HEALTH IMPACTS OF PER- AND POLYFLUOROALKYL SUBSTANCES: LITERATURE REVIEW

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INTRODUCTION

The per- and polyfluoroalkyl substances (PFAS) are a family of chemicals used in industrial manufacturing and consumer products. They have been widely employed since the 1950's. Between 5,000 and 10,000 different PFAS are now or have previously been in use. In the early 2000's there was compelling evidence that these compounds were accumulating in the environment and present in detectable levels in virtually all humans and wildlife across the globe. Recognizing this, regulatory agencies in the United States and Europe reached an agreement with manufacturers to phase out these agents by 2010. Manufacture of the long chain ($\geq$7 carbon atoms) PFAS has been discontinued, but they have been replaced by short-chain polyfluoroalkyl substances (4-6 carbon-fluoride molecules) including GenX, a 4-carbon chemical produced by Chemours, a subsidiary of Dupont. This review focuses upon the health impacts of PFAS with emphasis upon what we do and don't know about the newer short-chain chemicals.

CHEMICAL STRUCTURE AND PROPERTIES

All PFAS consist of a carbon backbone, typically 4-14 carbons in length, and a charged functional moiety (primarily carboxylate, sulfonate, or phosphonate). Hydrogen atoms are replaced by covalently bonded fluoride atoms. They can exist in the salt or acid form. Carbon-fluorine bonds are among the strongest in organic chemistry and are stable in air at high temperatures; nonflammable; not readily degraded by strong acids, alkalis, or oxidizing agents; and not subject to photolysis. PFAS do not exist in nature and there is no pathway for degradation in the environment, humans, or other life forms. Methods have been developed to remove the longer chain PFAS from water by adsorption, but shorter chains do not adsorb well to the columns and cannot be effectively and cost-efficiently removed by any large-scale technologies available at this time [15].

In general, long-chain refers to perfluorinated carboxylic acids (PFCAs) with eight or more carbons or polyfluorinated sulfonic acids (PFSAs) with six or more carbons. Short chain refers to PFCAs with seven or few carbons and PFSAs with five or few carbons. The most common PFAS are shown in Figure 1 adopted by the Organization for Economic Cooperation and Development and published by the Interstate Technology and Regulatory Council (ITRC) [14]:

Figure 1

Table 3-2. Short-chain and long-chain PFCAs and PFSAs

Short-chain PFCAs				Long-chain PFCAs				
PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA
PFBS	PFPeS	PFHxS	PFHpS	PFOS	PFNS	PFDS	PFUnS	PFDoS
Short-chain PFSAs		Long-chain PFSAs						

Perfluorohexane sulfonic acid (PFHxS), which contains six carbon atoms, occurs as a byproduct in the manufacture of long-chain PFAS and is frequently the shortest PFAS for which data are available in human health studies. Although the ITRC categorizes it as a long-chain PFCA, it will be included with data for short-chain PFAS for purposes of this review, since most studies analyze it in that category.

The most important short-chain alternatives among the perfluorocarboxylic acids (PFCAs) are perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA) and their precursors. The most important short-chain PFSAs include the perfluoroalkyl sulfonic acids such as perfluorobutane sulfonic acid (PFBS) and perfluoropolyether sulfonic acid (PFPeS), which also exist as various salts. Hundreds of derivatives of these compounds are in use.

EVALUATING THE HEALTH RISKS OF PFAS

While there is extensive evidence of elevated PFAS levels in humans, the impacts upon health are less well defined. Strategies for identifying health risks are generally based upon in vitro cell biology studies, animal models, and epidemiologic data. Epidemiologic data are usually based upon high-risk populations including workers with occupational exposure, people living in proximity to manufacturing facilities, or populations exposed to major contamination sites. General population studies are also studied and often used for comparisons. General population studies have been used more in recent years as researchers recognize the widespread exposure in water and in the food chain.

