occupation Ges wk at blood draw **Outcome:** Assoc. of PFOS w TTP **Major Findings:** Compared to TTP < 6 mos (n = 861), TTP 6-12 mos (n = 191), or ≥ 12 mos (n = 188) had **sig ↑ PFOS conc** (also PFOA)	**Major Limitations:** Stat analyses for PFOS do not appear to have controlled for PFOA Cohort included "partly planned" pregnancies. This results in uncertainty in determination of TTP PFOS exposure > 75th percentile US F >20 yrs old (NHANES 4th Biomonit Rpt) No data available on sperm quality. If PFOS reduces sperm quality, the paternal effect could confound the assessment of maternal fertility Because only eventual pregnancies included, unsuccessful at > 12 mos not included. If PFOS decreased fertility overall, this would result in underestimating effect of PFOS on fertility Potential for reverse causality because longer TTP would result in longer time for PFOS accum → assoc of ↑ TTP w ↑ PFOS **Other comments:** Except for the apparent failure to control PFOA concentrations in the PFOS analyses, the study appears to have adequately addressed issues of confounding The overall N is reasonably large although the n's for > 6 mos TTP are relatively small. Uncertainites about

Location: Denmark **Population:** Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected → 160 unplanned pregnancies or unknown time-to-pregnancy excluded → **N = 1240** 30% of TTP ≥ 6 mos 15% of TTP ≥ 12 mos Only eventual preg (i.e., at > 12 mos) included. Non-pregnancy at > 12 mos, not included Av. age = 30.6 yrs **Location:** Denmark **Related Studies:** Fei et al. (2007, 2008, 2010a, b; Fei and Olsen, 2011)		**Outcome:** Infertility (TTP > 12 mos) **Major Findings:** OR for infertility in 2nd, 3rd or 4th quart of PFOS **sig > 1.0** (1.7 2.34, 1.77 respectively) compared to 1st (ref) quart **p-trend sig** (p = 0.025) Odds of infertility ↑ 70-134% in 2nd, 3rd and 4th quarts Similar odds for PFOA **Outcome:** Fecundity **Major Findings:** FOR for PFOS **sig < 1.0** for 2nd, 3rd, and 4th quarts (compared to 1st) **p-trend sig** (p = 0.002)	"partially" planned pregnancies increase uncertainty about accurate TTP values.

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei et al. (2010a) Fei C, McLaughlin JK, Lipworth L, Olsen J. Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scand J Work Environ Health. 2010 Sep;36(5):413-21. Epub 2010 Mar 3. **Study Design:** Cross-sectional study nested in Danish National Birth Cohort Assoc of uration of *exclusive* breast feeding (i.e., no other nutrition source) w maternal PFOS plasma conc Single 1st trimester blood sample Info on infant breast feeding collected at 6 and 18 mo. Interviews (If conflict between reported termination of exclusive breastfeeding and date of first formula by > 2 wks (n = 50), date of first formula used) **Location:** Denmark	**Exposure Assessment:** ((Note: The following information is from Fei (2007), which used the same population and blood samples. The current publication provides less detail) Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml Sample < LOQ as LOQ/2 **Population-Level Exposure:** No PFOS samples < LOQ PFOS plasma conc 37. 0 - 32.3 ng/ml (conc ↓ with duration of breastfeeding - < 3 - ≥ 6 mos)	**Stat Method:** Cox proportional hazard analysis to est hazard ratio (HR) of early weaning and termination of exclusive breastfeeding over time Logistic reg w categorical analysis w cutpoints of 3 and 6 mos Stratification by parity <u>Confounders investigated</u> Maternal age at delivery Parity Pre-preg BMI Maternal SES Alcohol consumption Smoking Gest age at blood draw **Outcome:** Weaning at < 3 mos **Major Findigns** For women w first child, OR for each 10 ng/ml PFOS not sig For multiparous women, sig OR for each 10 ng/ml PFOS = 1.25 (PFOA also sig)` **Outcome:** Weaning at < 6 mos	**Major Limitations:** PFOS exposure > 75th percentile US F >20 yrs old (NHANES 4th Biomonit Rpt) For primaparoous (1st child) women, PFOS may be causal for reduced duration of breastfeeding, However, for multiparous women, plasma PFOS conc is reduced by previous breastfeeding. Therefore, higher PFOS concs may reflect shorter duration of breastfeeding w previous children and shorter duration of breastfeeding w previous children is likely to be correlated w duration of breastfeeding w subsequent children. Thus, causality of PFOS and shorter duration of breastfeeding in multiparous women is suspect. There were no data on non-biological factors that potentially could explain duration of breastfeeding (e.g. social, convenience-based choice). **Other comments:** Large N. The study could not adequately control directly for non-biological factors that could potentially influence duration of breastfeeding.

Population: Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected **Related Studies:** Fei et al. (2007, 2008, 2009, 2010b; Fei and Olsen, 2011)		**Major Findings:** For women w first child**, sig OR** for ea. 10 ng/ml PFOS = 1.20 For multiparous women**, sig OR** for ea 10 ng/ml PFOS = 1.20 (PFOA also sig) **Outcome:** Duration of any breastfeeding **Major Findings:** For women w first child, HR not sig For multiparous women, sig HR for three highest quart (1st quart as ref) of PFOS (1.42-1.55) and sig for trend	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei et al. (2010b) Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 2010 Nov;110(8):773-7. doi: 10.1016/j.envres.2010.08.004. Epub 2010 Aug 30. **Study Design:** Longitudinal cohort study Assoc. of maternal PFOS with early childhood hospitalization for infectious disease over 11 yrs following birth Av age at end of follow-up = 8.2 yrs (range = 5.8-10.7 yrs) Hospitalizations data from Danish Nat'l Hospital Registry Total hospitalizations (incl multiple hospitalizations per child) 11,350 person/yr of follow-up **Location:** Denmark	**Exposure Assessment:** ((Note: Parts of the following information are from Fei et al. (2007), which used the same population and blood samples. The current publication provides less detail) Plasma PFOS (PFOA) conc by HPLC-MS Isotope dilution in extraction procs PFOS CV for between batch spiked controls = 2.5-2.8% Repeat sample correlation – r = 0.993 LOQ = 1.0 ng/ml **Population-Level Exposure:** Mean PFOS = 35.3 ng/ml	**Stat Method:** Incident rate ratio (IRR) based on Poisson distribution <u>Covariates considered</u>: Maternal age at delivery Parity Pre-preg BMI Alcohol consumption during preg Smoking during preg Maternal SES Birth season Birth yr House density Number children in household Age diff w youngest sibling Child's gender Duration of breastfeeding Ges age at blood draw <u>Effect modification investigated by</u>: Gender Child's age at infection parity **Outcome:** IRR for hospitalization for infection **Major Findings:** No sig assoc for total cohort For total 0-1 yr, sig ↓ IRR at highest PFOS quart (marginally sig for neg trend)	**Major Limitations:** PFOS exposure > 75th percentile US F >20 yrs old (NHANES 4th Biomonit Rpt) Does not appear that PFOS analyses were controlled for PFOA. **Other comments:** The study is based on a large N. Outcome data are well defined and records are reliable and not subject to recall limiations Although no clear assoc is apparent, some weak assoc's are difficult to interpret.

Population: Danish Nat'l Birth Cohort 91, 827 preg F from 3/96-11/02 60% of Danish preg women Single live birth → no reported congenital malformation → 1st blood sample wks 4-14 → all interviews → 1,400/43,045 randomly selected **N = 1,400** 363 (25.9%) hospitalized ≥ one time for infectious disease 577 total hospitalizations for infectious disease **Related Studies:** Fei et al. (2007, 2008,. 2009, 2010a; Fei and Olsen, 2011)		For girls, sig ↑ IRR for 3rd (1.61) and 4th (1.59) quart PFOS, sig for trend (IRR = 1.18) (Also for PFOA) For boys, IRRs for all quart's neg (sig only for 3rd quart (IRR = 0.77) For primiparous, IRR ↑ w ↑ PFOS, but not sig at any quart or for trend	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fei (2012) Epidemiology. 2012 Mar;23(2):264-6. doi: 10.1097/EDE.0b013e3182467608. Commentary: perfluorinated chemicals and time to pregnancy: a link based on reverse causation? Fei C, Weinberg CR, Olsen J. **Study Design:** Re-investigation of Danish Nat'l Birth Cohort data on time-to-pregnancy (TTP) examined in Frei et al. (2009). In response to concerns about reverse causation. Analysis of TTP stratified on the basis of parity (nulliparous vs parous) women. See Fei et al (2009) **Location:** See Fei et al (2009) **Population:** Nulliparous preg women (n = 558) Parous preg women (n = 683) See Fei et al (2009) **Related Studies:** Fei et al. (2009)	**Exposure Assessment:** See Fei et al (2009) **Population-Level Exposure:**	**Stat Method:** Findings of delye TTP in Fei et al. (20090 was criticized as possibly reflecting reverse causation - longer TTP provides longer time for PFOS exposure leading to assoc of ↑ PFOS and ↑ TTP. Concept is plausible for parous women since pregnancy and nursing reduce PFOS body burden, thus allowing PFOS levels to increase post-natally. However, as nulliparous women are presumed to be at steady-state, early preg blood samples should reflect a preg-related change in PFOS regardless of TTP. **Outcome:** OR for TTP **Major Findings:** <u>Nullparous</u> OR (compared to 1st quart) sig for 3rd quart (2.50) and borderline sig for 4th quart (2.14 (95% CI = 1.0-4.60) Sig for trend (p = 0.036) <u>Parous</u> OR (compared to 1st quart) sig for 2nd and 3rd quart, but not 4th quart. Not sig for trend **Outcome:** OR for Fecundity (see Fei et al. (2009)	**Major Limitations:** **Other comments:** See Fei et al. (2009) Reasonable n for nulliparous and parous sub-pop's.

		Major Findings: <u>Nulliparous</u> OR (compared to 1st quart) sig (i.e., < 1.0) for 2nd-4th quart Sig fro trend (p = 0.006) <u>Parous</u> OR (compared to 1st quart) sig for 2nd-4th quart Not sig for trend	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fisher et al. (2013) Fisher M, Arbuckle TE, Wade M, Haines DA. Do perfluoroalkyl substances affect metabolic function and plasma lipids?--Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res. 2013 Feb;121:95-103. doi: 10.1016/j.envres.2012.11.006. Epub 2012 Dec 22. Erratum in: Environ Res. 2013 Oct;126:221. **Study Design:** Nested Cross-sectional Assoc of PFOS (PFOA, PFHxS) and metabolic function, plasma lipid levels Measured Trigylcerides Glucose HDL LDL Total cholesterol Insulin Insulin samples < LOD (72/1325) discarded HDL and total cholesterol on all samples LDL glucose, insulin and triglycerides on fasted samples only	**Exposure Assessment:** Fasted requested prior to blood samples PFOS measured in plasma PFOS by MS (apparently no HPLC) LOD = 0.3 ng/ml Samples < LOD = ½ LOD **Population-Level Exposure:** PFOS geom mean = 8.40 ng/ml PFOS consistent w US exposure for ≥ 20 yrs old (NHANES 4th Rpt) (PFOA geom mean = 2.46 ng/ml) PFOS-PFOA correlated, r = 0.36	**Stat Method:** Analyses presented as weighted and unweighted relative to sampling strategy in the original cohort Multiple linear reg to est assoc between log transf continuous outcomes and PFOS Potential co-variates considered: - Age - Gender - Marital status - Income adequacy - Race - Education - BMI - Smoking - Alcohol Co-variates included if sig in bivariate model w either outcome or exposure at $\alpha = 0.1$ and in > 1 multivariate mode, $\alpha = 0.05$ Multiple logistic regression for dichotomous outcomes Mandatory co-variates - Age - Sex Co-variates initially added with $p < 0.15$ and retained w Δ OR ≥ 10%	**Major Limitations:** Does not appear that PFOS analyses were controlled for PFOA or PFHxS Participants on cholesterol controlling drugs excluded. This may eliminate those w ↑ cholesterol resulting from ↑ PFOS Interpretation of weighted vs. unweighted analysis is unclear. **Other comments:** Large N. Reasonable statistical analysis (controlling) strategy. Rel modest PFOS exposure reducing power

Homoeostasis Model Assessment – Insulin Resistance (HOMA-IR) calc as function of glucose and insulin levels (formula not provided) Metabolic syndrome – occurrence of 3/5 of following: - Elevated abdominal waist circum - Elevated triglycerides - Reduced HDL-cholesterol - Elevated systole BP - Elevated fasting glucose **Location:** Canada **Population:** Canadian Health Measures Survey Designed to provide nationally rep sample of health conditions w ≥ 10% prevalence in Canadians 6-79 yrs old Self-reported questionnaire and mobile exam clinic 69.6% household response Current study incl non-preg 18-74 yrs old (M & F) **N = 2,700** (for clinical outcomes)		**Outcome:** HDL **Major Findings:** <u>Adj model</u> PFOS not sig assoc w HDL in unweighted or weighted model **Outcome:** Total cholesterol (TC) **Major Findings:** <u>Adj Model</u> PFOS **sig** assoc (pos) for TC in unweighted model, but **not in weighted model** **Outcome:** TC/HDL **Major Findings:** <u>Adj Model</u> PFOS **sig** assoc w TC/HDL (pos) in unweighted model, but **not in weighted model** **Outcome:** LDL	

Cholesterol lower med use excluded for cholesterol and metabolic syndrome determinations **N = 2366** **Related Studies:**		**Major Findings:** <u>Adj model</u> PFOS not sig assoc w LDL in either weighted or unweighted models **Outcome:** Non-HDL **Major Findings:** <u>Adj Model</u> PFOS **sig** assoc w non-HDL (pos) in unweighted model, but **not in weighted model** **Outcome:** Triglycerides (TRIG) **Major Findings:** <u>Adj model</u> PFOS not sig assoc w TRIG in either weighted or unweighted models **Outcome:** Insulin	

		Major Findings: <u>Adj model</u> PFOS not sig assoc w insulin in either weighted or unweighted models **Outcome:** Glucose **Major Findings:** <u>Adj model</u> PFOS not sig assoc w glucose in either weighted or unweighted models **Outcome:** HOMA-IR **Major Findings:** PFOS not sig assoc w HOMA-IR in either weighted or unweighted models **Outcome:** Metabolic syndrome (Y/N) **Major Findings:** <u>Adj model</u> PFOS not sig assoc w metabolic syndrome in either weighted or unweighted models	

		Outcome: High cholesterol (Y/N) **Major Findings:** <u>Adj model</u> PFOS not sig assoc w high cholesterol in either weighted or unweighted models **Outcome:** High cholesterol by quartile PFOS exposure **Major Findings:** <u>Adj model</u> Unweighted analysis - PFOS not sig assoc w high cholesterol for any quart of exposure (although borderline for 4th quart), but **sig for trend** Weighted analysis – PFOS not sig assoc w high cholesterol for any quart and not sig for trend	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fitz-Simon et al. (2013) Fitz-Simon N, Fletcher T, Luster MI, Steenland K, Calafat AM, Kato K, Armstrong B. Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology. 2013 Jul;24(4):569-76. doi: 10.1097/EDE.0b013e31829443ee. Erratum in: Epidemiology. 2013 Nov;24(6):941. **Study Design:** Longitudinal design Baseline PFOS, serum lipids at initial survey (2005/6) Follow up PFOS, serum lipids (2010) Mean interval between surveys = 4.4 yr Fasting status on blood draw recorded (but not required) Lipids measured enzymatically - total cholesterol - HDL cholesterol - triglycerides LDL cholesterol by Friedwald equation for triglycerides < 400 mg/dL	**Exposure Assessment:** Baseline sample analyzed by protein precip, reverse-phase HPLC-MS Follow-up sample analyzed by solid-phase extraction, reverse-phase HPLC, isotope dilution MS (NOTE: authors claim that both methods are essentially equivalent) **Population-Level Exposure:** Geom mean PFOS conc – baseline = 18.5 ng/ml Follow-up = 8.2 ng/ml	**Stat Method:** Linear regression models For log ratio (follow-up/baseline) PFOS conc Model structure eliminates co-variates that are constant between baseline and follow up Models adj for - age at baseline - fasting status - time between measurements - baseline BMI (in sens analysis) Analyses included joint PFOS, PFOA **Outcome:** Percent Δ in LDL cholesterol for 50% decrease in PFOS **Major Findings:** **Sig** (4.6-5.0%) **decrease** in LDL cholesterol for 50% ↓ in serum PFOS (Also sig when PFOA incl in model) **Outcome:** Percent Δ in total cholesterol for 50% decrease in PFOS **Major Findings:** **Sig** (2.8-3.2%) decrease in Total cholesterol for 50% ↓ in serum PFOS (Also sig when PFOA incl in model)	**Major Limitations:** Small N Inability to see change if initial effect of PFOS is irreversible **Other comments:** Longitudinal study Statistical analysis mechanism eliminates most issues of confounding

Serum creatinine measured. Used to calculate glomerular filtration rate Follow-up exclusions: - Lipid lowering drugs at baseline or follow-up - Exclusion for LDL when triglycerides > 400 mg/dL **Location:** OH, WV **Population:** C8 study cohort **N = 560** (for total cholesterol, HDL cholesterol, triglycerides) **N = 521** (for LDL cholesterol) F = 54% **Related Studies:**		**Outcome:** Percent Δ in HDL cholesterol for 50% decrease in PFOS **Major Findings:** Δ HDL cholesterol **not sig assoc** w 50% change in PFOS **Outcome:** Percent Δ in triglycerides for 50% decrease in PFOS **Major Findings**: Δ triglycerides cholesterol **not sig assoc** w 50% change in PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Frisbee et al. (2010) Frisbee SJ, Shankar A, Knox SS, Steenland K, Savitz DA, Fletcher T, Ducatman AM. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Arch Pediatr Adolesc Med. 2010 Sep;164(9):860-9. doi: 10.1001/archpediatrics.2010.163. **Study Design:** Cross-sectional community-based Participants in C8 study provided blood sample on enrollment (2005-2006) Time of last meal recorded Total cholesterol LDL cholesterol HDL cholesterol Triglycerides Lipid analysis in clinical laboratory (LabCorp) **Location:** W. Va and OH potentially exposed to PFC from DuPont Washington Works facility from public drinking water supplies	**Exposure Assessment:** Protein precip extraction, reverse phase HPLC-triple-quadrupole MS LOD not reported **Population-Level Exposure:** Mean PFOS = 22.7 (+/-12.6) ng/ml (mean PFOA = 69.2 (111.9) ng/ml	**Stat Method:** Co-variates (all considered in all models) - Age - Gender - BMI (z-score) - Fasting time (min) - Exercise (Y/N) Quantiles (where employed) age and gender-specific Multiple linear regression for lipids as continuous variables Logistic regression for odds of abnormal lipid levels (in children) - Total C -≥ 170 mg/dL - LDL-C ≥ 110 mg/dL - Triglycerides ≥ 150 mg/dL **Outcome:** Total-C **Major Findings:** <u>Continuous linear regression (adj model)</u> Sig pos assoc w PFOS (and PFOA) <u>Analysis of est. marginal mean (EMM) across quintiles of PFOS (adj model)</u> ↑ Trend sig for M, F and both for 1-11.9 yrs And 12-17 yrs	**Major Limitations:** Cross-sectional study Mean PFOS conc >95th percentile of 12-19 yr olds from NHANES 4th biomonitoring rpt Mean PFOA conc >>95th percentile of 12-19 yrs old from NHANES 4th biomonitoring rpt **Other comments:** The N of this study is large and statistical controls are reasonable. Although the study is cross-sectional exposure was consistent of the course of years.

Population: Children 1-17.9 yrs old in C8 Health Study N = 3,857 1-11.9 yrs M = 1,971 F = 1,886 N = 5,293 12-17.9 yrs M = 2,773 F = 2,520 ~40% overweight/obese (BMI > 85th percentile **Related Studies:** Geiger et al. (2014)		<u>OR for risk of abnormal level</u> Sig OR > 1.0 for 2nd-5th quintile (1st as ref) **Outcome:** LDL-C **Major Findings:** <u>Continuous linear regression (adj model)</u> Sig pos assoc w PFOS (and PFOA) <u>Analysis of est. marginal mean (EMM) across quintiles of PFOS (adj model)</u> ↑ Trend sig for M, F and both for 1-11.9 yrs And 12-17 yrs <u>OR for risk of abnormal level</u> Sig OR > 1.0 for 4th and 5th qunit (1st as ref) **Outcome:** HDL-C **Major Findings:** HDL-C pos assoc w PFOS (sig?)	

		Analysis of est. marginal mean (EMM) across quintiles of PFOS (adj model) ↑ Trend sig for M, and both for 12-17 yrs Marginally sig for F (p = 0.06) ↑ Trend sig for M and both (but not F) for 1-11.9 yr OR for risk of abnormal level Sig OR **<** 1.0 for 4th and 5th quint (1st as ref) **Outcome:** Triglycerides (fasting) **Major Findings:** Continuous linear regression (adj model) Not sig assoc w PFOS Analysis of est. marginal mean (EMM) across quintiles of PFOS (adj model) ↓ trend sig for F only OR for risk of abnormal level OR not sig for any quintile **Outcome:** Interaction of PFOS and PFOA **Major findings:** No sig interaction of PFOS and PFOA for any blood lipid outcome	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Fu et al. (2014) Fu Y, Wang T, Fu Q, Wang P, Lu Y. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf. 2014 Aug;106:246-52. doi: 10.1016/j.ecoenv.2014.04.039. Epub 2014 May 23. **Study Design:** Cross-sectional Total cholesterol (TC) Triglycerides (TG) HDL-C, LDL-C Measured **Location:** Yuangyang, China **Population:** Recruited randomly from patients at local hospital Age range – 0-88 yrs Mean = 34 yrs **N (for PFOS) = 133** **Related Studies:**	**Exposure Assessment:** Solvent extraction (MTBE) HPLC-triple quadrupole MS LOQ? **Population-Level Exposure:** PFOS mean conc = 1.68 ng/ml (sd = 1.20 ng/ml) 4th quart mean = 3.12 ng/ml (NOTE: exposure is only 18% of current overall US geom mean (NHANES 4th Rpt))	**Stat Method:** Linear regression analysis of ln-transformed: TC, TG, HDL-C and LDL-C (as quartiles) Also logistic regression for OR for abnormal lipids (Guidelines on Prevention and Treatment of Blood Lipid Abnormality in Chinese Adults (Zhao, 2008) Models (linear and logistic) controlled for age, gender, BMI) **Outcome:** TC **Major Findings:** (adj models) Change in TC per quartile PFOS **not sig** OR for abnormal TC **not sig >1.0** for any quartile **Outcome:** TG **Major Findings:** (adj models) Change in TG per quartile PFOS **not sig** OR for abnormal TG **not sig >1.0** for any quartile	**Major Limitations:** Very low PFOS exposure Modest N Large age range (unclear whether introduction of age co-variate into models is sufficient to address the age range of 0-88 yrs) Small suite of co-variates employed (e.g., smoking not considered) **Other comments:** Little power to detect results Minimal statistical analysis

		Outcome: HDL-C **Major Findings:** (adj models) Change in HDL-C per quartile PFOS **not sig** OR for abnormal HDL-C **not sig >1.0** for any quartile **Outcome:** LDL-C **Major Findings:** (adj models) Change in LDL-C per quartile PFOS **not sig** OR for abnormal LDL-C **not sig >1.0** for any quartile	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Gallo et al. (2012) Gallo V, Leonardi G, Genser B, Lopez-Espinosa MJ, Frisbee SJ, Karlsson L, Ducatman AM, Fletcher T. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environ Health Perspect. 2012 May;120(5):655-60. doi: 10.1289/ehp.1104436. Epub 2012 Jan 3 **Study Design:** C8 Study cohort Blood samples (at collection of questionnaire data) <u>Measured markers of liver function</u> AlT (alanine aminotransferase) GGT (Gamma-glutamyl transpeptidase) Direct bilirubin Measured in commercial clinical lab (LabCorp) Homeostasis model assessment of insulin resistanace (HOMA-IR) as measure of insulin resistanace Calculated as:	**Exposure Assessment:** Automated solid-phase extraction, reverse-phase HPLC-MS. Intra-laboratory CV for PFOS = 0.1 LOD = 0.5 ng/ml Non-detect (PFOS n = 230) = LOD/2 **Population-Level Exposure:** <u>PFOS median</u> - All - 20.3 ng/ml (IQR = 13.7-29.4 ng/ml) - F - 17.4 (IQR = 1.6-25.5) - M – 23.5 (IQR = 16.8-32.6) Levels consistent w National background (NHANES 4th Rpt)	**Stat Method:** Ln transformation of all outcome measures of linear regression <u>Potential confounders:</u> Age Physical activity BMI (underweight, normal, overweight, obese) Household income Educational level Race Alcohol Smoking HOMA-IR investigated as co-variate Logistic regression models for dichotomous assoc of PFOS w abnormal levels of outcome variables **Outcome:** Ln ALT (fully adj model) **Major Findings:** <u>Linear regression</u> PFOS stat sig assoc w ↑ <u>Logistic regression</u> OR for abnormal ALT stat sig > 1.0 for deciles > 5th Sig for ↑ trend	**Major Limitations:** PFOS outcomes were not controlled for PFOA conc, which was much higher than US average (NHANES 4th Rpt) Cross-sectional, but long-term exposure of pop. **Other comments:** Study is straightforward in design. Very large N. Although cross-sectional exposure can reasonably be assumed to have been constant for decades

(Basal glucose x insulin level)/2.25 **Location:** Mid-Ohio valley, WV. **Population:** C8 Study cohort Exposed to PFC contaminated drinking water for ≥ 1yr (prior to 2005-2006) 69,030 total cohort → adults ≥ 18 yrs old → **46,452** w complete co-variate information F - n = 24,171 M - n = 22,281 **Related Studies:** Frisbee et al. (2010)		**Outcome:** Ln GGT (fully adj model) **Major Findings:** <u>Linear regression</u> PFOS not sig assoc <u>Logistic regression</u> OR for abnormal GGT not sig for any decile **Outcome:** Ln direct bilirubin (fully adj model) **Major Findings:** <u>Linear regression</u> PFOS sig assoc w ↑ <u>Logistic regression</u> OR for abnormal direct bilirubin not sig for any decile Sig for ↑ trend	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Gallo et al. (2013) Gallo V, Leonardi G, Brayne C, Armstrong B, Fletcher T. Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study. BMJ Open. 2013 Jun 20;3(6). pii: e002414. doi: 10.1136/bmjopen-2012-002414. **Study Design:** Cross-sectional Exclusions for missing co-variate data Self-identified categorical short-term memory loss: "frequent," "sometimes," "rarely," "never" Analyses based on comparison of frequent/ sometimes vs. rarely/never **Location:** OH, WV **Population:** C8 study population ≥ 50 yrs old N = 21,024	**Exposure Assessment:** Solid-phase extraction, reverse-phase HPLC PFOS LOD = 0.5 ng/ml < LOD = LOD/2 (n = 101, 0.5%) **Population-Level Exposure:** Median PFOS conc ≈ 24 ng/ml (mean not given, median est as average of 3rd quintile range) (NOTE: median is ~ 2.4 x current US > 20 yr old conc (NHANES 4th Rpt)	**Stat Method:** Logistic regression Co-variates: - age (1 yr bands) - race - gender - education - income - physical activity - alcohol - smoking - BMI - diabetes PFOS as continuous variable – assoc based on doubling PFOS conc PFOS as quintiles Ordinal regression (outcome as 4 levels of memory loss) Sensitivity analyses: - ≥ 65 yrs old (n = 7,097) - full sample w outcome as *any* memory loss - geographic clustering of water districts	**Major Limitations:** Self-reported categorical assessment of memory loss **Other comments:** Cross-sectional study Length of exposure not controlled for in analyses Self-reported outcome status Unclear respondents used a consistent and objective scale of memory loss Large N

Related Studies:		**Outcome:** Assoc memory loss w serum PFOS **Major Findings:** OR for memory loss **not sig > 1.0** for any quintile PFOS Trend for continuous PFOS conc sig **neg assoc** w memory loss Memory loss not sig pos assoc w PFOS for any sensitivity analysis	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Geiger et al. (2013) Geiger SD, Xiao J, Shankar A. Positive association between perfluoroalkyl chemicals and hyperuricemia in children. Am J Epidemiol. 2013 Jun 1;177(11):1255-62. doi: 10.1093/aje/kws392. Epub 2013 Apr 3. **Study Design:** Cross-sectional Blood sample and personnel questionnaire data from NHANES Serum uric acid and serum PFOS from NHANES blood sample Uric acid analysis by clinical lab Assoc of PFOS w serum uric acid/hyperuricemia (elevated uric acid) (No std definition hyperuricemia for children– defined in study as ≥ 6 mg/dL **Location:**	**Exposure Assessment:** PFOS analysis by Nat'l Center Env. Health as part of NHANES analysis Automated solid-phase extraction, isotope dilution HPLC-MS LOD for PFOS 0.4 ng/ml (2003-4) 0.2 ng/ml (2005-8) **Population-Level Exposure:** Mean PFOS = 18.4 ng/ml (SE = 0.5 ng/ml) (Mean PFOA = 4.3 ng/ml (SE = 0.1 ng/ml)	**Stat Method:** Ln-PFOS as continuous and categorical variable <u>Co-variates in model</u> Age Sex Race BMI (categorical) Household income Moderate activity (Y/N) Serum total cholesterol Serum cotinine Logistic regression for OR hyperuricemia by PFOS quartile **Outcome:** Assoc uric acid relative and PFOS **Major Findings:** <u>Assoc uric acid and PFOS on continuous scale</u> <u>PFOS on linear scale</u> uric pos assoc w for 4th quart of PFOS exposure (1st quart as ref) **But for unadjusted model only** Uric acid **not** assoc w PFOS in adjusted model Trend not sig	**Major Limitations:** Cross-sectional PFOS analyses not controlled for PFOA (and other PFC) exposures **Other comments:** Large N Reasonable statistical control of confounders and co-variates (except PFOA, etc.) Equivocal findings

Population: NHANES 199-200, 2003-2008 data Children 12-18 yrs old completing sampling and interview portions of NHANES and complete information for critical variables N = 1,772 Mean age = 15.0 M = 51.9% F = 48.1% **Related Studies:**		<u>Ln-transformed PFOS</u> Uric acid pos assoc w ln-transform PFOS **Outcome:** Assoc of hyperuricemia and PFOS **Major Findings:** OR for hyperuricemia sig > 1.0 for 4th quart serum PFOS (adj and unadj models) (OR for Quart 2, 3 > 1.0, but not sig) ↑Trend stat sig Also, ln-transformed PFOS Similar results for alt cutoffs for definition hyperuricemia (5.5-7.7 mg/dL)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Geiger et al. (2014a) Geiger SD, Xiao J, Shankar A. No association between perfluoroalkyl chemicals and hypertension in children. Integr Blood Press Control. 2014 Jan 13;7:1-7. doi: 10.2147/IBPC.S47660. eCollection 2014. **Study Design:** Cross-sectional Data from NHANES - 1999-2000; 2003-2004; 2005-2006; 2007-2008 BP taken at examination portion of NHANES process (mean of ≤ 3 separate readings) Hypertension defined as BP ≥95th percentile Adj: age, height .sex **Location:** US **Population:** NHANES cohort 12-18 yrs old Excluding those w missing co-variate data **N = 1, 655**	**Exposure Assessment:** CDC-NHANES analytical proc **Population-Level Exposure:** Mean PFOS conc = 18.4 ng/ml	**Stat Method:** PFOS as continuous and categorical var linear regression Continuous PFC ln-transformed Co-variates: - age - sex - race/ethnicity - BMI - moderate physical activity (Y/N) - income - serum total cholesterol Categorical PFOS in quartiles Logistic regression OR of hypertension for ea quart Sample weights adj per NHANES **Outcome:** Assoc systolic BP/hypertension w PFOS **Major Findings:** (adj model) Systolic BP/hypertension **not sig assoc w PFOS** for either continuous or categorical (OR) regression	**Major Limitations:** PFOS analysis not adj for PFOA **Other comments:** Large N Reliable analytical methodology Cross-sectional study

Related Studies:		**Outcome:** Assoc diastolic BP/hypertension w PFOS **Major Findings:** (adj model) Diastolic BP/hypertension **not sig assoc w PFOS** for either continuous or categorical (OR) regression	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Geiger et al. (2014b) Geiger SD, Xiao J, Ducatman A, Frisbee S, Innes K, Shankar A. The association between PFOA, PFOS and serum lipid levels in adolescents. Chemosphere. 2014 Mar;98:78-83. doi: 0.1016/j.chemosphere.2013.10.005. Epub 2013 Nov 13. **Study Design:** Nested corss-sectional from NHANES 1999-2000, 2000-2008 Assoc PFOS w serum: Total cholesterol LDL-C HDL-C triglycerides **Location:** U.S. **Population:** Children 12-18 yrs Mean age = 15.1 yrs Completed laboratory and examination/ portions of NHANES Complete information on key variables N = 815	**Exposure Assessment:** PFC analysis by Nat'l Center Env. Health (CDC) Solid-phase extraction, isotope dilution HPLC-MS Non-detects as LOD/√2 LOD? **Population-Level Exposure:** PFOS detected in > 98% of samples Mean (SE) PFOS serum conc = 17.7 ng/ml (0.7 ng/ml)	**Stat Method:** PFOS as continuous and categorical variable w ln-transformed PFOS conc Models included: Age Sex Race-ethnicity Bw categories Household income Moderate activity (Y/N) Serum cotinine OR for dyslipidemia by Multivariate logistic regression **Outcome:** Total cholesterol **Major Findings:** (adj models) <u>Categorical analysis</u> Change in cholesterol conc (mg/dL) by PFOS tertile to 1st tertile (ref) ↑ cholesterol 2nd and 3rd tert Sig for 3rd tert , but not sig for 2nd tert Trend borderline sig <u>Continuous analysis (ln-PFOS)</u> Sig pos assoc (small)	**Major Limitations:** Cross-sectional study PFOS analyses did not control for PFOA **Other comments:** Relatively large N Reasonable statistical control for co-vartiates – except PFOA

