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Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous drinking water contaminants of concern due to mounting evidence implicating adverse health outcomes associated with exposure, including reduced kidney function, metabolic syndrome, thyroid disruption, and adverse pregnancy outcomes. PFAS have been produced in the U.S. since the 1940s and now encompass a growing chemical family comprised of diverse chemical moieties, yet the toxicological effects have been studied for relatively few compounds. Critically, exposures to some PFAS *in utero* are associated with adverse outcomes for both mother and offspring, such as hypertensive disorders of pregnancy (HDP), including preeclampsia, and low birth weight. Given the relationship between HDP, placental dysfunction, adverse health outcomes, and increased risk for chronic diseases in adulthood, the role of both developmental and lifelong exposure to PFAS likely contributes to disease risk in complex ways. Here, evidence for the role of some PFAS in disrupted thyroid function, kidney disease, and metabolic syndrome is synthesized with an emphasis on the placenta as a critical yet understudied target of PFAS and programming agent of adult disease. Future research efforts must continue to fill the knowledge gap between placental susceptibility to environmental exposures like PFAS, subsequent perinatal health risks for both mother and child, and latent health effects in adult offspring.

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Declaration of interests

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) comprise a diverse family of compounds used in a wide variety of industrial processes and the production of consumer goods (ITRC 2020). Since their genesis in the 1940s, PFAS have been detected in the ambient environment, wildlife, and human serum around the globe (Calafat et al. 2007; Domingo and Nadal 2019; Hanssen et al. 2013; Olsen et al. 2017). The environmentally and biologically persistent qualities of PFAS have raised concerns, further heightened by mounting scientific and clinical evidence associating PFAS exposure to multiple adverse health outcomes across all life stages.

1.1 Physicochemical Properties and Characterization of PFAS

Naming conventions for PFAS have evolved over time due in part to the increasing complexity and number of unique congeners, leading to inconsistencies across the scientific literature. For example, the term perfluorinated chemical, or PFC, has been widely used in the scientific literature, but it is inaccurate as it does not describe polyfluorinated substances, which are an important component of the PFAS family (ITRC 2020). Additionally, many PFAS exist in multiple ionic states (e.g. acids, cations, anions), with different names (e.g. perfluorooctane sulfonic acid and perfluorooctane sulfonate), and different physicochemical properties. Consistent and unified naming conventions for PFAS would provide clarity, consistency, and improved scientific communication.

PFAS contain 1 or more carbon atoms with fluorine atoms in place of hydrogen atoms, such that the compound contains the moiety $C_nF_{2n+1}-R$, where R represents additional functional groups (e.g. sulfonate, carboxylic acid; Figure 1) (ITRC 2020). The per- or polyfluoroalkyl moiety is highly chemically and thermally stable with extremely strong carbon-fluorine bonds, and it exhibits both lipophilic and hydrophilic properties (ITRC 2020). Together, these physical and chemical properties make PFAS ideal as surfactants and surface protection products.

PFAS can be broadly divided into polymers and non-polymers. PFAS polymers are thought to pose less of a risk to human and ecological health than some non-polymers and include fluoropolymers, polymeric perfluoropolyethers, and side-chain fluorinated polymers (ITRC 2020). However, the production of PFAS polymers contributes to the presence of non-polymers in the environment through: (1) release of non-polymers as waste byproducts in the production of polymers, (2) degradation of polymers into non-polymers (e.g. PTFE forms PFOA and *N*-methyl perfluorooctane sulfonamide; ITRC 2020). Non-polymer PFAS can be further separated into perfluorinated (fully fluorinated) and polyfluorinated (partially fluorinated) chemicals.

PFAS are individually characterized by carbon chain length and side group structure as well as their history of use. “Legacy” PFAS include compounds with a longstanding history of use and/or long biologic/environmental persistence, whereas replacement and alternative chemistry PFAS are generally referred to as “alternative PFAS” or “replacement PFAS”. However, certain short-chain legacy PFAS are now substituted in place of longer chain compounds to comply with government-derived standards, hence such PFAS could be

considered both a legacy and a replacement compound. For example, PFBS has been utilized as a substitute for PFOS in some manufacturing processes (Table 1 & Figure 2).

1.2 History of PFAS Usage in the U.S.

The earliest known PFAS compound in the U.S. was first synthesized as a fluoropolymer, called polytetrafluoroethylene (PTFE). Roy J. Plunkett is credited with the accidental discovery of PTFE in 1938 while employed by DuPont de Nemours, Inc. (Plunkett 1986). DuPont patented the discovery under the company Kinetic Chemicals in 1941 (Plunkett 1941) and registered the trademarked name, Teflon, in 1945 (Plunkett 1986). By 1948, DuPont was producing over 900 tons of PTFE per year at their facility in Parkersburg, West Virginia and another company, 3M, had begun production of their signature PFAS at their plant in Minnesota (Funderburg 2010).

Between the 1940s and 1950s, the 8-carbon perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) started to be used in the manufacturing of PTFE-based non-stick coatings. From the 1950s to the 1960s, PFOS and PFOA were also used to manufacture stain- and water-resistant products as well as protective coatings. By 1961, PTFE was applied to commercial cookware. From the 1960s to the 1970s, PFOS-based fire-fighting foam was manufactured, although it was later voluntarily phased out of Class A fire-fighting foams beginning in 2000 (US DOD 2000; 3M 2000).

Since their genesis in the mid-1900s, PFAS have since been incorporated into numerous other industrial and consumer products due to their excellent grease/water repellent properties, including: grease-resistant papers (e.g., fast food containers/wrappers, microwave popcorn bags, pizza boxes, candy wrappers); stain resistance either incorporated into or as spray-on coating applied to carpets and upholstery in home and vehicle products; water-resistant clothing and footwear, cleaning products, personal care products (e.g., shampoo, dental floss, cosmetics such as nail polish and eye makeup); paints, varnishes, ski wax and sealants; and Class B aqueous film forming foams (AFFF), which are used at airports and military facilities for firefighting and training exercises; and likely others (ATSDR 2019).

Now, in the 21st century, many consumer products and drinking water sources contain a mixture of PFAS. The specific mixture and volume of PFAS emitted into the environment varies based on the point source (Guelfo et al. 2019; Guelfo and Adamson 2018; Xu et al. 2016). For example, a water system near a military training area would likely have multiple species derived from AFFF present whereas a water system near a textile manufacturing plant would likely have a blend of PFAS precursor and legacy compounds present (e.g. both PFOSA, a PFOS precursor, and PFOS). Over time, the region-specific magnitudes and compositions of environmental contamination by PFAS has resulted in numerous U.S. states and communities with high levels of PFAS in their water, including states impacted by less well-studied alternative compounds [e.g. GenX and ADONA; for detailed reports see Hopkins et al. (2018) and Wang et al. (2013)].

1.4 Sources of PFAS Exposure—PFAS exposure is considered ubiquitous in the U.S., with PFOA and PFOS detectable in >90% of the population (Kato et al. 2011). Humans are exposed to PFAS through a variety of pathways, including through drinking water, air (both

indoor and outdoor), diet, dust, through maternal to fetal transfer *in utero*, and through breastfeeding as neonates (Figure 3; Sunderland et al. 2019). For adults, diet and drinking water are the main sources of exposure, however this may vary depending on lifestyle, diet, proximity to point and nonpoint sources, and local drinking water contamination levels. Formula-fed infants are thought to be the most highly exposed members of the human population due to their high water intake to body weight ratio (Goeden et al. 2019; Goeden 2018). Drinking water may be the largest source of PFAS exposure for communities impacted by high levels of PFAS contamination.

2. Adverse Health Effects Associated with PFAS

The desirable chemical properties of many PFAS paradoxically impart environmentally and biologically undesirable properties, including indefinite environmental persistence and long half lives in many living organisms, including humans. Unlike other common environmental pollutants, such as organochlorine pesticides, PFAS do not bioaccumulate in adipose tissue (Pérez et al. 2013). Some PFAS are structurally similar to natural fatty acids (Vanden Heuvel et al. 2006). Among the tissues evaluated, PFAS primarily accumulate in the serum, lungs, kidney, liver, and brain (Pérez et al. 2013). Human exposure to PFAS has been associated with adverse effects on the immune (NTP 2016), endocrine, metabolic, and reproductive systems (including fertility and pregnancy outcomes), and increased risk for cancer (ATSDR 2020). However, the weight of evidence supporting these associations varies by both outcome and specific PFAS examined.

2.1 History of PFAS-Related Human Health Concerns

Industrial usage of PFAS began in the 1940s, but exposure-related human health effects were not identified for another twenty years and were specific to occupationally exposed workers. These early concerns were largely contained within the industrial sphere for an additional forty years. Nearly six decades after PFAS began to be produced on an industrial scale, PFAS-related health concerns were raised by private citizens after the general public learned of connections between ecological observations and pollution potentially stemming from a nearby PFOA production site.

The first documented concerns regarding the toxicity of PFAS were mentioned in an internal memo at DuPont by Chief Toxicologist Dorothy Hood in 1961 (EWG 2019). By 1980, PFAS were measured in the serum of occupationally exposed workers and in 1981 concerns were raised internally at DuPont regarding birth defects in children born from occupationally exposed women (EWG 2019). Industrial and occupational exposure studies have since shown increased incidence of cancer including bladder (Alexander et al. 2003), kidney (Consonni et al. 2013), prostate (Gilliland and Mandel 1993; Lundin et al. 2009), and liver (Consonni et al. 2013), leukemia (Consonni et al. 2013), kidney disease (Steenland and Woskie 2012), and elevated cholesterol (Costa et al. 2009; Sakr et al. 2007).