In Vitro Assays

In vitro PFAS assays have been performed in an attempt to model toxicological risks. However, none of these have been shown to have any relevance to absorption, elimination, bioaccumulation, or toxicology in humans and will not be discussed in this review. Detailed information is available in several of the literature reviews and regulatory agency documents cited in this review and in the bibliography [4,5,15,24].

Animal Studies

Extensive studies have been performed in animal models, primarily rodent with some rabbit and monkey studies. Detailed information on studies in animal models is included in the literature reviews and regulatory agency publications. However, there is a growing consensus that animal models do not predict distribution, elimination, bioaccumulation or toxicity in

humans and that there are wide variations among the different PFAS formulations. Selected animal studies are highlighted in this review.

Human Studies

Prior to 2010 there were very few studies looking at human health risks. Lau et al published a review in 2007 looking at monitoring and toxicological findings [16]. They documented detectable levels of several PFAS in human serum, plasma, whole blood and milk. Even though these studies predated the development of short-chain PFAS, some studies detected elevated levels of PFBS (C4) and PFHxS (C6). These were presumed to be manufacturing byproducts or degradation products.

In the past 10 years there have been a growing number of human studies. Most of these have looked at high-risk populations, although some have used established population studies.

GenX

In 2010 DuPont introduced a new line of products intended to replace the phased out, longer chain PFAS. GenX is a brand name for a chemical process that uses ammonium 2,3,3,3-tetrafluoro2-(heptafluoropropoxy)-propanoate (FRD-903) to produce fluoroalkyls used in the production of a variety of substances including food packaging, paints, cleaning products, non-stick cookware, water-repellant clothing, cosmetics and fire-fighting foam. GenX is not covered by the consent decree with the EPA.

Chemours, a subsidiary of DuPont, published a stewardship flyer in 2010 [6]. Toxicity studies were performed for GenX, its raw materials, and one of its degradation products (PFHxA). Genetic toxicity was analyzed by the Ames method (bacterial reverse mutation) and assays of chromosomal alterations in mammalian cells. Mammalian studies were performed on monkeys, mice, rats and rabbits. They concluded that none of the products assayed was expected to be harmful to human health or the environment at environmentally relevant concentrations.

Chemours' bioelimination studies looked at rates of elimination of PFBS (4-carbon acid) and PFHxA (6-carbon sodium salt) in rats and monkeys. Both compounds were rapidly eliminated in rats. However, the monkey study demonstrated that while PFHxA was more rapidly eliminated, PFBS persisted at significant levels at 48 hours. The stewardship document states that short-chain perfluoroalkyls are not bioaccumulative, and this is also stated on the EPA website. However, this assumption has been challenged by subsequent reports noted later in this review. It is also complicated by the large number of PFAS compounds currently in use. Chemours looked at only one of these (6:2 FTS) in their analysis.

Since publication of this document, DuPont has submitted over 70 reports to the EPA. These are available on the EPA website (https://hero.epa.gov/hero/index.cfm/project/page/isws/false/search/true/project_id/2627/ [10]. None of the adverse data in these reports have been updated in the stewardship

document. They included several findings that were not reported in the initial report. These were based upon analysis of male and female rodents exposed to doses ranging from 0.1 mg/kg/day to 5mg/kg/day and included:

- Verification that biodegradation of the test substance was 0%
- Body weight increases in male rodents that were attributed to increases in liver weight
- Increases in bile acids and liver enzymes
- Abnormalities in liver cells
- Increased numbers of platelets
- Decreased cholesterol
- Adrenal hypertrophy
- Increased kidney weight with minimal tubular epithelial hypertrophy
- Reduced spleen weight
- Dermal sensitization