Related Studies: Frisbee et al. (2010)		Risk of dyslipidemia ↑ OR across tertiles Stat sig for 3rd tert Sig for trend Ln-PFOS sig in continuous analysis **Outcome**: LDL-C **Major Findings:** (adj models) Categorical analysis ↑ in LDL-C in 2nd and 3rd tert (1st as ref) Sig for 2nd and 3rd tert Sig for trend Continuous analysis (ln-PFOS) Sig pos assoc Risk of dyslipidemia ↑ OR across tertiles Stat sig for 3rd tert Sig for trend Ln-PFOS sig in continuous analysis	

		Outcome: HDL-C **Major Findings:** (adj models) <u>Categorical analysis</u> Inconsistent Sig pos assoc for 2nd, but not 3rd tert Trend not sig <u>Risk of dyslipidemia</u> ORs not sig Trend not sig Ln-PFOS not sig in continuous analysis **Outcome:** Triglycerides **Major Findings:** (adj models) <u>Categorical analysis</u> No sig assoc Trend not sig <u>Risk of dyslipidemia</u> ORs not sig Trend not sig Ln-PFOS not sig in continuous analysis	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Ghisari et al. (2014) Ghisari M, Eiberg H, Long M, Bonefeld-Jørgensen EC. Polymorphisms in phase I and phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environ Health. 2014 Mar 16;13(1):19. doi: 10.1186/1476-069X-13-19. **Study Design:** Further investigation of Bonefeld-Jorgensen (2011) examining assoc of spec SNPs w PFOS and breast cancer Case-control study **N = 31 breast cancer cases** Cases matched by age and district of residence to **controls (n = 115)** Blood samples at breast cancer diagnosis Questionnaire data for Demographic, lifestyle PCR for SNPs of multiple CYP polymorphisms	**Exposure Assessment:** (from Bonefeld-Jorgensen et al. Environ Health. 2011; 10: 88. Published online 2011 October 6. doi: 10.1186/1476-069X-10-88) Ion-pairing extraction LC-MS-MS) with electrospray ionization LOD = 0.1 to 0.4 ng/ml **Population-Level Exposure:** (from Bonefeld-Jorgensen et al. Environ Health. 2011; 10: 88) Median PFOS conc: Cases = 45.6 ng/ml Controls = 21.9 ng/ml	**Stat Method:** Unconditional logistic regression for interaction of CYP SNPs, PFOS and breast cancer risk PFOS ln-transformed Co-variates: - age - cotinine (other variables not included due to small n for cases) PFOS as categorical (high/low relative to control median) var and Continuous variable Analysis stratified by genotypes OR calculated for > median (high) vs. < median (low) PFOS (**Outcome:** OR for assoc PFOS (high/low) w breast cancer **Major Findings:** For all CYP genes tested**, OR sig > 1.0** for high PFOS for at least one SNP (for all other SNPs, OR could not be calculated due to lack of cases or controls)	**Major Limitations:** Small n **Other comments:** Largely a mechanistic assessment of PFOS influence on breast cancer through assoc PFOS w spec SNPs Case-control methodology Clear ascertainment of endpoint

Location: Greenland - Nuuk, Upernavik, Qeqertensuaq, Narsaq, Tarsilaq, Qaqortoq, Sisimiut, Assiat, Nanortalik **Population:** Inuit women **Related Studies:** Bonefeld-Jorgensen et al. (2011)			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Gleason et al. (2015) Gleason JA, Post GB, Fagliano JA. Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environ Res. 2015 Jan;136:8-14. doi: 10.1016/j.envres.2014.10.004. Epub 2014 Nov 19. **Study Design:** NHANES 2007-2008, 2009-2010 combined databases PFOS measured in random 1/3 of sample ≥ 12 yrs old Liver enzymes: ALT GGT AST ALP Total bilirubin Uric acid **Location:** U.S. **Population:** Hepatitis B/C carriers excluded N = 4,333	**Exposure Assessment:** Solid-phase extraction, HPLC-MS > LOD as LOD/√2 **Population-Level Exposure:** PFOS geom mean = 11.0 ng/ml (95% CI = 10.2-11.8) median = 11.3 (IQR = 7.0-8.0) (PFOA Geom mean = 3.5 ng/ml) Also PFNA, PFOS and PFHxS measured	**Stat Method:** Outcomes non-normal based on visual assessment ln-transformed PFOS ln-transformed Multiple-linear regression <u>Co-variates:</u> Age Gender Race/ethnicity BMI (dichotomized) Poverty (dichotomized) Smoking (dichotomized on cotinine) Alcohol (categorical) Ln-serum creatinine <u>Logistic regression-OR</u> PFOS as quartiles Outcomes dichotomized on 75th percentile **Outcome:** uric acid **Major Findings:** (fully adj models) <u>Linear regression</u> Sig pos assoc w PFOS ($p < 0.01$) <u>Logistic regression</u> OR < 1.0	**Major Limitations:** Cross-sectional PFOS not controlled for other PFCs **Other comments:** Large N Reasonable statistical analysis (except for other PFCs)

Related Studies: Geiger et al. (2013) (Uric acid and PFOS in adolescents from NHANES)		**Outcome:** Ln-ALT **Major Findings:** (fully adj models) <u>Linear regression</u> Not sig assoc w PFOS <u>Logistic regression</u> OR < 1.0 **Outcome:** Ln-GGT **Major Findings:** (fully adj models) <u>Linear regression</u> Not sig assoc w PFOS <u>Logistic regression</u> OR < 1.0 **Outcome:** Ln-AST **Major Findings:** (fully adj models) <u>Linear regression</u> Not sig assoc w PFOS <u>Logistic regression</u> OR < 1.0	

		Outcome: Ln-ALP **Major Findings:** (fully adj models) <u>Linear regression</u> Not sig assoc w PFOS <u>Logistic regression</u> OR < 1.0 **Outcome:** Total bilirubin **Major Findings:** (fully adj models) <u>Linear regression</u> Not sig assoc w PFOS <u>Logistic regression</u> OR quart 2,3, 4 (1 as ref) sig > 1.0 (~ 1.4-1.7 – visually from graphic) P trend = 0.026	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Goudarzi et al., 2017 Goudarzi, H., C. Miyashita, E. Okada, I. Kashino, C. J. Chen, S. Ito, A. Araki, S. Kobayashi, H. Matsuura and R. Kishi (2017). "Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age." Environ Int **104**: 132-138. **Study Design:** Prospective birth cohort **Location:** Japan **Population:** N=1558 mother-child pairs who were enrolled in the Hokkaido Study on Environment and Children's Health **Outcome Assessment:** Participant characteristics were obtained from medical birth records and self-administered questionnaires during pregnancy and after delivery and 4 years post-delivery.	**Exposure Assessment:** PFAAs measured in maternal plasma taken at 28-32 weeks of gestation **Population-Level Exposure:** Mean PFOS=5.5 ng/mL 25^{th} = 3.67 50^{th} = 4.93 75^{th} = 6.65 PFOA=2.7 ng/mL	**Stat Method:** PFAAs analyzed by quartiles and asses in crude and adjusted logistic regression analyses. Trend in the p-value was estimated. Potential confounders and covariates considered include maternal age, number of older siblings, maternal smoking during pregnancy, maternal education, infant sex, and breast-feeding period. Day care attendance and environmental tobacco smoke at 4 years were included for sensitivity analysis. **Outcome:** Total infectious disease= Otitis media, Pneumonia, Respiratory syncytial infection, varicella **Major Findings:** Q2 v Q1 OR=1.44 (95% CI 1.06, 1.96) Q3 v. Q1 OR=1.28 (95% CI 0.95, 1.73) Q4 v Q1 OR=1.61 (95% CI 1.18, 2.21) **P for Trend=0.008** **Similar findings by stratification for boy and girl, only P for trend for girls was statistically significant, but overall findings were comparative to boys.**	**Major Limitations:** Did not control for other co-occurring environmental contaminants as potential confounders. **Other comments:** Study design is a strength and serum PFAA collection at potential vulnerable developmental window

Reference and Study Design	Exposure Measures	Results	Comment
Study: Grandjean et al. (2012) [w. erratum 2012] Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, Heilmann C. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012 Jan 25;307(4):391-7. doi: 10.1001/jama.2011.2034. Erratum in: JAMA. 2012 Mar 21;307(11):1142. **Study Design:** Prospective birth cohort (1997-2000) **Location:** Faroe Islands (National Hospital) **Population:** n=656 consecutive singleton births recruited 1997-2000 and 587 followed-up through 2008. **Outcome Definition:** Serum antibody concentrations against tetanus and diphtheria toxoids at ages 5 years prebooster, approximately 4 weeks after the booster, and at age 7 years. Measurement of specific antibodies <u>Tetanus</u> – by enzyme-linked immunosorbent assay <u>Diphtheria</u> – by cell-based neutralization assay	**Exposure Assessment:** Gestational maternal serum PFOS exposure from last maternal ant-natal exam (32 wks) Post-natal PFOS exposure from child's serum 5 (pre-booster) Solid-phase extraction, HPLC-MS w/in and between batch imprecision (by CV) < 3.0%, 5.2% (respectively) **Population-Level Exposure:** PFOS Geometric mean (IQR): Maternal – 27.3 (23.2-33.1) 5 yrs old – 16.7 (13.5-21.1)	**Stat Method:** Correlations were determined by pairwise Pearson correlation coefficients. Linear regression, covariates and confounders considered include sex and age. For 5-year pre-booster data models adjusted for time since vaccination, possible PCB exposure, birth weight, maternal smoking during pregnancy, and duration of breastfeeding, and booster type. Structural equation models were generated to determine the joint association of PFCs with the overall antibody concentrations. Also controlled for PFCs in maternal pregnancy serum in some of these structural models. PFCs were also categorized when greater than 0.1 IU/mL – and odds ratios were estimated. PFCs and antibodies were log-transformed. **Outcome:** **Major Findings:** **<u>Tetanus % difference (2-fold)</u>** <u>Maternal PFC</u> (Year 5 Pre): -10.1 (95% CI -31.9, 18.7) (Year 5 Post): -2.3 (95% CI -28.6, 33.6); (Year 7): 35.3 (95% CI -3.9, 90.6) **(Year 7 adj. for 5): 33.1 (95% CI 1.5, 74.6);** not significant when controlled for PCBs <u>Child (age 5) PFC</u> (Year 5 Pre): -11.9 (95% CI -30.0, 10.9) **(Year 5 Post): -28.5 (95% CI -45.5, -6.1)** (Year 7): -23.8 (95% CI -44.3,4.2);* **significant when controlled for PCBs** (Year 7 adj for 5): -11.4 (95% CI -30.5, -12.8)	**Major Limitations:** Maternal PFOS concs at ~75th percentile US female conc (4th Nat'l Rpt) Combined sig neg assoc of tetanus and diphtheria antibodies in structural equation models suggest that est of independent PFOS effect is influenced by overall PFC effect. Possible confounding due to unmeasured variables, and other environmental contaminants **Other comments:** The prospective study design is powerful. The N's are reasonable, but larger n's may have yielded more definitive results.

		Structural Eq. (PFOA, PFOS, and PFHxS combined) *Age 5 pre booster* (Maternal): 20.0 (95% CI-49.2, 25.2) (Age 5): -20.5 (95% CI-44.4, 13.6) (Age 5 adj for maternal): -17.2 (-42.1, 18.5) *Age 7* (Maternal): 35.1 (95% CI -25.4, 144.6) (**Age 5): -55.2 (95% CI -73.3, -25.0)** **(Age 5 adj for maternal): -58.8 (-76.0, -29.3)** Odds Ratio Maternal serum Age 5: OR=1.16 (95% CI 0.71, 1.89) Age 7: OR=0.53 (95% CI 0.16, 1.79) Child serum Age 5: OR=1.16 (95% CI 0.77, 1.74) Age 7: OR=2.61 (95% CI 0.77, 8.92) **Diphtheria % difference (2-fold)** Maternal PFC **(Year 5 Pre): -38.6 (95% CI -54.7, -16.9);* not significant when controlled for PCBs** (Year 5 Post): -20.6 (95% CI -37.5, 0.9) (Year 7): -19.7 (95% CI -41.8, 10.7) (Year 7 adj. for 5): -10.0 (95% CI -32.6, 20.0 Child (age 5) PFC (Year 5 Pre): -16.0 (95% CI -34.9, 8.3) (Year 5 Post): -15.5 (95% CI -31.5, 4.3) **(Year 7): -27.6 (95% CI -45.8, -3.3);** (Year 7 adj for 5): -20.6 (95% CI -38.2, 2.1) *Structural Eq. (PFOA, PFOS and PFHxS combined)* *Age 5 pre-booster* **(Maternal): -47.9 (95% CI -67.7, -15.9)**	

		(Age 5): -7.9 (95% CI -38.0, 37.0) (Age 5 adj for maternal): -1.2 (-33.6, 46.8) *Age 7* **(Maternal): -42.0 (95% CI -66.1, -0.8)** (**Age 5): -44.4 (95% CI -65.5, -10.5)** **(Age 5 adj for maternal): -45.5 (-66.9, -10.3)** Odds Ratio Maternal serum **Age 5: OR=2.48 (95% CI 1.55, 3.97)** Age 7: OR=2.33 (95% CI 0.88, 6.14) Child serum **Age 5: OR=1.60 (95% CI 1.10, 2.34)** Age 7: OR=2.38 (95% CI 0.89, 6.35) **Structural equation – joint** **For the structural equation model the joint change in antibody showed decreased association with PFCs at age 5 and at age 5 with adjustment for PFC in maternal pregnancy serum (non-significant) and significant association with Age 7 joint vaccine.**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Granum et al. (2013) Granum B1, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, van Loveren H, Løvik M, Nygaard UC. J Immunotoxicol. 2013 Oct-Dec;10(4):373-9. doi: 10.3109/1547691X.2012.755580. Epub 2013 Jan 25. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. **Study Design:** Nested cross-sectional Voluntary recruitment from MoBa maternal-child cohort <u>Exclusion criteria</u> - maternal autoimmune disease - Use of steroids - Use of ant-inflammatory drugs - Use of anti-epileptic drugs - children not following Norwegian vaccination program Maternal blood at 0-3 days post-partum (P'FOS) Child blood at 3 yrs (mean = 35 mos) (Abs)	**Exposure Assessment:** PFOS plasma conc by LC-MS/MS LOQ = 0.05 ng/ml < LOQ = 0.035 ng/ml PFOS conc as integrated area under linear and branched isomer peaks **Population-Level Exposure:** Mean PFOS conc in maternal plasma = 5.6 ng/ml (median = 5.5 ng/ml) (NOTE: median PFOS conc ~71% of US F (NHANES 4th Rpt)	**Stat Method:** Poisson regression analysis for outcomes with counts (e.g., number of episodes of colds) Logistic regression for binary outcomes Linear regression for continuous outcomes Multivariate regression for bivariate regression w $p < 0.1$ Potential confounders selected for $p \leq 0.25$ for bivariate regression bet confounder and PFOS and bet confounder and outcome <u>Potential confounders:</u> - Older sibling - previous breastfeeding - maternal, paternal allergies - paternal asthma - maternal educ - income - birth season - gender - age at 3-yr follow-up For all regression models, backward elimination of least sig var until all vars $p \leq 0.05$	**Major Limitations:** Low n for most childhood conditions, but nearly 100 % for colds PFOS analyses not adj for other PFCs **Other comments:** Cross-sectional design Small-moderate n for antibody and health outcome analysis PFOS analyses not controlled for other PFCs although other PFCs also sig neg assoc w rubella vaccine antibody

Vaccine antibody levels measured for: - Measles - tetanus - rubella - *hoemophilus influenza*-b (Hib) Serum samples for allegen-specific IgE Cutoff for pos response at 0.35 PAU/l Questionnaire at 1, 2, 3 yrs on children's 12 mo history of: <u>infectious diseases</u> - cold/upper resp - otitis media - pneumonia - gastroenteritis w vomiting/diarrhea - urinary tract infect <u>Allergy/asthma</u> - diagnosis asthma/asthma bronchitis - > 10 d dry cough, chest tightness, wheeze - eczema/itches in face or joints - diagnosis ectopic eczema - diagnosis of allergy **Location:** Oslo and Akershus, Norway **Population:** BraMat cohort (est. 4/2007-3/2008) Nested in MoBa maternal-child cohort **N (antibody) = 49-51** **N (health outcomes) = 65-93** **Related Studies:**		**Outcome:** PFOS assoc w vaccine antibody level **Major Findings:** (multivariate model) PFOS sig assoc only w rubella antibodies **PFOS sig neg assoc** w rubella vaccine antibody levels (p = 0.007) (n = 50) (NOTE: PFOA, PFNA, PFHxS also sig neg assoc w rubella anitbodies) **Outcome:** Episodes/diagnosis of health outcomes **Major Findings:** **PFOS not sig assoc** w any health outcomes	

Reference and Study Design	Exposure Measures	Results	Comment
Study: **Grice et al. (2007)** Grice MM, Alexander BH, Hoffbeck R, Kampa DM. Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. J Occup Environ Med. 2007 Jul;49(7):722-9. **Study Design:** Self-reported medical conditions. Included yr of first diagnosis for each condition. Preg outcomes (F only) Attempted follow-up of diagnosis with subjects' physicians. **Location:** 3M facility, Dacatur, AL **Population:** All current, retired, and former employees with cumulative employment ≥1 yr eligible 1,400 participated with returned questionnaire – 74% of eligibile.	**Exposure Assessment:** Based on biomonitoring sample (n = 186) reported in Olsen et al. (2003b) (AIHA J (Fairfax, Va). 2003 Sep-Oct;64(5):651-9.) Job titles characterized according to characteristic serum PFOS levels (ppm). Each employee assigned to an exposure category based on job history by title Categories – 1. No direct exposure (0.11-0.29 ppm) 2. Low (0.39-0.89 ppm) 3. High (1.30-1.97 ppm) **Population-Level Exposure:** No exposure – 25% Low – 30% High – 45%	**Stat Method:** Logisitcal regression of exposure categories against reported outcomes. "No exposure" category as referent category. Adjustment for age and gender. Associations with exposure examined based on - Ever exposed in a given category - Exposed >1 yr in a given category - Ever exposed - Weighted exposre (No =1; Low =3; H = 10) **Outcome:** **Major Findings:** <u>Cancer</u> No association with exposure category for any reported cancer (colon, prostate). Breast cancer risk not calculated because denominator too small for each exposure cateogroy.	**Major Limitations:** Exposure classification based on correspondence of job category to exposure levels (serum PFOS). However, correspondence was based on a sample of 186 = 13% of the number of questionnaire respondents. Variability for some job categories was high including some with high PFOS exposure (95% UCI/geom.mean ≈ 3) (Olsen et al. 2003b)). "No-exposure" category is 5.5 times the median serum PFOS reported by NHANES = 0.02 ppm (Fourth National Report on Human Exposure to Environmental Chemicals; http://www.cdc.gov/exposurereport/pdf/fourthreport.pdf) Thus, use of "no-exposure" category as referent will bias against finding associations with medical conditions. Females accounted for only 19% of returned questionnaires. Significant co-exposure to PFOA (and less to other PFCs) not reported here, but based on Olsen et al. (2003b). Ability to detect exposure-related cancer is diminished by significant percentage of employees with <20 yrs of employment in this facility. **Other comments:** This study is weak both with respect to accurate exposure classification and with respect to chronic disease ascertainment, particularly cancer, given the relatively short exposure period relative to cancer latency. The use of "no-exposure" category with

58% of respondents worked: <20 yrs 42% <10 yrs; 31% <5 yrs. **Related Studies:** Olsen et al.(2003a) Olsen et al. (2003b) Alexander et al. (2003) Olsen et al.(2004) Alexander et al. (2007) Olsen et al. (2012)		<u>Non-cancer conditions</u> No association with exposure categories for commonly reported conditions: Cystitis Prostate hypertrophy Prostatitis Colon polyps Cholelithiasis (gallstones) Gastric ulcers Or for any other reported condition. <u>Birth outcomes</u> - Birthweight lowest in no-exposure category and not different across exposure categories - No association of exposure categories with stillbirths	significant exposure relative to NHANES pop. Median biases against finding association at higher exposure categories. Weak exposure assessment, disease ascertainment, and biased statistical structure.

Reference and Study Design	Exposure Measures	Results	Comment
Study: Gump et al. (2011) Gump BB1, Wu Q, Dumas AK, Kannan K. Environ Sci Technol. 2011 Oct 1;45(19):8151-9. doi: 10.1021/es103712g. Epub 2011 Jun 17. Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. **Study Design:** Cross-sectional nested in Pb study cohort PFOS from Pb blood draw Testing of assoc of differential reinforcement of low-rates of responding (DRL) w PFOS (other PFCs) - Money reward for learning correct hidden time interval (20 s) between computer level presses - Positive response corresponds to response inhibition (neg. results indicate impulsivity) Brief Mood Introspection Scale (BMIS) subsequent to DRL test (measurement of emotional response)	**Exposure Assessment:** PFOS in whole blood Extraction by ion-pairing HPLC-electrospray tandem-MS (HPLC-ESI-MS/MS) Quantification by isotope dilution – 98 +/- 5% recovery LOQ PFOS = 0.2 ng/ml **Population-Level Exposure:** Mean PFOS = 9.90 ng/ml (SD = 6.09 ng/ml) (NOTE: PFOS levels are low compared to NHANES 12-19 yrs old, mean = 19.3 ng/ml)	**Stat Method:** <u>Potential confounders investigated:</u> Age (child, mother, father) Family income "Parent's"(?) education "Parent's"(?) occupational class BMI (child, mother, father) Child's gender Child's race Family history of chronic illnesses Blood Pb Blood Hg Confounders included in model if bivariate relationship w outcome $p < 0.2$ PFOS conc log-transformed **Outcome:** Median IRT (Inter-response time – time between lever pushes) (5 min bins) (NOTE: Learning is indicated by ↑ IRT in successive 5 min bins – total bins = 4) **Major Findings:** For total PFCs, β neg for all bins) and sig for bins 2-4 For PFOS, all β neg, but sig for only bin 3	**Major Limitations:** Exposure to PFOS ~ ½ that in general US pop 12-19 yrs old (NHANES, 4th Rpt.) Cross-sectional design PFOS assoc not controlled for other PFCs. However, IRT effect most sig for total PFCs, suggesting possible confounding of specific PFOS effect **Other comments:** Relatively small N. Lack of stat controlling of PFOS results for other PFCs Equivocal results, small N, lack of controlling for other PFCs

Location: Oswego, NY **Population:** Children 9-11 yrs old N = 83 F = 30 M = 53 Mean age = 10.13 yrs Exclusions: - Use of medication for cardiovascular function on day of testing - Developmental disorders affecting test outcome **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Halldorsson et al. (2012) Halldorsson TI1, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, Henriksen TB, Olsen SF. Environ Health Perspect. 2012 May;120(5):668-73. doi: 10.1289/ehp.1104034. Epub 2012 Feb 3. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. **Study Design:** Longitudinal nested in birth cohort Face-to-face interview at wk 30 of gestation and blood sample collected Maternal health and birth outcomes from hospital records Offspring at ~20 yrs (2008-2009) web-based questionnaire health status, lifestyle, dietary habits, height, wt Clinical/anthropometric exam (incl. BMI and waist circum data) for partial N Clinical BMI/waist circum from clinical exam, n = 423 Self reported n = 242	**Exposure Assessment:** Column switching-LC-triple quadropole MS (not in this MS, but in J Chromatogr A. 2009 Jan 16;1216(3):385-93) LOQ for PFOS (and others) = 0.05 ng/ml **Population-Level Exposure:** Median PFOS = 21.5 ng/ml (IQR = 9.1) Consistent with US female pop (NHANES 4th report)	**Stat Method:** NOTE: co-variates reported for PFOA, but not PFOS. It is assumed that these co-variates were at least investigated for PFOS Maternal age Maternal education Smoking (categorical) Pregnancy BMI Parity Infant birth wt Offspring age at follow-up **Outcome:** Offspring BMI **Major Findings:** (adj model) No sig assoc w PFOS **Outcome:** Offspring waist circumference **Major Findings:** (adj model) No sig assoc w PFOS	**Major Limitations:** Did not account for offspring PFOS exposure post-natal. **Other comments:** Reasonable cohort size (although only moderate for each sex) Longitudinal follow-up Lack of investigation for confounding by post-natal (and older) exposure PFOS Stat control for other PFCs in analyses

Adiponectin and leptin by immunofluorescence Plasma insulin by commercial lab **Location:** Aarhus, Denmark **Population:** Birth cohort recruited 4/88-1/89 N = 665 M = 320 F = 325 **Related Studies:**		**Outcome:** Risk of overweight (BMI > 25 kg/m^2) **Major Findings:** (adj model) Rel risk (RR) not significantly > 1.0 for PFOS **Outcome:** Waist circum > action level (> level 2 – value not specified) **Major Findings:** (adj model) RR not significantly > 1.0 for PFOS **NOTE:** Positive assoc were seen for several outcomes with PFOA. Authors state that models for PFOA effects that included other PFCs (incl. PFOS) did not change the relationship between PFOA and outcomes	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Hamm et al. (2010) Hamm MP1, Cherry NM, Chan E, Martin JW, Burstyn I. J Expo Sci Environ Epidemiol. 2010 Nov;20(7):589-97. doi: 10.1038/jes.2009.57. Epub 2009 Oct 28. Maternal exposure to perfluorinated acids and fetal growth. **Study Design:** Cross-sectional maternal-child study Maternal cohort screened at 15-18 wks gestation Blood samples collected 12/2005-6/2006 <u>Outcomes</u> Birth wt Small for gestational age Length of gestation Pre-term delivery **Location:** Edmonton, Alberta, Canada	**Exposure Assessment:** Solid-phase extraction HPLC-triple quadrupole linear ion trap MS PFOS % recovery = 91.1 +/- 13.9 LOD = 0.125 ng/ml < LOD as LOD/2 **Population-Level Exposure:** PFOS mean = 9.0 ng/ml Geom mean = 7.4 (geom SD = 2.0) NOTE: geom mean PFOS conc < ½ US female geom mean (NHANES 4th report)	**Stat Method:** PFOS concs as untransformed and ln-transformed Birth wt, length of gestation by linear regression Small for gestational age, preterm-delivery as risk ratio (RR) by Poisson regression <u>Potential confounders</u> Maternal age Maternal wt (dichotomized for high and low) Maternal ht (dichotomized) Smoking during preg (Y/N) Infant gender Maternal race parity **Outcome:** Birth wt **Major Findings:** (adj model) PFOS not sig assoc w birth wt (PFOA and PFHxS not sig assoc)	**Major Limitations:** Small N PFOS analyses not controlled for other PFCs PFOS exposure low compared to US female pop **Other comments:** Good analytical methodology and statistical control (except for PFC co-exposure), but small N and low expsorue

Population: Preg women > 18 yrs old Live, singleton births No evidence of malformation Delivery ≥ 22 wks gestation Initial N = 1588 252 serum samples selected for analysis **Related Studies:**		**Outcome:** Length of gestatsion **Major Findigs:** PFOS (PFOA,) not sig assoc w. length of gest (PFHxS sig assoc w ↑ length gest) **Outcome:** Small for gest age (SGA) **Major Findings:** 3rd tertile (but not 2nd (1st as ref)) PFOS sig assoc w ↓ risk of SGA **Outcome:** Preterm delivery **Major Findings:** PFOS not sig assoc w risk preterm delivery	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Hardell et al. (2014) Hardell E, Kärrman A, van Bavel B, Bao J, Carlberg M, Hardell L. Environ Int. 2014 Feb;63:35-9. doi: 10.1016/j.envint.2013.10.005. Epub 2013 Nov 16. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. **Study Design:** Case-control prostate cancer Controls matched to cases on Age Location (county) Cases = 201 Controls = 186 Blood samples from cases and controls drawn during "same time period" Analysis blinded to case-control status Reporting of Gleason Score (prostate cancer stage), prostate spec antigen (PSA) from medical records Information on first degree relatives w prostate cancer (Y/N)	**Exposure Assessment:** UPC, E-MS/MS PFOS LOD = 0.1-? ng/ml (upper limit not clear due to typo in MS) <LOD → LOD/2 **Population-Level Exposure:** PFOS (mean) Cases = 11 ng/ml Controls = 10 ng/ml (NOTE: exposure level ~ ½ the geom mean for US mean > 20 yrs old (NHANES 4th Rpt))	**Stat Method:** OR by unconditional logistic reg <u>Co-variates</u> Age BMI Year of sampling **Outcome:** OR for prostate cancer **Major Findings:** OR for PFOS not sig > 1.0 **Outcome:** Gleason score **Major Findings:** OR for score 2-6 (n = 70) and 7-10 (n = 123) not sig > 1.0 **Outcome:** PSA **Major Findings:** OR for PSA ≤ 10 (n = 110) and PSA ≥ 11 (n = 91) Not sig > 1.0	**Major Limitations:** PFOS analyses not controlled for other PFCs Exposure is relatively low compared to adult US males (NHANES 4th Rpt) N is moderate for a case-control study **Other comments:** Although the number of cases (and controls) is only moderate this does not appear to add uncertainty to the finding of an increased risk for PFOS under conditions of hereditary risk **However, similar hereditary associations were found for all other PFCs in this study. Lack of control for other PFCs in PFOS analysis of heredity raises concerns about specificity of the PFOS finding**

Location: Õrebro, Sweden **Population:** Prostate cancer patients admitted 2007-2011 to University Hosp, Õrebro Controls from Swedish pop registry **Related Studies:**		**Outcome:** PFOS-heredity interaction (heredity = first order relative w prostate cancer) **Major Findings:** No heredity, PFOS ≤ median as ref Heredity, PFOS ≤ median – OR not sig No heredity PFOS > median – OR not sig **Heredity, PFOS > median – OR sig (2.7)**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Hoffman et al. (2010) Hoffman K1, Webster TF, Weisskopf MG, Weinberg J, Vieira VM. Environ Health Perspect. 2010 Dec;118(12):1762-7. doi: 10.1289/ehp.1001898. Epub 2010 Jun 15. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. **Study Design:** Cross-sectional, case-control study of assoc of PFOS and ADHD Children 12-15 yrs old NHANES data 1999-2000; 2003-2004 -Parental report of prior ADHD diagnosis -Alternative (more stringent definition) parental report of prior ADHD diagnosis AND parental identification of child's taking medication approved for ADHD **Location:** U.S.	**Exposure Assessment:** Solid-phase extraction, reverse-phase HPLC-MS PFOS LOD = 0.2 ng/ml LOD → LOD/√2 **Population-Level Exposure:** Median PFOS conc 22.6 ng/ml (IQR = 15.9 ng/ml)	**Stat Method:** <u>Potential confounder/co-variates</u> Age Sex Race/ethnicity NHANES sample cycle SES Routine health care provider (Y/N) Health insurance coverage (Y/N) Pb ETS Birth wt Admittance to NICU Maternal preg smoking Pre-school Loistic regression (PFOS as continuous variable) Variables added to model if p < 0.1 in bivariate regression or > 10% chnge model relationship between PFOS and ADHD OR Simultaneous inclusion of PFOS w PFOA, PFNA and PFHxS also principle component analysi **Outcome:** Risk of ADHD	**Major Limitations:** Total n is moderate Case n is relatively small Overall effect (OR) is relatively small **Other comments:** Data set is well vetted. PFOS analysis is well conducted Control of PFOS analysis for other PFCs provides evidence for independent PFOS effect Self (parental) identification of cases introduces uncertainty

Population: National data (NHANES) children 12-15 yrs old PFOS sample from children's serum. N = 571 -Parental rpt of ADHD diagnosis n = 48 -Parental rpt ADHD + ADHD medication n = 21 **Related Studies:**		**Major Findings:** (adj model) OR = 1.03 (sig) for each 1 ng/ml ↑ in PFOS based on parental reporting of diagnosis OR = 1.05 (sig) for each 1 ng/ml ↑ in PFOS based on parental reporting of diagnosis + ADHD medication OR = 1.60 for each IQR ↑ in PFOS (which case definition?) **Outcome:** Risk of ADHD for PFOS in combined PFC model **Major Findings:** Principle component analysis showed combined PFCs accounted for 58% of variability for individual PFCs For logistic regression including combined PFC variable and individual PFCs (incl PFOS), combined PFC variable sig, also PFOS (and PFOA, and PFHxS; but not PFNA) sig.	