Community-based exposure concerns in the U.S. were first raised in 1998 by Wilbur Tennant, a cattle farmer whose land was downstream from and bordered a landfill used by the DuPont Washington Works plant near Parkersburg, WV (Rich 2016). Tennant enlisted legal counsel from Rob Bilott after documenting unusual phenomenon, such as foamy,

discolored water in the creek from which Tennant's cattle drank, atypical behavior of the cattle, and a wide array of physical abnormalities in the cattle including black teeth, skin lesions and tumors, discolored organs, malformed hooves, and calves born with profound birth defects (Rich 2016). One hundred and fifty-three of Tennant's original herd of two hundred cattle died, and Tennant believed the pollution dumped by DuPont into the landfill upstream of his cattle farm was the cause (Rich 2016). What began as a legal case on behalf of a private citizen ultimately led to a larger class-action lawsuit filed by Bilott on behalf of over 70,000 citizens in the Mid-Ohio Valley whose drinking water sources were affected by the contamination stemming from DuPont's plant (Rich 2016).

The class-action lawsuit resulted in a settlement that funded a biomonitoring effort of unprecedented size and scale, called the C8 Health Study (Frisbee et al. 2009). The results of the C8 Health Study identified a probable link between exposure to PFOA and increased cholesterol, kidney cancer, testicular cancer, ulcerative colitis, thyroid disease, and pregnancy-induced hypertension (C8 Science Panel). Since the genesis of the C8 Health Study, numerous epidemiological studies of the adverse health effects associated with PFAS exposure have been conducted across the globe, including many other regions in the U.S., Europe, and China.

Multiple organizations and regulatory agencies in the U.S. have also generated statements based on systematic reviews of the available literature regarding the most well-supported human health effects associated with PFAS. The Center for Disease Control (CDC)/Agency for Toxic Substances and Disease Registry (ATSDR) states that PFAS might increase the risk of cancer, affect the immune system, increase cholesterol levels, act as endocrine disruptors, reduce female fertility, and adversely impact infant/early childhood growth and development (ATSDR 2019). The U.S. Environmental Protection Agency (EPA) states that elevated cholesterol in adults is the most consistent health effect associated with PFAS, but less consistent data suggest PFAS might be associated with low infant birth weight (Johnson et al. 2014; Koustas et al. 2014; Lam et al. 2014), effects on the immune system, increased risk for cancer (specific to PFOA), and disrupted thyroid hormones (specific to PFOS) (822-R-16-005 US EPA). The World Health Organization (WHO)/International Agency for Research on Cancer (IARC) states that PFOA is possibly carcinogenic to humans (IARC 2016). The National Toxicology Program (NTP) states that PFAS might have effects on metabolism, pregnancy, neurodevelopment, and the immune system, with both PFOA and PFOS presumed to be immune hazards (NTP 2016). Such statements only address the possible health effects associated with legacy PFAS as there are little to no data on health outcomes associated with replacement PFAS.

Although many biological systems are adversely impacted by PFAS, this review focuses primarily on PFAS-associated, placenta-based adverse pregnancy outcomes and how they may increase susceptibility for latent health effects including metabolic dyshomeostasis, thyroid disruption, and kidney effects (Figure 4).

2.2 Challenges of Studying PFAS-Related Human Health Effects

There are many challenges in studying PFAS-related human health effects, including ones specific to studying PFAS exposure and effects in the human population as well as those that are specific to the study of PFAS toxicology in animal models.

First, humans are exposed to PFAS as complex mixtures that differ based on the source of exposure (e.g. near a military base or an industrial site, consumer product use) and differ temporally within the same geographic location (e.g. an industrial site phasing out PFOA and PFOS and utilizing alternative PFAS instead; seasonal patterns in rainfall/drought affecting the extent to which PFAS in water systems are diluted or concentrated). States have authority to develop statewide regulations on consumer product labels and contents, which also influences the extent to which individuals are potentially exposed to PFAS (e.g. consumers can choose to avoid PFAS-containing products under regulations such as Proposition 65). Thus, different individuals and populations are exposed to different levels and mixtures of PFAS. Little is known regarding the potential synergistic effects of PFAS mixtures. Human exposure to PFAS also varies by demographic factors, and they likely differ by individual PFAS congener within a given population. Lifelong exposure, combined with variable exposure to temporally stochastic mixtures and congener-specific demographic factors, presents a complex set of challenges to understanding the relationship between PFAS exposure and adverse health outcomes.

A second challenge in epidemiologic studies of PFAS-related health effects is the lack of an unexposed control population. All humans born after the year 1950, when PFAS were first introduced at the industrial scale, have potentially been exposed to PFAS their entire lives, including *in utero*. This precludes the ability to design and conduct cohort studies with a no-exposure comparison group. Instead, such cohort studies of PFAS must make comparisons between low and high exposure groups to estimate disease risk based on exposure status.

Third, it is possible that PFAS-related adverse health outcomes observed in adulthood are latent manifestations of perturbations during sensitive periods of development (Barker 2004). It is also possible that some PFAS-related adverse health outcomes observed in adulthood are due to chronic, lifelong exposure. In order to address these hypotheses, a population of humans without PFAS exposure during early development would be required. However, such a population only existed prior to 1950, making such a study extremely difficult if not impossible.

Fourth, while experimental toxicology studies provide invaluable information, the elimination kinetics, sensitive endpoints, and biological mechanisms of action in target tissue(s) demonstrate interspecies variation between experimental models (rats vs mice) as well as compared to humans (e.g. serum half-life elimination of PFHxS in mice is ~25-30 days, ~2-7 days in rats, and 5-8 years in humans; Fenton et al. 2020). Many of the interspecies differences regarding toxicological effects and toxicokinetics of PFAS are not fully understood and are further complicated by sex differences in elimination rates (e.g. the human serum half-life of GenX has yet to be fully characterized but it is 3 hours in male rats, 8 hours in female rats, and 18-20 hours in male or female mice; Fenton et al. 2020).

2.3 The Placenta as a Target of PFAS and Critical Driver of Adverse Pregnancy Outcomes

The placenta is a critical organ that exists exclusively during pregnancy and serves as a conduit between mother and developing offspring, mediating the maternal-fetal transfer of nutrients, oxygen, blood, waste, and xenobiotics. The healthy development and function of the placenta is paramount to not only the healthy development of the fetus, but also the health of the mother. Indeed, many adverse pregnancy outcomes are due to placental insufficiency (PI), a condition in which the functional capacity of the placenta is limited or deteriorates, resulting in reduced transplacental transfer of oxygen and nutrients to the fetus (Gagnon 2003). Previous studies have demonstrated that the placenta is vulnerable to environmental insults (Laine et al. 2015; Leclerc et al. 2014; Pedersen et al. 2014).

Adverse pregnancy outcomes associated with PFAS exposure in humans include increased time to pregnancy (Bach et al. 2016; Lum et al. 2017), hypertensive disorders of pregnancy (which includes pregnancy induced hypertension [PIH] (Huang et al. 2019; Savitz et al. 2012b), and preeclampsia [PE] (Stein et al. 2009; Wikström et al. 2019)), gestational diabetes (GD) (Matilla-Santander et al. 2017; Zhang et al. 2015), excess gestational weight gain (Ashley-Martin et al. 2016), and low birth weight of the infant (Johnson et al. 2014; Li et al. 2017; Marks et al. 2019; Meng et al. 2018; Sagiv et al. 2018; Starling et al. 2017; Wikstrom et al. 2019; Xu et al. 2019). Independent of PFAS exposure, deficiencies in placental development and/or function are involved in the etiology of many of these adverse pregnancy outcomes (Hutcheon et al. 2012; Risnes et al. 2009; Thornburg et al. 2010). Thus, it is possible the placenta is a target of PFAS such that PFAS-induced reductions in placental function significantly contribute to adverse pregnancy and birth outcomes.

The placenta is a plausible target of PFAS as it is exposed to PFAS via maternal circulation, shares biologic features in common with other known target tissues of PFAS, such as kidney and liver, and plays a role in the etiology of pregnancy disorders related to PFAS exposure. It is well documented in humans and animal models that PFAS readily pass from maternal serum to the developing embryo via the placenta (Chen et al. 2017a; Chen et al. 2017b; Wang et al. 2019; Yang et al. 2016a; Yang et al. 2016b) and that PFOA transplacentally transfers to the mouse offspring (Fenton et al. 2009). During early stages of development, the placenta functions as the liver, lungs, and kidneys for the embryo; the placenta is responsible for xenobiotic metabolism, supplying oxygen to the fetus, and eliminating waste from embryonic/fetal circulation. Placental trophoblasts express multiple types of ATP-binding cassette (ABC) and organic anion transporter (OAT) proteins in order to shuttle nutrients, xenobiotics, and waste between the maternal and fetal compartments. Many of the transporter proteins expressed in placental trophoblasts are also expressed in liver or kidney, where they excrete substrates into the bile or urine, respectively (Joshi et al. 2016). An *ex vivo* study of human placenta showed the involvement of OAT4 in the placental transport of PFOA and PFOS (Kummu et al. 2015).