One of these submissions was a 4,000-page report submitted by DuPont looking at chronic toxicity and cancers in mice (H-28548: Combined Chronic Toxicity/Oncogenicity Study 2-Year Oral Gavage Study in Rats). The no-observed-adverse-effect level (NOAEL) for chronic disease for males was 1mg/kg/day and for females 50 mg/kg/day. The toxicities in males included hepatic (focal cystic degeneration, focal necrosis, centrilobular necrosis, increased liver enzymes), pancreatic acinar cell tumors, and testicular interstitial cell tumors. In females they included mild decreases in red cell mass, liver disease, hyperplasia and/or inflammation in non-glandular stomach and tongue and hepatocellular adenomas and carcinomas. The toxicities increased with increasing dose and exposure. They concluded that these risks were not a concern in the doses to which humans would be exposed, and that non-genotoxic peroxisome proliferators (i.e., chemicals that do not directly damage DNA) likely had no relevance in humans. This last assertion does not conform with the literature. As an example, a review conducted by Soeteman-Hernandez et al found that human non-genotoxic carcinogens were present in 12% (45/371) of the IARC's Groups 1, 2A and 2B carcinogens, and that a potential hazard was associated with 27% (12/45) of them [22].

Another study done by Gomis et al in 2018 challenged these toxicokinetic studies [11]. They evaluated to what extent differences in toxicological effect thresholds for PFAAs and fluorinated alternatives were confounded by differences in their distribution and elimination kinetics. Using their toxicokinetic model for male rats, they ranked toxicity for serum (GenX>PFOA>PFHxA>PFBA) and liver (GenX>PFOA≈PFHxA≈PFBA) concentrations. They concluded that some fluorinated alternatives have similar or higher toxic potency than their predecessors when correcting for differences in toxicokinetics.

CONTAMINATION SITES

Parkersburg, West Virginia

The largest study of a high-risk population was carried out by the C8 Science Panel between 2005-2013 [5]. This panel was created as part of a class action settlement against DuPont, whose Washington Works plant in Parkersburg, West Virginia, emitted PFOA into the air and water from the 1950s until the early 2000's. The C8 Health Project interviewed and collected blood samples from about 69,000 people living in the affected area. They then analyzed the data and determined whether there were "probable links" to a list of disease processes. Their analysis included an extensive literature review, which factored into their determinations. Detailed information and references are posted on their website (www.c8sciencepanel.org). They found probable links to the following diseases:

- Elevated cholesterol
- Kidney cancer
- Testicular cancer
- Ulcerative colitis
- Thyroid disease
- Pregnancy-induced hypertension (although not reproduced)
- Preeclampsia (weak association that increased with more recent pregnancies)

They did not find links to these diseases:

- Hypertension
- Coronary artery disease
- Chronic kidney disease
- Liver disease
- Osteoarthritis
- Parkinson's disease
- Other autoimmune diseases (rheumatoid arthritis, lupus, type 1 diabetes, Crohn's disease, multiple sclerosis
- Common infections (including influenza)
- Neurodevelopmental disorders in children
- Asthma or chronic obstructive pulmonary disease
- Stroke
- Birth defects
- Miscarriage and stillbirths
- Pre-term birth and low birth weight

The West Virginia registry has been used for several subsequent studies that have, in addition to the above, supported other findings suggesting alterations in humoral immunity.

Steenland and Woskie did a follow-up study of this cohort in 2010 [23]. They looked at the mortality of 5,791 workers at the DuPont plant using a job exposure matrix based on serum data for 1,308 workers from 1979-2004. The estimated average serum PFOA level was 350 ng/mL. In comparison to other DuPont workers, the standardized mortality ratio (SMR) was elevated for mesothelioma (SMR 2.85, 95% CI), diabetes mellitus (SMR 1.90, CI 1.35), and chronic renal disease (SMR 3.11, 95% CI). Significant positive exposure-response trends occurred for both malignant and non-malignant renal disease (12 and 13 deaths, respectively). The authors noted that results were limited by small numbers and restriction to mortality.

Barry et al did another study of this cohort in 2013 looking specifically at cancers among adults living near the plant [1]. They found that PFOA exposure was associated with kidney and testicular cancer in this population. They alluded to studies showing both renal and testicular toxicity in rodent studies. The investigators noted that this was largely a survivor cohort. As they were not able to link PFOA levels and deaths in this cohort and did not include them in this study, the possibility of links to other more uniformly fatal cancers, such as lung and pancreatic cancers, could not be excluded.