		Although combined PFCs appear to be pos assoc w risk ADHD, PFOS appears to be independently sig associated w ADHD.	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Humblet et al. (2014) Humblet O1, Diaz-Ramirez LG, Balmes JR, Pinney SM, Hiatt RA. Environ Health Perspect. 2014 Oct;122(10):1129-33. doi: 10.1289/ehp.1306606. Epub 2014 Jun 6. Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). **Study Design:** Cross-sectional Self-reported asthma status: - wheezing/whistling in chest past 12 mos - Yes to wheezing + still have symptoms = current asthma - physician-diagnosed asthma (ever) = ever asthma Comparison group for “current asthma” = never diagnosis of asthma **Location:** US	**Exposure Assessment:** CDC analysis For PFOS 100% > LOD **Population-Level Exposure:** Mean PFOS conc = 16.7-17.2 ng/ml (conc presented by asthma status category)	**Stat Method:** NHANES weighting factors not applied – oversampling instead addressed by co-variates OR for assoc PFOS w asthma status vars <u>Co-variates</u> - NHANES cycle - Age - sex - Race/ethnicity - poverty income ratio (income/poverty income definition) - ever smoking - health insurance Analysis by 3 models: - linear - ln-linear - tertiles (ln-linear model gives OR for doubling PFOS conc)	**Major Limitations:** Cross-sectional design PFOS analyses not adj for other PFCs **Other comments:** Cross-sectional design Large overall n, but moderate n for asthma outcomes Lack of control of PFOS analyses for other PFCs

Population: NHANES 1999-2000; 2003-2004; 2005-2006; 2007-2008 12-19 yrs old **N – never asthma = 1,559** **N – ever asthma = 318** **N – no wheeze past 12 mos = 1,660** **N – wheeze past 12 mos = 217** **N – no current asthma = 1,559** **N – current asthma = 191** **Related Studies:**		**Outcome:** OR for PFOS and Ever asthma **Major Findings:** **OR not sig <> 1.0** for any model **Outcome:** OR for PFOS and wheeze **Major Findings:** **OR not sig <>1.0** for any model **Outcome:** OR for PFOS and current asthma **Major Findings:** **OR not sig <> 1.0** for any model	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Impinen et al. 2018 Impinen, A., U. C. Nygaard, K. C. Lodrup Carlsen, P. Mowinckel, K. H. Carlsen, L. S. Haug and B. Granum (2018). "Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood." Environ Res **160**: 518-523. **Study Design:** Nested prospective birth cohort study **Location:** Oslo, Norway **Population:** Selected from healthy newborns in the Environment and Childhood Asthma cohort recruited between 1992 and 1993 (n=3754). N=641 participants with exposure measured **Outcome Assessment:** Assessed at 2 and 10 years of age and included reported obstructive airways disease (wheeze by 10 years; asthma by 2 and 10 years; reduced lung function at birth; allergic rhinitis by 10 years), atopic dermatitis by 2 and 10 years, lower respiratory tract infections by 10 years.	**Exposure Assessment:** Cord blood serum PFASs concentrations **Population-Level Exposure:** Mean concentration (ng/mL) PFOS=5.6 PFOA=1.8 PFOSA=0.4 PFHxS=0.3 PFNS=0.2 PFUnDA=0.1	**Stat Method:** Differences in health outcomes between boys and girls were tested using chi-square tests. Binomial logistic regression models were computed for binary health outcomes. PFAS was log transformed. Count data were analyzed using Poisson regression. Estimates are based on doubling of PFAS concentration. Bonferroni correction was applied to estimated p-values. Possible confounders examined were sex, birth weight, birth month, breastfeeding at 6 months and at 12 months, maternal smoking during pregnancy, household smoking at birth, at preschool age and at school age, parental asthma, AD and allergic rhinitis, parental education and household income. Final models were adjusted for sex only. **Outcome:** Asthma **Major Findings:** Current @ 10y OR=1.14 (95% CI 0.84, 1.54) Ever @ 10y OR=1.32 (95% CI 0.89, 1.97) **Outcome:**	**Major Limitations:** Only controlled for sex in final adjusted models. Did not control for other co-occurring environmental contaminants as potential confounders. **Other comments:** Study population is complicated, number of cases versus controls is not stated. Potential for over-recruitment of children with BO into the 10- year study group.

Collected from questionnaires at birth and every 6 months until 2 years, parental interview and clinical investigation. At 10 years of age clinical investigation including parental interview		Wheeze **Major Findings:** Before 3y, OR=1.26 (95% CI 0.83, 1.90) After 3y, OR=1.08 (95% CI 0.66, 1.77) Throughout, OR=1.41 (95% CI 0.95, 2.08) **Outcome:** Severity of obstructive airways (2 years) OSS score 1 through 12 **Major Findings:** **OSS 1-5 v. 0 OR=1.71 (95% CI 1.16, 2.53)** OSS 6-12 v. 0 OR=1.15 (95% CI 0.71, 1.84) **Outcome:** Reduced lung function at birth **Major Findings:** OR=0.86 (95% CI 0.43, 1.72) **Outcome:** Atopic dermatitis **Major Findings:** 0-2 years, OR=1.15 (95% CI 0.88, 1.52) 10 years – ever OR=0.68 (95% CI 0.38, 1.20) **Outcome:** Rhinitis & IgE **Major Findings:**10 years ever, OR=1.05 (95% CI 0.74, 1.48) Rhinits ever and spes IgE>0.35 OR=1.02 (95% CI 0.71, 1.47) At least one pos spes IgE>0.35 OR=0.88 (95% CI 0.66, 1.17) **Outcome:**	

		Rhinoconjunctivitis **Major Findings:** at 10 years (ever), OR=1.02 (95% CI 0.72, 1.45) **Outcome:** Allergic sensitization (skin prick test - SPT) **Major Findings:** Any pos 10 y OR=0.87 (95% CI 0.65, 1.17) SPT+ and/or sIgE>0.35 10 y OR=0.91 (95% CI 0.69, 1.19) **Outcome:** Number of episodes of common cold by 2 years **Major Findings:** β=-0.03 (95% CI -0.08, 0.01) **Outcome:** Number of episodes of lower respiratory tract infections by 10 years **Major Findings:** **β=0.50 (95% CI 0.42, 0.57)**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Innes et al. (2011) Am J Epidemiol. 2011 Aug 15;174(4):440-50. doi: 10.1093/aje/kwr107. Epub 2011 Jun 27. Innes KE, Ducatman AM, Luster MI, Shankar A. Association of osteoarthritis with serum levels of the environmental contaminants perfluorooctanoate and perfluorooctane sulfonate in a large Appalachian population. **Study Design:** Cross-sectional Assoc of osteoarthritis and PFOS (PFOA) in 6 water districts w known drinking water contamination by PFOA Baseline data 8/2005-8/2006 Medical history incl. diagnosis of osteoarthritis self-reported by questionnaire **Location:** **Population:** Subset of C8 cohort OH, WV.	**Exposure Assessment:** Protein precip extraction, reverse-phase HPLC-triple quadrupole MS LOD? **Population-Level Exposure:** Mean PFOS = 23.5 ng/ml (SD = 16.2 ng/ml), median = 20.3 ng/ml (consistent w US pop – NHANES 4th Rpt) Mean PFOA = 87.4 ng/ml (high – local contamination)	**Stat Method:** PFOS as categorical and continuous variables <u>Co-variates</u> Age BMI Age Gender Race/ethnicity Marital status SES Exercise prog (Y/N) Vegetarian diet (Y/N) Smoking Alcohol Menopausal status Hormone replacement Specific co-morbidity (by condition) Treatment for hypertension Treatment for hyperlipidemia Serum uric acid Serum cholesterol C-reactive protein Estradiol Other PFCs	**Major Limitations:** No validation of self-reporting data for osteoarthritis Cross-sectional **Other comments:** Large N allowed detailed model w numerous co-variates

Adults ≥ 21 yrs old at time of baseline → exclude rheumatoid arthritis → exclude missing data for PFOA or PFOS → exclude missing data for other co-variates of interest → N = 49.432 Cases (osteoarthritis) = 3,731 Controls = 45.701 **Related Studies:**		**Outcome:** Risk of osteoarthritis **Major Findings:** (adj model) PFOS sig **neg** assoc w risk of osteoarthritis p (trend) = 0.00001 (PFO sig pos assoc w risk of osteoarthritis) No evidence of modifying effect of age or BMI for PFOS assoc w osteoarthritis	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Jain (2013a) Jain RB. Effect of pregnancy on the levels of selected perfluoroalkyl compounds for females aged 17-39 years: data from National Health and Nutrition Examination Survey 2003-2008. J Toxicol Environ Health A. 2013;76(7):409-21. **Study Design:** Cross-sectional NHANES 2003-4; 2005-6; 2007-8 **Location:** U.S. (nationwide) **Population:** US pregnant and non-preg women 17-39 yrs old (Preg women oversampled in NHANES 2003-4 and 2005-6 (not 2007-8)) **pregnant** women in NHANES, age 17-39 N = 180 - 1st trimes n = 32 - 2nd trimes n = 59 -3rd trimes n = 70	**Exposure Assessment:** Solid-phase extraction, HPLC-turbo ion spray, MS-MS LOD? **Population-Level Exposure:** PFOS conc (median) - Pregnant 10.07 (95% CI = 7.90-12.20) ng/ml - Non-preg 12.11 (11.14-13.09)	**Stat Method:** Linear regression Log transformed PFCs <u>Co-variates</u> Ethnicity/race Pregnancy status (Y/N) Breast feeding (Y/N) Age $(Age)^2$ NHANES cycle Parity BMI Serum albumin Serum cotinine Serum creatinine Serum cholesterol Serum protein Backward elimination to achieve all terms w $p \leq 0.1$ Age as mandatory **Outcome:** (combined preg + non-preg) Serum cholesterol **Major Findings:** PFOS sig pos assoc w serum cholesterol	**Major Limitations:** Preg n is small, not permitting conclusions re adverse outcomes (cholesterol, triglycerides) for preg pop alone **Other comments:** Reasonable consideration of co-variates in model. However, study is largely focused on factors assoc w PFOS (and PFC) levels rather than outcomes Relatively small preg N precludes conclusions for preg-specific outcomes

Non-pregnant women in NHANES, ages 17-39 N = 899 **Related Studies:**		**Outcome:** (combined preg + non-preg) Serum triglycerides **Major Findings:** PFOS not sig assoc w serum triglycerides	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Jain et al (2013b) Jain RB. Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008. Environ Res. 2013 Oct;126:51-9. doi: 10.1016/j.envres.2013.08.006. Epub 2013 Sep 18. **Study Design:** Cross-sectional Thyroid function variables TSH (thyroid stimulating hormone) FT4 (free thyroxine) TT4 (total thyroxine) FT3 (free triiodothyroxine) TT3 (total triiodothyroxine) TGN (thyroglobulin) **Location:** US (nationwide) **Population:** NHANES 2007-8 ≥ 12 yrs old <u>Exclusions</u> - Pregnant - Diagnosed thyroid problems - TPOAb (thyroid autoantimbodies) ≥ 35 UI/ml	**Exposure Assessment:** PFC (PFOS) analytical methodology for NHANES cited Thyroid function variables analytical methodology for NHANES cited **Population-Level Exposure:** Not reported (but presumably close to NHANES 4th Rpt but differing by exclusions)	**Stat Method:** <u>Co-variates considered</u> Age Gender Race/ethnicity Smoking Iodine status (deficient/replete) C-reactive protein BMI Fasting time before blood draw Calories in prev 24 hrs Thyroid and PFOS (PFC) variables log-transformed Each thyroid variable examined separately. Interaction terms among age, race, gender investigated *a priori* and non-sig interaction terms eliminated PFCs as continuous variables (alternatively as categorical if continuous not sig) **Outcome:** FT3 **Major Findings:** PFOS **not sig assoc** w FT3	**Major Limitations:** Cross-sectional design Does not appear that PFOS analyses not controlled for other PFCs, however, description of stat approach is ambiguous Exposure statistics not reported (cannot be precisely derived from NHANES due to exclusions) **Other comments:** The structure of the statistical analysis is not entirely clear. Large n Reliable (CDC) PFOS and thyroid variable analyses

- TgAB (thyroglobin antibody) ≥ 20 UI/ml - prescription thyroid med - “Other” race/ethnicity category - missing data N = 1,540 **Related Studies:**		**Outcome:** FT4 **Major Findings:** PFOS **not sig** assoc w FT4 **Outcome:** TT3 **Major Findings:** PFOS **not sig** assoc w TT3 **Outcome:** TT4 **Major Findings** PFOS **not sig** assoc w TT4 **Outcome:** TSH **Major Findings:** PFOS **not sig** assoc w TSH **Outcome:** TGN **Major Findings:** PFOS **not sig** assoc w TGN	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Ji et al.(2012) Ji K, Kim S, Kho Y, Paek D, Sakong J, Ha J, Kim S, Choi K. Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environ Int. 2012 Sep 15;45:78-85. doi: 10.1016/j.envint.2012.03.007. Epub 2012 May 9. **Study Design:** Nested cross-sectional Blood sampled July-Aug, 2008 Demographic and dietary questionnaire T4 (total) TSH By commercial chemoluminescence immunoassay. CV ≤ 11% **Location:** Siheung, S. Korea	**Exposure Assessment:** $^{13}C_4$-internal PFOS standard HPLC-triple quadrupole-MS in electrospray negative ionization mode Recovery = 100.2 +/- 6.6% LOD = 0.04 ng/ml CV = 6.6% **Population-Level Exposure:** PFOS Median (inter-quartile range) M – 9.58 (6.54 -14.00) ng/ml F – 7.16 (5.02-10.60) ng/ml	**Stat Method:** <u>Co-variates considered</u> Age Sex BMI PFOS, T4, TSH log-transformed < LOD as LOD/√2 Bonferroni correction for sig PFOS considered in model containing other PFCs **Outcome:** T4 (total) **Major Findings:** PFOS **not sig** assoc w T4 **Outcome:** TSH **Major Findings:** PFOS **not sig** assoc w TSH	**Major Limitations:** Cross-sectional; Minimal co-variates considered Exposure ~50% of US (NHANES 4thRpt) N relatively small **Other comments:** Rel low exposure and rel low N result in low power Compared to other studies, few co-variates were controlled for in the models

Population: Portion of previously established Siheung cohort ≥ 12 yrs old Total = 633 M – 258 F - 375 **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Jiang et al. (2014) Jiang W, Zhang Y, Zhu L, Deng J. Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environ Int. 2014 Mar;64:40-7. doi: 10.1016/j.envint.2013.12.001. Epub 2013 Dec 20. **Study Design:** Pregnant women 8-12 wks gest (1st trimest) samples collected 8-9/2012 (NOTE: text specified serum samples collected, but whole blood was used to obtain RBC count) **Subject recruitment??** **Subject demographics??** Hematological assessments/serum chem: - WC count - RBC count - Hb - platelet - total bilirubin - total protein - albumin - glucose - AST - ALT	**Exposure Assessment:** Examination of linear and branched PFOS - "n" specifies linear - "iso" specifies branched - "m_x" specified degree of branching - Nm (e.g., 4m) refers to carbon on which branch occurs Solid phase extraction Samples spiked with labeled internal stds HPLC-MS/MS analysis RSD (CV): - linear PFOS < 5% - branched PFOS isomers <10% (except 4m-PFOS, 1m-PFOS, and $\sum m_2$-PFOS < 30%) LOD (all PFAs = 0.1-19.0 ng/ml PFOS detected in 100% of samples **Population-Level Exposure:** Mean n-PFOS = 4.75 ng/ml Mean iso-PFOS = 0.74 ng/ml Mean ∑PFOS = 7.32 ng/ml (NOTE: PFOS conc appear to be consistent w US F pop (NHANES 4th Rpt)) n-PFOS = 66.7% of ∑PFOS	**Stat Method:** PFOS conc and blood metrics log-transformed Outcomes based on Pearson correlation coeff between ∑PFOS isomers, or proportion PFOS isomers; and hematological/serum chem parameters **Outcome:** WBC count **Major Findings:** (unless specified PFOS forms not sig correlated w outcome) 1m-PFOS **sig pos corr** w WBC count ($r = 0.2$, $p \leq 0.05$) 4m-PFOS **sig pos corr** w WBC count ($r = 0.187$, $p \leq 0.05$) 3 + 5m-PFOS **sig pos corr** w WBC count ($r = 0.183$, $p \leq 0.05$) % n-PFOS **sig neg corr** w WBC couont ($r = -0.254$, $p \leq 0.01$)	**Major Limitations:** No information provided on subject recruitment No information on subject demographics (e.g., age, BMI) PFOS analysis not adj for PFOS or other PFCs **Other comments:** Moderate N Correlation analysis rather than regression No information on subject recruitment or demographics

Location: Tianjin, China **Population:** N = 141 **Related Studies:**		**Outcome:** RBC count **Major Findings:** (unless specified PFOS forms not sig correlated w outcome) n-PFOS **sig pos corr** w RBC count (r = 0.205, p ≤ 0.05) iso-PFOS **sig pos corr** w RBC count (r = 0.284, p ≤ 0.01) 3 +5m-PFOS **sig pos corr** w RBC count (r = 0.172, p ≤ 0.05) **Outcome:** Hb **Major Findings:** (unless specified PFOS forms not sig correlated w outcome) n-PFOS **sig pos corr** w Hb (r = 0.279, p ≤ 0.01) iso-PFOS **sig pos corr** w Hb (r = 0.325, p ≤ 0.01) 1m-PFOS **sig pos corr** w Hb (r = 0.233, p ≤ 0.01)	

		4m-PFOS **sig pos corr** w Hb (r = 0.235, p ≤ 0.01) 3 + 5m-PFOS **sig pos corr** w Hb (r = 0.258, p ≤ 0.01) ∑m_2-PFOS **sig pos corr** w Hb (r = 0.182, p ≤ 0.05) **Outcome:** Platelet count **Major Findings:** (unless specified PFOS forms not sig correlated w outcome) Iso-PFOS **sig pos corr** w platelet count (r = 0.207, p ≤ 0.05) **Outcome:** Glucose **Major Findings:** PFOS **not sig corr** w glucose **Outcome:** Total protein **Major Findings:** PFOS **not sig corr** w total protein	

		Outcome: Albumin **Major Findings:** PFOS **not sig corr** w albumin **Outcome:** Total bilirubin **Major Findings:** $\sum m_2$-PFOS **sig pos corr** w total bilirubin ($r = 0.201$, $p \leq 0.05$) **Outcome:** AST **Major Findings:** PFOS **not sig corr** w AST **Outcome:** ALT **Major Findings:** PFOS **not sig corr** w ALT	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Joensen et al. (2009) Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jørgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect. 2009 Jun;117(6):923-7. doi: 10.1289/ehp.0800517. Epub 2009 Mar 2. **Study Design:** Nested case-control (high testosterone, low testosterone) Subset of cohort selected on basis of testosterone level Semen and blood samples collected Analysis of repro hormones: -Testosterone -Estradiol -Sex hormone binding globin (SHBG) -Luteinizing hormone (LH) -Follicle stimulating hormone (FSH -Inhibin B -Free androgen index (testosterone x 100/SHBG) Semen analysis: -vol by wt -sperm conc	**Exposure Assessment:** $^{14}C_4$-PFOS internal isotope spike HPLC-MS-MS tandem triple quadrupole w electro-spray ionization **Population-Level Exposure:** Median PFOS = 24.5 ng/ml (consistent w US pop (NANES 4th Rpt))	**Stat Method:** PFOS < LOD = 0 ng/ml Sperm conc, semen vol, total sperm count adj for duration of ejaculation abstinence period Sex hormone variables adj for hour of sampling PFOS comparison Goup 1 vs.2 investigated for BMI, smoking status Semen and hormone variables (except morph) ln-transformed Assoc analyzed as PFOS and PFOA separately and as PFOS + PFOA **Outcome:** Sperm morphology **Major Findings:** Number and percent morph normally spermatozoa **sig neg** assoc with **sum of PFOS + PFOA,** but **not sig for PFOS alone**	**Major Limitations:** Relatively small N Few co-variates examined **Other comments:** Few co-variates and small N

-total sperm count -percent motile spermatozoa -sperm morphology **Location:** Copenhagen, Denmark **Population:** Military recruits (compulsory) 2003 Med age = 19 yrs N = 105 - Group 1 High testosterone (median = 31.8 nmol/L, range = 30.1-34.8) N = 53 - Group 2 Low testosterone (median = 14.0 nmol/L, range = 10.5-15.5) N = 52 Thawed serum samples analyzed 2008 **Related Studies:**		**Outcome:** Sperm vol, conc, total count, motility, **Major Findings:** **not sig assoc** w PFOS (or PFOS + PFOA) serum conc **Outcome:** Sex hormones: (Testosterone, Estradiol, SHBG, LH, FSH, Inhibin B, Free androgen index **Major Findings:** PFOS (and PFOS + PFOA) **not sig assoc** w any sex hormones	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Joensen et al. (2013) Joensen UN1, Veyrand B, Antignac JP, Jensen MB, Petersen JH, Marchand P, Skakkebaek NE, Andersson AM, Le Bizec B, Jørgensen N. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum Reprod. 2014 May 8. **Study Design:** Cross-sectional 2008-9 247 M undergoing compulsory Danish military physical randomly selected Abstinence from ejaculation for 48 hrs Blood sample at time of semen collection FSH, LH and SHBG (sex hormone binding globin) by fluoroimmunoassay	**Exposure Assessment:** Solid-phase extraction HPLC-MS PFOS LOD = 0.05 ng/ml LOQ = 0.15 ng/ml **Population-Level Exposure:** Mean PFOS conc = 8.46 ng/ml (median = 7.79 ng/ml) PFOS detected in 100% samples	**Stat Method:** Repro hormones (and ratios bet hormones and serum vol) - ln-transformed Sperm conc, total sperm count – cubic root transformed Progressively motile values – squared Morphologically normal counts = sq root transformed PFOS as continuous var in linear regress Co-variates incl if sig predictor of individual outcome and → Δ outcome > 10% - BMI in models for T, E, SHBG, FAI, T/LH, T/E - smoking in models of T and FT (BMI and smoking incl in all models of all repro hormones) - abstinence time in models of semen vol, conc., total count <u>Co-variates considered but not included</u> - time of day of blood sample - ethnicity - alcohol	**Major Limitations:** Cross-sectional study **Other comments:** Cross-sectional design Moderate N Small effects (βs) Good statistical control

Total testosterone (T) and estradiol (E) by radioimmunoassay Inhibin-B by double antibody enzyme immunometric assay FAI (free androgen index) as T x 100/SHBG FT (free testosterone) from T and SHBG <u>Semen parameters</u> - semen volume - sperm conc (in duplicate) - total sperm count (volume x conc) - % progressively motile sperm - % motile sperm (in duplicate) - morphology (two analysts) **Location:** Denmark **Population:** M undergoing compulsory military physical N = 247 Mean age = 19.6 yr **Related Studies:** Joensen et al. (2009)		- in utero exposure to smoking - previous/current disease - recent fever - recent medication **Outcome:** Serum/sperm parameters **Major Findings:** PFOS **not sig assoc** with any serum or sperm parameters (vol, conc, total count, progressively motile, morph normal, total normal count) **Outcome:** testosterone **Major Findings:** PFOS **sig neg assoc** w serum testosterone β = -0.010 **Outcome:** FAI **Major Findings:** PFOS **sig neg assoc** w serum FAI β = -0.20	

		Outcome: FT **Major Findings** PFOS **sig neg assoc** w serum FT β = -0.016 **Outcome:** FT/LH **Major Findings** PFOS **sig neg assoc** w serum FT/LH β = 0.022 **Outcome:** FAI/LH **Major Findings:** PFOS **sig neg assoc** w serum FAI/LH β = -0.025 **Outcome:** T/LH **Major Findings:** PFOS **sig neg assoc** w serum T/LH β = -0.016	

		Outcome: Other sex hormones **Major Findings:** PFOS **not sig assoc** w: E, T/E, SHBG, LH, FSH, inhibin-B, inhibin-B/FSH	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Jørgensen et al. (2014) Jørgensen KT, Specht IO, Lenters V, Bach CC, Rylander L, Jönsson BA, Lindh CH, Giwercman A, Heederik D, Toft G, Bonde JP. Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environ Health. 2014 Dec 22;13:116. doi: 10.1186/1476-069X-13-116 **Study Design:** Cross-sectional, multiple cohorts Enrollment during anti-natal visits 3/2002-2/2004 Questionnaire and blood sample at enrollment Exclusion: - pregnant while using birth control (not time-to preg (TTP)) - no information on TTP - no blood sample - primaparous <u>Questionnaire info:</u> - Starting Time = intercourse w/out birth control in order to conceive - How long from Starting Time until preg?	**Exposure Assessment:** PFOS by LC-MS PFOS LOD = 0.2 ng/ml PFOS detected in 100% of samples PFOS CV (dup samples) = 8% **Population-Level Exposure:** F - PFOS pooled median conc = 10.6 ng/ml - Greenland median = 17.17 ng/ml - Poland median = 6.98 ng/ml - Ukraine median = 3.98 ng/ml (NOTE: PFOS conc for Greenland ~2.2 x US F Poland consistent w US F Ukraine ~ 52% of US F (NHANES 4th Rpt))	**Stat Method:** Fecundity ratio (FR) ([$prob_{exposure\ group}$ conceiving/time]/[$prob_{ref\ group}$conceiving/time]) Calculated: Country specific tertiles Country specific continuous log-transformed Pooled sample continuous log-transformed <u>Co-variates (F)</u> - maternal age - gest wk at interview - smoking - parity - maternal BMI - country (pooled analysis) Logistic regression – OR for infertile (TTP > 13 mo) Same vars as analysis of fecundity ratio <u>Co-variates (M)</u> - paternal age - paternal BMI - maternal age - country (pooled sample)	**Major Limitations:** PFOS analyses not adj for PFOA (or other PFCs) although PFOS corr w PFOA – r_s = 0.50 Moderate N for individual countries Measurement of serum PFOS during preg may not represent serum conc at time of conception despite adj for gest age Time point for attempting preg may not be precisely defined **Other comments:** Use of F and M serum PFOS Control for reverse causation by primaparous sens analysis Reasonable N Multiple country cohorts w diff exposure levels

Location: Greemland, Poland (Warsaw), Ukraine (Kharkiv) **Population:** INUENDO cohort ≥ 18 yrs old Born in country of study **Total N (F) = 938** - Greenland = 448 - Poland = 203 - Ukraine = 287 **Total (M spouses) = 401** - Greenland = 160 - Poland = 146 - Ukraine = 95 **Related Studies:**		**Outcome:** FR (fecundity ratio) **Major Findings:** FR **not sig assoc** w maternal PFOS for pooled or individual countries Restriction to primaparous (N = 59% of total) – FR **not sig assoc** w maternal PFOS for pooled or individual countries **Outcome:** OR infertility **Major Findings:** OR infertility **not sig > 1.0** for any tertile, or for continuous analysis for pooled or individual countries Restriction to primaparous (N = 59% of total) – OR infertility **not sig > 1.0** for any tertile, or for continuous analysis for pooled or individual countries **Outcome:** Assoc TTP w PFOS for M **Major Findings:** ↑ TTP **not sig assoc** w M serum PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Kielsen et al (2016) Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E, Heilmann C. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol. 2016;13(2):270-3. doi: 10.3109/1547691X.2015.1067259. Epub 2015 Jul 16. **Study Design:** Prospective Booster vaccination w. tetanus-diphtheria vaccine – antibody response during 1 month follow-up Serum PFOS 10 d post-vaccination Pre-vaccine Ab determination. Post vaccine Ab determined day-2, 4, 7, 10, 14, 30 Ab measurement by ELISA **Location:** Copenhagen, Denmark	**Exposure Assessment:** On-line solid-phase extraction, HPLC-tandem MS **Population-Level Exposure:** Median PFOS conc = 9.52 ng/ml	**Stat Method:** PFOS and Ab concs. log-transformed Relationship of Ab and PFOS conc over time estimated assuming 4-d lag in Ab response, (log)linear increase 4-10 d and constant > 10 d Model calculates Δ model prediction of Ab conc for doubling PFOS conc <u>Co-variates in model</u> Age Sex (co-variates allowed to affect intercept and linear slope day 4-10) **Outcome:** Increase in diphtheria Abs **Major Findings:** Doubling of PFOS predicted to account for 11.90% decrease in expected linear increase (d 4-10) p = 0.044 (adj for sex and age → slightly stronger effect)	**Major Limitations:** Small n Simultaneous background exposure to a variety of PFCs, PFOS yielded second strongest effect (PFHxS had stronger effect, but borderline sig). **Other comments:** Samll n, but longitudinal study w close temporal monitoring PFOS effect could not be clearly dissociated from other PFCs (PFOS effect not controlled for other PFCs)

Population: Healthy adult hospital staff volunteers (n = 12) with no history of tetanus-diphtheria booster vaccination in prev. 5 yrs Childhood initial vaccination median age = 37.9 yrs 50% M **Related Studies:**		(NOTE: PFHxS accounted for 13.31% decrease, but borderline sig (p = 0.055) **Outcome:** Increase in tetanus Abs **Major Findings:** **Not sig assoc**. Doubling of PFOS predicted to account for 3.59% decrease in expected linear increase (d 4-10)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Kim et al. (2011) Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, Kim S, Park S, Hwang I, Jeon J, Yang H, Giesy JP. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol. 2011 Sep 1;45(17):7465-72. doi: 10.1021/es202408a. Epub 2011 Aug 12. **Study Design:** Cross-sectional Blood samples collected - Most (n = 27) during 3rd trimest, N = 7 during late 2nd trimest Cord blood - Total n = 43 - From matched maternal-child pairs N = 35 Breast milk at hospital at ~1 mo. Post-partum Questionnaire: Current/prev preg history Med history Demographic parameters Infant sex	**Exposure Assessment:** HPLC-triple quadruple MS in electrospray neg ion mode Quantification w ^{13}C-PFOS stds All > LOD for PFOS **Population-Level Exposure:** <u>Median PFOS (IQR) (ng/ml)</u> <u>Maternal blood</u>: (mean) All – 2.93 (2.08-4.36) 20-29 yrs old – 2.02 (1.57-3.66) 30-39 yrs old – 2.91 (2.25-4.16) 40-49 yrs old – 7.85 (n = 2) **NOTE** – exposure levels < 50% those reported for US women (CDC-NHANES 4th Rpt) <u>Fetal cord blood</u> All – 1.26 (0.81-1.82) Maternal 20-29 yrs – 0.94 (0.5-1.19) Maternal 30-39 yrs – 1.52 (1.08-2.01) Maternal 40-49 yrs – 1.95 (n =2)	**Stat Method:** Thyroid hormones log-transformed <u>Adj for</u> T3: Maternal age Gestational age T4 and TSH: Maternal age Gest age Maternal BMI Analysis for PFOS and ΣPFCs **Outcome:** T3 - maternal serum **Major Findings:** (adj model) **Sig** neg correlated w PFOS ($p < 0.05$) **Sig** neg correlated w ΣPFCs ($p < 0.05$) **Outcome:** T3 – fetal serum **Major Findings:** (adj model) **Non-sig** neg correlated w PFOS and ΣPFCs	**Major Limitations:** Limited information on statistical methodology Small N Overlap of effects between PFOS and ΣPFCs makes determination of PFOS-specific effects uncertain Low exposure relative to US pop **Other comments:** Small N Statistical methodology not well described Low exposure

Thyroid hormone analysis data in Suppl Information **Location:** Souel, Cheongju, and Gumi, S. Korea **Population:** Preg women in three hospitals 8/2008-3/2009 N = 44 **Related Studies:**		**Outcome:** T4 – maternal serum **Major Findings:** (adj model) **Non-sig** neg correlated w PFOS and ΣPFCs **Outcome:** T4 – fetal serum **Major Findings:** (adj model) **Non-sig** neg correlated w PFOS and ΣPFCs **Outcome:** TSH – maternal serum **Major Findings:** (adj model) **Non-sig** neg correlated w PFOS and ΣPFCs **Outcome:** TSH – fetal serum **Major Findings:** (adj model) **Non-sig** neg correlated w PFOS and ΣPFCs	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Knox et al. (2011) Knox SS, Jackson T, Frisbee SJ, Javins B, Ducatman AM. Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. J Toxicol Sci. 2011 Aug;36(4):403-10. **Study Design:** Cross-sectional Analysis of clinical <u>parameters by LabCorp</u> Total T4 T3 uptake (TBG saturation) TSH Serum albumin **Location:** WV and OH **Population:** C8 Health Project ≥ 20 yrs old No thyroid dieseae N = 50,044	**Exposure Assessment:** Protein precipition, reverse-phase HPLC-triple quadrupole MS LOQ = 0.5 ng/ml **Population-Level Exposure:** (NOTE; no overall statistic reported) Mean (by water district) = 20.97-26.15 ng/ml (**NOTE**: corresponds to 75-90th percentile US distribution (NHANES 4th Rpt)	**Stat Method:** Regression analyses Separate analysis of M, F and two age groups ≥ 20-50, >50 yrs old Log-PFOS as quintiles <u>Co-variates</u>: Age Serum estradiol Alcohol Stratification of analyses by BMI (< ≥30) **Outcome:** Total T4 **Major Findings:** PFOS **sig pos** assoc w T4 For M and F and all ages in study Sig higher in F compared to M	**Major Limitations:** Cross-sectional ↓ T3 uptake w ↑ total T4 suggests ↑ TBG levels. However, TBG was not measured **Other comments:** Large N

M = 25,026 F = 25, 018 **Related Studies:**		**Outcome:** TSH **Major Findings:** PFOS **not sig** assoc w TSH for M or F for any age **Outcome:** T3 uptake **Major Findings:** PFOS **sig neg** assoc w T3 uptake in M, F all age groups Sig lower in F compared to M	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Kristensen et al. (2013) Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, Halldorsson TI, Becher G, Haug LS, Toft G. Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum Reprod. 2013 Dec;28(12):3337-48. doi: 10.1093/humrep/det382. Epub 2013 Oct 15. **Study Design:** Longitudinal, nested cohort–mother/daughter Enrollment in cohort at 30-wk routine visit Questionnaire: Age Parity Height Pre-preg wt Smoking Alcohol Blood sample at enrollment (preg wk 30) Perinatal data from birth cert and hosp records	**Exposure Assessment:** Column-switching LC/MS LOQ 0.05 ng/ml **Population-Level Exposure:** Median maternal PFOS = 3.6 ng/ml (IQR = 2.8-4.8 ng/ml) (**NOTE**: exposure ~ 1/2 US F NHANES 4th Rpt)	**Stat Method:** PFOS in tertiles: Low – 0.1-3.0 ng/ml Med – 18.0-23.6 High – 23.6-53.1 <u>Outcomes</u> Age at menarchy Menstrual cycle length Number of follicles Level of reprod hormones (total testosterone, SHBG, DHEAS, FSH, LH, FAI (free androgen index), estradiol, AMH) PFOS regression analyses w and w/out PFOA entered in model <u>Co-variates</u> (selected a-priori based on literature and included in models w/out prior testing of effect on models) Age of menarchy: Maternal preg smoking (Y/N) Social class BMI Menstrual cycle length; reprod hormones; follicle number: Maternal smoking (Y/N) Social class Daughter's BMI	**Major Limitations:** Low exposure compared to US Retrospective/recall for determination of age at menarchy **Other comments:** Longitudinal design Relatively small n for contraceptive and non-contraceptive groups Relatively low median PFOS exposure compared to US pop., but relatively large range (high PFOS 23.6-53.1 ng/ml)