There is growing evidence of the mechanisms of PFAS toxicity towards the placenta. A study in mice showed dose-dependent necrotic changes in placenta after gestational exposure to 10 mg/kg/day and 25 mg/kg/day PFOA (Suh et al. 2011). In rats, gestational exposure to 20 mg/kg/day PFOS reduced fetal and placental weight, inhibited placental 11 β -hydroxysteroid dehydrogenase 2 activity, and down-regulated 45 gene corresponding to

growth factors and hormones, transporters, and signal transducers, among others (Li et al. 2016). More recently, it was demonstrated that gestational exposure to either PFOA or GenX induced placental lesions in CD-1 mice, with disrupted placental weights and fetal:placental weight ratios and histopathological changes in the placental labyrinth, including congestion and atrophy (Blake et al. 2020).

A study of *ex vivo* term placental cytotrophoblasts exposed to PFOS demonstrated concentration-dependent suppression of estradiol, human chorionic gonadotropin, and progesterone, reduced cell viability, and dysregulation of apoptotic proteins in favor of proapoptosis (Zhang et al. 2015). *In vitro* studies of JEG-3 human placental trophoblasts showed PFOS, PFOA, and PFBS inhibit aromatase (CYP19) activity and an exposure to a mixture of eight different PFAS caused an increase in certain lipid classes (Gorrochategui et al. 2014). In the JEG-3 human placental trophoblast line, exposure to PFOA, PFOS, or GenX altered gene expression profiles towards an anti-proliferative and pro-apoptotic state after exposure to 10 µg/mL in cell culture media (Bangma et al. 2020). However, JEG-3 internal cellular accumulation of GenX was far lower than that of PFOS or PFOA, which suggests a higher sensitivity of placental cells towards GenX as compared to PFOS or PFOA (Bangma et al. 2020). These findings suggest GenX, PFOA, and PFOS may adversely affect placental development and function via disrupted balance of apoptosis and proliferation of the trophoblasts.

Human-derived first trimester trophoblast HTR-8/SV_{neo} cells have been used to demonstrate that PFOS, PFOA, and GenX disrupt inflammatory signaling, decrease trophoblast migration, and decrease trophoblast invasion (GenX only), which indicates a potential mechanism through which PFAS exposure could disrupt placental development and function (Szilagyí et al. 2020). Another study utilizing HTR-8/SV_{neo} trophoblasts similarly showed concentration-dependent decreased migration and invasion after exposure to PFBS, in addition to altered expression of genes implicated in PE (Marinello et al. in press). Additionally, PFOS exposure in HTR-8/SV_{neo} trophoblasts increased levels of miR29-b, a microRNA associated with PE, altered downstream epigenetic processes including global DNA hypomethylation and hyperacetylation of protein, and increased reactive oxidative species production, suggesting a mechanism for increased placental oxidative stress due to PFAS exposure which could ultimately lead to preeclampsia (Sonkar et al. 2019).

Taken together, these studies suggest PFAS induce adverse outcomes in the placenta via mechanisms involving endocrine/lipid/sterol disruption, oxidative stress, disrupted balance of pro- and anti-apoptotic signaling, impaired trophoblast function, and epigenetic alterations. It is possible that mechanisms of PFAS toxicity towards the placenta are compound-specific and may involve numerous overlapping aspects of biology. Improper development or function of the placenta is associated with an array of adverse pregnancy outcomes (including hypertensive disorders of pregnancy), many of which are also associated with maternal exposure to PFAS. It is possible that adverse pregnancy outcomes associated with PFAS exposure are mediated in part by the negative impact of PFAS on the development and function of the placenta. Understanding the risk of environmental insults on the health and development of the placenta is critical for protecting both short and long-

term maternal and offspring health outcomes because the placenta is a driver of latent disease risk for both postpartum women and their offspring.

2.3.1 PFAS Exposure, Hypertensive Disorders of Pregnancy, and the

Placenta—Hypertensive disorders of pregnancy (HDP) are a family of disorders that include PE, eclampsia, HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome, and PIH, all of which are related to deficiencies in placental development and/or function (Pijnenborg et al. 1991). This family of disorders not only endangers the health of both the mother and developing fetus during pregnancy, but also increases the risk of post-pregnancy hypertension, heart disease, and stroke in affected women as well as increased risk for adverse cardiometabolic outcomes in adult offspring (Pinheiro et al. 2016).

During normal pregnancy, at 13 weeks gestation the placental cytotrophoblasts begin to invade the maternal uterine wall and remodel the maternal spiral arteries to establish blood flow to the growing embryo (Shah 2020). This process of vasculogenesis and angiogenesis by placental cytotrophoblasts remodels the maternal spiral arteries to become low-resistance, low-capacitance vessels so that blood flow and sufficient nutrients and oxygen are supplied to the growing embryo (Shah 2020). This carefully coordinated process requires an appropriate balance of proangiogenic and antiangiogenic signals (Shah 2020). Disruption of this process can lead to abnormal placental vascularization, resulting in suboptimal pregnancy hemodynamics which can give rise to HDP as well as impaired fetal growth (Shah 2020).

There is growing evidence from human studies implicating maternal PFAS exposure in the development of HDP. Multiple epidemiologic studies have found positive associations between maternal exposure to PFAS and PE (Huang et al. 2019; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009; Wikström et al. 2019a) as well as PFAS and PIH (C8 Science Panel; Darrow et al. 2013; Holtcamp 2012). It is possible that reduced or impaired placental function as a consequence of PFAS exposure results in or significantly contributes to the development of HDP. Indeed, a recent study in mice showed increased placenta weight, reduced fetal:placental weight ratios, and placental lesions following gestational exposure to PFOA or GenX, suggesting a role for placental toxicity by PFAS (Blake et al. 2020).

Furthermore, it is well documented that the placenta itself is strongly associated with cardiometabolic disease risk in adults. Indeed, the size of the placenta is strongly associated with both perinatal and adult outcomes. In a study of 87,600 Canadian singleton births, fetuses with abnormally small placentas were at increased risk for stillbirth (OR 2.0, 95% CI 1.4-2.6) while fetuses with abnormally large placentas were at increased risk for adverse neonatal outcomes, including seizures, respiratory morbidity, and low Apgar score (odds ratio [OR] 1.4, 95% confidence interval [CI] 1.2-1.7) (Hutcheon et al. 2012). Bigger is not necessarily better; it has been hypothesized that an enlarged placenta is a biomarker for impaired nutrient supply to the fetus. This reduced capacity could be explained by restricted or impaired blood flow through the placenta. It is possible that impaired blood flow, which would in turn disrupt the normal flow of nutrients, oxygen, and waste between maternal and fetal compartments, during critical windows of *in utero* development may result in long-term adverse function of the cardiometabolic system. A U.S. cohort study of nearly 30,000 births

showed that large placenta relative to birth weight, but not birth weight itself, is associated with high blood pressure in childhood (Hemachandra et al. 2006). In a study of 31,307 Norwegian adults, disproportionately large placentas at birth were associated with increased risk of death associated with cardiovascular disease (CVD), and this association remained positive when considering placenta weight alone (Risnes et al 2009).

While mounting epidemiologic evidence suggests PFAS exposure as a risk factor for the development of HDP, a specific mechanism has yet to be validated in experimental settings. Such experimental systems are critical in addressing the hypothesis that maternal exposure to PFAS increases the risk of developing HDP via disruption of the placenta. Due to the complex and multifactorial nature of HDPs, and interspecies differences in maternal spiral artery structure and formation, current animal models are unable to adequately recapitulate the multiple manifestations of this complex group of disorders (Cushen and Goulopoulou 2017; Marshall et al. 2017). Indeed, there is no “standard” or preferred animal model of HDP, and it is unlikely that such an animal model will ever exist (Cushen and Goulopoulou 2017; Marshall et al. 2017). Thus, the development of sensitive laboratory tools for assessing clinically relevant biomarkers of HDPs in animal models is a necessary next step towards determining mechanisms of PFAS toxicity.

2.3.2 PFAS Exposure and Low Birth Weight—Low birth weight is the most consistently reported adverse pregnancy outcome associated with gestational exposure to PFAS in human epidemiologic and animal studies. It is also well established in the literature that placental insufficiency contributes to the etiology of low birth weight (Audette and Kingdom 2018; Cuffe et al. 2017; Henriksen and Clausen 2002). What remains unaddressed by the current body of literature is the relationship between gestational exposure to PFAS, placental function, and birth weight.

In 2014, a three-paper series performed systematic reviews of the existing human and animal literature on the relationship between *in utero* exposure to PFOA and low birth weight (Johnson et al. 2014; Koustas et al. 2014; Lam et al. 2014). After gathering primary data sets and detailed descriptions of study designs from numerous research teams, the authors were able to calculate estimates corresponding to the predicted reduction in birth weight in humans [−18.9 g birth weight per 1 ng PFOA/mL maternal serum, 95% CI: −29.8, −7.9; (Johnson et al. 2014)] and in mice [−23.0 mg birth weight per 1 mg PFOA/kg maternal body weight/day, 95% CI: −29.0, −16.0; (Koustas et al. 2014)]. The authors concluded from their systematic analyses of the literature that PFOA is indeed associated with reduced birth weight in both humans and mice. The effect estimate corresponding to birth weight in mice after gestational exposure to 1 mg PFOA/kg maternal body weight/day generated by Koustas et al. (2014) was consistent with findings in a recent study that measured near-term fetal body weights after gestational exposure to 1 mg PFOA/kg maternal body weight/day (−28 mg fetal weight at embryonic day 17.5, 95% CI: −114, 59; Blake et al. 2020).