2008 Follow-up of F offspring at 20 yrs old N = 436 Questionnaire: - Age at menarchy - History of hormonal contraception N = 367 Clinical examination of daughters Partial exclusions (for some analyses) for: - menstrual cycle length (?) - reproductive hormone levels (?) - Follicle number (?) - Breast feeding - Signs of premature ovarian failure - incomplete data (incl. contraceptive hormones) Final N varied by outcome (147-246) **Location:** Denmark **Population:** 1988-9 Danish Pregnancy Cohort Original n = 1,212 Daughters' mean age = 19.6 yrs old (sd = 0.4 yrs) **Related Studies:**		Daughter's smoking Menstrual cycle phase at exam (FSH LH, estradiol) Analyses stratified on contraceptive hormone use at exam (except age at menarchy) – FSH, LH and estradiol analyses on non-users only **Outcome:** Age at menarchy **Major Findings:** PFOS **not sig** assoc w age at menarchy (Low PFOS n = 110 Med PFOS n = 113 High PFOS n = 114) **Outcome:** <u>Reproductive parameters</u> Cycle length Total testosterone SHBG FAI DHEAS AMH Number of follicles/ovary FSH LH estradiol	

		Major Findings: PFOS **not sig** assoc w any reprod parametrs (contraceptive (n = 50-66) and non-contraceptive (n = 17-30) users)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Kvist et al. (2012) Kvist L1, Giwercman YL, Jönsson BA, Lindh CH, Bonde JP, Toft G, Strucinski P, Pedersen HS, Zvyezday V, Giwercman A. Reprod Toxicol. 2012 Dec;34(4):644-50. doi: 10.1016/j.reprotox.2012.09.007. Epub 2012 Oct 5. Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland. **Study Design:** Blood and semen samples collected (48 hr sexual abstinence) Analysis of PFOS in serum Lifestyle factors by interview Sperm X and Y chromosome microscopic analysis by fluorescent-bound nucleic acid hybridization probes **Location:**	**Exposure Assessment:** Labeled internal standard Analysis by LC/MS/MS LOD? **Population-Level Exposure:** (mean (95% CI) PFOS conc) Greenland (Inuit) – 51.65 ng/ml (48.04-55-26) Poland – 12.12 ng/ml (17.19-19.05) Ukraine – 8.20 ng/ml (7.52-8.88)	**Stat Method:** Y:X chromosome ratio calculated as mean +/- sd Analysis of assoc w continuous PFOS in linear regression. Also, MANOVA w categorical (quartile) PFOS conc. Analysis w full dataset And w data set w extremem and influential data points removed <u>Mandatory confounders included</u> Age Abstinence time Alcohol intake PCB-153 **Outcome:** Assoc PFOS and Y:X chromosome ratio **Major Findings:** <u>Linear regression analysis</u> Full dataset Pooled data: PFOS **sig assoc** (pos) w Y:X ratio ($p = 0.026$, $r^2 = 0.016$	**Major Limitations:** 41% exclusion rate from original collected sample pool Relatively small overall N and individual country n (Note; exact n for individual countries not provided) Relationships are not consistent across countries or by type of analysis (continuous regression, categorical MANOVA) (although note that Greenland exposure much larger than Poland or Ukraine) **Other comments:** Relatively small N (and individual n's) High non-participation rate possibly resulting in bias Lack of consistency across populations (although note exposure diff)

Population: M spouses of pregnant women in Greenland (Inuit), n = 201; Warsaw, Poland, n = 198; and Kharkiv, Ukraine, n = 208 3/2002-2/2004 <u>Exclusions</u> Insufficient semen (n = 98) Insufficient sperm (n = 95) Lack of exposure data (n = 55) Final N = 359 **Related Studies:**		Individual Countries: PFOS **not sig assoc** w Y:X ratio Dataset excluding outliers, influential pts PFOS **not sig assoc** w Y:X ratio for pooled or individual data sets <u>MANOVA</u> Full dataset Pooled data: **Sig diff** in Y:X ratio between 2nd and 4th quart of PFOS (p = 0.006) **Pos trend** Y:X ratio (p = 0.017) Individual Countries: <u>Inuit</u> – **Sig diff** in Y:X ratio between 2nd-4th and 3rd-4th quart PFOS exposure **Neg trend** (p = 0.028) Dataset excluding outliers, influential pts Pooled data: **Sig diff** in Y:X ratio between 2nd and 4th quart of PFOS (p = 0.043) **Pos trend** in Y:X ratio (p = 0.039) Individual Countries: <u>Inuit</u> –**Neg trend** (p = 0.044)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: La Rocca et al. (2014) La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F, Bergamasco B, Stecca L, Perra G, Mancini FR, Marci R, Bordi G, Caserta D, Focardi S, Moscarini M, Mantovani A. Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas. Int J Environ Res Public Health. 2014 Sep 29;11(10):10146-64. doi: 10.3390/ijerph111010146. **Study Design:** Population data from Italian Nat'l Inst Statistics 1/2009-12/2011 **Location:** Italy Rome ("metropolitan area), Ferrara ("urban area"), Sora ("rural area") **Population:** Women	**Exposure Assessment:** PFOS measurement in whole blood Extraction with liquid-liquid extraction, HPLC- electrospray ionization-MS PFOS LOD = 0.4 ng/ml < LOD = LOD/2 **Population-Level Exposure:** Mean PFOS conc for total pop: - infertile = 3.5 ng/ml - fertile = 2.2 ng/ml Median (both categories) = < 0.4 ng/ml (NOTE: mean PFOS conc = 29-36% of US F (NHANES 4th Rpt))	**Stat Method:** Diff between fertile and infertile F by Wilcoxon-Mann-Whitney test (non-parametric equivalent of 2-sample t-test) Bonferroni adj for multiple comparisons Analyses stratified by geographic area **Outcome:** Assoc of PFOS with fertile/infertile status **Major Findings:** PFOS **not sig assoc** w fertility status for any geographic study area	**Major Limitations:** PFOS measurement in whole blood (vs. serum) is unusual. Unclear how this could affect exposure assessment Small overall N and smaller for each geog area. This is particularly a limitation given the geog stratification of the analysis. No indication of co-variate adj of statistical analysis PFOS analysis not controlled for PFOA **Other comments:** Unusual PFOS analysis in whole blood Small overall and area N's No apparent co-variate adjustment of statistical analysis

Total: - 110 infertile, 43 fertile Metropolitan: - 49 infertile; 13 fertile Urban: - 38 infertile, 22 fertile Rural: 23 infertile, 8 fertile Fertile: - regular menstrual cycle - spontaneous preg in prev yr - stopped breastfeeding ≥ 6 mos before entry into study Infertile: - diagnosis of primary infertility, or unexplained infertility - enrolled in study prior to infertility treatment Inclusion criteria: - residence in one of study areas - 18-40 yrs old - BMI < 30 - PBMC (periph blood mononuclear cells) in normal range Exclusion criteria: - occupational exposure to PFOS (or other study substs) - smoking - vegetarian diet - BMI > 30 - evidence of inflammatory or infectious disease **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Liew et al. (2014) Am J Epidemiol. 2014 Sep 15;180(6):574-81. doi: 10.1093/aje/kwu179. Epub 2014 Aug 19. Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. Liew Z, Ritz B, Bonefeld-Jørgensen EC, Henriksen TB, Nohr EA, Bech BH, Fei C, Bossi R, von Ehrenstein OS, Streja E, Uldall P, Olsen J. **Study Design:** Case-control cohort study Two blood samples for most, 1st and 2nd trimester Inclusion criteria: - singleton births - telephone interview 14-19 wks t gest - blood sample during 1st or 2nd tri-mest Source pop = 83,389 mother-child pairs	**Exposure Assessment:** Solid-phase extraction LC-MS **Population-Level Exposure:** PFOS median **maternal** serum conc. by sex of child: Boys - cases = 28.90 ng/ml - controls = 27.60 Girls - cases = 27.50 - controls = 26.20 (NOTE: PFOS med conc ~ 3.5 x US F (NHANES 4th Rpt)) PFOS detected in 100% of samples	**Stat Method:** 1st trimester blood sample used preferentailly PFOS as continuous var w and w/out log-transform Also quartiles based on control disturb Risk ratios from GLM w Poisson distrib Generalized additive models to examine non-linear assoc bet PFOS and CP Analyses stratified by sex, term and pre-term birth status <u>Adjustment for potential confounders</u> - maternal age at birth - parity - SES - smoking - alcohol - education - maternal psychiatric illnesses - child's sex	**Major Limitations:** Different times of maternal blood sample during gest **Other comments:** Case-control design Adj of PFOS for all PFCs analyzed Clear case ascertainment Blood samples from either 1st or 2nd tri-mest CP is likely to be an umbrella rubric for several diff conditions

Location: Denmark **Population:** Danish National Birth Cohort (1996-2002) Source pop = 83,389 mother-child pairs <u>Cerebral palsy</u> (CP) cases in source pop identified from Danish Nat'l CP Re **N = 156** <u>Controls</u> Random selection from source pop **N = 550** M = 440 F = 110 **Related Studies:**		<u>Co-variates included</u> - fish consumption - organic food consumption - housing attributes - bisphenol-A exposure - phthalate exposure <u>Co-variates investigated, but not included</u> - gest wk blood sampling - birth yr - father's age at birth - maternal pre-preg BMI - season of conception - maternal preg illness **Outcome:** CP - Boys **Major Findings:** <u>All Boys (n = 86)</u> Risk ratio **sig > 1.0** (= 1.7 (1.0-2.8) Risk ratio **sig >1.0** for quarts 1 and 3 (but not quart 2) Adj for other PFCs did not sig affect outcome <u>Boys born at term (n = 65)</u> Risk ratio **sig >1.0** (= 2.1 (1.2-3.8)	

		Outcome: CP – Girls **Major Findings:** <u>All Girls (n = 66)</u> Risk ratio **not sig > 1.0** <u>Girls born at term (n = 45)</u> Risk ratio **not sig > 1.0**	

Reference and Study Design	Exposure Measures	Results	Comments
Study: Liew et al. (2015) Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, Bossi R, Henriksen TB, Bonefeld-Jørgensen EC, Olsen J. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect. 2015 Apr;123(4):367-73 **Study Design:** Nested case-control Recruitment at 6-12 wks gest Exclusion - not fluent in Danish - non-singleton births Telephone interviews - 2 x during preg - ~ 12 wk; - timing of 2nd interview? - 2 postpartum (dates?) 1-2 blood samples (1st and/or 2nd trimester)	**Exposure Assessment:** Plasma samples Solid phase extraction LC-MS LLOQ PFOS = 0.28 ng/ml 100% PFOS analyses > LOD **Population-Level Exposure:** Median PFOS conc: - controls = 27.40 ng/ml - ADHD cases = 26.80 ng/ml - autism cases = 25.40 ng/ml	**Stat Method:** Risk ratio by generalized linear models - PFOS continuous conc ln-transformed - Gen. additive models to investigate non-linear relationships OR by unconditional logistic regression - categorized in quartiles <u>Potential confounders in final model</u> (a priori) - maternal age at delivery - parity - SES - smoking - alcohol - self-reported psychiatric illness - gest wk of blood draw - birth yr - sex Multiple PFAS model considered **Outcome:** ADHD	**Major Limitations:** Most PFOS analyses from 1st trimester sample 13% from 2nd trimester sample – possible exposure misclassification Moderate N in general Weighted toward boys because of higher risk of autism, however, results in low power for girls **Other comments:** Case-control Mostly 1st trimmest exposure analysis – unclear as to predictive value Also, possible confounding by partial 2nd trmest sampling

- 87% of samples analyzed were from 1st trimester Singleton births ADHD, autism diagnosis through Danish Nat'l Hosp reg based on 10.7 yr follow-up of birth cohort Cases and controls matched on sex **Location:** Denmark **Population:** Danish National Birth Cohort 1996-2002 60% participation ADHD - N = 220 - M = 179 - F = 41 Autism - N = 220 - M = 187 - F = 33 control - N = 550 - M = 440 - F = 110 **Related Studies:**		**Major Findings:** (adj model) RR not sig > 1.0 No quart sig > 1.0 (1st quart as ref) **Outcome:** autism **Major Findings:** (adj model) RR not sig > 1.0 No quart sig > 1.0 (1st quart as ref)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lin et al. (2009) Lin CY, Chen PC, Lin YC, Lin LY. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care. 2009 Apr;32(4):702-7. doi: 10.2337/dc08-1816. Epub 2008 Dec 29. **Study Design:** Cross-sectional Data from NHANES 1999-2000; 2003-2004 Serum total cholesterol and triglycerides by enzymatic assay HDL cholesterol by dedicated instrument (?) Serum C-reactive protein (SCRP) by latex enhanced neflalometry Plasma insulin by immunoendymatic assay Insulin resistance (HOMA-IR) by homeostasis model assessment (HOMA2)	**Exposure Assessment:** Solid-phase extraction, HPLC, negative ion turbo-ion spray ionization tandem MS Isotope-labeled internal standards LOD(?) **Population-Level Exposure:** Mean (SE) 12-20 yrs = 22.42 ng/ml (1.15) > 20 yrs = 24.29 ng/ml (0.99)	**Stat Method:** Stratification of analyses by age - 12-20 yrs - > 20 yrs Multiple linear reg models for assoc PFOS w glucose, insulin, HOMA-IR OR for metabolic syndrome by logistic regression <u>Covariates – linear regression</u> - Age - Sex - Race - Smoking - Alcohol - Household income - Waist meas - CRP - Insulin/glucose/HOMA - Medications (antihypertensive, antidepressive, antihyperglycemic <u>Covariates – logistic regression</u> As above + other components of metabolic syndrome **Outcome:** Glucose	**Major Limitations:** Corss-sectional PFOS analyses not controlled for PFOA or other PFCs Incomplete alcohol consumption data for adolescents **Other comments:** Large N Thorough consideration of co-variates (although incomplete alcohol data for 12-20 yrs)

Metabolic syndrome determined based on: - Waist measurement (↑) Serum triglyceride (↑) - serum HDL (↓) - BP (SBP, DBP) (↑) (or anti-hypertensive med) **Location:** US **Population:** US sample (NHANES) ≥ 12 yrs old, blood sample for PFCs (3,695) → Morning exam, fasting glucose, insulin, triglyceride data (1,788) → No other missing data → **N = 1,443** 12-20 yr old n = 474 > 20 yrs old n = 969 **Related Studies:** Fisher et al. (2013) (Canada)		**Major Findings:** (fully adj models) <u>12-20 yrs</u> Glucose **not sig assoc** w PFOS > <u>20 yrs</u> Glucose **not sig assoc** w PFOS **Outcome:** Insulin **Major Findings:** (fully adj models) <u>12-20 yrs</u> Insulin **not sig assoc** w PFOS ><u>20 yrs</u> Insulin **sig pos assoc** w PFOS ($p < 0.01$) **Outcome:** HOMA-IR **Major Findings:** (fully adj models) <u>12-20 yrs</u> HOMA-IR **not sig assoc** w PFOS	

		>20 yrs HOMA-IR **sig pos assoc** w PFOS ($p < 0.01$) **Outcome:** β cell function **Major Findings:** (fully adj models) 12-20 yrs β cell function **not sig assoc** w PFOS > 20 yrs β cell function **sig pos assoc** w PFOS ($p < 0.01$) **Outcome:** Metabolic syndrome **Major Findings:** (fully adj model) 12-20 yrs OR for metabolic syndrome (waist) **sig < 1.0** (OR = 0.37, $p < 0.05$) OR for full metabolic syndrome and other components **not sig diff from 1.0**	

		<u>> 20 yrs</u> OR for metabolic syndrome (HDL cholesterol) **sig > 1.0** (OR = 1.61, $p < 0.05$) OR for full metabolic syndrome and other components **not sig diff from 1.0**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lin et al. (2011) Lin CY, Lin LY, Wen TW, Lien GW, Chien KL, Hsu SH, Liao CC, Sung FC, Chen PC, Su TC. Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. Int J Cardiol. 2013 Oct 9;168(4):3309-16. doi: 10.1016/j.ijcard.2013.04.042. Epub 2013 May 7 **Study Design:** Cross-sectional Cohort of hypertensive (and non-hypertensive) school age children drawn from school pop-based urine screening (gr 1-12) 1992-2000 2006-2008 follow-up → 707 hypertensive, 690 non-hypertens Demographic, medication, income by interview Blood draw after ≥ 8 hr fasting	**Exposure Assessment:** PFOS (PFCs) by UPLC-triple quadrupole MS PFOS LOQ = 0.22 ng/ml < LOQ (1.7% for PFOS) = LOQ/2 **Population-Level Exposure:** PFOS median conc (total) = 8.93 ng/ml (range (max-min) = 67.14 ng/ml) M = 11.82 ng/ml (range = 67.14) F = 8.10 ng/ml (range = 28.34) **Note:** - PFOS conc consistent w US pop (NHANES 4th Rpt)	**Stat Method:** Linear regression models with categorical PFOS (< 50th, 75th-89th, > 90th percentiles) Ln-transform of adiponectin, CRP, HOMA-IR, triglyceride to produce normal distrib <u>Co-variates</u> Age Gender Smoking Alcohol Income Waist circum SBP Total cholesterol HOMA-IR creatinne **Outcome:** Glucose homeostasis **Major Findings:** Glucose homeostasis **not sig assoc** w PFOS **Outcome:** Adiponectin	**Major Limitations:** Small N (n for 12-19 yrs old is only 78) PFOS analyses not adjusted for other PFCs **Other comments:** Small n – especially for adolescents raises issues of power to detect relatively subtle associations

Triglycerides, plasma cholesterol, LDL, HDL, glucose by autoanalyzer Adiponectin and Insulin by commercial kit C-reactive protein (CRP) by enzyme-immunoassay HOMA-IR calculated BP measured twice Height, wt → BMI Metabolic syndrome determination based on ≥ 3 of: - ↑ waist circum - ↑ serum triglyceride - ↓ HDL - ↑ SBP or ↑DBP or anti-hypertensive med - ↑ glucose or anti-hyperglycemic med **Location:** Tapei, Taiwan **Population:** Exclusion for insuff vol, budgetary constraints, diabetes meds → **N = 287** M = 121 F = 166		**Major Findings:** Adiponectin levels **not sig assoc** w PFOS **Outcome:** Lipid profile **Major Findings:** Lipid profile **not sig assoc** w PFOS **Outcome:** Inflamatory markers **Major Findings:** Inflammatory markers **not sig assoc** w PFOS	

Hypertensive = 17 Non-hypertens = 270 12-19 yrs, n = 78 20-30 yrs n = 209 **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lin et al. (2013a) Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Hsu SH, Chien KL, Liao CC, Sung FC, Chen PC, Su TC. The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. J Hazard Mater. 2013 Jan 15;244-245:637-44. doi: 10.1016/j.jhazmat.2012.10.049. Epub 2012 Nov 2. **Study Design:** Cross-sectional Interview: Age Gender Med history Household income Questionnaire: Alcohol Smoking Measurement: - Wt, height → BMI - BP → ↑ BP (or reported BP med)	**Exposure Assessment:** Serum PFOS UPLC-triple quadrupole MS LOQ = 0.22 ng/ml < LOQ (1.6% of PFOS samples) = LOQ/2 **Population-Level Exposure:** <u>Geom mean (geom sd)</u> Total – 7.78 ng/ml (2.42) M – 8.82 ng/ml (2.60) F – 7.18 ng/ml (2.29) 12-19 yrs – 7.04 (2.38) 20-30 yrs – 8.28 (2.44) (**Note**: consistent w US pop (NHANES 4th Rpt))	**Stat Method:** PFOS as categorical variable (<50th, 50-75th, 75-90th, > 90th percentiles) Linear regression (TSH and FT4 as dependent vars): - TSH ln-transformed - Analyses stratified by sex and age categories Logistic regression (OR for TSH > normal range: - stratified by BMI, smoking, hypertension <u>Co-variates</u> Age Gender Smoking alcohol **Outcome:** FT4 **Major Findings:** (adj model) FT4 **not sig assoc** w PFOS (for total N or for subgroups – smoking, BMI, hypertension)	**Major Limitations:** CVs for TSH and FT4 reported twice w different values PFOS analyses not adj for other PFCs **Other comments:** Moderate N for age subgroups. Power may not be sufficient to discern diff in thyroid function w age

Blood sample (when?): - Fasting glucose (or reported insulin med→ diabetes - Thyroid (immunoluminescence assay) - TSH (CV = 2.09%, 3.34% **?**) - FT4 (CV = 1.37%, 4.51% **?**) **Location:** Tapei, Taiwan **Population:** School children (gr 1-12) participants in pop-wide urine screening Nested cohort from urine screening 1992-2000 w and w/out ↑ BP ↑ BP Nested cohort – 707 → **n = 40** Normal BP Nested cohort – 6,390 w → **n = 505** M - n = 214 F – n = 337 12-19 yrs old – n = 212 20-30 yrs old – n = 339 **Related Studies:** Lin et al. (2011)		**Outcome:** TSH **Major Findings:** (adj model) TSH **not sig assoc** w PFOS **Outcome:** OR for TSH > normal range **Major Findings:** OR TSH > normal range not sig > 1.0 for PFOS conc categories	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lin et al. (2013b) Lin CY, Lin LY, Wen TW, Lien GW, Chien KL, Hsu SH, Liao CC, Sung FC, Chen PC, Su TC. Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. Int J Cardiol. 2013 Oct 9;168(4):3309-16. doi: 10.1016/j.ijcard.2013.04.042. Epub 2013 May 7 **Study Design:** Cross-sectional Interview: Age Gender Med history Household income Questionnaire: Alcohol Smoking Measurement: - Wt, height → BMI - BP → ↑ BP (or reported BP med) - Heart rate - cholesterol	**Exposure Assessment:** Serum PFOS UPLC-triple quadrupole MS LOQ = 0.22 ng/ml < LOQ (1.6% of PFOS samples) = LOQ/2 **Population-Level Exposure:** (geom mean (95% CI on geom mean)) Total = 7.85 ng/ml (5.13-11.78) M = 8.97 ng/ml (3.24-12.72) F = 7.21 ng/ml (4.41-11.75) 12-19 yrs = 7.25 ng/ml (2.44-23.69) 20-30 yrs = 8.21 ng/ml (6.27-34.71)	**Stat Method:** To correct for multiple comparisons among 4 PFCs, Bonferoni correcton applied to p-value (α = 0.025) for sig Linear regression models PFOS treated as categorical (< 25^{ht}, 25th 50th-75th, >75th percentile) assoc between [SBP, BMI, LDL, CRP, triglycerides (TG), HOMA-IR] and PFOS (PFCs) Ln-transformation (for CRP, HOMA-IR, TG) Co-variates: Gender Age Smoking SBP BMI LDL CRP HOMA-IR For analysis of assoc CIMT and PFOS, PFOS analyzed separately and adj for other PFCs	**Major Limitations:** Moderate N Authors identify limitation resulting from original urine screening cohort consisting of subjects w abnormal urinalysis (proteinuria, glucosuria, hematuria). However, it is not clear if all subjects were abnormal in urine screen. Does not appear that urine screen positives will necessarily bias CIMT outcomes. **Other comments:** Moderate N – particularly for adolescents PFOS investigated as individual factor and adjusted for other PFCs Pop may not be normal w respect to urinalysis. This may introduce a bias

- triglycerides - HDL - LDL - glucose - insulin (commercial kit) - C-reactive protein (chemoluminescence-immunoassay) - HOMA-IR (glucose x insulin) - Diabetes (↑ glucose or diabetes med) - Uric acid (UA) (reported but not in Methods) CIMT (Carotid artery intima-media thickness) - sub-clinical marker of atherosclerosis - by ultrasonography - computer assisted, 150 measurements of 10 mm section of common carotid artery - repeat measurement of record of 30 random samples after 2 wks → 98.5-98.8% coeff correlation reliability Apiloprotein E (APOE) genotypes measured by sequence specific PCR **Location:** Taipei, Taiwan		<u>Logistic regression</u> OR of ↑ CIMT w 50% ↑ in PFOS conc **Outcome:** Cardiovascular risk factors (SBP, BMI, LDL, TG, UA, HOMA-IR) **Major Findings:** Cardiovascular risk factors **not sig assoc** w PFOS **Outcome**: CIMT – linear regression **Major Findings:** (fully adj model) <u>PFOS individual model</u> CIMT **sig pos assoc** w PFOS <u>PFOS model adj for other PFCs</u> CIMT **sig pos assoc** w PFOS	

Population: School children (gr 1-12) participants in pop-wide urine screening Nested cohort from urine screening 1992-2000 – 790 → full PFC analysis only → **N = 644** M - n = 250 F – n = 394 12-19 yrs old – n = 231 20-30 yrs old – n = 413 **Related Studies:**		<u>PFOS individual model stratified by subpopulations (as indicated)</u> Sex – CIMT **sig pos assoc** w PFOS for F CIMT **not sig assoc** w PFOS for M Age – CIMT **sig pos assoc** w PFOS for 12-19 yrs CIMT **not sig assoc** w PFOS for 20-30 yrs BMI – CIMT **sig pos assoc** w PFOS for BMI = < 24 kg/m^2 CIMT **not sig assoc** w PFOS for BMI > 24 24 kg/m^2 Smoking – CIMT **sig pos assoc** w PFOS for never smoked CIMT **not sig assoc** w PFOS for has smoked HOMA-IR – CIMT **not sig assoc** w PFOS for HOMA-IR ≤ 0.93 CIMT **sig assoc** w PFOS for HOMA-IR > 0.93	

		APOE genotype – CIMT **sig assoc** w PFOS for E2 carrier and E3/E3 CIMT **not sig assoc** w PFOS for E4 carrier **Outcome:** OR of ↑ CIMT w 50% ↑ in PFOS – logistic regression **Major Findings:** OR sig > 1.0 (2.93) for APOE E2 carriers OR sig > 1.0 (1.84) for APOE E3/E3	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lin (2014) Lin LY, Wen LL, Su TC, Chen PC, Lin CY. Negative association between serum perfluorooctane sulfate concentration and bone mineral density in US premenopausal women: NHANES, 2005-2008. J Clin Endocrinol Metab. 2014 Jun;99(6):2173-80. doi: 10.1210/jc.2013-3409. Epub 2014 Feb 28 **Study Design:** Cross-sectional F ≥ 12 yr old Dual x-ray absorptiometry (DXA) measurement over lumbar and spine for bone mineral density (BMD) Self-reported fractures Exclusion: - pregnant - radiographic contrast material use in past 7 d - nuclear med study past 3 d - wt > 300 lb **Location:** US	**Exposure Assessment:** CDC analytical proc PFOS LOD = 0.2 ng/ml **Population-Level Exposure:** Geom mean PFOS serum conc M = 19.23 ng/ml F = 12.09 < 40 yrs old = 11.95 < 60 = 15.22 ≥ 60 = 21.13	**Stat Method:** <u>Co-variates</u> - age - race - BMI - smoking - alcohol - osteoarthritis - daily use of prednisone or cortisone - prior osteoporosis treatment Separate models for: - men - women non-menopausal - women menopausal NHANES sample weights Multiple linear regression And Logistic regression of OR for self-reported fractures w unit increase in ln- PFOS **Outcome:** Total lumbar spine BMD (g/cm^2) **Major Findings:** <u>M</u> – lumber spine BMD **not sig assoc** w PFOS	**Major Limitations:** Cross-sectional design Self-reported fracture **Other comments:** Large N Careful statistical design and analysis

Population: Premenopausal women in NHANES (2005-6; 2007-8) N = 2339 (w PFOS and DXA measurement) **Related Studies:**		F- Non-menopausal – lumber spine BMD **sig neg assoc** w PFOS **sig for trend across quartiles** F - Menopausal – lumber spine BMD **not sig assoc** w BMD **Outcome:** Total hip BMD (g/cm^2) **Major Findings:** M – hip BMD **not sig assoc** w PFOS F- Non-menopausal – hip BMD **not sig neg assoc** w PFOS F - Menopausal – hip BMD **not sig assoc** w BMD **Outcome:** OR for bone fracture as function of unit incr in ln-PFOS **Major Findings:** For all groups (M, F-non-menopausal/menopausal) OR **not sig <>1.0**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lind et al. (2014) Lind L, Zethelius B, Salihovic S, van Bavel B, Lind PM. Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia. 2014 Mar;57(3):473-9. doi: 10.1007/s00125-013-3126-3. Epub 2013 Dec 14. **Study Design:** Cross-sectional Fasting ≥ 8 hrs prior to sampling Questionnaire: - med history - edu - exercise - smoking - regular medication - diagnosis of diabetes (Y/N) Measure plasma proinsulin and insulin by ELISA Proinsulin/insulin ratio as measure of insulin secretion HOMA-IR as index of insulin resistance	**Exposure Assessment:** Rapid protein precip,automated column-switching UPLC-MS/MS Electrospray interface in neg ion mode LOD (all PFAS) = 0.01-0.17 ng/ml **Population-Level Exposure:** Mean PFOS plasma conc (linear) = 13.2 ng/ml (NOTE adult geiom mean PFOS = 9.7 ng/ml (NHANES 4rh Rpt))	**Stat Method:** Logisitic regression for assoc PFOS and prevalent diabetes (OR) PFOS as linear and squared forms For continuous analysis adj for: - sex - serum cholesterol - triglycerides - BMI - smoking - exercise - energy intake - alcohol - education Linear regression for assoc PFOS w proinsulin/insuln ratio and HOMA-IR (analysis for non-diabetic subjects only) Bonferroni correction for p-values for prevalent diabetes due to 7-PFAS, α = 0.0071 No Bonferroni correction for proinsul/insulin ratio or HOMA-IR (i.e., α = 0.05)	**Major Limitations:** Cross-sectional design Low-moderate n for diabetes Confined to spec, elderly pop. **Other comments:** Moderate n for diabetes Reasonable stat analysis

Location: Upsala, Sweden **Population:** PIVUS cohort 2001-2004 Age = 70 yrs **N = 1, 016** **N w diabetes = 119** (mean duration diabetes = 8.9 yrs) **Related Studies:**		**Outcome:** Prevalent diabetes **Major Findings:** (adj model) OR for assoc PFOS w prevalent diabetes **not sig <> 1.0** **Outcome:** Proinsulin/insulin ratio **Major Findings:** (adj model) PFOS **not sig assoc** w proinsulin/insulin ratio **Outcome:** HOMA-IR **Major Findings:** (adj model) PFOS **not sig assoc** w HOMA-IR	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Looker et al. (2014) Looker C1, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, Fletcher T. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci. 2014 Mar;138(1):76-88. doi: 10.1093/toxsci/kft269. Epub 2013 Nov 27. **Study Design:** Longitudinal (?) 2010- 2011 Part of C8-Science Panel Interview of subset 2010 Participants (not already vaccinated) received influenza vaccine (FLUVIRIN) 1st serum sample collected at vaccination 2nd serum sample 21 +/- 3 days post-vaccination Serum testing for influenza-specific antibody by hemaglutination inhibition (HI)	**Exposure Assessment:** Solid-phase extraction, reverse-phase HPLC, isotope dilution tandem MS PFOS LD = 0.2 ng/ml Inter-day precision (CV for 60 repeat measurements) = 7.3-7.6% Intra-day precision (CV 5 measurements) = 4.9-5.8% **Population-Level Exposure:** Log_{10} median PFOS conc = 0.96 = 9.12 ng/ml (linear) IQR = 5.75-14.45 ng/ml (linear)	**Stat Method:** Antibody titer ↑ post-vaccination = post vaccine – pre-vaccine (value log-transformed) Ratio Post-vaccination/Pre-vaccination (value log-transformed) PFOS analyzed as log-transformed and categorical (quartiles) <u>Linear regression</u> Co-variates: - Age (obligatory) (as non-linear cubic spline) - Gender (obligatory) Retained if p in model ≤ 0.05: - smoking - previous (> 3 mos) influenza vaccine - day of serum collection - co-existing medical conditions - anti-inflamatory/pain-relief meds - mobility (no. of address since 1970)	**Major Limitations:** Moderate N PFOS analyses not controlled for PFOA Influenza vaccinations in prev yrs was found to be a sig determinant of these outcomes, but was self-reported. This raises possibility uncertainty w respect to control by this variable. However, unclear if this is directional **Other comments:** Study is well designed with clear cut determination of outcomes. Co-variattes appear to be reasonably complete. The N is moderate

assay for A/H3N2, A/H1N1 and influenza B Influenza-specific titer measured **Location:** WV, OH **Population:** Adult (> 18 yrs) C8- study participants who had not received influenza vaccine in prev 3 mos N = 403 (titer studies) N = 755 (self-reported cold/influenza in past yr) **Related Studies:**		<u>Logistic regression</u> OR of achieving Seroconversion (4 x ↑ in titer) seroprotection (≥ 40 x absolute titer ↑) Co-variates retained in model if $p < 0.05$ Age (obligatory) as categorical variable (10 yr bands) OR of self-reported cold/influenza in past yr - Age (obligatory), gender (obligatory) - smoking, alcohol, BMI, diabetes, educatin – considered, but rejected **Outcome:** Antibody titer ↑; antibody titer ratio post-vaccine **Major Findings:** (adj model) Titer ↑ or ratio **not sig assoc** w PFOS conc	