Since the publication of these systematic reviews in 2014, the human epidemiologic literature on the relationship between PFAS and birth weight has expanded to include understudied PFAS (e.g. PFNA, PFDA, PFHxS). In a recent report, an analysis of 3,535 mother-infant pairs in the Danish National Birth Cohort examined six different PFAS and

reported increased risk for preterm birth associated with maternal serum levels of PFOA, PFOS, PFNA, PFDA, and perfluoroheptane sulfonate (Meng et al. 2018). Low birth weight risk was also elevated, but estimates were less precise in this analysis (Meng et al. 2018). In a study of 1,533 Swedish mother-infant pairs, increased maternal exposure to PFOS, PFOA, PFNA, PFDA, and perfluoroundecanoic acid were associated with lower birth weight and size, but associations were only significant in girls (Wikstrom et al. 2019). Conversely, a study of 457 British mother-infant dyads showed inverse associations between maternal PFOS and measures of both birth weight and size (Marks et al. 2019), which had previously been shown in mother-daughter dyads in the same cohort (Maisonet et al. 2012). A study of 1,645 mother-infant pairs in Massachusetts showed that PFOS and PFNA were weakly inversely associated with birth weight and positively associated with higher odds of preterm birth (Sagiv et al. 2018). In another U.S. cohort, maternal serum PFOA and PFNA was inversely associated with birth weight in an analysis of 628 mother-infant pairs (Starling et al. 2017). A smaller study of 98 Chinese mother-infant pairs also found PFOS was negatively associated with birth weight, and that both PFOS and PFHxS were negatively associated with birth size (small for gestational age, SGA) (Xu et al. 2019). Taken together, these epidemiological studies further underscore the potential for PFAS other than PFOA to adversely affect fetal growth *in utero*.

There are limited animal studies supporting the association between PFAS, other than PFOA and PFOS, and reduced birth weight. Studies of both mice (Chang et al. 2018) and rats (Ramhoj et al. 2018) have shown reduced offspring body weight after developmental exposure to PFHxS. In rats, developmental exposure to perfluoroundecanoic acid has been shown to reduce offspring weight (Takahashi et al. 2014). Previous animal studies of the developmental effects of PFNA have reported adverse neonatal outcomes but did not report effects on birth weight (Das et al. 2015; Wolf et al. 2010). Similarly, a previous study of the developmental toxicity of PFDA in mice found no reductions in birth weight (Harris and Birnbaum 1989). Studies in mice have demonstrated a modest effect of prenatal exposure to GenX on near-term (Blake et al. 2020) and early postnatal offspring body weights, with effects becoming more significant throughout lactation (DuPont-18405-1037).

The association between PFAS exposure and low birth weight is well supported in humans, as is the causal link between placental insufficiency and low birth weight. This, in combination with the biologically plausible proposed mechanisms of PFAS toxicity towards the placenta, further underscores the need to test the hypothesis that the PFAS-mediated placental effects are a critical driver of adverse pregnancy and birth outcomes.

2.4 PFAS Exposure and Metabolic Effects

Metabolic syndrome (also referred to as cardiometabolic syndrome) describes a group of disorders that affects approximately one third of the U.S. adult population and includes obesity, dyslipidemia, elevated blood pressure, and impaired glucose tolerance (Eckel et al. 2005; Ervin 2009). The liver is a central hub in the regulation of pathways controlling systemic metabolic homeostasis, and liver dysfunction is a major component of metabolic syndrome (D'Amore et al. 2014). While the liver regulates metabolic homeostasis, lifelong metabolic health is influenced by placental health and function during critical periods of *in*

utero fetal programming (Nugent & Blake, 2016; Rinaudo & Wang, 2012). Therefore, environmental exposures impacting placental health and function, such as PFAS, may prime the system for increased susceptibility to metabolic syndrome, which could be further exacerbated by continued PFAS exposure across the lifespan.

A recent review of the literature examined 69 epidemiological studies evaluating the association between PFAS exposure and a variety of metabolic outcomes, including lipid homeostasis, diabetes, overweight/obesity, and cardiovascular disease (Sunderland et al. 2019). Across the human literature, Sunderland et al. (2019) reported relatively consistent and modest positive associations between PFAS exposure and serum lipids, such as total cholesterol and triglycerides. Although associations were less consistent across studies, Sunderland et al. (2019) also reviewed epidemiologic studies examining the adverse effect of PFAS exposure on insulin resistance, diabetes, hypertension, vascular disease, and stroke. Across these health outcomes, adverse effects were most consistently reported in association with exposure to PFOA (Sunderland et al. 2019). The effects of PFAS on metabolism of developmentally exposed offspring is the topic covered more in depth by Cope et al. (*this issue*).

More recently, additional studies of humans have explored the association between PFAS exposure and metabolic syndrome. Christensen et al. (2019) leveraged National Health and Nutrition Examination Survey (NHANES) data sets to investigate the relationship between PFAS exposure and metabolic syndrome in the general U.S. population between years 2007-2014 and found PFNA was consistently associated with increased risk of metabolic syndrome, while the highest levels of PFHxS exposure were associated with elevated triglycerides (Christensen et al. 2019). This study suggests that PFAS other than PFOA and PFOS may contribute to adverse metabolic health outcomes in humans.

Most animal studies investigating the effect of PFAS exposure on metabolic syndrome have focused on PFOA. Previous work in mice has shown that developmental exposure to PFOA disrupts weight gain, serum leptin, and insulin sensitivity later in life (Abbott et al. 2012; Hines et al. 2009). While data from adult-dosed animals initially suggested that peroxisome proliferator-activated receptor alpha (PPAR α) was the mechanism through which PFOA exerted deleterious effects on the liver and metabolic system (Elcombe et al. 2012; Kennedy et al. 2004; Klaunig et al. 2003; Rosen et al. 2007), later work using mice null for PPAR α revealed adverse liver effects persisted or worsened following perinatal exposures, suggesting PFOA can induce toxicity (e.g. tumor development) in the liver via PPAR α -independent mechanisms such as glycogen depletion and mitochondrial dysfunction (Filgo et al. 2015; Mashayekhi et al. 2015; Quist et al 2015) and that effects may have been mediated *in utero* (Filgo et al 2015; Quist et al 2015). However, more recently PFOA, PFOS, PFNA, and PFHxS were evaluated in mice and found to induce expression of hepatic genes involved in fatty acid and triglyceride synthesis, potentially causing steatosis through disruption of the balance between fatty acid accumulation and oxidation (Das et al. 2017). Similarly, in primary human and rat hepatocytes, PFOA and PFOS were shown to activate multiple nuclear receptors and the metabolic response shifted from carbohydrate metabolism to fatty acid accumulation and oxidation (Bjork et al. 2011). In an *in vitro* 3D spheroid model of mouse liver AML12 cells, PFOA, GenX, and another PFOA alternative, 3,5,7,9-

tetraoxadecanoic perfluoro acid (PF04DA), were shown to induce PPAR α targets, oxidative stress, and lactate dehydrogenase (LDH) leakage, suggesting PFOA alternatives have the potential to induce liver injury (Sun et al. 2019). Across the human, animal, and *in vitro* literature, there is consistent evidence to suggest PFAS disrupt metabolic homeostasis through damaging the liver. Whether PFAS-mediated effects on the placenta may have a role in programming latent liver and metabolism-related outcomes is the focus of on-going work.

2.5 PFAS Exposure and Thyroid Effects

PFAS are hypothesized to target the thyroid by influencing multiple biological mechanisms involved in thyroid homeostasis, including thyroid hormone biosynthesis, transport, metabolism, and interference with thyroid receptors in target tissues (Boas et al. 2009). Proposed mechanisms of action of thyroid disruption by PFAS include reduced circulating levels of thyroxine (T4) due to competitive binding to thyroid hormone transporter (THT) proteins (Weiss et al. 2009), increased T4 metabolism in the thyroid and liver (Chang et al. 2009; Yu et al. 2009; Yu et al. 2011), reduced thyroid production of T4 (Webster et al. 2014), or reduced thyroid peroxidase (TPO) activity (Coperchini et al. 2015). During pregnancy, the placenta regulates the exchange of thyroid hormone (TH) from the maternal to fetal compartments and must maintain appropriate TH levels in the fetal circulation throughout gestation to ensure healthy development (Chan et al. 2009). Fetal TH levels are modulated by the placenta through plasma membrane THTs, metabolism, and binding to proteins in trophoblast cells (Chan et al. 2009). Thus, any PFAS implicated as TH disrupters may perturb the ability of the placenta to appropriately modulate fetal thyroid hormone balance during development.

Previous epidemiologic studies have examined the association between exposure to PFAS and THs, with inconsistent findings across the literature. Several studies leveraged the NHANES database and showed positive associations between PFOA and total triiodothyronine (T3) (Jain 2013; Webster et al. 2016; Wen et al. 2013), TSH (Jain 2013; Lewis et al. 2015), and self-reports of current thyroid disease (Melzer et al. 2010). PFHxS has been associated with increases in total T4 across the general U.S. population (Jain 2013) as well as with sex-specific positive associations in women (Wen et al. 2013). A recent meta-analysis of twelve epidemiologic studies found that PFAS are generally negatively associated with T4, but certain associations may be non-monotonic (Kim et al. 2018). For example, PFOS was positively associated with T4 and TSH only in the intermediate exposure group (Kim et al. 2018). Positive associations between serum PFOS and TSH were similarly reported in a longitudinal study of adults with serum levels of PFOS within the range of the general U.S. population (Blake et al. 2018), and PFAS were generally found to be positively associated with TSH in pregnant women in a systematic review (Ballesteros et al. 2017). However, it is possible that inconsistencies across the human literature are attributable to differences in the overall range of PFAS exposure across populations studied, which may help explain the non-monotonic dose-response described in Kim et al. (2018).