		Outcome: OR seroconversion **Major Findings:** (adj model) OR for seroconversion **not sig assoc** w PFOS conc **Outcome:** OR seroprotection **Major Findigns:** OR for seroprotection **not sig assoc** w PFOS conc **Outcome:** OR self-reported cold/influenza in past yr **Major Findings:** OR for self-reported cold/influenza past yr **not sig assoc** w PFOS conc	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lopez-Espinosa et al. (2011) Lopez-Espinosa MJ, Fletcher T, Armstrong B, Genser B, Dhatariya K, Mondal D, Ducatman A, Leonardi G. Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environ Sci Technol. 2011 Oct 1;45(19):8160-6. doi: 10.1021/es1038694. Epub 2011 May 2. **Study Design:** Cross-sectional C8 Science Panel enrolled 8/2005-7/2006 **Location:** WV, OH **Population:** C8 Science Panel 8-18 yrs old at recruitment N = 6,007 (F = 2.931 M = 3076)	**Exposure Assessment:** "Liquid chromatography separation" (HPLC?)-tandem MS Precision +/- ~10% in multiple replicates LOD = 0.5 ng/ml < LOD = LOD/2 (n = 11) **Population-Level Exposure:** Median PFOS conc M – 20 ng/ml F – 18 ng/ml (**NOTE**: levels are 2-3 x US levels for 12-19 yr old (NHANES 4th Rpt))	**Stat Method:** Assoc of pubertal status and PFOS by logistic regression <u>Covariates considered</u> Age at survey (mandatory) BMI Height Annual household family income Ethnicity (non-Hisp white/other) Smoking (ever Y/N) Alcohol (ever Y/N) Time of sample collection (mo, hr) Only age included (BMI and height in sensitivity analyses) PFOS as categorical (quartiles) and continuous ln-transformed PFOS analysis adj for PFOA in model **Outcome:** M Age at puberty assoc w PFOS **Major Findings:** (full adj model – incl PFOA) PFOS **sig assoc w delay** in onset of puberty for quartiles	**Major Limitations:** Cross-sectional For F, uncertainty regarding measurement of onset of puberty due to: 1. Confounding of estradiol conc by hormone contraceptive use; 2. Self-reporting of onset of menarche. Authors consider menarche basis more reliable. 3. Variable offset between PFOS sample and puberty Potential reverse causation bias for F. Blood loss due to menstruation would result in lower PFOS conc. Later menarche would allow greater retention of PFOS – later menarche → ↑ PFOS; early menarche → ↓ PFOS However, does not appear to have parallel for M **Other comments:** Large N Objective hormone measure + self-reported menarche data Reasonable statistical controls Large effect level

Hormone determination in clinical lab Estradiol (LOD = 7 pg/ml) , total testosterone (LOD = 10 ng/dL) by electrochemiluminesscent immunoassay Free testosterone by radioimmunoassay (LOD = 0.2 pg/ml) F w estradiol < LOD = 149 M w total, free testosterone < LOD = 158, 608 Questionnaire: - Residential history - Employment history - Lifestyle (?) - Family medical history - Health variables (?) - F – age at first menstruation (don't know → exclusion) M - free testosterone levels dichotomized as indicators of sexual maturation F – estradiol levels confounded by contraception medication. Therefore, sexual maturation based on estradiol cutoff or menarche **Related Studies:**		3 and 4 (1st Q as ref) and for continuous model. Delays for Q3 (compared to Q1) = 118, 122 days based on total, free testosterone Delays for Q4 (compared to Q1) = 187, 123 days (total, free testosterone Delay for ln unit PFOS in continuous model = 128, 76 d **Outcome:** F Age at puberty assoc w PFOS **Major Findings:** (fully adj model incl PFOA) <u>Based on age at menarche:</u> PFOS **sig assoc w delay** in puberty for Q3, Borderline sig assoc w delay for Q4 PFOS **sig assoc w delay** for continuous model Delay for Q3 (compared to Q1) = 117 d Delay for ln unit PFOS in continuous model = 94 d <u>Based on estradiol levels</u> PFOS **sig assoc w delay** in puberty for Q3 and Q4 (1st Q as ref) And for continuous model	

		Delay for Q3 (compared to Q1) = 175 d Delay for Q4 (compared to Q1) = 268 d Delay for ln unit PFOS in continuous model = 76 d	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lopez-Espinosa et al. (2012a) Lopez-Espinosa MJ, Mondal D, Armstrong B, Bloom MS, Fletcher T. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ Health Perspect. 2012 Jul;120(7):1036-41. doi: 10.1289/ehp.1104370. Epub 2012 Mar 27. **Study Design:** Cross-sectional TSH by electrochemiluminescence immunosassay total T4 (TT4) by cloned enzyme immunodonor assay Sub-clinical hypothyroidism defined as TSH > age-specific normal range *and* TT4 w/in normal range (N = 365) Sub-clinical hyperthyroidism defined as TSH < age-specific normal range *and* TT4 w/in normal range (N = 78)	**Exposure Assessment:** Liquid chromatography (HPLC?) – MS PFOS precision +/- 10% w multiple replicates LOD = 0.5 ng/ml < LOD (PFOS = 16) as LOD/2 **Population-Level Exposure:** Median PFOS = 20 ng/ml (IQR = 15-28 ng/ml) (Note; ~ 3 x most recent NHANES levels for 12-19 yrs old (NHANES 4th Rpt))	**Stat Method:** <u>Co-variates considered</u> Age Sex Race/ethnicity BMI Month of sampling Household income Ever smoking Ever alcohol <u>Co-variates employed</u> (> 10% change when omitted) Age Sex Month of sampling TSH ln-transformed <u>Linear regression of TSH or T4</u> (exclusion of clinical thryroidism) Regression w continuous ln-transformed PFOS (stratified by sex and age group) Regression w (non-transformed) categorical (quartile) PFOS concs. PFOS analyzed w and w/out adj for other PFCs	**Major Limitations:** Cross-sectional **Other comments:** Large N Reasonable statistical controls Measurement of clinical and sub-clinical endpoints Note, however, that the magnitude of endpoints assoc w PFOS were small, ≤ 2%

Clinical hypo/hyperthyroidism based on self-reported diagnosis or medication (n = 61) (**NOTE**: In addition to measured serum PFOS in 1-17 yr olds at time of entry into study, Lopez-Espinosa et al. also modeled *in utero* PFOS exposure. As this is not empirical, those results are not reported here) **Location:** WV, OH **Population:** 2005-6 C8 cohort Children 1-17 yrs N = 10,657 w serum PFOS measurement (N =4, 713 matched to maternal serum PFC) **Related Studies:**		<u>Logistic regression</u> OR for: - Clinical hypo-hyperthyroidism - sublinical hypo- - sublicinical hyper- **Outcome:** TSH level **Major Findings:** (adj model) PFOS **borderline sig pos assoc** w TSH level for 4th Q (1st Q as ref) for full cohort For M, PFOS **sig pos assoc** w TSH levels 1-5 yrs old (NOTE: results for PFOS similar in models adj for PFOA) **Outcome:** TT4 level **Major Findings:** (adj model) PFOS **sig pos assoc** w TT4 level for 4th Q (1st Q as ref) for full cohort	

		PFOS **sig pos assoc** w TT4 for full cohort And for 6-10 yrs and > 10 yrs – continuous analysis For M, PFOS **sig pos assoc** w TT4 for full cohort And for >10 yrs For F, PFOS **sig pos assoc** w TT4 for full cohort And for 6-10 yrs and >10 yrs (NOTE: results for PFOS similar in models adj for PFOA) **Outcome:** Clinical thyroid disease/hypothyroidism **Major Findings:** OR for clinical thyroid disease or hypothyroidism **not sig** for PFOS **Outcome:** Sub-clinical hypothyroidism **Major Findings:** OR for sub-clinical hypothyroidism **not sig** for PFOS	

		Outcome: Sub-clinical hyperthyroidism **Major Findings:** OR for sub-clinical hyperthyroidism **not sig** for PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Louis et al. (2012) Louis GM, Peterson CM, Chen Z, Hediger ML, Croughan MS, Sundaram R, Stanford JB, Fujimoto VY, Varner MW, Giudice LC, Kennedy A, Sun L, Wu Q, Kannan K Perfluorochemicals and endometriosis: the ENDO study.. Epidemiology.2012ov;23(6):799-805.doi:10.1097/EDE.0b013e31826cc0cf. **Study Design:** Case-control Baseline interview by nurses 2 mos before surgery (cases) or MRI (controls) Std anthropometric assessment Non-fasting blood sample MRIs read by 2 radiologists **Location:** Salt Lake City, UT San Francisco, CA **Population:** Women scheduled for surgery (laparoscopy, laparotomy) **N = 473** (79% eligible participation)	**Exposure Assessment:** Ion-pair extraction w $^{13}C_4$-PFOS spike Recovery 98-140% RSD for duplicate analyses < 5% HPLC-MS + tandem electrospray MS (?) PFOS 100% > LOQ LOD (LOQ) ? **Population-Level Exposure:** PFOS geom mean conc (endometriosis – operated, non-operated) = 6.11-7.41 ng/ml (Note: consistent w US F pop (NHANES 4th Rpt))	**Stat Method:** OR for endometriosis relative to PFOS by logistic regression PFOS conc log-transformed <u>Co-variates</u> Age (*a priori)* BMI (*a priori*) Investigated in sens analyses: - Parity (conditioned on gravidity) - restriction of endometriosis to stage 3 and 4 - restricting cases to post-operative finding of (otherwise) normal pelvis **Outcome:** OR for endometriosis per log-unit change in PFOS conc (operative sample, non-operative sample) **Major Findings:** (adj model) OR for endometriosis **not sig assoc** w PFOS log-unit change for either operative or non-operative sample	**Major Limitations:** Small N for endometriosis (190, operative + 14, non-operative) Moderate N for non-endometriosis (283, operative + 113, non-operative) LOD/LOQ not reported for PFOS (or other PFCs) **Other comments:** N (depending on category) was small to moderate Categorization of status (operative positive, operative neg, non-operative pos, non-operative neg, normal pelvis, non-normal pelvis) is complicated and not clearly explained and makes interpretation relative to cases and controls difficult

Non-surgery pop identified through UT Pop Database and phone directory age-matched surgery pop limited to menstruating women in referent pop to same clinical facilities (50 mile radius) Exclusions (non-surgery): -Pelvic MRI to exclude unknown cases - previous case of endometriosis - <18, > 44 yrs - history of cancer - injectable hormones in ≤ 2 yrs prev - current breastfeeding ≥ 6 mos **N = 127** (81% eligible participation) Surgery pop → **N = 190** endometriosis cases Non-surgery → **N = 113** non-endometriosis (based on MRI) **Related Studies:**		**Outcome:** OR for endometriosis per log-unit change in PFOS conc Operative sample restricted to endometriosis stage 3 and 4 **Major Findings:** **OR (1.86) sig** for PFOS <u>adj for age, BMI</u> OR (1.50) **not sig** for PFOS <u>adj for age, BMI and parity</u> **Outcome:** OR for endometriosis per log-unit change in PFOS conc Comparison pop = operative sample w normal pelvis **Major Findings:** (adj model) OR **not sig** for PFOS (w or w/out parity adj)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Louis et al. (2015) Louis GM, Chen Z, Schisterman EF, Kim S, Sweeney AM, Sundaram R, Lynch CD, Gore-Langton RE, Barr DB. Perfluorochemicals and human semen quality: the LIFE study. Environ Health Perspect. 2015 Jan;123(1):57-63. doi: 10.1289/ehp.1307621. Epub 2014 Aug 15. **Study Design:** Yr sample collection? Data and sample collection in participants' homes - blood - BMI - ejaculate 2 sample following 2-day abstinence - 80% provided 2 samples - General characteristics e.g., vol - Motility measures - sperm head measures - morphology measures - chromatin stability measures **Location:** MI, TX	**Exposure Assessment:** Analyses by NIEHS-CDC Isotope dilution HPLC-MS < 1% PFOS samples < LOD **Population-Level Exposure:** MI - geom mean = 17.39 ng/ml - median = 19.15 TX - geom mean = 21.23 ng/ml - median = 21.6 ng/ml (NOTE: PFOS conc ~ 42% (MI) and 75% larger than current US M (NHANES 4th Rpt))	**Stat Method:** Linear mixed models to investigate assoc semen/sperm parameters w Δ 1 unit ln-PFOS <u>Co-variates</u> - age (a priori) - BMI (a priori) - smoking (a priori) - abstinence time (a priori) - study site (a priori) - sample age (a priori) (Note; only sig outcomes are noted here) **Outcome:** Motility (distance migrated in straw) **Major Findings:** PFOS **sig pos assoc** w distance migrated **Outcome:** Morphology (coiled tail) **Major Findings:** PFOS **sig neg assoc** w % sperm w coiled tail	**Major Limitations:** There were 35 parameters assessed w α = 0.05. No Bonferroni correction. Therefore ~ 2 sig associations expected by chance **Other comments:** Modest size N Good analytical methodology Multiple comparisons w chance outcome (~2 sig findings expected, 2 sig outcomes observed) PFOS spec findings are not a priori biologically plausible.

Population: LIFE cohort - MI, n = 96 - TX, n = 366 M of couples discontinuing contraception to achieve preg Recruiting through marketing database in MI; Hunting/fishing licensing in TX M ≥ 18 yrs old No medical diagnosis of sterility **Related Studies:** Joensen et al. (2009) Raymer et al. (2012) Toft et al. (2012)			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Lyngsø et al. (2014) Lyngsø J1, Ramlau-Hansen CH, Høyer BB, Støvring H, Bonde JP, Jönsson BA, Lindh CH, Pedersen HS, Ludwicki JK, Zviezdai V, Toft G. Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study. Hum Reprod. 2014 Feb;29(2):359-67. doi: 10.1093/humrep/det390. Epub 2013 Oct 25. **Study Design:** Cross-sectional questionnaire Menstrual cycle characteristics pre-preg w intercourse w/birth control Length from one "bleeding" to next "bleeding" as average cycle length (if given as range, average was calculated) **Location:** Ukraine, Poland, Greenland	**Exposure Assessment:** LC-MS LOD = 0.2 ng/ml 100% samples > LOD for PFOS CV for repeat analyses (diff days) = 9% **Population-Level Exposure:** Median PFOS conc Greenland – 20.2 ng/ml Poland – 8.0 ng/ml Ukraine – 5.0 ng/ml (Note: Poland and Ukraine PFOS concs are consistent w US pop, Greenland PFOS ~ 3 x current US F population (NHANES 4th Rpt.))	**Stat Method:** <u>Co-variates/confounders investigated</u> Age BMI Parity Smoking Education Alcohol Imputation of missing data by replacement of missing values by random plausible values through model using following data as predictors: - PFOS, PFOA levels - mean length of cycle - irregular cycle - age at menarche - age at pregnancy - pre-preg BMI - smoking - parity - education level <u>A priori variables</u> Age at menarche Age at preg Parity Pre-preg BMI Smoking (Y/N) 100 data complete data sets created by imputation	**Major Limitations:** Recall of menstrual cycle length at some unspecified number of months in past Imputation of missing data based on predictive models for missing data. However, analysis with complete datasets only gave comparable results (but with smaller N (48-56% of N w imputed data) PFOS analyses not controlled for PFOA (and other PFCs) **Other comments:** Cross-sectional Large N for pooled analyses Reasonable statistical controls Uncertain error/bias due to recall of cycle length Uncertainty/bias in imputed analyses (non-imputed analyses w smaller N)

Population: INJENDO cohort (?) Enrolled 6/2002-5/2004 During ante-natal visits ≥ 18 yrs Born in country in which enrolled 1,735 interviewed Exclusions: - oral contraceptives ≥ 2 mos prior to preg - reported menstrual cycle < 16 days (interpreted as error) N = 1,623 Greenland = 528 Poland = 452 Ukraine = 643 **Related Studies:**		PFOS association w cycle length by mult logistic regression Stratification by country and pooled analysis (adj for country) PFOS as tertiles Also as continuous (log-transformed) varaible OR for short and long cycles (separate analyses) **Outcome:** Menstrual cycle **Major Findings:** (adj model) PFOS **not sig assoc** w irregular, short, or long cycles By categorical (H, M, L) or continuous analysis Similar results w imputed datasets and full data sets-only	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Maisonet et al. (2012) Maisonet M, Terrell ML, McGeehin MA, Christensen KY, Holmes A, Calafat AM, Marcus M. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect. 2012 Oct;120(10):1432-7. doi: 10.1289/ehp.1003096. Epub 2012 Jul 10. **Study Design:** Longitudinal Sample as sub-sample of nested cohort selected for menarche onset case-control study - Cases = menarche < 11.5 yrs (n = 218) - Controls = random sample w menarche ≥ 11.5 yrs (n = 230) Maternal serum sample during preg (median = 15 wks) Full N = 447 N for each analysis varied due to missing maternal data	**Exposure Assessment:** Analysis by CDC LOD for PFOS = 0.2 ng/ml Precison of measurement = 8-13% **Population-Level Exposure:** Maternal PFOS median conc = 19.6 ng/ml (Note: this is ~2.5 x current U.S. F exposure (NHANES 4t Rpt))	**Stat Method:** <u>Co-vairates/confounders considered</u> Gestational age Maternal education Preg BMI Maternal age at delivery Prev live births Maternal preg smoking (Y/N) Maternal ethnicity Breast feeding to 4 wks (Y/N) Gestational age at blood sample Sample is subsample of previously selected sample of larger cohort for study of onset of menarche. To correct potential sampling bias, current sample was weighted based on menarche onset parameter Linear regression of birth wt, birth wt, gestational age, ponderal index (wt/length x 100) on maternal PFOS Backward elimination with exclusion for $p > 0.2$ in model Trends sig at $\alpha < 0.05$	**Major Limitations:** Use of nested cohort originally based on onset of menarche potentially biases outcomes. It is not clear to what extent this potential bias has been corrected by weighting procedure. Self-reporting of maternal characteristics **Other comments:** Longitudinal study Moderate size N

Birth wt and gestational age from med records Wt, height at 2 and 20 mos from routine health surveillance prgm Maternal characteristics self-reported during preg Breast feeding info from questionnaires at 4 wks post-delivery **Location:** Avon County, UK **Population:** ALSPAC cohort Pregnant women w expected delivery 4/1991-12/1992 → 14,610 offspring → 11,820 at 13 yrs old → 5,756 F → 3,682 w ≥ 2 assessments of pubertal status 8-13 yrs → sample of 447 **Related Studies:**		**Outcome:** Birth wt (n = 422) **Major Findings:** (adj for maternal preg smoking, maternal pre-preg BMI, prev live births, gest age) PFOS **sig neg assoc** w birth wt p-trend 0.0053 **Outcome:** Birth length (N = 356) **Major Findings** (adj for maternal preg smoking, maternal pre-preg BMI, maternal educ, prev live births, gestational age) PFOS **sig neg assoc** w birth length p-trend = 0.013 **Outcome:** Gestational age (N = 444) **Major Findings:** PFOS **not sig assoc** w gest age	

		Outcome: Ponderal index (N = 360) **Major Findings:** PFOS **not sig assoc** w ponderal index **Outcome:** Wt at 20 mos (N = 320) **Major Findings:** (adj for maternal age at delivery, maternal educ, prev live births, ht at 20 mos, birth wt) PFOS **sig pos assoc** w wt at 20 mos p-trend < 0.0001 When stratified by tertile of PFOS *and* tertile of birth wt (n = 107) PFOS **sig pos assoc** w wt at 20 mos **only for highest tertile of birth wt** (borderline sig for lowest tertile birth wt) (adj for maternal educ, maternal age at delivery, prev live births, birth wt as continuous variable)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Melzer et al. (2010) Melzer D1, Rice N, Depledge MH, Henley WE, Galloway TS. Environ Health Perspect. 2010 May;118(5):686-92. doi: 10.1289/ehp.0901584. Epub 2010 Jan 7. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. **Study Design:** Nested cohort NHANES interview - ever been told had thyroid problem – did they still have the problem? Current thyroid disease → taking thyroid med To determine thyroid specificity, assoc examined between PFOS and other NHANES disease categories (ischemic heart disease, diabetes, arthritis, current asthma, COPD, bronchitis, emphysema) **Location:** U.S.	**Exposure Assessment:** Solid-phase extraction, HPLC, turbo ion spray ionization, tandem MS with isotope-labeled internal stds PFOS LOD = 0.2 ng/ml **Population-Level Exposure:** Geom mean M = 25.08 ng/ml F = 19.14 ng/ml	**Stat Method:** Sample weighting by NHANES weighting factors Multivariate logistic regression - OR disease outcome by pop-weighted quartile PFOS conc Stratification of analysis by sex <u>Confounders and co-variates considered</u> Age Sex Race/ethnicity Education Smoking BMI alcohol **Outcome:** Self-reported thyroid disease - ever **Major Findings:** F - OR for thyroid disease (ever) **not sig > 1.0 f**or PFOS M - OR for thyroid disease (ever) **not sig > 1.0 f**or PFOS	**Major Limitations:** Small n for cases – especially M Self-identification of thyroid diagnosis and current condition PFOS analyses not controlled for PFOA Single serum sample – unknown temporal relation to “ever diagnosed” status **Other comments:** Good analytical methodology Potential temporal disconnect between serum sample and reporting (especially “ever diagnosed w thyroid condition”) Definition of “current thyroid disease” category as taking thyroid med makes revere causation unlikely (medication restores normal thyroid function and therefore thyroid dysfunction should not → ↑ PFOS

Population: NHANES 1999-2000, 2003-2004, 2005-2006 1/3 random sample of ≥ 12 yrs old NHANES participants Participants < 20 yrs excluded due to no information on disease prevalence N-total = 3,966 Cases (ever thyroid disease) F = 292 (adj % = 16.08%) M = 69 (ad % = 3,06%) Cases (current thyroid disease) F = 164 (adj n = 9.89%) M = 46 (adj n = 1.18%) **Related Studies:**		**Outcome:** Self-reported thyroid disease – current **Major Findings:** F - OR for thyroid disease (current) **not sig > 1.0** for PFOS M – OR for thyroid disease (current) not sig > 1.0 for OR for 4th Q vs. Q 1 and Q2 (i.e., below median) **sig > 1.0** (OR = 2.68 (1.03–6.98), p = 0.043)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Nelson et al. (2010) Nelson JW1, Hatch EE, Webster TF. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect. 2010 Feb;118(2):197-202. doi: 10.1289/ehp.0901165 **Study Design:** Cross-sectional Serum samples at NHANES interview Total cholesterol (TC), HDL, non-HDL, LDL, - TC measured enzymatically - HDL measured after precip of apoliprotein B - non-HDL as TC-HDL - LDL only measured in fasting subset of participants based on “Friedwald formula” - Weight - height - BMI - Waist Circumf - insulin resistance by homeostatic model assessment (HOMA)	**Exposure Assessment:** By CDC-NCEH, isotope dilution HPLC-tandem MS Automated solid-phase extraction **Population-Level Exposure:** PFOS median conc = 21.0 ng/ml	**Stat Method:** <u>Co-variates</u> (A priori) Age Sex Race SES Saturated fat intake Exercise (past 30 d) Time in front of TV/monitor Alcohol (> 20 yrs old) Smoking (> 20 yrs old) Regression analyses for PFCs separately HOMA log transf PFOS as quartiles for total pop and for age/sex categories NHANES weighting factors not used **Outcome:** Total cholesterol (TC) (20-80 yrs) **Major Findings:** PFOS **sig pos assoc** w TC (p-trend = 0.01) 0.27 µg/dL ↑ in TC/ng/ml ↑ in PFOS	**Major Limitations:** PFOS analyses not controlled for other PFCs TC and non-HDL analyses are linked since non-HDL = 70-80% of TC Cross-sectional Potential for reverse causality (however, controlling for albumin did not change outcomes) **Other comments:** Cross-sectional Rel large N Large number co-variates in model Stratification by age

Location: US **Population:** NHANES cohort ≥ 12 yrs old Exclusions: - > 80 yrs - Pregnant - Breast feeding - Insulin medication - Dialysis - Cholesterol lowering med (for cholesterol analyses) N for PFOS analyses = 860 **Related Studies:**		**Outcome:** Non-HDL (20-80 yrs) **Major Findings:** PFOS **sig pos assoc** w non-HDL (p-trend = 0.02) 0.25 µg/dL ↑ in non-HDL/ng/ml per µg/L ↑ in PFOS **Outcome:** HDL (20-80 yrs) **Major Findings:** PFOS **not sig assoc** w HDL **Outcome:** LDL (20-80 yrs) **Major Findings:** PFOS **not sig assoc** w LDL **Outcome:** BMI **Major Findings:** For M 12-19 yrs; 20-59 yrs, PFOS **sig neg assoc** w BMI (p-trend = 0.004)	

		For M 60-80 yrs PFOS **sig pos assoc** w BMI (p-trend ?) PFOS **not sig assoc** w BMI for F **Outcome:** HOMA **Major Findings:** PFOS **not sig assoc** w HOMA	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Ode et al. (2014) Ode A, Källén K, Gustafsson P, Rylander L, Jönsson BA, Olofsson P, Ivarsson SA, Lindh CH, Rignell-Hydbom A. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One. 2014 Apr 23;9(4):e95891. doi: 10.1371/journal.pone.0095891. eCollection 2014. **Study Design:** Case-control design Children born and living in Malmo 1978-2000 w clinical diagnosis of ADHD in study hospital ADHD cases linked to Swedish Nat'l Birth Reg for demographic, obstetric data Banked cord serum collected from Malmo Maternal Unit Serum Bloodbank Controls matched on yr of birth and maternal country of birth **Location:** Malmo, Sweden	**Exposure Assessment:** Isotopically labeled internal std LC/MS-MS LOD (all PFCs) = 0.2 ng/ml Results as aver of 2 samples on diff days CV for dup samples PFOS = 11% **Population-Level Exposure:** PFOS median conc Cases = 6.92 ng/ml Controls = 6.77 ng/ml	**Stat Method:** Conditional logistic reg OR calc based on: - unit incr in PFOS - ≥75th percentile of PFOS conc of controls Co-variates (based on literature) - smoking (cotinine) - parity - gestational age at birth- **Outcome:** OR for ADHD **Major Findings:** OR for ADHD **not sig <> 1.0** for Unit ↑ PFOS Or ≥ 75th percentile control PFOS conc	**Major Limitations:** PFOS analyses not controlled for PFOA **Other comments:** Case control design Clear diagnostic records and diagnostic criteria Mod large n for cases PFOS analyses not controlled for PFOA

Population: N (study and control) = 206 **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Okada et al. (2012) Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, Konishi K, Ito YM, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res. 2012 Jan;112:118-25. doi: 10.1016/j.envres.2011.10.003. Epub 2011 Oct 24. **Study Design:** Prospective cohort Women self-admin questionnaire in 2nd trimester: - Med history - education - household income - smoking - alcohol - caffeine - food intake freq From med records: - maternal age - maternal height - pre-preg wt - Preg complications - gestational age - parity - infant gender - birth wt	**Exposure Assessment:** Serum analyzed by column-switching LC-MS PFOS LOD = 0.5 ng/ml **Population-Level Exposure:** Mean maternal PFOS conc = 5.6 ng/ml (median = 5.2 ng/ml) PFOS detect = 100% (NOTE: PFOS exposure ~30% lower than US F pop (NHANES 4th Rpt))	**Stat Method:** <u>Analysis of IgE and PFOS assoc</u> PFOS, IgE log-transformed Polynomial regression Co-variates/confounders considered: (vars in full model in bold) **Maternal age** **Maternal allergy history** **Infant gender** **Birth season** **Home distance to highway** **Sampling period** **Parity** Deep sea fish preg intake Also stratification by infant gender <u>Analysis of infant allergies and infect diseases</u> Binomial logistic regression OR for risk of allergies/infectious diseases with PFOS levels Co-variates in full model: Maternal age Maternal educ Pre-preg BMI	**Major Limitations:** Small N for full cohort sample – esp for M-only and F-only Allergy/disease outcomes based on maternal self-identification **Other comments:** Prospective cohort design Self-identification of allergy disease outcome Limited power due to small N

Self admin questionnaire at 18 mos post-natal: - breastfeeding - current infant wt, length - smoking (both parents) - ETS - pets - "living environment" - day care - vaccinations - infant med history allergies, infectious diseases Assessment of infant allergies based on maternal questionnaire responses at 18 mos Maternal blood sample after 2nd trimester (post-delivery if maternal anemia) IgE from cord blood by enzyme-linked immunosorbant assay - mean cord IgE conc = 0.62 IU/ml (median = 0.21 IU/ml) **Location:** Sapporo, Hokkaido, Japan		Maternal/paternal allergy history (Y/N) Parity (prima/multiparous) Infant gender Breast feed (< ≥ 4 mos) ETS (Y/N) Day care (Y/N) Maternal blood sampling period (pre-post birth) **Outcome:** IgE **Major Findings:** <u>Full cohort</u> IgE **not sig assoc** w log PFOS <u>M-only</u> IgE **not sig assoc** w log PFOS <u>F-only</u> IgE **not sig assoc** w log PFOS **Outcome:** Allergies/infectious diseases at 18 mos	

Population: Birth cohort from Sapporo 7/2002-10/2005 1796 eligible → 514 agreed to participate → 10 excluded due to stillbirth, miscarriage, relocation withdrawal → 13 excluded due to infant death, or withdrawal ≤ 18 mos → **N = 343 for PFOS;** **N = 231 for IgE** **Related Studies:**		**Major Findings:** <u>Full cohort</u> OR for allergies/diseases as function of PFOS **not sig < > 1.0** <u>M-only</u> OR for allergies/diseases as function of PFOS **not sig < > 1.0** <u>F-only</u> OR for allergies/diseases as function of PFOS **not sig < > 1.0**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Okada et al. (2014) Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, Itoh K, Ikeno T, Tamakoshi A, Kishi R. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int. 2014 Apr;65:127-34. doi: 10.1016/j.envint.2014.01.007. Epub 2014 Jan 29 **Study Design:** Prospective birth cohort Mothers and children born in Hakkaido, 2003-2009 Exclusions: - no baseline questionnaire - no 3rd trimmest blood sample - stillbirth - congenital malformation - multiple births Self-administered questonnaires - 1st trimest - 4, 12, 24 mos post-natal Infant allergies developing 12-24 mos - eczema - wheezing	**Exposure Assessment:** Blood samples 28-32 wks of gest PFOS in plasma by ultra-HPLC-triple quadrupole MS MDL = 0.3 ng/ml PFOS detect in 100% of samples PFOS median conc = 5.02 ng/ml (mean = 5.56 ng/ml) **Population-Level Exposure:**	**Stat Method:** Categorical analysis by quartile PFOS OR as quart 2-4 compared to 1st quart (ref) <u>Potential confounding vars</u> - maternal age* - education* - parental allergy history - infant gender* - gest age - birth season - breast feeding* - siblings* - ETS* - pets - day care* * = final model **Outcome:** Total allergic diseases **Major Findings:** (adj model) OR not sig < > 1.0 for total cohort or M/F separately **Outcome:** Eczema	**Major Limitations:** PFOS analyses not adj for other PFCs **Other comments:** Prospective design Large N Outcome data from self-admin questionnaires No adjustment for other PFCs

Location: Hokkaido, Japan **Population:** Birth cohort from Hokkaido hospitals Pop meeting all criteria = 6,335 → 300/yr 2003-2008 + 295 in 2009 → 2,095 Excluded late observed congenital malformation and blood samples prior to 26 wks gest → **N = 2,063** Mean maternal age = 30.4 yrs **Related Studies:**		**Major Findings:** (adj model) OR not sig < > 1.0 (except 3rd quart F sig < 1.0)	

<table>
<tr><th>Reference and Study Design</th><th>Exposure Measures</th><th>Results</th><th>Comment</th></tr>
<tr>
<td>Olsen et al. (1999)

Study Design:
Cross-sectional, across two years (1995, 1997)

Location:
Decatur, AL (USA); Antwerp, Belgium

Population:
3M workers at two PFC manufacturing plants
1995 – total n = 178
Decatur n = 90
Antwerp n = 88
1997 – total = 149
Decatur n = 84
Antwerp n = 65

Outcome Definition:
Hematology and serum chemistry

Related studies:
Follow-up of one or both populations in:
Olsen et al.(2003)
Alexander et al. (2003)
Olsen et al.(2004)
Alexander et al. (2007)
Grice et al. (2007)
Olsen et al. (2012)</td>
<td>Exposure Assessment:
Subjects provided blood samples as part of voluntary medical exam. Serum PFOS was measured by LC/MS

Population-Level Exposure:
Exposure levels are combined for both locations.

<table>
<tr><th colspan="4">Exposure levels in 1995</th></tr>
<tr><th>Exposure level</th><th>ppm</th><th>n</th><th>%</th></tr>
<tr><td>1</td><td>0-<1</td><td>45</td><td>25</td></tr>
<tr><td>2</td><td>1-<3</td><td>91</td><td>51</td></tr>
<tr><td>3</td><td>3-<6</td><td>35</td><td>20</td></tr>
<tr><td>4</td><td>≥6</td><td>7</td><td>4</td></tr>
</table>

<table>
<tr><th colspan="4">Exposure levels in 1997</th></tr>
<tr><th>Exposure level</th><th>ppm</th><th>n</th><th>%</th></tr>
<tr><td>1</td><td>0-<1</td><td>60</td><td>40</td></tr>
<tr><td>2</td><td>1-<3</td><td>63</td><td>43</td></tr>
<tr><td>3</td><td>3-<6</td><td>21</td><td>14</td></tr>
<tr><td>4</td><td>≥6</td><td>5</td><td>3</td></tr>
</table>
</td>
<td>Results are combined for both locations.