Pregnant women and their developing offspring are particularly sensitive to disruptions in THs, which are critical endocrine modulators of early neurodevelopment (de Escobar et al. 2004; Porterfield 1994). PFAS have been documented as thyroid disrupters in pregnant

women in some epidemiologic studies (Ballesteros et al. 2017; Berg et al. 2015; Wang et al. 2014; Webster et al. 2014). Generally, maternal PFAS levels during pregnancy are associated with shifts in TH levels consistent with hypothyroidism (e.g. elevated TSH), which is associated with increased risk for low birth weight (Alexander et al. 2017; Belet et al. 2003). In rodent models, gestational exposure to GenX resulted in reduced maternal serum T3 and T4 levels in rats (Conley et al. 2019) and increased placental T4 levels in mice (Blake et al. 2020), demonstrating the potential for this replacement compound to act as a TH disruptor during pregnancy.

A current challenge in studying the effect of PFAS on THs is the inherent negative bias of common analog methods used to measure THs (e.g. radioimmunoassay, chemiluminescent assay), but this artifact is likely specific to rodent studies. Reduction in TH binding protein or displacement of TH via competitive binding, such as displacement of T4 by PFOS in rats, reduces serum binding capacity for TH and analog and result in negatively biased measurements of free THs (for an in-depth explanation, see Chang et al. 2007). In order to avoid such negative bias, equilibrium dialysis or high-performance liquid chromatography-based methods should be employed in animal studies. However, a follow up study conducted using human samples demonstrated no such negative bias for either PFOA or PFOS with respect to free T4 whether assayed using the analog or equilibrium dialysis method (Lopez-Espinosa et al. 2012).

Due to the critical role that THs play in human health and development, disruptions at any life stage warrant further study to understand potential underlying mechanisms. Both hyperthyroidism and hypothyroidism are suboptimal health outcomes and increase risk for adverse effects on the function of TH-responsive tissues, such as kidney and the cardiovascular system. Therefore, continued study of the effects of PFAS on thyroid function are warranted, especially as it pertains to other associated health effects. The role of placenta-specific TH action on fetal development and metabolism needs additional research; how PFAS may affect this target is poorly understood.

2.6 PFAS Exposure and Kidney Effects

The kidney is considered a target tissue of PFAS, evidenced by a growing body of human epidemiologic data further supported by animal studies and *in vitro* models. In humans, PFAS exposure has been associated with adverse kidney outcomes such as reduced kidney function, chronic kidney disease (CKD), and kidney cancer, including mortality from kidney cancer. Human epidemiologic studies have shown an association between low birth weight and adverse kidney outcomes in later life (Dotsch et al. 2011; Hershkovitz et al. 2007). It is possible that placental dysfunction due to PFAS resulting in low birth weight may result in lifelong increased susceptibility and reduced kidney function. However, similar to metabolic syndrome, it is difficult to determine what proportion of disease susceptibility is programmed during development and how much is exclusively a consequence of adult exposures. Due to lifelong PFAS exposure stemming from their ubiquity in the environment, it is likely that increased susceptibility resulting from exposure during critical periods of development and continued exposure through adulthood interact in complex ways to influence disease risk.

The primary elimination route for PFAS is via urinary excretion. Given that the most well-studied PFAS do not undergo biotransformation prior to urinary excretion, one hypothesized mechanism of PFAS-induced kidney injury is via reabsorption of PFAS across the renal tubules which causes localized damage, potentially through oxidative stress (Han et al., 2011). It has been demonstrated in humans that PFAS, once absorbed, distribute primarily to serum, liver, and kidney (Fabrega et al. 2014; Perez et al. 2013). It is yet to be determined if the kidney is a sensitive target of PFAS due to high accumulation of PFAS within the renal tissue, creating a high internal dose in kidney, or if the kidney is particularly sensitive to PFAS-induced effects. The extent to which PFAS distribute and/or accumulate in different tissues likely varies by individual congener. For example, perfluorododecanoic acid (PFDoA), perfluorodecanoic acid (PFDeA), and perfluorobutyrate have been shown to highly accumulate in kidney relative to other tissues (Perez et al. 2013).

Elimination rates also vary widely by individual congener with human serum half-lives spanning from about 24 hours to ~15 years which generally (though not always) corresponds to carbon chain length and functional groups (Fenton et al. 2020). Such differences in elimination rate and serum half-life are hypothesized to result from different rates of secretion and reabsorption by the kidney proximal tubules. This process, driven by renal tubule efflux transporters, actively transports PFAS back into systemic circulation, thus contributing to their long half-lives in the human body (Han et al., 2011). Uptake of PFAS into proximal tubules, both apically and basolaterally, is mediated by solute-carrier protein family transporters such as organic anion transporters (OAT). OAT1 and OAT3 have been shown to transport PFAS basolaterally in proximal tubules with apical transport mediated by OAT4 and urate transporter 1 (URAT1) (Worley et al. 2017; Yang et al. 2010; Zhang et al. 2013).

In a recent scoping review of the effect of PFAS on kidney health, Stanifer et al. (2018) identified 74 studies comprised of epidemiologic, pharmacokinetic, and toxicological studies of humans, animals, and *in vitro* models (Stanifer et al. 2018). Across 21 epidemiologic studies of occupationally and non-occupationally exposed populations, Stanifer et al. (2018) reported consistent associations between PFAS exposure and adverse kidney outcomes, which included reduced kidney function and kidney cancer (Stanifer et al. 2018). Studies published since this scoping review have similarly provided evidence suggesting human exposure to PFAS is detrimental to kidney health (Blake et al. 2018, Jain and Ducatman 2019a).

In addition to concerns over reduced kidney function, compensatory increases in kidney function (e.g. glomerular filtration rate) are associated with increased risk for cardiovascular morbidity and mortality. Such hyperfiltration has been observed in patients with prediabetes and prehypertension (Palatini 2012; Shastri and Sarnak 2011). A positive association between methyl-perfluorosulfonic acid (Me-PFOSA) and increased kidney filtration was reported (Blake et al. 2018). Indeed, the relationship between PFAS exposure and kidney function (glomerular filtration rate) is nonmonotonic (Jain and Ducatman 2019a). It has been hypothesized that altered balance between glomerular secretion of PFAS into the urine for excretion (e.g. via OAT1 and OAT3) and renal reabsorption (e.g. via OAT4) with increasing severity of kidney disease progression may explain the inverse U-shaped relationship. Based

on these findings, Jain and Ducatman (2019a) hypothesized that PFAS reabsorption in renal tubules decreases with advancing stages of renal failure. In a follow up study, Jain and Ducatman (2019b) demonstrated that increased albuminuria may provide further explanation for decreasing serum PFAS concentrations with increasing kidney failure. Excessive albumin proteins in the urine may essentially off-load the body burden of PFAS with high binding affinity to albumin proteins (Jain and Ducatman 2019b). However, these studies were conducted using cross-sectional data from NHANES and the reported findings would be greatly strengthened if validated in longitudinal cohorts.

Animal studies and *in vitro* models have provided further evidence for the adverse effect of PFAS on kidney health (Stanifer et al. 2018). Studies conducted in rats and mice have reported increased kidney weight (Butenhoff et al. 2012; Curran et al. 2008; Ladics et al. 2005), increased blood urea nitrogen (BUN) (Butenhoff et al. 2012; Seacat et al. 2003; Takahashi et al. 2014), renal tubular atrophy (Klaunig et al. 2015) or hypertrophy (Ladics et al. 2005), and tubular epithelial hyperplasia (Kim et al. 2011), among other adverse kidney findings (see Stanifer et al. 2018 for a comprehensive review). *In vitro* systems have provided further evidence to suggest oxidative stress as a mechanism of PFAS-induced kidney damage (Chung 2015; Wen et al. 2016). A recent study by Gong et al. (2019) used rat mesangial cells as an *in vitro* model of diabetic kidney disease to determine the effect of PFAS exposure in the diabetic condition, and similarly reported PFOA or PFOS exposure resulted in increased oxidative stress, fibrosis, and inflammation in this *in vitro* model (Gong et al. 2019). Studies using rats must be interpreted with some caution due to interspecies differences in PFAS elimination rates, with PFAS excretion rates being more rapid in rats (especially female rats) compared to mice (Loccisano et al. 2013; Lou et al. 2007; Pizzurro et al. 2019; Russell et al. 2013).

Overall, there is consistent evidence across human epidemiologic, animal, and *in vitro* studies to support the claim that PFAS are damaging to kidney health, however the relationship between *in utero* exposure to PFAS, placental dysfunction, and latent kidney disease, failure, or cancer is less clear and should be investigated further.

2.7 The Interplay Between Biologic Systems Affected by PFAS Exposure

The adverse health effects of PFAS exposure on human metabolic homeostasis, thyroid function, kidney function, and pregnancy outcomes are likely interrelated due to the extensive overlap between these biologic systems and how each of these systems are influenced by placental health in the context of pregnancy. For example, the liver is responsible for thyroid hormone metabolism and transport, both kidney and liver express type 1 deiodinase (an enzyme that converts T4 to T3) (Sanders et al. 1997), and both the liver and placenta express enzymes of the type 3 deiodinase system, which converts active T4 to inactive rT3, and/or active T3 or inactive rT3 to inactive T2 (Bianco et al. 2002; Darras et al. 1999). During pregnancy, the placenta regulates the degree to which maternal thyroid hormones pass to the developing fetus and maintains the optimal balance of thyroid hormones throughout *in utero* development (Chan et al. 2009).

There is human clinical evidence to suggest shared pathogenic mechanisms for non-alcoholic fatty liver disease and chronic kidney disease (Musso et al. 2016), thyroid disease

(both hyperthyroidism and hypothyroidism) and liver injury (Malik and Hodgson 2002), and hypothyroidism and chronic kidney disease (Rhee 2016). The proposed shared pathogenic mechanisms for these overlapping biologic systems also coincide with hypothesized mechanisms of PFAS toxicity, including disruption of lipid metabolism (e.g. cholesterol and triglycerides), nuclear receptor activation (e.g. peroxisome proliferator-activated receptors, retinoid X receptor), and oxidative stress (Malik and Hodgson 2002; Musso et al. 2016; Rhee 2016), among others. In the context of pregnancy, chronic kidney disease increases the risk of PE and low birth weight (Fischer 2007), and non-alcoholic fatty liver disease increases risk of gestational diabetes and pregnancy-induced hypertension (Hershman et al. 2019).

The clinical evidence underscores the careful balance between multiple biologic systems, and the shared pathogenic mechanisms affecting these systems coincides with toxicologic mechanisms through which PFAS affect these same systems. The complex interplay between the placenta, liver, kidney, and thyroid is further complicated by the physiological demands of pregnancy, such as increased metabolic rate, oxygen consumption, blood volume, weight gain and fluid retention. Furthermore, *in utero* exposure to PFAS occurring during critical periods of development may increase susceptibility for latent onset of chronic adult diseases, such as obesity, diabetes, problems in kidney function, and fatty liver disease. Some of these latent effects have been demonstrated in the context other exposures, such as maternal malnutrition, to be directly associated with poor fetal growth and placental abnormalities (Barker 2004), suggesting a potential shared mechanism across different maternal/developmental/placental stressors and latent health outcomes. Given the complex interplay between placenta, PFAS-sensitive organ systems influencing maternal health, and PFAS-sensitive latent health outcomes in adulthood, pregnant women and their developing offspring should continue to be considered the most sensitive populations when conducting PFAS health and risk assessments.

3. Health Effects-Based Risk Evaluations of PFAS in the U.S.

Human health risk assessments of PFAS have been hindered by a paucity of data, which was first remedied by reports and publications resulting from the C8 Health Study. These data provided the foundation for preliminary risk evaluations and subsequent decision-making processes. The first U.S. actions taken to limit the use and emission of PFAS began in 2000 when the company 3M voluntarily phased PFOS out of production and use. Then in 2006, the U.S. EPA invited eight major fluoropolymer and fluorotelomer manufacturing companies to participate in a global stewardship program to commit to a 95% reduction of PFOA emissions and product by the year 2010 and a complete elimination by 2015 (EPA 2006). To meet these program goals, companies ceased manufacturing and importation of long-chain PFAS and/or transitioned to alternative chemicals while some companies exited the PFAS industry altogether. The impact of this phaseout can be appreciated by temporal declines in human serum PFOA and PFOS concentrations in datasets such as the NHANES; between 1999 and 2014, average PFOA and PFOS serum levels in the general U.S. population declined by ~60% and ~80%, respectively (ATSDR 2017).

Despite the reduction in serum burden of PFOA and PFOS since the genesis of the stewardship program, a recent biomonitoring effort (Hu et al. 2016) determined that drinking water sources for as many as six million U.S. citizens exceed the U.S. EPA-derived lifetime drinking water Health Advisory Level (HAL) for PFOA alone or in combination with PFOS of 70 parts per trillion (ppt) (822-R-16-005 US EPA). However, this number likely underestimates the number of U.S. citizens with drinking water exposure above the PFOA/PFOS HAL as the sampling method used by Hu et al. (2016) was conducted under the scope of the third Unregulated Contaminant Monitoring Rule (UCMR3), which limits drinking water PFAS measurements to public systems serving over 10,000 people. The exposure levels for U.S. citizens on private well water or public water systems serving under 10,000 people have yet to be characterized.

The PFOA/PFOS HAL represents a non-enforceable exposure level under which no adverse health outcomes would be expected given a lifetime of exposure, based on the U.S. EPA's risk assessment of PFOA, which used a weight of evidence approach to examine existing human epidemiologic and animal toxicity studies. Data from a carefully conducted developmental and reproductive toxicity study was selected to obtain the reference dose (RfD) used in calculation of the lifetime HAL for PFOA, in order to generate the most protective exposure level (822-R-16-005 US EPA; Lau et al. 2006).

The lack of federally enforceable regulations on any PFAS in the U.S., as well as increasing concerns over local contamination levels has led several states to implement their own regulatory actions based on varied health effects (Figure 5). The states of California, Connecticut, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, North Carolina, and Vermont have each proposed various drinking water actions to address PFAS contamination (ASDWA 2020). New Hampshire and New Jersey have formally adopted statewide regulations, mandating drinking water levels for PFOA (NH: 12 ppt), PFOS (NH: 15 ppt), PFHxS (NH: 18 ppt), and PFNA (NH: 11 ppt; NJ: 13 ppt) substantially lower than the U.S. EPA HAL of 70 ppt for PFOA alone or combined with PFOS (ASDWA 2020). Other states, such as North Carolina and Michigan, have proposed health advisories or maximum contaminant levels (MCLs) for replacement PFAS, such as GenX (NC: 140 ppt; MI: 370 ppt) (ASDWA 2020) while Ohio and Washington are in the process of developing draft health advisories for various PFAS (OH EPA 2019; WA DOH 2020). In August 2020, Michigan set new limits for several more PFAS: PFNA (6-ppt); PFOA (8-ppt); PFOS (16-ppt); PFHxS (51-ppt); PFBS (420-ppt); PFHxA (400,000-ppt).

Regulation of PFAS as the statewide level has resulted in disparate regulatory standards with regards to both the drinking water limit and extent to which regulations may be enforced. PFAS contamination in the environment and drinking water is driven by point sources of pollution, such as industrial/manufacturing sites, airports, biosolids fields, and military training bases. At each site, different levels of a multitude of PFAS are used in complex mixtures, depending on the application. The heterogeneity of the exposure mixture as well as relative concentrations of individual PFAS adds to the complexity of both studying PFAS effects of human health and setting appropriate standards to adequately protect the most vulnerable members of society. There is little to no toxicity data for the vast majority of

PFAS currently in use, therefore there is great need for efficient methods to evaluate these compounds.

4. Conclusions

The complex family of PFAS compounds presents unique challenges to toxicologists and risk assessors. The effects of PFAS on human health differ based on compound, impact multiple overlapping biological systems, affect health outcomes at all life stages, and exposure levels (and mixtures) differ temporally and geographically. Importantly, early life PFAS insults may in fact disrupt placental growth and function, thereby increasing susceptibility for later life chronic health conditions, which may be further exacerbated by lifelong PFAS exposure. Thus, it is critical to improve our understanding of the developmental and adult health consequences associated with PFAS exposure over time, identify emerging PFAS threats to sensitive subpopulations, and develop tools to efficiently evaluate and characterize PFAS toxicity on sensitive targets such as the placenta. Future research is needed to determine if latent health effects of PFAS exposure are programmed or mediated by the placenta. Going forward, experimental toxicology studies designed to formally assess the placenta are needed to determine the sensitivity of this tissue towards PFAS and explore the molecular mechanisms of placental toxicity.