Stat Method:
Regression models; covariates and confounders considered included age, body mass, current alcohol consumption, and cigarettes smoked/day

p-value (Bonferroni adjusted) based on comparison to low exposure group

Outcome: Total bilirubin

Major Findings:
<u>For 1995</u>
↓ for exposure levels 2 and 3 ($p<0.05$)
Overall ↓ trend was statistically significant

<u>For 1997</u>
↓ for exposure level 2 only ($p<0.05$)
Overall ↓trend was statistically significant

Outcome: Direct bilirubin

Major Findings:
<u>1997 only</u>
↓ for exposure level 2 only ($p<0.05$)
Overall ↓ trend was statistically significant</td>
<td>Major Limitations:
There is no true control group and PFOS-related effects in lowest exposure group could confound a dose-response relationship in higher exposure groups.

Only males in the study populations.

Different serum PFOS analytical methods in 1995 and 1997 r = 0.92 for individual samples across sampling periods

No detection limit reported for either year.

Change in total bilirubin was not significant in either year when results were stratified by plant location.

Other comments:

The study was well conducted and used serum concentration as an unambiguous measure of relative total exposure. However, the absence of a true control group can lead to underestimating PFOS-exposure-related effects. Despite the two year of the study, there was significant turnover in the worker population and the comparison across the two years cannot be considered a longitudinal measure. The number of workers in each exposure category, especially the two highest, is relative small.

Suggestive, but inconsistent associations between PFOS exposure and decreased bilirubin; increased cholesterol, LDL.</td>
</tr>
</table>

		Outcome: Total Cholesterol **Major Findings:** 1997 only ↑ for exposure level 3 only ($p < 0.05$) Overall ↑ trend was statistically significant **Outcome: LDL** **Major Findings:** 1997 only ↑ for exposure level 3 only ($p < 0.05$) Overall ↑ trend was statistically significant **Outcome: HDL** **Major Findings** Overall trend **sig** ↓ 1995 only **Outcome: Triglycerides** **Major Findings** no sig trend	

Reference and Study Design	Exposure Measures	Results	Comment
Olsen et al. (2003b) **Study Design:** Cross-sectional Longitudinal (1994/1995 and/or 1997 compared with 2000) Longitudinal based on repeated medical surveillance, but no details Longitudinal analyses for cholesterol and triglycerides only **Location:** Decatur, AL (USA) Antwerp (Belgium)	**Exposure Assessment:** Serum PFOS and PFOA from participants in voluntary PFC medical surveillance. 73-75% participation +/- 20% precision (most +/- 10%) Analyzed for: Total organic fluorine (TOF) (PFOS + PFOA only for longitudinal analyses) - Perfluorohexanesulfonate - N-ethyl perfluorooctane-sulfonamidoacetate - N-mthyl perfluorooctane-sulfonamidoacetate - perfluorooctane-sulfonamidoacetate - perfluorooctane-sulfonamide Detected at "1-3 order of magnitude below PFOS and PFOA" – not reported.	**Statistical Method** **<u>Cross-Sectional Analysis</u>** <u>Covariates considred</u> Age BMI Alcohol Smoking Yrs employment Job title Controlled for PFOA and TOF **<u>Longitudinal Analysis</u>** As repeated measures <u>Covariates conosidred</u> Yrs of follow-up Age BMI Smoking Alcohol Yr of entry Location Baseline yrs worked Triglycerides (for hepatic chem) Controlled for PFOA and TOF	**Major Limitations** Limit of detection not reported No detail about design of longitudinal study No non-factory controls Lowest exposure category is till elevated **Other comments:** Partial R^2 for PFOS for endpoints in multiple regression models were relatively small = <0.01-0.27) High exposure No non-factory controls – can reduce power to detect effect Most outcomes are cross-sectional

Population

Cross-sectional analysis (2000)

	M	F
Antwerp	206	49
Decatur	215	48

No non-factory controls

	M	F
Antwerp		
production	73%	12%
Non-production	27%	88%
Decatur		
production	75%	63%
Non-production	25%	37%

Longitudinal Analysis

(Employees participating in 1994/5 and/or 1997 and 2000

- 1994/5 and 2000, n = 64
-1997 and 2000, n = 69
-1994/5, 1997 and 2000, n = 41
(sex not specified)

Outcome Definition:

Standard hematology and clinical chemistry.

Urinalysis - glucose, albumin and RBCs (Decatur only)

Population-Level Exposure:
(data presented for 2000 only)

Serum conc. (ppm)

	Mean	Geom. mean	Range
Antwerp			
PFOS	0.80	0.44	0.04-6.24
PFOA	0.84	0.33	0.01-7.04
Decatur			
PFOS	1.32	0.91	0.06-10.06
PFOA	1,78	1,13	0.04-12.70

Quartiles of Serum ppm

	Quartile 1	Q 2	Q3	Q4
PFOS	0.21	0.59	1.17	2.46
PFOA	0.25	0.86	1.20	2.43
TOF	0.43	1/14	1.88	4.06

Outcome:
Cholesterol

Major Findings:
not sig assoc
cross-sectional or long models

Outcome:
HDL

Major Findigs:
Not sig assoc
(cross-sectional)

Outcome:
Triglycerides

Major Findings:
Sig ↑ M only
For 4th quart

Not sig assoc for F in cross-sectional
Or in longitudinal analysis

Outcome:
Alkaline phosphatase

Major Findings:
Sig ↑ M and F

Outcome:
GGT

Major Findings:
Sig ↑ F 4th quart only
M – not sig assoc

Related studies Olsen et al. (1999) Alexander et al. (2003) Olsen et al. (2004) Alexander et al. (2007) Grice et al. (2007) Olsen et al. (2012)		**Outcome:** AST **Major Findings:** <u>Not sig assoc</u> **Outcome:** ALT **Major Findings:** **Sig** ↑ - <u>M only</u> **Outcome:** Total bilirubin **Major Findings:** **Sig** ↓ M & F **Outcome:** TSH **Major Findings:** <u>Not sig assoc</u> **Outcome:** T4 **Major Findings:** <u>Not sig assoc</u> **Outcome:** Free T4 **Major Findings:** <u>Not sig assoc</u> **Outcome:** T3 **Major Findings:** **Sig** ↑ - M only – 4th quart	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Olsen et al. (2004) Marshall JC, Burris JM, Mandel JH. Analysis of episodes of care in a perfluorooctanesulfonyl fluoride production facility. Olsen GW, Burlew MM, J Occup Environ Med. 2004 Aug;46(8):837-46. **Study Design:** 3M workers in PFC facility. Use of "episodes of care" (one or more health claims defined by ICD code for related medical conditions (through company's health care insurance system) to identify exposure related health effects. Chemical plant (direct PFC exposure), and film plant (no direct PFC exposure) workers. **Location:** Decatur, AL **Population:** All active and disability inactive (short and long-term disability to 18 mos.) workers in employment history database 1993-1998.	**Exposure Assessment:** H, L, and "minimal" (film plant) exposure categories (as per Alexander et al. (2003) based on job title with PFOS exposure within title based on Olsen et al. 2003(b) measurements. **Population-Level Exposure:** - H = (geom mean) 0.6-2.0 ppm - L = 0.4 ppm - Minimal = 0.1-0.2 ppm	**Stat Method:** Comparison of all PFC plant employees (n = 652) to all film plant employees (n = 659) Comparison of all workers in H exposure category for 10 yrs solely in PFC plant (n = 211), to film plant workers for 10 yrs (n = 345). Observed number of cases for health condition compared to expected on basis of age and sex. Risk ratio based on $claims_{PFC}/claims_{film}$ **Outcome:** **Major Findings:** Total episodes of care PFC plant = 10,608 Film plant = 11,957 **All Employees** >2.0 or stat. sig. (Risk Ratios)	**Major Limitations:** Exposure classification for PFC plant employees based on correspondence of job category to exposure levels (serum PFOS). However, correspondence was based on a sample of 186 = 29% of the number of respondants. Variability for some job categories was high including some with high PFOS exposure (95% UCI/geom.mean ≈ 3) (Olsen et al. 2003b)). "Minimal" category (for film plant employees) mean 0.1-0.2 ppm is approx. 10 times the median serum PFOS reported by NHANES = 0.02 ppm (Fourth National Report on Human Exposure to Environmental Chemicals; http://www.cdc.gov/exposurereport/pdf/fourthreport.pdf) Thus, use of "minimal" category as referent will bias against finding associations with medical conditions. Sig. co-exposure to PFOA. **Other comments:** The study was well designed and conducted. However, it suffers from using an indirect measure of disease – episodes of care. In addition, the use of episodes of care results in counting multiple episodes in one worker equally with individual episodes among multiple workers. It is likely that risk ratios for causally related endpoints were underestimated due to above-background PFOS exposure in the Film Plant workers.

Related Studies: Olsen et al. (2003) Alexander et al. (2003) Alexander et al. (2007) Grice et al. (2007) Olsen et al. (2012)		<u>Cancers and benign tumors</u> Malignant neoplasms of colon = 5.4 (not sig.) Malignant neoplasms of lower resp tract = 2.7 (not sig.) Malignant melanomas of skin = 12 (not sig.) Malignant neoplasms of prostate = 79 (not sig.) <u>Gastrointestinal</u> Cholelithiasis/Acute cholecystitis (gallbladder inflammation) = 8.6 (**sig**.) Acute pancreatitis = 2.6 (not sig.) *(Note: due to 6 episodes from 1 employee)* <u>Reproductive/Developmental</u> Preterm labor = 3.9 (not sig.) **<u>Long-Term (≥10 yrs) Workers Only</u>** (High Exposure PFC Workers Compared to Film Plant Workers) >2.0 or stat. sig. (Risk Ratios)	On the other hand, co-exposure to PFOA may have confounded risk ratios that may have been causally related to PFOA, but not PFOS.

		<u>Cancers and benign tumors</u> Malignant neoplasms of colon = 12 (not sig.) Malignant neoplasms of rectum = 11 (not sig.) Benign colonic polyps = 2.4 (**sig**) Malignant melanomas of skin = 10 (not sig.) Malignant neoplasms of prostate = 8.2 (not sig.) <u>Gastrointestinal</u> Biliary tract disorders = 2.6 (**sig**) Cholelithiasis/Acute cholecystitis = 25 (**sig**) Cholelithiasis/Chronic cholecystitis = 2.5 (not sig.) Acute pancreatitis = 5.5 (not sig) *(Note: due to 6 episodes from 1 employee)* <u>Urologic</u> Cystitis = 2.4 (**sig**) Urinary tract infection (unspec.) = 2.1 (**sig**)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: **Olsen et al. (2012)** Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. Olsen GW, Ehresman DJ, Buehrer BD, Gibson BA, Butenhoff JL, Zobel LR. J Occup Environ Med. 2012 Aug;54(8):974-83 **Study Design:** Study of workers involved in demolition of two 3M PFC plants. Baseline and end-of-project medical assessments – clinical chemistry. Blood collected at each medical assessment for serum PFOS and PFOA. **Location:** Cottage Grove, MN Decatur, AL **Population:** 179 workers with baseline and end-of-project assessment, without lipid lowering medication 14 3M employees 165 contract workers	**Exposure Assessment:** Serum PFOS (and PFOA) Mean time between baseline and end-of-project assessments = 164 days (38.5% >180 d) **Population-Level Exposure:** <u>Increase in contract workers *****</u> Mean = 1.0 ng/ml <u>Decrease in 3M employees *</u> Mean = 101.3 ng/ml <u>Matched-Pair Change in PFOS *****</u> (for workers with baseline PFOS *and* PFOA <95th percentile) Median = +0.7 ng/ml Mean = +4.2 IQR = -1.0-4.7 ***** Authors do not provide independent data for PFOS increases or decrease across the population except as stratified by PFOA changes Increases were almost all for low baseline worker. Workers with highest baseline mostly experienced decrease due to high baselines and longer time between baseline and end-of-project. Consistent with elimination T1/2.)	**Stat Method:** Matched-pair and linear regression analysis of changes in clinical chem. from baseline. Regression co-variates: sex, baseline age, BMI, alcohol, time between assessments. **Outcome:** <u>Matched pair analyses</u> **Major Findings:** No sig change in: - Total cholesterol - Non-HDL - HDL - Total cholesterol/HDL - Alkaline phosphatase - AST - ALT Sig, but very small change (mean = -0.05 mg/dL) in total bilirubin. **Outcome:** <u>Linear regression analyses *****</u>	**Major Limitations:** Significant co-exposure to PFOA Unclear if regression of clinical chem outcomes against PFOS change controlled for PFOA change. **Other comments:** From the standpoint of assessing PFOS effects, this paper suffers from sig co-exposure to PFOA. Furthermore, changes in PFOS between baseline and end-of-project are not clearly presented for PFOS *per se*. Regression analyses are problematic as it is not clear if coefficients for changes in PFOS are controlled for PFOA changes.

Related Studies:		**Major Findings:** No sig changes except for ↓ ALT for full dataset (No sig change when stratified by low baseline PFOS and PFOA) ***** Unclear from paper if regression analyses for PFOS controlled for PFOA	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Osuna et al. (2014) Osuna C, Grandjean P, Weihe P, El-Fawal HA. Toxicol Sci. 2014 Nov;142(1):158-66. doi: 10.1093/toxsci/kfu163. Epub 2014 Aug 14. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. **Study Design:** Birth cohort - longitudinal Cord blood Inclusion – donated blood sample at age ~7 yrs PFOS in cord blood and serum Assoc auto-antibodies rel to prenatal and age-7 PFOS Measurement serum auto-antibodies to neurotypic and glyotypic proteins, NF-L, NF-M, NF-H, GFAP, actin, keratin, desmin, choline acetyltransferase **Location:** Faroe Is.	**Exposure Assessment:** Online solid-phase extract, HPLC-MS **Population-Level Exposure:** Geom mean PFOS conc - cord blood = 3.1 ng/ml - serum 7 yrs = 27 ng/ml (NOTE: 7 yr serum conc ~ 4 x NHANES 12-19 yr old geom mean (NHANES 4th Rpt))	**Stat Method:** Assoc PFOS w auto-antibodies by linear regression Auto-antibody levels ln-transformed PFOS conc ln-transformed (to give % change in auto-antibodies per Δ 2x change in PFOS **Outcome:** Auto-antibody levels **Major Findings:** PFOS **not sig pos assoc** w any auto-antibody levels – either prenatal or 7 yrs Prenatal PFOS neg assoc w actin-specific IgG	**Major Limitations:** PFOS LOD not provided PFOS analyses not adj for PFOA Relatively small N **Other comments:** Longitudinal design Analytically specific outcomes Rel small N

Population: Birth cohort 1986-7 **N = 37 (cord blood)** **N = 34 (serum 7 yrs)** M = 16 F = 22 Mean age at post-natal sampling = 6.6 yrs **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Power et al. (2013) Power MC1, Webster TF, Baccarelli AA, Weisskopf MG. Neuroepidemiology. 2013;40(2):125-32. doi: 10.1159/000342310. Epub 2012 Oct 24. Cross-sectional association between polyfluoroalkyl chemicals and cognitive limitation in the National Health and Nutrition Examination Survey. **Study Design:** **Total N = 1,766** Primary outcomes Self-reported limitations (Y/N) in: - Memory - Periods of confusion 13% (one or both) Secondary outcomes (sens analyses) - Difficulties in daily activities due to senility (Y/N) **n =17** - performance on digit symbol substitution test **n = 275** **Location:** US	**Exposure Assessment:** CDC HPLC-MS internal spiked stds CV-repeat samples = 10-15% **Population-Level Exposure:** Geom mean PFOS conc = 22.63 ng/ml	**Stat Method:** Data for "small number" persons missing data on potential confounder vars imputed <u>Co-variates</u> Main analyses: - Age - Race - Gender - NHANES cycle - Education - Poverty-income ratio - Food security (Y/N) - Health insurance - Social support (Y/N) - Moderate phys activity (Y/N) - Smoking - alcohol Sensitivity analyses: <u>Metabolic syndrome factors</u> - hypercholesterolemia (self-report, measured, or med) - hypertension ((self-report, measured, or med) - diabetes (self-report, or med) - BMI - osmolality - glumerular filtration rate - fish consumption in past 30 d	**Major Limitations:** Self-reported status for outcomes Self-evaluation of mental status may be biased by actual mental status **Other comments:** Large N Good PFOS measurement Detailed statistical analysis Uncertain determination of outcomes status

Population: NHANES cohort 60-85 yrs old 1999-2000; 2003-2004; 2005-2006; 2007-2008 **Related Studies:**		Adjustment for co-variates used in NHANES weights rather than weights *per se* PFOS conc log-transformed **Outcome:** Difficulty remembering or periods of confusion **Major Findings:** OR for outcomes **not sig < > 1.0** for doubling of PFOS Not affected by adjustment for diabetes, metabolic syndrome factors, fish consumption, or artifact due to changes in serum vol or kidney function Not sig affected by stratification by diabetes OR for outcomes **sig < 1.0** for doubling PFOS conc for diabetics w/out medication (n = 54) **Outcome:** Difficulties w daily life/senility **Major Findings:** OR for outcomes **not sig < > 1.0** for doubling of PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Raymer et al. (2012) Raymer JH1, Michael LC, Studabaker WB, Olsen GW, Sloan CS, Wilcosky T, Walmer DK. Reprod Toxicol. 2012 Jul;33(4):419-27. doi: 10.1016/j.reprotox.2011.05.024. Epub 2011 Jun 29. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. **Study Design:** Cross-sectional 2002-2005 In conjunction with IVF screen Routine sperm analyses (e.g., viscosity, volume, pH) Tests of functional motility Semen sample ≤ 7 d of last ejaculation, but after 48 hr abstinence Delivery to lab ≤ 1 hr post collection	**Exposure Assessment:** Solid-phase extraction, negative elcectrospray ionization, HPLC-MS/MS Field blanks, field controls, lab method blanks, lab method control samples Calibration check sample every 10 samples 30 plasma samples to interlaboratory QA analysis CV for replicate extraction and analysis plasma samples for PFOS = 16% CV for replicate extraction and analysis semen samples for PFOS = 21% PFOS LOD = 0.4 ng/ml (semen and plasma) **Population-Level Exposure:** Mean plasma PFOS conc = 37.4 ng/ml (median = 32.3 ng/ml)	**Stat Method:** Semen and plasma variables kept un-logged Logistic and linear modeling Full model w age, duration abstinence, tobacco use (as mandatory co-variates) Forward selection model w age, duration of abstinence, tobacco use incl. if $p < 0.5$ OR for categorical outcomes **Outcome:** Semen vol **Major Findings:** (adj models) Semen vol **not sig assoc** w plasma or semen PFOS conc OR for abnormal vol not sig <>1.0 **Outcome:** Semen pH	**Major Limitations:** PFOS analyses not adj for PFOA **Other comments:** Mod large N Good measurement precision and control for PFOS and semen characteristics Large number of semen characteristics and hormone variables investigated Well-designed statistical analyses Failure to control PFOS analyses for PFOA conc

Spermatozoa conc by Neubauer hemacytometer - Total testosterone Free testosterone - Follicle stimulation hormone (FSH) - luteinizing hormone (LH) - prolactin - estradiol - T3 - T4 - TSH Reprod health questionnaire: - reprod history - sexual activity - duration of abstinence prior to sample **Location:** Durham, NC **Population:** N = 252 men for PFOS analyses At Duke U. Fertility Center **Related Studies:** Joensen et al. (2009)	(NOTE: PFOS conc ~ 2.7 x current NHANES for M (NHANES 4th Rpt))	**Major Findings:** Semen pH **not sig assoc** w plasma or semen PFOS conc **Outcome:** Sperm conc (x 10^6/ml) **Major Findings:** Sperm conc **not sig assoc** w plasma or semen PFOS conc OR for abnormal sperm conc not sig <>1.0 **Outcome:** WBC conc (x 10^5/ml) **Major Findings:** WBC conc **not sig assoc** w plasma or semen PFOS conc **Outcome:** % motile sperm **Major Findings:** % motile sperm **not sig assoc** w plasma or semen PFOS conc **Outcome:** Initial total motile sperm (x 10^6/ml)	

		Major Findings: Initial total motile sperm **not sig assoc** w plasma or semen PFOS conc **Outcome:** % swim-up overnight sperm motility **Major Findings:** % swim-up overnight sperm motility **not sig assoc** w plasma or semen PFOS conc **Outcome:** Swim-up conc (x 10^6/ml) **Major Findings:** Swim-up conc **not sig assoc** w plasma or semen PFOS conc **Outcome:** % swim-up motility **Major Findings:** % swim-up motility **not sig assoc** w plasma or semen PFOS conc **Outcome:** Swim-up total motility (x 10^6/ml)	

		Major Findings: Swim-up total motility **not sig assoc** w plasma or semen PFOS conc **Outcome:** OR for abnormal liquification **Major Findings:** OR not sig <>1.0 **Outcome:** OR for abnormal Viscosity **Major Findings:** OR not sig <>1.0 **Outcome:** OR for abnormal motility **Major Findings:** OR not sig <>1.0 **Outcome:** PFOS correlation w hormones **Major Findings** PFOS plasma conc **sig correlated w T3** (r = 0.138; p = 0.030) PFOS (semen or plasma) **not sig correlated** w any other hormones	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Robledo et al. (2015) Robledo CA1, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB, Louis GM. Environ Health Perspect. 2015 Jan;123(1):88-94. doi: 10.1289/ehp.1308016. Epub 2014 Aug 5. Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. **Study Design:** Longitudinal Investigation of Fertility and the Environment (LIFE) cohort Couples planning preg w/in 6 mos recruited 2005-2009 <u>Exclusion criteria:</u> - either couple sterile - contraception discontinued for > 2 mos - menstrual cycle not between 21-42 d - F received injectable contraceptive w/in 12 mos - could not communicate in English or Spanish - >12 mos attempted preg - non-singleton birth	**Exposure Assessment:** Pre-conception blood sample (when?) Analysis by CDC **Population-Level Exposure:** PFOS geom mean conc (Suppl info) F = 12.44 ng/ml M = 24.6 ng/ml	**Stat Method:** PFOS ln-transformed Multiple linear regression Separately for each parent Stratified by infant sex Outcomes (birth size characteristics) as continuous variables - Δ per 1 SD change in PFOS <u>A-priori adj for</u>: - maternal age - Δ maternal-paternal age - pre-preg BMI - infant sex - serum lipids - serum cotinine - non-PFOS PFCs - (other) partner's total serum PFC conc Sens analyses excluding gestational diabetes or hypertension – no difference , therefore all pregnancies meeting inclus criteria incl	**Major Limitations:** Rel small N **Other comments:** Prospective study Rel small N Power reduced by stratification by infant sex Good stat design

- non-live birth - birth wt not reported - birth wt > 99th perc - head circum > 99th perc Parental reporting of birth size characteristics; - sex - birth wt - length - head circum - Ponderal index Questionnaires to each parent separately - medical history - reprod history - alcohol - tobacco Parental BMI Date of conception from journal entries for intercourse and fertility monitor for peak LH (ovulation) Daily preg journals – wt gain, gravid diseases **Location:** MI, TX **Population:** N = 180-230 (for various parental reported birth size characteristics) **Related Studies:**		**Outcome:** Birth size characteristics **Major Findings:** PFOS **not sig assoc** w birth size characteristics for either maternal or paternal pre-preg serum conc	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Shankar et al. (2011a) Shankar A, Xiao J, Ducatman A. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am J Epidemiol. 2011 Oct 15;174(8):893-900. doi: 10.1093/aje/kwr171. Epub 2011 Aug 26. PMID: 21873601 [PubMed - indexed for MEDLINE] **Study Design:** Cross-sectional Est glomerular filtration rate (eGFR) calc from serum creatinine conc, age, gender Chronic kidney disease defined as GFR < 60 mL/min/1.73 m^2 Prevalence of chronic kidney disease in sample ≈ 5% (depending on quart of PFOS) N ≈ 230 Serum total cholesterol (enzymatically)	**Exposure Assessment:** Automated solid-phase extraction, isotope dilution HPLC-MS PFOS LOD = 0.2 ng/ml PFOS Inter-assay CV = 13% **Population-Level Exposure:** PFOS median conc = 18.7 ng/ml	**Stat Method:** PFOS as continuous (log-transformed) and categorical (quartiles) variable Multivariate linear reg for assoc PFOS w eGFR Also stratified by: - age - race/ethnicity - gender - education - BMI Categorical regression - OR for chronic kidney disease for each quart PFOS <u>Co-variates</u> Age Sex Race/ethnicity Education Smoking Alcohol SBP DBP Diabetes Total serum cholesterol % glycohemoglobin (NHANES?) sample weights applied	**Major Limitations:** Analysis of PFOA adj of PFOS (but no vice-versa) did not change sig. Not clear if this indicates lack of confounding of PFOS analyses by PFOA Moderate sample size (~ 230) for chronic kidney disease subjects **Other comments:** Analysis for PFOS assoc w eGFR stratified by chronic kidney disease status shows ↑ assoc for <u>non-kidney disease status</u>. Suggests that ***a priori*** kidney disease does not influence PFOS function. Large overall N allows in-depth statistical investigation However, only mod N for chronic kidney disease Good analytical confidence Strong prob of assoc PFOS w outcome, but risk (OR) is only moderate

Serum glucose BP **Location:** **Population:** NHANES 1999-2000; 2003-2004; 2005-2006; 2007-2008 ≥ 20 yrs old 5,717 → exclusions for CV disease, missing data on serum creatinine, or covariates → **N = 4,587** Prevalence of chronic kidney disease in sample ≈ 5% (depending on quart of PFOS) N ≈ 230 F = 51.8% **Related Studies:**		**Outcome:** mean change in eGFR/increment PFOS **Major Findings:** (full adj model) <u>Total sample</u> PFOS **sig neg assoc** w eGFR for Q 3 and 4 (compared to Q1) p-trend = < 0.0001 <u>stratified – age</u> (Q4 vs. Q1) PFOS **sig neg assoc** w eGFR < 60 yrs old Borderline neg sig for ≥ 60 yrs <u>Stratified – sex</u> (Q4 vs. Q1) PFOS **sig neg assoc** w eGFR for M and F <u>Stratified – race/ethnicity</u> (Q4 vs. Q1) PFOS **sig neg assoc** w eGFR for all categories <u>Stratified – education</u> (Q4 vs. Q1) PFOS **sig neg assoc** w eGFR for all categories	

		<u>Stratified – BMI</u> (Q4 vs. Q1) PFOS **sig neg assoc** w eGFR for BMI < > 30 **Outcome:** OR for chronic kidney disease by quart PFOS **Major Findings:** (full adj model) OR for chronic kidney disease **sig > 1.0** for all quarts PFOS (Q2-4 vs. Q1) Max OR (Q4) = 1.82 p-trend = 0.019 inclusion of C-reactive protein in model to address inflammation – no sig change reverse causation investigated by modeling eGFR w stratification for chronic kidney disease – assoc PFOS and eGFR stronger for non-chronic kidney disease	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Shankar et al. (2011b) Shankar A, Xiao J, Ducatman A. Perfluoroalkyl chemicals and elevated serum uric acid in US adults. Clin Epidemiol. 2011;3:251-8. doi: 10.2147/CLEP.S21677. Epub 2011 Sep 30. PMID: 22003309 **Study Design:** Cross-sectional NHANES Exclusion: - missing data for PFC s - missing data for uric acid - missing data on included co-variates Serum total cholesterol measured enzymatically Hypertenstion = BP-S ≥ 140 and/or BP-D ≥ 90 BP-S, BP-D Outcomes: - uric acid conc in serum - presence of hyperuricemia = M – uric acid > 6.8 mg/dL F – uric acid >6.0 mg/dL	**Exposure Assessment:** CDC analyses < LOD = LOD/√2 **Population-Level Exposure:** Median PFOS conc = 17.2 ng/ml (i.e., upper range of 2nd quartile)	**Stat Method:** PFOS as continuous and categorical var Linear regression: Continuous – PFOS log (base-2) transformed Categorical – quartiles Logistic regression: OR for hyperuricemia <u>Co-variates</u> - sex - age - race/ethnicity - educ - smoking - alcohol - hypertension (Y/N) - diabetes (Y/N) - serum total cholesterol NHANES sampling weights applied **Outcome:** Uric acid level **Major Findings:** PFOS **sig pos assoc** w serum uric acid by quartile, sig for trend, and sig for continuous model (log-transformed PFOS)	**Major Limitations:** PFOS analyses not adj for PFOA **Other comments:** Cross-sectional design Large N Reasonable statistical design PFOS analyses not adj for PFOA (PFOA also pos assoc) Although overall summary statistics are consistent with a pos assoc w PFOS, not all analyses are sig.