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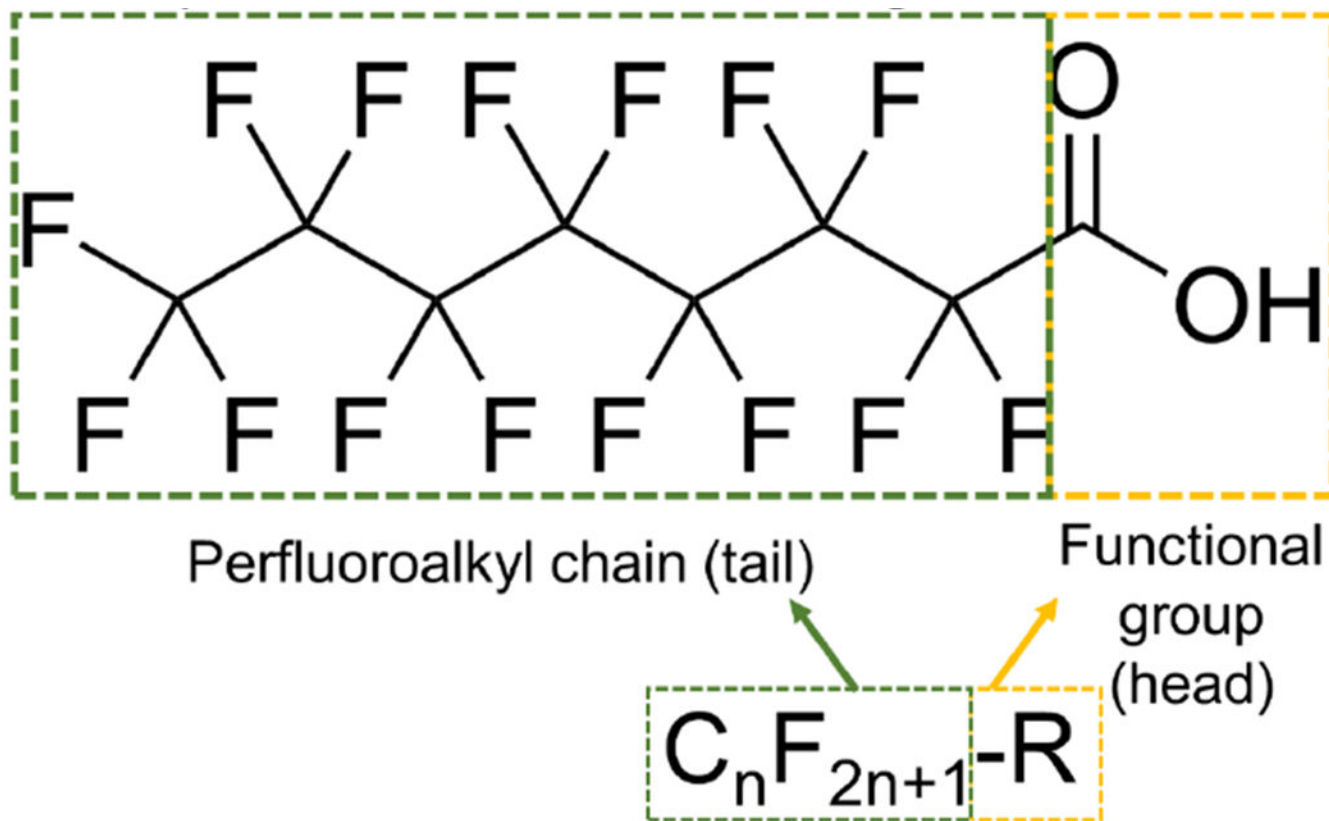
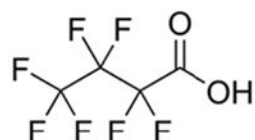
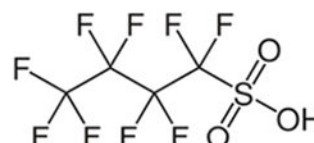


Figure 1. Basic structural features of a perfluoroalkyl substance (PFAS).

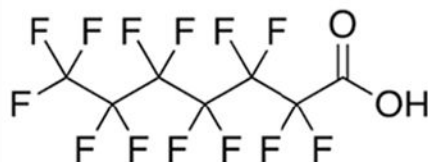
The compound perfluorooctanoic acid (PFOA) is shown here as an example. The perfluoroalkyl chain (tail) is indicated by a dashed green outline, while the functional group (head) is indicated by a dashed yellow outline. Legacy PFAS share these structural features while replacement, or “alternative chemistry” PFAS, contain substitutions along the carbon tail.



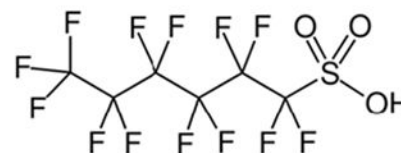
Perfluorobutanoic acid (PFBA)



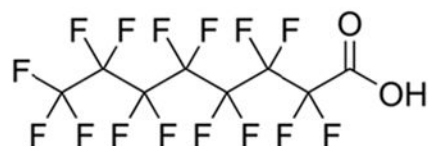
Perfluorobutane sulfonic acid (PFBS)



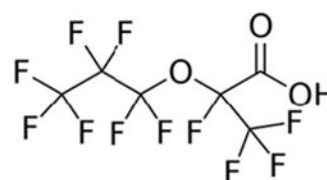
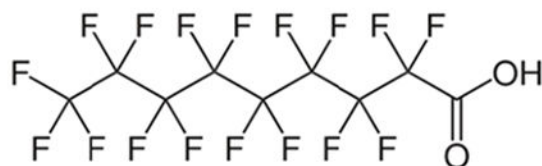
Perfluoroheptanoic acid (PFHpA)



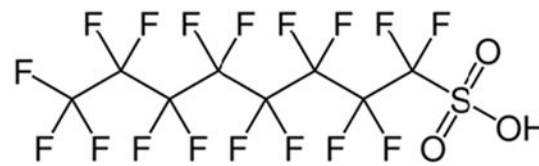
Perfluorohexane sulfonic acid (PFHxS)



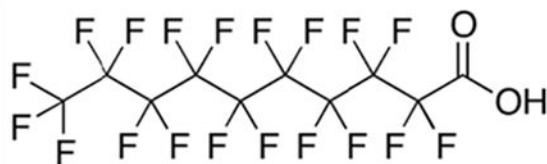
Perfluorooctanoic acid (PFOA)

Hexafluoropropylene oxide dimer acid
(HFPO-DA or GenX)

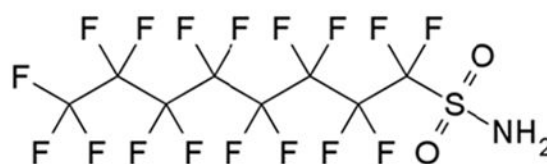
Perfluorononanoic acid (PFNA)



Perfluorooctane sulfonic acid (PFOS)



Perfluorodecanoic acid (PFDA)



Perfluorooctanesulfonamide (PFOSA)

Figure 2. Structures of common legacy perfluoroalkyl substances (PFAS) and replacement PFAS. The ether substitution in the carbon tail of hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX) is thought to favorably alter the toxicokinetic profile of the compound. Perfluorobutane sulfonic acid (PFBS) has classic structural features but has been selected as a replacement compound for longer-chain PFAS.

(2019) and Hu et al. (2016).

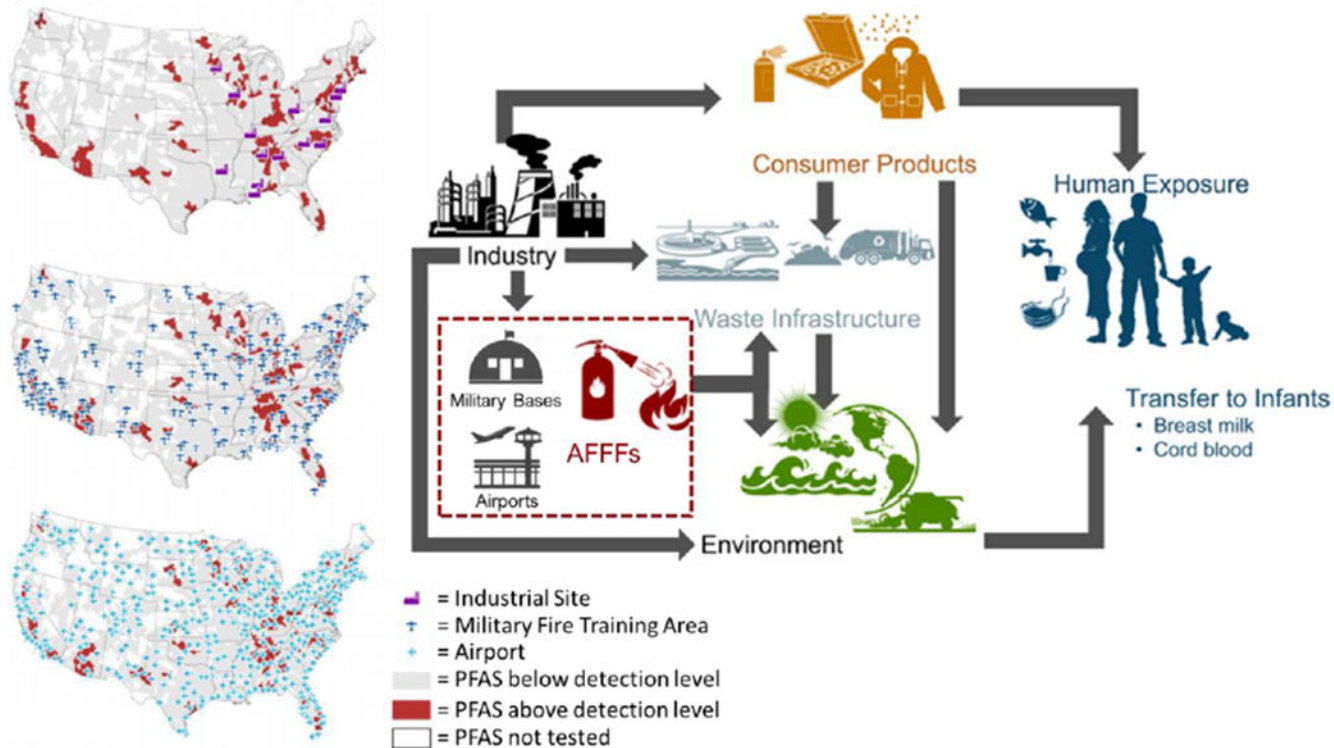


Figure 3. Major sources of human exposure to per- and polyfluoroalkyl substances. Humans are directly exposed to PFAS through the ambient environment (e.g. air), consumer products, house dust, drinking water, and diet. For developing humans, exposure can occur transplacentally *in utero* and through breastmilk. Environmental PFAS contamination is caused by waste and pollution generated by industrial complexes in the manufacturing or use of PFAS, including in the manufacturing of downstream products containing PFAS, such as aqueous filmforming foams (AFFFs). Environmental PFAS contamination is also caused by run-off of PFAS-containing AFFFs at military training bases and airports. Figure adapted from Sunderland et al. (2019) and Hu et al. (2016).