Location: US **Population:** NHANES 1999-2000, 2003-2004, 2005-2006 ≥ 20 yrs **N = 3,883** F = 51.7% **Related Studies:**		<u>By sex</u> M – borderline sig pos assoc F – **sig pos assoc** by quartile and for trend. Borderline sig (dependent on model) for continuous model (log-transformed PFOS) <u>By BMI</u> BMI <30 kg/m^2 - **sig pos assoc** by quart, for trend, and for continuous model (log-trans PFOS) BMI >30 kg/m^2 – **not sig assoc** **Outcome:** OR for hyperuricemia **Major Findings:** OR **sig > 1.0** for quarts. Borderline sig for trend (dependent on model), **sig pos assoc** for continuous model (log-transformed PFOS)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Shrestha et al. (2015) Shrestha S, Bloom MS, Yucel R, Seegal RF, Wu Q, Kannan K3, Rej R4, Fitzgerald EF Environ Int. 2015 Feb;75:206-14. doi: 10.1016/j.envint.2014.11.018. Epub 2014 Dec 5. Perfluoroalkyl substances and thyroid function in older adults. **Study Design:** Cross-sectional study M, F 55-74 yr old Recruitment 2000-2002 Blood sample at recruitment ≥ 25 yrs residency in Fort Edward, Hudson Falls, Glens Falls, NY Cohort originally estab for study of GE PCBs Exclusion criteria: - residence in target towns ≤25 yrs - worked in PCB job ≥ 1 yr - stroke - head injury - Parkinson's - Alzheimer's - severe cognitive impairment - TH hormone therapy - sex hormone therapy	**Exposure Assessment:** Ion-pairing extraction HPLC-MS Isotopically labeled internal stds LOQ = 0.5-1.0 ng/ml PFOS detected in 100% of samples **Population-Level Exposure:** Geom mean PFOS conc = 31.60 ng/ml (Note this is 3.25 x NAHNES value for > 20 yrs old(NHANES 4th Rpt))	**Stat Method:** Multivariate linear regression <u>Co-variates</u> - age - sex - educ - ∑serum PCBs **Outcome:** TSH **Major Findings:** (full adj model) PFOS **not sig assoc** w serum TSH **Outcome:** fT4 **Major Findings:** (full adj model) PFOS **sig pos assoc** w fT4 (p = 0.044 – borderline) NOTE: assoc ↓ w PFOA incl in model	**Major Limitations:** Rel small N **Other comments:** Cross sectional design Small N PFOS analyses adj for PFOA

Thyroid function serum markers: - TSH - fT4 (free T4) - T4 - T3 By immunoelectro-chemiluminometric assy Mean inter-run C V = 2.5% **Location:** Warren, Saratoga, Washington counties, NY **Population:** N = 87 **Related Studies:**		**Outcome:** T4 **Major Findings:** (full adj model) PFOS **sig pos assoc** w T4 (p = 0.001) NOTE: assoc persists w PFOA incl in model **Outcome:** T3 **Major Findings:** PFOS **not sig assoc** w T3	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Specht et al. (2012) Specht IO, Hougaard KS, Spanò M, Bizzaro D, Manicardi GC, Lindh CH, Toft G, Jönsson BA, Giwercman A, Bonde JP. Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances - a study of spouses of pregnant women in three geographical regions. Reprod Toxicol. 2012 Jul;33(4):577-83. doi: 10.1016/j.reprotox.2012.02.008. Epub 2012 Mar 15. **Study Design:** Recruitment at first ante-natal visit Inclusion: - ≥ 18 yrs old - born in country of study Interview: - lifestyle - occupation - reprod history Blood and semen samples 5/2002-2/2004 w/in 1 wk of each other **Location:** Greenland, Poland (Warsaw), Ukraine (Kharkiv)	**Exposure Assessment:** LC-MS/MS Radiolabeled internal stds PFOS LOD? 100% of samples > LOD **Population-Level Exposure:** Mean PFOS serum conc: Greenland = 51.9 ng/ml Poland = 18.6 Ukraine = 8.1 ng/ml (NOTE: Greenlan PFOS conc = 4.5 x US M; Poland = 1.6 x US M Ukraine = 0.7 x US M (NHANES 4th Rpt))	**Stat Method:** Analysis by generalized linear models (GLM) PFOS as tertiles Outcome vars on continuous scale Analyses stratified by country/region <u>Co-variates</u> - period sexual abstinence - age - BMI - caffeine - cotinine - fever in past 3 mos - self-reported genital infection (Y/N) - testicular disorder (Y/N) - spillage of semen sample <u>Interactions w PFOS</u> - age - smoking status at preg - serum cotinine - PFOA **Outcome:** Sperm chromatin/DNA fragmentation **Major Findings:** (adj model) PFOS **not sig assoc** w chromatin/DNA fragmentation	**Major Limitations:** Modest N for each location (Note analyses stratified by location) Greenlad serum samples ~ 1 yr before semen samples **Other comments:** Cross-sectional design Modest N High PFOS exposure in Greenland increases power to detect effect Reasonable statistical controls

Population: M partners of preg F Greenland – N = 199 Poland – N = 197 Ukraine – N = 208 **Related Studies:**		**Outcome:** TUNEL assay positive (terminal deoxynucleotidyl transferase driven dUTP nick end labeling) a measure of apoptosis **Major Findings:** PFOS **not sig assoc** w TUNEL pos outcome **Outcome:** Apoptotic markers (DFI, Fas, Bcl) **Major Findings:** PFOS **not sig assoc** w apoptotic markers (trend sig pos for Fas for Poland only, but tertiles not sig diff) **Outcome:** Sex hormone binding globin (SHBG) **Major Findings:** PFOS **not sig assoc** w SHBG **Outcome:** Testosterone	

		Major Findings: PFOS **not sig assoc** w serum testosterone **Outcome:** Estradiol **Major Findings:** PFOS **not sig assoc** w serum estradiol **Outcome:** Gonadotrophin hormones **Major Findings:** PFOS **not sig assoc** w serum gonadotrophins	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Starling et al. (2014a) Starling AP, Engel SM, Richardson DB, Baird DD, Haug LS, Stuebe AM, Klungsøyr K, Harmon Q, Becher G, Thomsen C, Sabaredzovic A, Eggesbø M, Hoppin JA, Travlos GS, Wilson RE, Trogstad LI, Magnus P, Longnecker MP. Am J Epidemiol. 2014 Apr 1;179(7):824-33. doi: 10.1093/aje/kwt432. Epub 2014 Feb 20. Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study. **Study Design:** Nested case-control in MoBa cohort Recruitment during first trimest preg 2003-2007 Inclusion criteria: - preg w singleton - no prev births or stillbirths - no chronic hypertension pre-preg - mid-preg plasma sample Non-fasting blood sample	**Exposure Assessment:** HPLC-MS LOQ = 0.05 ng/ml PFOS as linear + branched 100% > LOQ **Population-Level Exposure:** PFOS median conc = 12.87 ng/ml (NOTE: This is ~1.7 times current median in US F (NHANES 4th Rpt))	**Stat Method:** OR by weighted Cox proportional hazard models Weights as inverse prob selection into study PFOS as quartiles and ln-transf continuous <u>Co-variates</u> - maternal age at delivery - BMI - maternal educ - smoking at mid-preg (Y/N) - creatinine (sens analysis) - cystatin C (sens analysis) - HDL (sens analysis) **Outcome:** OR for preeclampsia **Major Findings:** **OR for preeclampsia not sig <> 1.0** for any PFOS quartile or for ln-unit incr in PFOS	**Major Limitations:** PFOS analyses not adj for PFOA Preeclampsia is assoc w kidney disease. Although direction of causality is not clear, if sub-clinical preeclampsia conditions are present pre-preg, then changes in kidney function → changes in plasma PFOS **Other comments:** Case-control design Objective case ascertainment Restricted to nulliparous F to eliminate confounding due to ↓ PFOS conc in preg Hypothetical kidney function/preeclampsia link partly addressed by sens analysis for plasma creatinine and cystatin in 1st trimmest plasma

preeclampsia determined at ante-natal visit based on following criteria determined at same visit: - BP-S ≥ 140, or BP-D ≥ 90 after 20 wks gest - urine proteinuria (dipstick ≥ 1+ **Location:** Norway **Population:** Norwegian Mother and Child Study (MoBa) Cases - N = 466 (random selection) Controls – N = 510 (random selection) **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Starling et al. (2014b) Starling AP1, Engel SM, Whitworth KW, Richardson DB, Stuebe AM, Daniels JL, Haug LS, Eggesbø M, Becher G, Sabaredzovic A, Thomsen C, Wilson RE, Travlos GS, Hoppin JA, Baird DD, Longnecker MP. Environ Int. 2014 Jan;62:104-12. doi: 10.1016/j.envint.2013.10.004. Epub 2013 Nov 2. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. **Study Design:** Cross-sectional MoBa sub-cohort originally created for study of subfecundity (Whitworth et al. 2012b). Blood draw at 12-37 wks gest (99% at 14-26 wks, second trimest; 73% at 17-20 wks) Measurement of plasma lipids and PFOS	**Exposure Assessment:** HPLC-MS PFOS as linear + branched CV = 11.3% PFOS measured in 100% of samples **Population-Level Exposure:** PFOS median conc = 13.03 ng/ml (NOTE: PFOS conc = 1.7 x US F conc (NHANES 4th Rpt))	**Stat Method:** <u>Co-variates</u> - maternal age - pre-preg BMI - parity/inter-preg interval - duration breastfeeding mostrecent child - maternal educ - smoking status at mid-preg - gest wk at blood draw - daily oily fish consumption atmid-preg - For HDL, plasma albumin conc Wt gain as (self-reported) current – pre-preg wt Multiple linear regression of assoc PFOS w outcomes (weighted by inverse prob of inclusion in study) PFOS as quartiles or ln-transf continuous var Lipids as continuous outcomes Triglycerides ln-transformed (to normalize residuals) Multi-PFAS (7) model **Outcome:** Total cholesterol	**Major Limitations:** Non-fasting plasma lipid measurements **Other comments:** Cross-sectional design Non-fasting lipids Large N Adequate stat adj Rel high PFOS exposed pop ↑ HDL not an adverse effect. Potential adverse effect for PFOS limited to equivocal assoc w total cholesterol

Outcomes: - total cholesterol - HDL cholesterol - LDL cholesterol - triglycerides Maternal characteristics/lifestyle info from questionnaire data **Location:** Norway **Population:** Norwegian Mother and Child Cohort study (MoBa) Enrolled in MoBa 2003-2004 Delivered live birth Provided mid-preg plasma sample Provided complete questionnaire info on time-to-preg **N = 891** **Related Studies:** Whitworth et al. (2012b)		**Major Findings:** Total cholesterol **pos assoc** w ln-PFOS as continuous var and for ↑ of interquart range (However, not sig assoc w any quart PFOS) **Outcome:** HDL cholesterol **Major Findings:** HDL cholesterol **sign pos assoc** w PFOS for 4th quart (borderline for 3rd quart) and for ln-PFOS as continuous var and for ↑ of IQR β for ln-PFOS ↓ ~50% when adjusted for 6 other PFA **Outcome:** LDL cholesterol **Major Findings:** LDL cholesterol **not sig assoc** w PFOS for any quart, as continuous var, or for ↑ of IQR	

		Outcome: Triglycerides **Major Findings:** triglycerides **not sig assoc** w PFOS for any quart, as continuous var, or for ↑ of IQR	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Steenland et al. (2009) Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am J Epidemiol. 2009 Nov 15;170(10):1268-78. doi: 10.1093/aje/kwp279. Epub 2009 Oct 21. **Study Design:** Cross-sectional Consumers of water from any of 6 contaminated districts for ≥ 1 yr before 12/2004 Blood sample (fasting not required) Lipid analysis: - Total cholesterol (TC) - LDL cholesterol (LDL-C - HDL cholesterol (HDL-C) - Triglycerides - Non-HDL cholesterol (non-HDL-C) = TC-HDL-C **Location:** OH, WV	**Exposure Assessment:** LC-MS Precision "generally" w/in 10% for multiple replicates **Population-Level Exposure:** Mean PFOS conc = 22.4 ng/ml	**Stat Method:** Ln-transformation for lipid vars <u>Co-variates</u> Based on relation to 1 or more lipids (indep of PFOS) - age - gender - BMI - education - smoking - exercise - education Co-variates maintained in all models Fasting incl only for triglyceride models (did not sig affect other models) Linear regression: PFOS as continuous and categorical var (deciles) Also, logistic regression model for dichotomous hypercholesterolemia (cholesterol ≥ 240 mg/dL) - PFOS as quartiles - also PFOS as continuous var PFOS analyses w and w/out adjustment for PFOA	**Major Limitations:** Cross-sectional design PFOS analyses not controlled for PFOA (PFOA and PFOS gave similar results for all lipid vars) **Other comments:** Large n Good analytical precision Good statistical analysis Specific analyses for influence of age, BMI Specific consideration of reverse causation. PFOS analyses w and w/out adj for PFOA gave similar results

Population: Adults > 18 yrs old In C8 Health Project 2005-2006 46,494 ≥ 18 yrs → exclusion for cholesterol lowering meds → **n = 46,294** **Related Studies:**		<u>Linear regression</u> **Outcome:** TC **Major Findings:** PFOS **sig pos assoc** w TC for deciles 2-10 (dec 1 as ref) And trend for continuous var Stratification by gender gave similar results Models w and w/out BMI (under hypothesis that BMI is an intermed var for TC) gave similar results Model w PFOS as dep variable w cholesterol lowering med (Y/N) as indep var (under hypothesis of reverse causation – higher cholesterol → higher PFOS) Cholesterol lowering med (Y/N) not sig predictor of PFOS **Outcome:** HDL-C **Major Findings:** PFOS **not sig** assoc w HDL-C	

		Outcome: LDL-C **Major Findings:** PFOS **sig pos assoc** w LDL-C (continuous var, categorical not shown) **Outcome:** Triglycerides **Major Findings**: PFOS **sig pos assoc** w triglycerides (continuous var, categorical not shown) **Outcome:** HDL-C/TC **Major Findings** PFOS **sig pos assoc** w HDL-C/TC (continuous var, categorical not shown)	

		Outcome: Non-HDL-C **Major Findings:** PFOS **sig pos assoc** w non-HDL-C (continuous var, categorical not shown) <u>Logistic Regression</u> **Outcome:** Hypercholesterolemia **Major Findings:** OR for hypercholesterolemia **sig > 1.0** for Q2-4 (Q1 as referent) P-trend <0.0001	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Steenland et al. (2010) Steenland K, Tinker S, Shankar A, Ducatman A. Environ Health Perspect. 2010 Feb;118(2):229-33. doi: 10.1289/ehp.0900940. Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. **Study Design:** Cross-sectional Blood sample at enrollment Fasting not required for blood samples **Location:** OH, WV **Population:** C8 study population Est participation (≥ 20 yrs old) = 81% ≥ 18 yrs old Median age ~ 40-49 yrs **N = 53,454**	**Exposure Assessment:** Std C8 methodology (LC-MS) Precision (multiple replicates generally +/- 10% LOD = 0.5 ng/ml < 1% < LOD < LOD = LOD/2 **Population-Level Exposure:** Median = 20.2 ng/ml	**Stat Method:** <u>Linear regression w uric acid as dep var</u> Analysis by deciles (1st decile as ref) <u>Co-variates</u> (a priori) - age - sex - BMI - educ - smoking - alcohol - creatinine (logged) Model w and w/out PFOA <u>Logistic regression for dichotomous outcomes</u> Hyperuricemia (uric acid > 6 mg/dL - F; > 6.8 mg/dL- M Same co-variates as linear regression **Outcome:** Uric acid **Major Findings:** (full adj model) **Stat sig pos** associated w PFOS (sig pos trend w PFOA in model, but max effect diminished ~ 50%)	**Major Limitations:** Results are stronger for PFOA than PFOS. Also serum PFOA ~ 4x serum PFOS. Although PFOS analyses controlled for PFOA in alternative analyses, possibility of incomplete adjustment. **Other comments:** Very large N Adj for PFOA Sens analysis w exclusion of elevated creatinine (suggestive of kidney disease)

Related Studies:		**Outcome:** hyperuricemia **Major Findings:** OR **sig > 1.0** for quartiles 2-4 (OR remains sig pos w PFOA in model)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Stein et al. (2009) Stein CR, Savitz DA, Dougan M. Am J Epidemiol. 2009 Oct 1;170(7):837-46. doi: 10.1093/aje/kwp212. Epub 2009 Aug 19. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. **Study Design:** Cross-sectional Self-reported outcomes ≤ 5 yrs prior to enrollment Self-reported preg outcomes: - miscarriage - premature birth - low birth wt - preeclampsia - reported birth defects **Location:** OH and WV **Population:** C8 study cohort pregnant women Incl all: - singleton miscarriages - stillbirths - live births	**Exposure Assessment:** Solid-phase extraction, reverse-phase-HPLC LOD = 0.5 ng/ml < LOD = LOD/2 **Population-Level Exposure:** Mean PFOS conc = 15.0 ng/ml (Median = 13.6) 90th percentile = 23.2 ng/ml (NOTE: median PFOS conc ~ 1.8 x F conc in most recent NHANES (4th Rpt)). However, 90th percentile ≈ NHANES F 90th percentile	**Stat Method:** Logistic regression models OR for outcomes relative to change in PFOS = IQR (9.0-17.7 ng/ml) Also OR based on PFOS category (quartiles) PFOS analyses adjusted for PFOA <u>Mandatory co-variates</u> - maternal age - parity - maternal educ - smoking **Outcome:** Miscarriage **Major Findings:** (adj models) OR for miscarriage **not sig <>1.0** for either Δ IQR, or individual quarts **Outcome:** Preeclampsia **Major Findings:** (adj model)	**Major Limitations:** Cross-sectional Self-reported outcomes Outcome data ≤ 5 yrs offset from exposure data (although sens analysis conducted for ≤ 3 yr offset w similar results) **Other comments:** Cross-sectional design Large N Reasonable stat control of co-variates PFOS analyses adj for PFOA Self-reported outcomes Outcome-exposure offset may be sig (However, exposure misclassification would tend to reduce observed assoc)

Exclusion: - non-white F - missing covariate data - preg diabetes **N = 5,282-4,512** (depending on spec outcome) **Related Studies:**		OR for preeclampsia **sig > 1.0** (= 1.6) for > 90th percentile PFOS exposure **Outcome:** Premature birth (< 37 wks) **Major Findings:** (adj model) OR for premature birth **sig > 1.0** for Δ IQR (OR = 1.3), and for Q3 (OR = 1.6), and Q4 (>90th percentile) (OR = 1.8) **Outcome:** Birth defects **Major Findings:** (adj model) OR for birth defeces **not sig <>1.0** for either Δ IQR, or individual quarts	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Stein et al. (2016) Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatr Res. 2016 Mar;79(2):348-57. doi: 10.1038/pr.2015.213. Epub 2015 Oct 22. **Study Design:** Cross-sectional Rubella, mumps, measles serum IgG by ELISA Allergy status by questionnaire for prev. 12 mos Ever diagnosed w asthma Current asthma (spec. diagnosis or attack in past yr) Total and Allergy-specific IgE Sensitization = allergy-specific IgE **Location:** US – NHANES	**Exposure Assessment:** NHANES methodology < LOD as LOD/√2 (<1%) **Population-Level Exposure:** Vaccine geom mean = 20.8 ng/ml Allergy Geom mean = 15.0 ng/ml	**Stat Method:** Recommended NHANES sample wts incl in all stat analyses <u>All models adj for</u> (a-priori factors) Age Sex Race <u>Vaccine models</u> NHANES survey yr <u>Allergy models</u> Cotinine Age/sex spec BMI % <u>For vaccine study</u> – PFOS and Ab conc ln-transforned Linear reg → % change for doubling PFOS, also % change by PFOS quartile <u>For allergy study</u> – - OR for Δ 25-75%tile by quartile PFOS by logistic reg - linear reg for %Δ for total and spec IgE for doubling PFOS conc **Outcome:** Measles Ab levels **Major Findings:** Measles Ab level not assoc with PFOS	**Major Limitations:** Cross-sectional study No data on whether children had been vaccinated – stratification to sero-positive is used as surrogate for vaccination **Other comments:** Large N Spec Ab assessment

Population: NHANES 1999-2000; 2003-2004 for vaccine Abs NHANES 2005-2006 for allergy study Children 12-19 yrs N (vaccine) = 1,188 N (allergy) = 640 **Related Studies:**		**Outcome:** Mumps Ab **Major Findings:** Mumps Ab **<u>sig neg assoc</u>** w PFOS doubling PFOS → 7.4% ↓ (5.9% ↓ for sero positive children only) **Outcome:** Rubella Ab **Major Findings:** **<u>Sig neg assoc</u>** 13.3% ↓ for doubling PFOS (but for sero positives only) **Outcome:** Asthma **Major Findings:** Not sig assoc w PFOS **Outcome:** Wheeze **Major Findings:** Not sig assoc w PFOS	

		Outcome: Allergy (reported) **Major Findings:** Not sig pos assoc w PFOS **Outcome:** Rhinitis **Mafor Findings:** Not sig assoc w PFOS **Outcome:** Allergic sensitization (by total and spec IgE) **Major Findings:** **Sig pos assoc** w mold allergen (sig neg assoc w “any”, plants, cockroach, dust mites, rodents, foods	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Stein and Savitz (2011) Stein CR, Savitz DA. Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environ Health Perspect. 2011 Oct;119(10):1466-71. doi: 10.1289/ehp.1003538. Epub 2011 Jun 10. **Study Design:** Cross-sectional/case control ADHD determination based on self-reporting of physician diagnosis of ADHD or ADD, plus self-reported ADHD med use Cases = 5.1% Self-reported learning problems **Location:** OH, WV **Population:** C8 Study cohort (n = 69,030) Children 5-18 yrs old With PFC measurements (n = 11,046) Non-Hispanic white (**n = 10, 546**)	**Exposure Assessment:** Solid-phase extraction, reverse phase HPLC-MS (?) PFOS detected in 100% of samples **Population-Level Exposure:** Mean (sd) PFOS conc = 22.9 ng/ml (12.5 ng/ml) (NOTE; even though PFOS exposure is noted by the authors to be consistent w NHANES exposure, w respect to current exposure, exposure of 12-15 yr old segment of cohort is ~ 2x that of current exposure in this NHANES age range (NHANES 4th Rpt))	**Stat Method:** PFOS categorized in quartiles <u>Co-variates considered</u> (**bold in final model)** - **age** - **sex** - race/ethnicity - BMI - aver household income Logistic regression OR of ADHD for given quart PFOS PFOS model adjusted for other PFCs (PFOA, PFHxS, PFNA) **Outcome:** ADHD (phys diagnosis plus med) **Major Findings:** OR for ADHD **not sig <> 1.0** for any quart PFOS (Q1 as referent) **Outcome:** Learning problems **Major Findings:** OR for learning problems **sig < 1.0** for Q2-3 PFOS, borderline sig for Q4 (OR = 0.74-0.85)	**Major Limitations:** Cross-sectional design Self-reported outcomes Unclear at what age responses were provided by 5-18 yr olds vs. parents **Other comments:** Large N Reliable PFOS analytical measurements Reasonable statistical control incl adjustment of PFOS analyses for other PFCs Cross-sectional design Self-reported outcome data (some by ≤18 yrs old)

Reference and Study Design	Exposure Measures	Results	Comment
Study: Strom et al. (2014) Strøm M, Hansen S, Olsen SF, Haug LS, Rantakokko P, Kiviranta H, Halldorsson TI. Environ Int. 2014 Jul;68:41-8. doi: 10.1016/j.envint.2014.03.002. Epub 2014 Apr 2. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up. **Study Design:** Prospective pregnancy cohort 22 yrs follow-up Pre-birth cohort Recruitment at wk 30 of gest 1988-89 Questionnaire and interview at recruitment – lifestyle, SES, health Serum sample at recruitment Outcome assessment through linkage to Danish pop-based registries: - <u>ADHD</u> – based on Rx for psychostimulant med; or in/outpatient for hyperkinetic disorder	**Exposure Assessment:** PFOS by column-switching isotope dilution LC-MS/MS LOQ = 0.05 ng/ml Intra-sample CV = 2.8% **Population-Level Exposure:** Median PFOS conc = 21.4 ng/ml (NOTE: median PFOS conc = 2.7 times US F median (NHANES 4th Rpt))	**Stat Method:** PFOS as tertiles For ADHD and depression, analysis by Cox proportional hazards regression model → hazard ratio (HR) (age as underlying scale) – dichotomous model For academic achiev, analysis by linear regression-continuous model <u>Co-variates</u> - maternal age - parity - pre-preg BMI - maternal educ - maternal smoking in preg - maternal cholesterol - maternal triglycerides - offspring sex **Outcome:** ADHD **Major Findings:** (adj model) ADHD **not sig <> 1.0** for PFOS for either tertile (1st tert as reference)	**Major Limitations:** Outcomes for ADHD, depression defined on clinical basis, less severe conditions would not be detected **Other comments:** Prospective study design Long (22 yr) follow-up Large N Objective and precise case ascertainment Relatively crude measures for ADHD and depression Reasonable statistical analysis

- Depression – based on Rx for anti-depression med; or in/outpatient for depression - Academic achievement – based on score on standardized 9th grade achievement test **Location:** Aarhus, Denmark **Population:** Danish Fetal Origins 1988 (DaFO88) Cohort N (offspring) = **876** for ADHD, depression **822** for academic achievement **Related Studies:**		**Outcome:** Depression **Major Findings:** (adj model) Depression **not sig <> 1.0** for PFOS for either tertile (1st tert as reference) **Outcome:** Academic achievement **Major Findings:** (adj model) Academic achievement **not sig assoc** w PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Taylor et al. (2014) Taylor KW, Hoffman K, Thayer KA, Daniels JL. Environ Health Perspect. 2014 Feb;122(2):145-50. doi: 10.1289/ehp.1306707. Epub 2013 Nov 26. Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). **Study Design:** Cross-sectional NHANES questionnaire data on age at menopause Menopause = No menstrual period in last 12 mos (not due to med condition, preg, breastfeeding, irreg periods) Pre-menopause = regular periods, or preg, or breastfeeding <u>Reverse causation</u> (potential higher PFOS serum conc due to menopausal retention of blood) addressed by: 1. examining assoc PFOS conc w hysterectomy (i.e., artificial menopause → ↑ PFOS?) 2. examining assoc bet time since menopause and serum PFOS conc	**Exposure Assessment:** NHANES-CDC analysis **Population-Level Exposure:** Median PFOS conc Pre-menopausal = 10.3 ng/ml Menopausal = 14.03 ng/ml Hysterectomy = 17.5 ng/ml	**Stat Method:** PFOS as tertiles Hazard ratio (HR) for normal menopause as function of age and serum PFOS by proportional NHANES sample weights not used but sample weight categories included in models <u>Co-variates</u> - age - race - parity - educ - smoking Assoc between time since menopause and PFOS conc by gen additive models (GAM) and linear regress **Outcome:** menopause **Major Findings:** (adj model) HR for menopause **sig > 1.0** for 2nd tert (1.22), but not for 3rd tert	**Major Limitations:** PFOS analyses not adj for other PFCs **Other comments:** Cross-sectional design Rel large N across categories PFOS not adj for other PFCs Assoc. of menopause w PFOS are modest Analyses for reverse causality suggest that modest assoc of menopause w PFOS may reflect reverse causality

(i.e.,↓ time since menopause → ↓ PFOS serum conc?) **Location:** US **Population:** NHANES 1999-2000, 2003-2004, 2005-2006, 2007-2008, 2009-2010 F ≥ 18-65 yrs old Pre-menopause - N = 1,800 Menopause – N = 502 Hysterectomy – N = 431 **Related Studies:**		**Outcome:** hysterectomy **Major Findings:** (adj model) HR for hysterectomy **sig >1.0** for tert-2 (1.44) and tert-3 (2.56) **Outcome:** Time since menopause **Major Findings:** ∆ PFOS conc for 1 yr ↑ in time since menopause is pos, but **not sig**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Timmermann et al. (2014) Timmermann CA, Rossing LI, Grøntved A, Ried-Larsen M, Dalgård C, Andersen LB, Grandjean P, Nielsen F, Svendsen KD, Scheike T, Jensen TK. Adiposity and glycemic control in children exposed to perfluorinated compounds. J Clin Endocrinol Metab. 2014 Apr;99(4):E608-14. doi: 10.1210/jc.2013-3460. Epub 2014 Feb 25. **Study Design:** Nested-cross-sectonal Nested in Danish component of European Youth Heart Study Measurement of: - height - wt - waist circum - skinfold thickness Aerobic fitness test – peal Watts rel to bw Pubertal status Overweight = age/sex adj BMI at 18 yrs old > 25 kg/m^2	**Exposure Assessment:** NHANES-CDC **Population-Level Exposure:** Median PFOS conc = 41.5 ng/ml (NOTE: median PFOS conc is 6 x US 12-19 yrs old (NHANES 4^{th} Rpt))	**Stat Method:** Linear regression w PFOS as continuous variable Adiposity outcome vars ln-transformed (for normality of residuals) <u>Co-variates</u> - sex - age - ethnicity - paternal income - fast food consumption - height (waist circum endpoint) - BMI (glycemic control endpoints) - skinfold thickness (glycemic control endpoints) - waist circum ((glycemic control endpoints) **Outcome:** BMI **Major Findings:** (adj model) BMI **not sig assoc** w PFOS **Outcome:** Skinfold thickness **Major Findings:** (adj model) Skinfold thickness **not sig assoc** w PFOS	**Major Limitations:** Cross-sectional design **Other comments:** Cross-sectional design Moderate N Reasonable statistical control Rel high exposure PFOS analyses not adj for PFOA

Questionnaire to child and parents: - birthweight - breastfeeding - ethnicity - dietary intake - daily TV watching - parental BMI - parental educ - income **Location:** Odense, Denmark **Population:** Children 8-10 yrs old Attending public school Cluster sampling from 25 schools **N = 590** M = 279 F = 311 **Related Studies:**		**Outcome:** Waist circum **Major Findings:** (adj model) Waist circum **not sig assoc** w PFOS **Outcome:** Adiponectin **Major Findings:** (adj model) Adiponectin **not sig assoc** w PFOS **Outcome:** Leptin **Major Findings:** (adj model) Leptin **not sig assoc** w PFOS **Outcome:** Insulin **Major Findings:** (adj model) Insulin **not sig assoc** w PFOS <u>for normal wt</u> Insulin **sig pos assoc** w PFOS <u>for overweight</u>	

		Outcome: HOMA-β **Major Findings:** (adj model) HOMA-β **not sig assoc** w PFOS <u>for normal wt</u> HOMA-β **sig assoc** w PFOS <u>for overweight</u> **Outcome:** HOMA-IR **Major Findings:** (adj model) HOMA-IR **not sig assoc** w PFOS <u>for normal wt</u> HOMA-IR **sig assoc** w PFOS <u>for overweight</u> **Outcome:** glucose **Major Findings:** (adj model) glucos **not sig assoc** w PFOS <u>for normal wt or overweight</u>	

		Outcome: triglycerides **Major Findings:** (adj model) triglycerides **not sig assoc** w PFOS <u>for normal wt</u> triglycerides **sig assoc** w PFOS <u>for overweight</u>	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Toft et al. (2012) Toft G, Jönsson BA, Lindh CH, Giwercman A, Spano M, Heederik D, Lenters V, Vermeulen R, Rylander L, Pedersen HS, Ludwicki JK, Zviezdai V, Bonde JP. Exposure to perfluorinated compounds and human semen quality in Arctic and European populations. Hum Reprod. 2012 Aug;27(8):2532-40. doi: 10.1093/humrep/des185. Epub 2012 May 30. **Study Design:** Cross-sectional Abstinence from sexual activity for ≥ 2 d Analysis of semen samples w/in 1 hr of ejaculation for 83% ofsamples Analysis for conc, motility, morphology CV for conc, motility = 8.1, 11% Semen/sperm outcome measures ln-transformed **Location:** Greenland, Poland (Warsaw), Ukraine (Kharkiv)	**Exposure Assessment:** PFOS serum conc PFOS by LC//MS/MS PFOS LOD = 0.2 ng/ml **Population-Level Exposure:** Total - PFOS median = 18.4 ng/ml - P66 = 27.3 ng/ml Greenland - PFOS median = 44.7 ng/ml - P66 = 56.1 ng/ml Poland - PFOS median = 18.5 ng/ml - P66 = 21.2 ng/ml Ukraine - PFOS median = 7.6 ng/ml - P66 = 8.5 ng/ml (NOTE: PFOS conc total, Greenland, and Poland larger than current US M pop. (median = 11.8). Poland less than US M pop (NHANES 4th Rpt)).	**Stat Method:** Combined and pop-stratified analyses Analyses w PFOS categorized as tertiles PFOS ln-transformed <u>Co-variates:</u> (a priori) - Abstinence time - age - spillage (Y/N) - smoking (Y/N) - ever urogenital infection - BMI - country (combined analyses) Adj of PFOS for other PFCs in sensitivity analysis Analyses of vol and count restricted to no spillage Analyses of motility restricted to analysis w/in 1 hr Also, analyses w generalized additive mode (GAM) to capture non-linear relationships **Outcome:** Sperm conc **Major Findings:** (adj model)	**Major Limitations:** Cross-sectional Small n for individual countries Low participation from cohort in Poland and Ukraine Temporal relation bet blood sample and semen sample unknown **Other comments:** Rel small n's for each individual pop. Given large differences in PFOS conc across pops, small individual n's could reduce power to see differences. Pops differences in PFOS conc makes interpretation of combined analyses unclear Good statistical control Good sample QC Temporal blood/semen relationship unknown

Population: INJENDO cohort <u>participation</u> Greenland - 79% Poland - 29% Ukraine – 36% M ≥ 18 yrs old **N = 588** Greenland = 196 Poland = 189 Ukraine = 203 **Related Studies:** Kvist et al (2012)		Sperm conc **not sig diff** across PFOS tertiles, combined or for any pop **Outcome:** Semen vol **Major Findings:** (adj model) Semen vol **not sig diff** across PFOS tertiles, combined or for any single pop **Outcome:** Sperm total count **Major Findings:** (adj model) Sperm count **sig diff** between 1st and 2nd tert for Polan (but not 1st and 3rd tert) **Not sig diff** for combined or any other pop **Outcome:** Percent motile sperm **Major Findings:** (adj model) % motile sperm **not sig diff** across PFOS tertiles, combined or for any single pop	

		Outcome: Percent normal cells **Major Findings:** % normal cells **sig diff** between 1st and 2nd and 1st and 3rd terts for combined analysis only (not for any single pop) p-trend (combined) borderline sig (p = 0.06)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Uhl et al. (2013) Uhl SA, James-Todd T, Bell ML. Environ Health Perspect. 2013 Apr;121(4):447-52. doi: 10.1289/ehp.1205673. Epub 2013 Feb 7. Association of Osteoarthritis with Perfluorooctanoate and Perfluorooctane Sulfonate in NHANES 2003-2008. **Study Design:** Cross-sectional Osteoarthritis self-reported by questionnaire ("Had doctor/health professional ever told you…"). If Y, type of arthritis (DK, or non-osteo, excluded Missing data on ≥ 1 co-variawte → exclusion **Location:** US **Population:** NHANES cohort 2003-2008 20-84 yrs old	**Exposure Assessment:** CDC - Solid-phase extraction, HPLC-MS **Population-Level Exposure:** Mean PFOS conc = 21.23 ng/ml	**Stat Method:** PFOS characterized by quartiles Q1 = ≤ 2.95 ng/ml Q2 = > 8.56-13.59 ng/ml Q3 = >13.59-20.97 ng/ml Q4 = > 20.97 ng/ml <u>Co-variates considered</u> **(selected for full model based on p < 0.05 in model)** - **age** - **sex** - **poverty status** - **race/ethnicity** - daily fat intake - daily calorie intake - **BMI** - **history bone fractures (self-reported)** - **participation in sports/fitness/recreational physical activities** - **smoking** - parity (F) Multivariate logistic regression for odds assoc osteoarthritis w PFOS CDC-recommended NHANES sampling weights applied Analyses for combined and separate M and F	**Major Limitations:** Cross-sectional study design Self-reported osteoarthritis status PFOS analyses not adj for PFOA Small n (365) for cases, esp stratified by sed (F = 238, M = 127) **Other comments:** Cross-sectional design Large N, but rel small N for cases, especially stratified by sex Good statistical control of analyses Good analytical precision Suggestive, but ambiguous findings of PFOS-osteoarthritis assoc

N = 3,809 Cases n = 365 - M = 127 - F = 238 **Related Studies:** Innes et al. (2011)		**Outcome:** OR for osteoarthritis for specified ↑ in PFOS **Major Findings:** (full adj model) <u>M + F</u> OR **sig > 1.0** for Q3 (OR = 1.99) and Q4 (OR = 1.77) (Q1 as ref) OR **not sig > 1.0** for continuous (unit incr) analysis <u>M</u> OR **not sig > 1.0** for any PFOS quart or for unit ↑ in PFOS <u>F</u> OR **not sig > 1.0** for any PFOS quart or for unit ↑ in PFOS (**borderline sig** OR = Q3-1.92; Q4-1.73; unit ↑-1.22) (OR sig > 1.0 for Q3-4 and unit ↑ in PFOS for *crude* analysis)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Vagi et al. (2014) Vagi SJ, Azziz-Baumgartner E, Sjödin A, Calafat AM, Dumesic D, Gonzalez L, Kato K, Silva MJ, Ye X, Azziz R BMC Endocr Disord. 2014 Oct 28;14:86. doi: 10.1186/1472-6823-14-86. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol a in polycystic ovary syndrome: a case-control study. **Study Design:** Case-control design Study of polycystic ovary syndrome (PCOS) Self-provided information on: - age - race - ethnicity - BMI - virilization (M sex-related characteristics)	**Exposure Assessment:** Solid-phase extraction, HPLC-MS/MS < LOD = LOD/√2 **Population-Level Exposure:** Geom mean PFOS conc: - cases = 8.2 ng/ml - controls = 4.9 ng/ml (NOTE: case PFOS conc is consistent with latest NHANES F data. Control PFOS ~ 67% of current NHANES F (4th Rpt))	**Stat Method:** PFOS as tertiles Multivariate logistic regression of PCOS outcome <u>Co-variates</u> - age - BMI - white vs. other race **Outcome:** PCOS **Major Findings:** (adj model) **PFOS conc in cases (8.2 ng/ml) sig higher than in controls (n = 4.9), p = 0.01.** **OR for PCOS sig > 1.0** for Tert-3 (5.79) P = 0.005 OR for T2 (3.43) borderline sig P = 0.062	**Major Limitations:** Small sample size for cases (n = 52) and controls (n = 50) POCS is associated with reduced menstruation. Therefore cases may have higher body burdens of PFOS compared to those with regular menstruation (and greater elimination of PFOS). Therefore, there is a potential for reverse causation. **Other comments:** Case-control design Small N Since PCOS is under hormonal control, there is potential for reverse causality if hormones mediate PFOS storage/elimination. Also PCOS necessarily corresponds to reduced menstruation which would bias toward higher PFOS conc.

Exclusion criteria: - current preg - use of hormones (incl contraceptives) or “other medication” in prev 3 mos - diabetes - menopause Case definition: - anovulation or oligo ovulation (cycle > 35 d) - hirsutism score > 6 - lab evidence of hperandrogenism - exclusion of related disorders (thyroid, hyperprolactinemia, non-classic adrenal hyperplasia, androgen secreting tumors) Single spot urine and blood samples **Location:** CA (Los Angeles area) **Population:** F 52 cases 50 controls Recruited through specialty clinics and advertisements 18-45 yrs old **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Vested et al. (2013) Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, Halldorsson TI, Becher G, Haug LS, Ernst EH, Toft G. Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 2013 Apr;121(4):453-8. doi: 10.1289/ehp.1205118. Epub 2013 Jan 23. **Study Design:** Longitudinal Semen sample, Self-measured testicle vol Blood sample Semen analysis w/in 1 hr of ejaculation for 86% 100% w/in 2 hr - vol - motility - concentration PFOS analysis in maternal and sons' blood	**Exposure Assessment:** Column-switching isotope dilution, LC-MS PFOS LOD = 0.05 ng/ml CV for in-house QC samples for PFOS = 4.4% PFOS Interlab comparison w/in 1 SD of consensus values **Population-Level Exposure:** PFOS median conc = 21.2 ng/ml (NOTE: PFOS median conc ~ 2x most recent adult M conc (NHANES 4th Rpt))	**Stat Method:** PFOS as tertiles Multivariate regression analysis w PFOS as continuous var Outcome vars ln-transformed <u>Co-variates</u> (a priori) - history of reprod tract disease - BMI - smoking status - maternal smoking - SES at birth - abstinence time (for applicable outcomes) - spillage (Y/N) **Outcome:** (as function of maternal PFOS at preg wk 30) Sperm concentration **Major Findings:** Maternal PFOS **not sig assoc** w sperm conc	**Major Limitations:** Small sample size Self-measurement of testicular volume PFOS analyses not controlled for PFOA (PFOA analysis adj for PFOS is sens analysis, but unclear if this is predictive for PFOS adj for PFOA) **Other comments:** Longitudinal design Good analytical performance Small sample size Lack of statistical control for PFOA confounding

Serum sex hormone binding globin (SHBG Reproductive hormones: - testosterone - estradiol - LH - FSH - inhibin B - free androgen index (FAI) **Location:** Denmark **Population:** 2008-2009 follow-up of sons of mothers in 1988-1989 cohort from Aarhus, Denmark Semen sample, Self-measured testicle vol Blood sample 468 invited → 176 consented → **169 PFOS analysis** Additional 45 excluded from analysis of sperm count and semen vol due to spillage **Related Studies:** Toft et al. (2012); Raymer et al. (2012); Joensen et al. (2009)		**Outcome:** (as function of maternal PFOS at preg wk 30) Total sperm count **Major Findings:** Maternal PFOS **not sig assoc** w sperm count **Outcome:** (as function of maternal PFOS at preg wk 30) Semen vol **Major Findings:** Maternal PFOS **not sig assoc** w semen vol **Outcome:** (as function of maternal PFOS at preg wk 30) % progressive spermatozoa **Major Findings:** Maternal PFOS **not sig assoc** w % progressive spermatoza	

		Outcome: (as function of maternal PFOS at preg wk 30) Mean testicular vol **Major Findings:** Maternal PFOS **not sig assoc** w mean testicular vol **Outcome:** (as function of maternal PFOS at preg wk 30) Testosterone serum conc **Major Findings:** Maternal PFOS **not sig assoc** w testosterone serum conc **Outcome:** (as function of maternal PFOS at preg wk 30) Estradiol serum conc **Major Findings**: Maternal PFOS **not sig assoc** w estradiol serum conc	

		Outcome: (as function of maternal PFOS at preg wk 30) LH **Major Findings:** Maternal PFOS **not sig assoc** w LH serum conc **Outcome:** (as function of maternal PFOS at preg wk 30) FSH **Major Findings:** Maternal PFOS **not sig assoc** w FSH serum conc In multivar regression w PFOS as continuus var, maternal PFOS borderlins assoc w FSH (p-trend = 0.06), however β is minimal and categorical analysis is not sig **Outcome:** (as function of maternal PFOS at preg wk 30) Inhibin B **Major Findings:** Maternal PFOS **not sig assoc** w inhibin B serum conc	

		Outcome: (as function of maternal PFOS at preg wk 30) SHBG **Major Findings:** Maternal PFOS **not sig assoc** w SHBG serum conc **Outcome:** (as function of maternal PFOS at preg wk 30) FAI **Major Findings:** Maternal PFOS **not sig assoc** w FAI serum conc	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Vestergaard et al. (2012) Vestergaard S1, Nielsen F, Andersson AM, Hjøllund NH, Grandjean P, Andersen HR, Jensen TK. Hum Reprod. 2012 Mar;27(3):873-80. doi: 10.1093/humrep/der450. Epub 2012 Jan 13. Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. **Study Design:** Prospective Sample collection - 1992-1995 Enrollment with cessation of contraception Followed for 6 menstrual cycles or until preg achieved Questionnaire at enrollment: - Demographic - medical - occupational - reproductive - Lifestyle M – semen sample F – blood sample	**Exposure Assessment:** LC-MS/MS w/in batch CV = < 3% between batch CV = < 5.2% LOQ = 0.03 ng/ml 100% of samples detectable for PFOS **Population-Level Exposure:** Median PFOS conc - No pregnancy = 35.75 ng/ml - Preg = 36.29 ng/ml (NOTE: Median PFOS conc. ~ 5 x US F pop, and > 90th perecentile (NANES 4th Rpt))	**Stat Method:** <u>Co-variates</u> - age - BMI - smoking - caffeine consumption - cycle length - last contraception method - diseases related to fecundity (self-report) - sperm conc (oligospermia Y/N) PFOS conc dichotomized at median OR for subfecundity by logistic regression Diff in TTP by high-low PFOS determined by fecundity ratio (FR - prob of preg/time) analyzed by discrete time-survival models Also w log-transformed and continuous PFOS models **Outcome:** OR subfecundity for PFOS > median **Major Findings:** (adj model) OR subfecundity for PFOS > median **not sig <> 1.0**	**Major Limitations:** Moderate sample size PFOS analyses not controlled for PFOA **Other comments:** Prospective study design High PFOS exposure Good statistical control and sens analyses Precise analytical determination Not subject to reverse causation arising from reduced serum PFOS due to previous pregnancies

Outcome – time-to-preg (TTP) over ≤ 6 mesntrual cycles Menstrual cycle log books Cycle-spec information on freq of sexual intercourse Subfecundity = TTP > 6 menstrual cycles **Location:** Denmark **Population:** Women attempting preg for first time Couples w/out prev reproductive experience planning to break contraception 430 couples enrolled → **N = 222** w blood samples 20-35 yrs old **Related Studies:**		**Outcome:** Monthly FR for PFOS > median compared to < median **Major Findings:** (adj model) Monthyly FR for > PFOS median compared to < PFOS med **not sig dif from 1.0**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Versterholm-Jensen et al. (2014) Vesterholm Jensen D1, Christensen J, Virtanen HE, Skakkebæk NE, Main KM, Toppari J, Veje CW, Andersson AM, Nielsen F, Grandjean P, Jensen TK. Reproduction. 2014 Mar 2;147(4):411-7. doi: 10.1530/REP-13-0444. Print 2014. No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. **Study Design:** Nested case-control study Preg women recruited 1997-2001 (Denmark) and 1997-1999 (Finland). Additional *cases* recruited in Finland 1999-2002) <u>Denmark</u> - Children examined at birth and 3 mos <u>Finland</u> – M w cryptorchidism and every 10th M of cohort + 2 controls/case matched on: - date of birth - gest age - parity - maternal diabetes - smoking	**Exposure Assessment:** Umbilical cord serum On-line solid-phase extraction, LC-MS/MS LOQ = 0.03 ng/ml PFOS quantified in 100% of samples **Population-Level Exposure:** Median **total** PFOS cord serum conc= 9.1 ng/ml **Danish** - controls =10.2 ng/ml Cases = 8.9 ng/ml **Finnish** - controls = 5.5 n/ml Cases = 4.8 ng/ml	**Stat Method:** PFOS ln-transformed Ln-PFOS as tertiles and continuous vars Sens analysis for primapara Multiple logistic regress for OR cryptorchidism for continuous and tertiles Co-variates: - bw - gest age - parity Danish and Finish cohorts separately **Outcome:** OR for cryptorchidism **Major Findings:** (adj model) OR **not sig <>1.0** for PFOS as continuous var or for any tertile. Trend not sig.	**Major Limitations:** Mod low exposure **Other comments:** Prospective case-control design Mod large (for case-control) Ns

Followed for 18 mos **(timing of examination(s)?)** Testicular position determined at birth and dichotomized on cryptorchidism Gest age from sonogram or last menstruation **Location:** Denmark, Finland **Population:** Danish-Finish birth cohort N cases cryptorchidism = 107 N controls = 108 **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Wang et al. (2011b) Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, Chiang CF, Wu TN, Chen PC. Environ Res. 2011 Aug;111(6):785-91. doi: 10.1016/j.envres.2011.04.006. Epub 2011 May 23. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. **Study Design:** Prospective case-control Cord blood → PFOS analysis Parental lifestyle/demographic questionnaire Hospital neonate health records: - head circum - birth wt - birth ht - wks gestation - type of delivery 2-yr questionnaire: - duration of breastfeeding - < 1 yr egg consumption - < 1 yr wheat consumption - <1 yr soy bean consumption - <1 yr shrimp consumption - older siblings - furry pets	**Exposure Assessment:** UHPLC – triple quadrupole MS PFOS LOQ = 0.22 ng/ml < LOQ = LOQ/2 PFOS 99.6% detect **Population-Level Exposure:** Cord blood PFOS median conc = 5.5 ng/ml	**Stat Method:** Cord blood IgE, 2-yr serum IgE and PFOS log-transformed Linear regression IgE on unit ↑ in PFOS Also categorical PFOS (quartiles) Assoc of PFOS and AD by multivariate linear regression <u>Co-variates ingestigates</u> Gender Gestational age Parity Delivery type Maternal age Maternal education Maternal occupation Preg alcohol Preg smoking Income Parental history atopy Duration breastfeeding Post-natal ETS Incense use Home carpet Fungi/mold on walls Co-variates included w 10% in est	**Major Limitations:** Small number (43) of cases Assessment of AD at 2 yrs as function of gestational exposure could be confounded by post-natal exposure **Other comments:** Prospective study Reasonable analytical precision Comprehensive modeling Small sample size – especially cases

- home carpet - fungi on walls - incense use at home - post-natal ETS IgE in cord blood and serum at 2 yrs **Location:** Taiwan **Population:** Preg F in 3rd trimester w prenatal exams recruited Cases of AD defined by questionnaire data on children at 2 yrs – presence of atopic dermatitis AD - recurrent rash for ≥ 6 mos - location of rash - ever diagnosed AD by Dr. Exclusion criteria: - multiple gestation (twins etc) - inability to answer questions (in Chinese) - relocate prior to delicery **N = 244** AD cases = 43 Non-AD = 201 **Related Studies:**		**Outcome:** Cord blood IgE **Major Findings:** (adj model) Cord blood IgE **sig pos assoc** w cord blood PFOS (p = 0.017) Stratified by gender, **assoc is spec to M** **Outcome:** 2-yr blood IgE **Major Findings:** (adj model) 2-yr old blood IgE **not sig assoc** w cord blood PFOS **Outcome:** OR for AD by PFOS cord blood quartile **Major Findings:** (adj model) OR for AD **not sig <> 1.0** for any quart PFOS (trend is pos, and Q4 is sig in crude analysis only)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Wang et al. (2013) Wang Y1, Starling AP, Haug LS, Eggesbo M, Becher G, Thomsen C, Travlos G, King D, Hoppin JA, Rogan WJ, Longnecker MP. Environ Health. 2013 Sep 8;12(1):76. doi: 10.1186/1476-069X-12-76. Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. **Study Design:** Cross-sectional Norwegian Mother and Child Cohort Study (MoBa) Recruited 2003-2004 Questionnaire preg wk 13-17 Blood sample preg wk 17-18 TSH by immunoassay Minimal detection limit = 0.01 µU/ml Intra-inter assay CV < 10% **Location:** Norway	**Exposure Assessment:** HPLC-MS PFOS LOQ = 0.05 ng/ml Intra-assay CV < 10% Inter-assay CV < 15% **Population-Level Exposure:** PFOS median conc = 12.8 ng/ml (IQR = 10.1-16.5 ng/ml) (NOTE: PFOS median conc ~1.6 times US F median (NHANES 4th Rpt))	**Stat Method:** TSH ln-transformed Sub-fecund and fecund pops not sig diff for TSH and were combined Assoc TSH w PFOS by linear regression Also, logistic regression for PFOS dichotomized at 95th percentile <u>Co-variates examined</u> - age (*a priori*) - gestational age at blood draw (*a priori)* - pre-preg BMI - preg smoking - parity - time between prev birth and current preg - duration of prev breastfeeding - total seafood intake (mid-preg) - plasma HDL - plasma albumin Vars incl in models if p < 0.1 in bivariate models w PFOS *and* TSH **Outcome:** TSH **Major Findings:** (adj model) TSH **sig pos assoc** w PFOS (p = 0.03)	**Major Limitations:** Cross-sectional PFOS analyses not adj for PFOA **Other comments:** Reasonable N PFOSCross-sectional design (subject to reverse causation if (e.g.) TSH affects glomerular filtration rate → high TSH → low serum PFOS (therefore, low TSH assoc w rel ↑ PFOS) Reasonable stat control

Population: Norwegian Mother and Child Cohort Study (MoBa) Recruited 2003-2004 Radom selection among subfecund F (> 12 mos to preg) **N = 400** Additional random selection (w/out prior condition) **N = 550** Exclusion for reported thyroid abnormality, missing co-variate data **N (total) = 903** **Related Studies:**		0.8% ↑ in TSH for ea ng/ml ↑ in serum PFOS When stratified by fecundity status, TSH sig assoc w PFOS only for fecund group (NOTE: PFOS was only PFC sig assoc w TSH in adj models)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Wang et al. (2014b) Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, Longnecker MP, Wang SL. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect. 2014 May;122(5):529-34. doi: 10.1289/ehp.1306925. Epub 2014 Feb 21. **Study Design:** Longitudinal birth cohort study Blood samples during 3rd trimest Umbilical cord blood at delivery Exclusion: - missing PFOS mes - Missing thyroid horm mes - thyroid disease - Free-T4 - Total T4 - Total T3 - TSH All by radioimmunoassay (commercial kits) Intra-assay CV = < 5% Inter-assay CV < 10%	**Exposure Assessment:** HPLC-triple quadrupole MS LOQ? 100% PFOS sample > LOQ Intra-assay CV (all PFASs) = 0.83-7.94% Inter-assay CV (all PFASs) = 1.57-24.7% **Population-Level Exposure:** Maternal serum PFOS conc = 12.73 ng/ml (NOTE: This is ~1.6 x US F PFOS median (NHANES 4th Rpt))	**Stat Method:** Linear regression of thyroid hormones (w and w/out ln-transformation) <u>Co-variates considered</u> - maternal age (a priori) - maternal educ - prev live births - income - pre-preg BMI - fish consumption - neonate sex (for models of maternal PFOS and cord blood hormones) - method of delivery (for models of maternal PFOS and cord blood hormones) **Outcome:** Maternal free-T4 **Major Findings:** (adj model) Maternal free-T4 **not sig assoc** w maternal serum PFOS **Outcome:** Maternal total-T4 **Major Findings:** (adj model) Maternal total-T4 **not sig assoc** w maternal serum PFOS	**Major Limitations:** PFOS analyses not adj for other PFCs Other factors potentially influencing thyroid hormones (e.g., iodine status) not controlled **Other comments:** Longitudinal study design Moderate size N Incomplete co-variate control (e.g., iodine status)

Location: Central Taiwan **Population:** Pregnant women recruited 12/2000-11/2001 **N = 285** **Related Studies:**		**Outcome:** Maternal total-T3 **Major Findings:** (adj model) Maternal total-T3 **not sig assoc** w maternal serum PFOS **Outcome:** TSH **Major Findings:** (adj model) Maternal TSH **not sig assoc** w maternal serum PFOS **Outcome:** Cord blood free-T4 **Major Findings:** (adj model) Cord blood free-T4 **not sig assoc** w maternal PFOS **Outcome:** Cord blood total-T4 **Major Findings:** (adj model) Cord blood total-T4 **not sig assoc** w maternal PFOS	

		Outcome: Cord blood total-T3 **Major Findings:** (adj model) Cord blood total T3 **not sig assoc** w maternal PFOS **Outcome:** Cord blood TSH **Major Findings:** (adj model) Cord blood TSH **not sig assoc** w maternal PFOS	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Washino et al. (2009) Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009 Apr;117(4):660-7. doi: 10.1289/ehp.11681. Epub 2008 Nov 4. **Study Design:** Prospective cohort Self-admin questionnaire after 2nd trimmest - dietary - smoking - alcohol - caffeine - income - educ Blood sample after 2nd trimester – 72.4% Blood sample after delivery – 27.6% **Location:** Sapporo, Hokkaido, Japan	**Exposure Assessment:** LC-MS/MS Spike recovery = 97.5- 99.3% CV = 3.0-6.3% LOD = 0.5 ng/ml PFOS detect in 100% of samples **Population-Level Exposure:** Mean maternal PFOS serum sampling during preg conc. = 5.6 ng/ml (med = 5.2 ng/ml) Mean maternal PFOS serum conc Sampling post-delivery = 3.8 ng/ml (NOTE: during-preg PFOS conc ~73% of US F mean conc (NANES 4th Rpt))	**Stat Method:** <u>Co-variates investigated</u> (**in full model**) - **maternal age** - **maternal age** - **Preg BMI** - **preg smoking** - **gestational age** - **gender** - **parity** - **blood sampling time (preg or post preg)** - infant disease - birth wt - birth size - preg complications - **delivery mode** (for head cirum outcome) PFOS conc log-transformed Multiple regression model **Outcome:** Birth wt **Major Findings:** (adj model) Birth wt **sig neg assoc** w PFOS P = 0.046 **Not sig when stratified for M only** **Sig when stratified for F only** P = 0.007	**Major Limitations:** PFOS analyses not adj for PFOA Although regression analysis controlled for during vs. post-preg blood sampling for PFOS, not clear that model can completely adjust since diff is large (during preg = 1.5 x post preg PFOS) **Other comments:** Prospective cohort design Moderate sample size Good analytical performance Reasonable stat analysis (except failure to adj PFOS analyses for PFOA) Self-administered questionnaire, but during preg likely to reduce recall bias

Population: 7/2002-10/2005 F in wks 23-35 of preg during routne GYN checkup Native Japanese 1,796 eligible → 514 participated → 10 excluded for birth outcome, or volunatary withdrawal, preg-induced hypertension, diabetes, fetal heart failure, twins **N = 428** **Related Studies:**		**Outcome:** Birth length **Major Findings:** (adj model) PFOS **not sig assoc** w birth length Bordeline sig (p = 0.055) when stratified for F only **Outcome:** Chest circum **Major Findings:** PFOS **not sig assoc** w chest circum **Outcome:** Head circum **Major Findings:** PFOS **not sig assoc** w head circum	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Watkins et al. (2013) Watkins DJ, Josson J, Elston B, Bartell SM, Shin HM, Vieira VM, Savitz DA, Fletcher T, Wellenius GA. Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect. 2013 May;121(5):625-30. doi: 10.1289/ehp.1205838. Epub 2013 Mar 7. **Study Design:** Cross-sectional Questionnaire on -enrollment: - Demographics - Personal health history - Residential history - lifestyle Blood sample on enrollment - fasting not required Est glomerular filtration rate (eGFR) based on serum creatinine and height **Location:** OH, WV	**Exposure Assessment:** (Note explicitly provided, but same as for other C8 study reports) **Population-Level Exposure:** Median serum PFOS = 20.0 ng/ml (NOTE: median PFOS conc ~ 2 x current US levels (NHANES 4th Rpt))	**Stat Method:** Multiple imputation for missing co-variates Multiple linear regression for assoc PFOS and eGFR PFOS as continuous variable PFOS conc log-transformed Also as categorical analysis (quart PFOS) <u>Co-variates</u> - age - sex - race - smoking - income - regular exercise - BMI - total cholesterol **Outcome:** Assoc eGFR w PFOS **Major Findings:** (full adj model) eGFR **sig neg assoc** w PFOS $p < 0.0001$ **Sig neg trend** across quartiles PFOS	**Major Limitations:** Cross-sectional design Multiple imputation used for missing variables: - 21% missing income - 0.8% missing BMI Potential for reverse causality of ↓ GFR results in ↑ retention of PFOS Failure to adj PFOS analyses for PFOA **Other comments:** Large N Missing/imputed co-variate data

Population: C8 Health Study cohort 8/2006-8/2006 1 - < 18 yrs old at enrollment N = 9,783 → exclusion for questionable data → **N = 9.660** F = 48% M = 52% **Related Studies:**			

Reference and Study Design	Exposure Measures	Results	Comment
Study: Webster et al. (2014) Webster GM, Venners SA, Mattman A, Martin JW. Environ Res. 2014 Aug;133:338-47. doi: 10.1016/j.envres.2014.06.012. Epub 2014 Jul 12. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. **Study Design:** Longitudinal cohort Blood sample 12/2006-6/2008 Collected twice ~15 and18 wks gest Free-T4 Total-T4 TSH Thyroid peroxidase antibody (TPOAb) (marker of autoimmune hypothyroidism) Thyroid hormones by Beckman Access 2 Thyroid peroxidase Ab immunoassay Claimed that this method is rel insensitive to bias from changing levels of serum-binding proteins during preg	**Exposure Assessment:** HPLC/MS/MS 100% > DL **Population-Level Exposure:** Mean maternal serum PFOS = 5.1 ng/ml (sd = 2.8 ng/ml) Median = 4.8 ng/ml (NOTE: PFOS conc ~62% of US F (NHANES 4th Rpt))	**Stat Method:** <u>Co-variates investigated</u> - maternal age - ethnicity - educ - income - current stress level - smoking - ETS - drug use - alcohol - prenatal vitamins (w iodine) - iodized salt - time of day of blood draw - wk of gest - gest age at delivery Mixed-effects models w random intercept Continuous vars for PFOS (as IQR) and thyroid hormones "Variance components" correlation structure for thyroid meas at 2 time points Models of all PFAs investigated but not reported due to dominance by PFOS **Outcome:** Free-T4 **Major Findings:** (adj model) Free-T4 **not sig assoc** w PFOS W or w/out strat for high/low TPOAb	**Major Limitations:** Rel small N and small N for high TPOAb Iodine sufficiency est by questionnaire **Other comments:** Longitudinal cohort design w two time points Rel small N and small N for high TPOAb subset Stratification by TPOAb (as indicator of thyroid autoantibody hypothyroidism) Consideration of total PFA effect Est of iodine sufficiency by questionnaire → uncertainty Apparent control (in thyroid hormone analytical method) for variable serum protein levels during preg

Location: Vancouver, Canada **Population:** 2007-2008 152 women ≤15 wks preg Inclusion criteria: - euthyroid (normal thyroid) - non-smokers - singleton preg - normal (non-hormonal) conception - no thyroid affected med - lived in N. America past 3 consec yrs - fluent in English - ≥ 19 yrs old **Related Studies:**		**Outcome:** TSH **Major Findings:** (adj model) TSH **sig assoc** w PFOS **only when interaction term (H/L) for TPOAb included** – sig for high TPOAb only, n = 14**)** **Outcome:** Total T4 **Major Findings:** (adj model) Total T4 **not sig assoc** w PFOS (w or w/out adj for TPOAb)	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Wen et al. (2013) Wen LL, Lin LY, Su TC, Chen PC, Lin CY. J Clin Endocrinol Metab. 2013 Sep;98(9):E1456-64. doi: 10.1210/jc.2013-1282. Epub 2013 Jul 17. Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010. **Study Design:** Cross-sectional Total T3 Free T3 Total T4 Free T4 TSH Thyroglobulin Thyroid hormones by immunoenzymatic assay Sub-clinical hyperthyroidism = TSH < 0.24 mU/L Sub-clinical hypothyroidism = TSH > 5.43 mU/L **Location:** US	**Exposure Assessment:** NHANES analytical methodology PFOS LOD = 0.2 ng/ml < LOD = LOD/√2 0.7% of PFOS samples **Population-Level Exposure:** PFOS geom mean conc = 14.2 ng/ml (95% CI = 13.59-14.86 ng/ml)	**Stat Method:** All thyroid measures log-transformed Except total T3 and total T4 PFOS log-transformed Analysis stratified by gender Multivariate linear regression of thyroid measures <u>Co-variates considered</u> - age - gender - race - alcohol - smoking - urinary iodine PFOS also modeled in multi-PFC analysis Also categorical analysis of PFOS in quartiles Analyses w and w/out NHANES sample weights Logistic regression for OR of sub-clinical hypo/hyperthyroidism	**Major Limitations:** Cross-sectional design Small N by gender for sub-clinical hypothyroidism (and presumably for sub-clinical hyperthyroidism (?)) Potential for reverse causality Exclusion of clinical cases reduces power of analysis **Other comments:** Large N in total, but small n's for M, F hypothyroidism Good analytical chem Cross-sectional Potential for reverse causality

Population: NHANES 2007-2008, 2009-2010 ≥ 20 yrs old Not preg Not nursing PFC and thyroid measures Exclusion: - Reported history thyroid disease - missing data on alcohol - missing data on urine iodine **N = 1,181** M = 672 F = 509 **Related Studies:**		**Outcome:** Total T4 **Major Findings:** (adj model) Total T4 **not sig assoc** w PFOS for M or F **Outcome:** Log free T4 **Major Findings:** (adj model) Log free T4 **not sig assoc** w PFOS for M or F **Outcome:** Total T3 **Major Findings:** (adj model) Total T3 **not sig assoc** w PFOS for M or F **Outcome:** Log free T3 **Major Findings:** (adj model) Log free T4 **not sig assoc** w PFOS for M or F	

		Outcome: Log TSH **Major Findings:** (adj model) Log TSH **not sig assoc** w PFOS for M or F **Outcome:** Log thyroglobulin **Major Findings:** Log thyroglobulin **not sig assoc** w PFOS for M or F **Outcome:** Sub-clinical hypothyroidism **Major Findings:** (adj model) OR for assoc of sub-clinical hypothyroidism w unit ↑ in PFOS **sig pos for M and F (**OR M = 1.98; OR F = 3.03) N = 23 (M = 15, F = 8) **Outcome:** Sub-clinical hyperthyroidism **Major Findings:** OR for assoc sub-clinical hyperthyroidism **not sig <> 1.0 for M or F**	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Whitworth et al. (2012a) Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, Thomsen C, Eggesbo M, Travlos G, Wilson R, Cupul-Uicab LA, Brantsaeter AL, Longnecker MP. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol. 2012 Jun 15;175(12):1209-16. doi: 10.1093/aje/kwr459. Epub 2012 Apr 19. **Study Design:** Nested cross-sectional MoBa Pregnancies linked to Norway Birth Reg - birth wt - gestational age Birth wt z-scores based on Norwegian births 1987-1998 Pre-term birth = < 37 wks Small for gestational age = < 10th percentile – gender and gest age specific	**Exposure Assessment:** HPLC-MS **Population-Level Exposure:** PFOS median conc = 19.3 ng/ml (NOTE: median exposure ~2.5 x current US F exposure (NHANES 4th Rpt)) LOD = 0.05 ng/ml 100% detect w/in batch CV for PFOS = 4.5% between batch CV = 11.3%	**Stat Method:** Linear regression <u>Co-variates considered</u> **(included in adj model)** - **fish consumption (lean**,oily) - **interpregnancy interval** - **maternal age** - **maternal albumin** - pregnancy wt gain at 17 wks - gestational age at blood draw - smoking - alcohol - **maternal education** - maternal diabetes - child's gender - income Weighted methods to address previous selection criteria (subfecundity) Regression analysis based on continuous PFOS conc, and on quartiles Birth wt z-scores adj for : (a-priori) - maternal age - preg BMI - parity Backwards elimination – retention in model w ≥ 10% change Also, logistic regression for OR for assoc PFOS w outcomes	**Major Limitations:** Cross-sectional design Small no. cases for small for gest age (n = 35) PFOS analyses not controlled for PFOA **Other comments:** Large N for birth wt z-scores Small number cases for pre-term birth Broad statistical controls

Large gest age = > 90th percent – gender, gest age specific Food freq questionnaire at preg wk 22 - consumption 15 kinds fish Data on interpreg interval (mos. From prev birth to current conception) **Location:** Norway **Population:** Norwegian mother-child cohort study (MoBa) Enrollment 2003-2004 At ~ 17 wks gestation Based on sub-cohort from MoBa subfecundity study - random sample n = 550 - cases n = 400 Exclusions: - missing preg BMI - missing gestational age at birth - twins - pre-term birth (excluded from analysis of birth wt z-score		- preterm birth - small for gest age - large for gest age Models included a-priori vars only **Outcome:** Birth wt z-scores **Major Findings:** (adj model) Birth wt z-scores **not sig assoc** w PFOS either by quarts or in continuous model (Crude regression sig neg assoc for quarts and continuous model) **Outcome:** OR for preterm birth **Major Findings:** (adj model) OR's **not sig <> 1.0** for any quart PFOS However, Q4 borderline sig **P-trend stat sig for neg trend** (ORs < 1.0) (p = 0.03)	

Birth wt z-score - **N = 866** Pre-term birth, small for gest age, large for gest age – **total N = 901** **Preterm birth cases, N = 35** **Small for gest age, N = 60** **Large for gest age, N = 125** **Related Studies:**		**Outcome:** OR for small for gest age **Major Findings:** (adj model) ORs **not sig <> 1.0** for any quart PFOS (Q3 borderline sig) P-trend not sig **Outcome:** OR for large for gest age **Major Findings:** (adj model) ORs **not sig <> 1.0** for any quart PFOS p-trend not sig	

Reference and Study Design	Exposure Measures	Results	Comment
Study: Whitworth et al. (2012b) Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, Thomsen C, Eggesbo M, Travlos G, Wilson R, Longnecker MP Perfluorinated compounds and subfecundity in pregnant women.. Epidemiology. 2012 Mar;23(2):257-63. doi: 10.1097/EDE.0b013e31823b5031. **Study Design:** Case-control design PFOS assoc w subfecundity by parous/nulliparous status Questionnaire on enrollment: - demographic factors - lifestyle factors - medical history - reprod history - breastfeeding - previous births - Was current preg planned? - How many mos. of non-contraception intercourse before preg? - if ≥ 3 mos, specific time Subfecundity = time to preg (TTP) > 12 mos Time since prev preg - from Nor. Birth Reg	**Exposure Assessment:** HPLC-MS PFOS LOQ = 0.05 ng/ml 100% of samples detect for PFOS Within batch CV = 4.5% Between batch CV = 11.3% **Population-Level Exposure:** PFOS median conc Cases = 14 ng/ml Controls = 13 ng/ml (NOTE: ~ 1.75 current median PFOS in US F (NAHNES 4th Rpt))	**Stat Method:** Logistic regression for OR subfecundity by quartile PFOS <u>Co-variates considered</u> - Maternal age (a priori) - Pre-preg BMI (a priori) - plasma albumin - yr of blood draw - smoking - alcohol - fish consumption - maternal education - selected maternal diseases - paternal age - paternal education - menstrual irregularities - freq sexual intercourse Vars retained in model if deletion → Δ OR > 10% (No a prior var met inclusion criterion) Analyses stratified by parity (nulliparous/ parous) Parous models adj for inter-preg interval **Outcome:** OR for subfecundity Stratified by parity (nullparous/parous) **Major Findings:** (adj model)	**Major Limitations:** PFOS analyses not controlled for PFOA **Other comments:** Case-control design Moderate N Reasonable statistical control of analyses Stratification by parity may offer better control of associations resulting from reverse causation than in Danish study (parity as model var) Failure to control for PFOA in PFOS analyses

Eligibility - live-born child - plasma sample at ~17 wks gest **Location:** Norway **Population:** Norwegian Mother and Child Cohort Study (MoBa) Enrollment 2003-2004 Random selection among planned preg, subfecund **N = 416** Random selection – no restriction **N = 484** **Related Studies:** Vestergaard et al. (2012) Fei et al. (2009)		<u>Nullparous</u> OR for subfecundity **not sig <> 1.0** <u>Parous</u> OR for subfecundity **sig > 1.0 for Q4 of PFOS** (≥16.61 ng/ml) OR = 2.1 (borderline sig for Q2, Q3 (OR = 1.5, 1.5) Outcome not affected by adjustment for duration of breastfeeding	