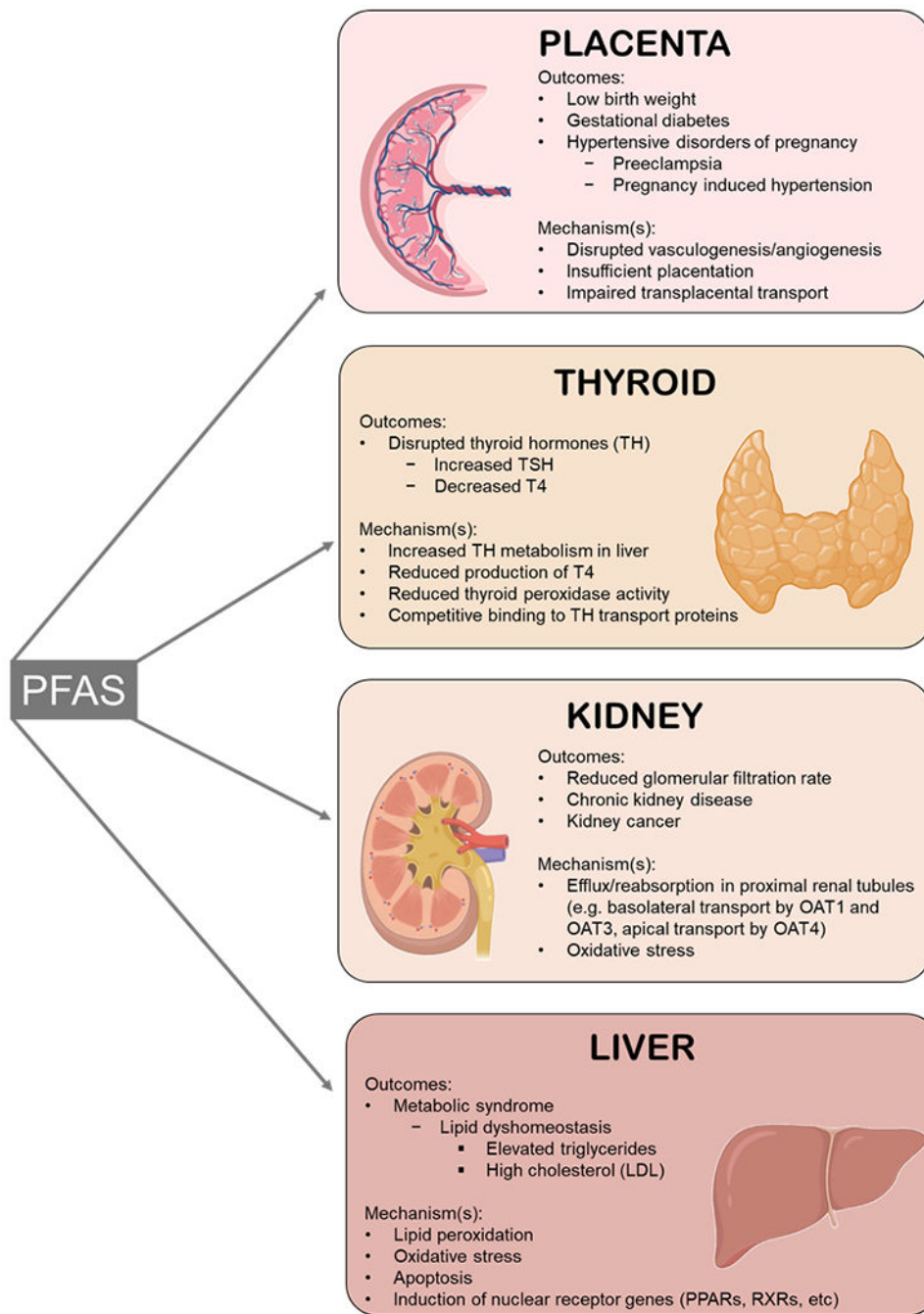


Figure 4. Summary of adverse health outcomes associated with PFAS exposure, the target tissue implicated by the outcomes, and hypothesized mechanism(s) of PFAS toxicity.
Image created using BioRender.

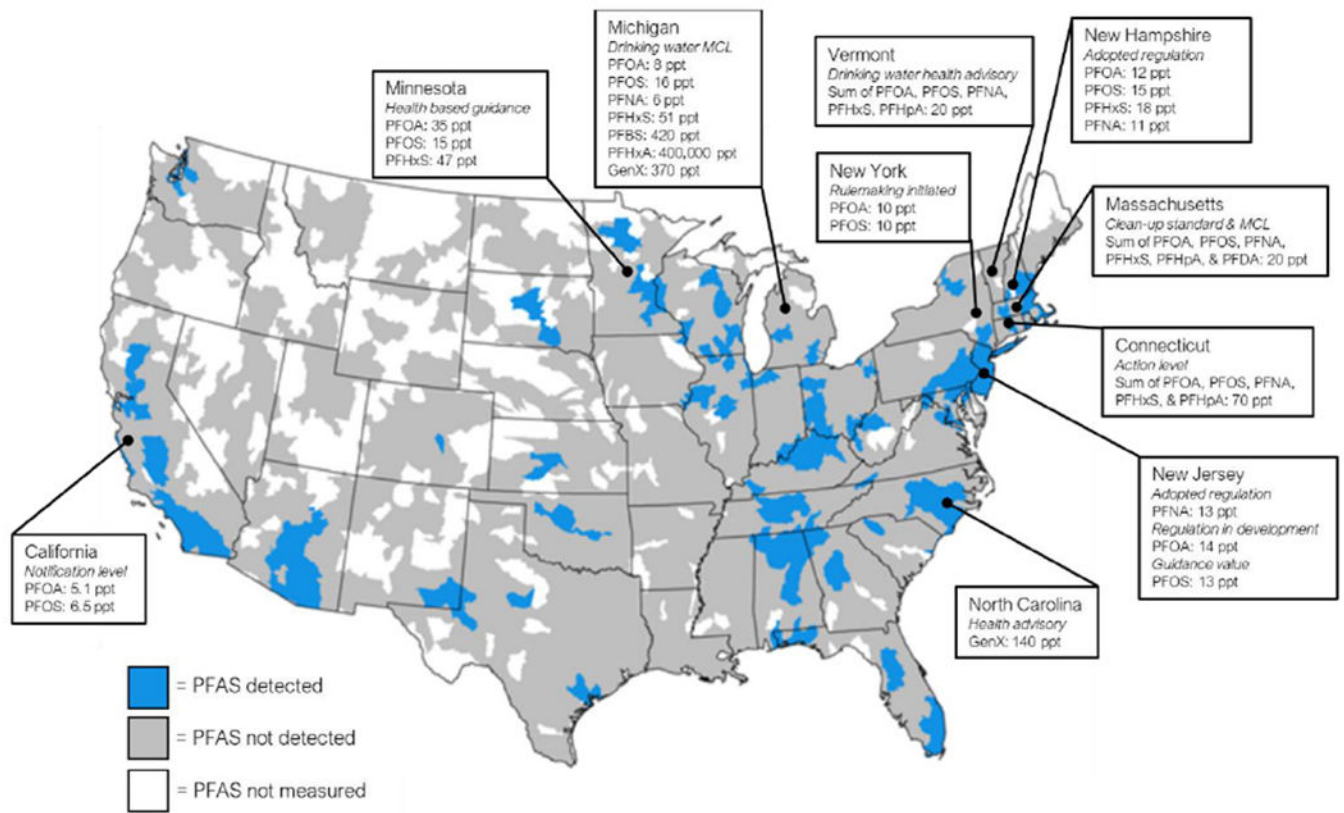


Figure 5. Responses of U.S. states to PFAS contamination compared to nationwide PFAS levels (inset).

Most state actions are non-enforceable and include notification levels, maximum contamination limits (MCLs), health advisories or guidance, and action levels. Several states have adopted enforceable regulation, such as New Hampshire and New Jersey, while other states are actively in pursuit of enforceable legislation, such as New York. There are some states with considerable PFAS contamination but no statewide action, including Alabama, New Mexico, and Kentucky. Figure adapted from Hu et al. (2018).

Table 1.

Common legacy per- and polyfluoroalkyl substances.

Chemical name	CAS	Abbr	Family	# Carbons w/fluorine	Carbon chain length	Type
Perfluorooctanoic acid	335-67-1	PFOA	PFCA	7	8	Legacy
Perfluorohexanoic acid	307-24-4	PFHxA	PFCA	5	6	Legacy
Perfluoropentanoic acid	2706-90-3	PFPeA	PFCA	4	5	Legacy
Perfluorheptanoic acid	375-85-9	PFHpA	PFCA	6	7	Legacy
Perfluorononanoic acid	375-95-1	PFNA	PFCA	8	9	Legacy
Perfluorodecanoic acid	335-76-2	PFDA	PFCA	9	10	Legacy
Perfluorobutanoic acid	375-22-4	PFBA	PFCA	3	4	Legacy & replacement
Perfluorobutane sulfonic acid	375-73-5	PFBS	PFSA	4	4	Legacy & replacement
Perfluorohexane sulfonic acid	355-46-4	PFHxS	PFSA	6	6	Legacy
Perfluorooctane sulfonic acid	1763-23-1	PFOS	PFSA	8	8	Legacy
Perfluorooctane sulfonamide	754-9-6	PFOSA	PFSA	8	8	Legacy

Abbr: Perfluorocarboxylic acid = PFCA, perfluorosulfonic acid or amide = PFSA