Looker et al studied immune function in a cross-section of the C8 cohort in 2010, at which time serum levels of PFOA were substantially reduced but still well above those found in the general population [17]. They found that elevated PFOA serum concentration was associated with a reduced rise in influenza virus vaccination titers and an increased risk of not attaining the antibody threshold considered to offer long-term protection.

The long-term human health risks of PFOS in general have not been well defined. Follow up for the C8 cohort is now out 5 years from completion, and studies of this population are ongoing.

Washington County, Minnesota

Drinking water in Washington County, Minnesota (east of St. Paul), was contaminated by a 3M Company plant in Cottage Grove that manufactured Scotchgard. Although the health impacts on this population have not been studied as extensively, some data are available. Grandjean and Clapp published a review in 2015 that included the 3M workers [12]. They noted that the DuPont cancer surveillance system has been monitoring cancer incidence in workers as far back as 1956 and that an internal report covering the years 1956 – 2002 showed excess kidney cancer (SMR 2.3), bladder cancer (SMR 1.93), and myeloid leukemia (SMR 2.25). There was also an elevated but not statistically significant risk of testicular cancer (SMR 2.3).

Since 2002, the Minnesota Department of Health has partnered with the Minnesota Pollution Control Agency to investigate PFAS in Minnesota. This work began with drinking water investigations near the 3M Cottage Grove plant and related legacy waste disposal sites in Washington County. In February of this year 3M settled a lawsuit with Minnesota for $850,000.

Wilmington, North Carolina

In 2017 residents of Wilmington, NC, discovered that the Chemours Plant in Fayetteville had been discharging GenX into the Cape Fear River since 1980. Chemours claimed that this was not due to discharge of the chemical but rather discharge of the byproduct of another industrial process in another area of the plant (not the area currently producing GenX and not covered under the EPA consent decree). This revelation has resulted in a series of investigations and litigation.

A group of North Carolina and Eastern Carolina University researchers obtained National Institutes of Environmental Health Sciences funding to test for PFAS levels in drinking water and in blood and urine in people living in the Cape Fear River basin. In the first phase, the GenX Exposure Study enrolled 345 people age 6 or older who had lived in New Hanover County since November 2016 [19]. Their primary drinking water source had to be provided by the Cape Fear Public Utility Authority. Pregnant women were excluded. Samples were obtained between November 3 and December 8, 2017. They looked at the following PFAS:

Table 1: Newly identified ether PFAS

	Short Name	Chemical Name	Chemical Formula	CAS Number
1	GenX	Perfluoro-2-propoxypropanoic acid	$C_6HF_{11}O_3$	13252-13-6
2	Nafion byproduct 1		C7HF13O5S	29311-67-9
3	Nafion byproduct 2	Ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-	$C_7H_2F_{14}O_5S$	749836-20-2
4	Nafion byproduct 4	2,2,3,3,4,5,5,5-4-(1,1,2,2-tetrafluoro-2-sulfoethoxy)pentanoate	$C_7H_2F_{12}O_6S$	No CAS
5	PFO2HxA	Perfluoro(3,5-dioxahexanoic) acid	$C_4HF_7O_4$	39492-88-1
6	PFO3OA	Perfluoro(3,5,7-trioxaoctanoic) acid	$C_5HF_9O_5$	39492-89-2
7	PFO4DA	Perfluoro(3,5,7,9-tetraoxadecanoic) acid	$C_6HF_{11}O_6$	39492-90-5
8	PFO5DoDA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	$C_7HF_{13}O_7$	39492-91-6
9	Hydro-EVE	2,2,3,3-tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy)propanoic acid	$C_8H_2F_{14}O_4$	773804-62-9
10	PMPA	Perfluoromethoxypropyl carboxylic acid	$C_4HF_7O_3$	13140-29-9
11	NVHOS	1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate	$C_4H_2F_8O_4S$	801209-99-4 (free acid)
12	PEPA	Perfluoroethoxypropyl carboxylic acid	$C_5HF_9O_3$	267239-61-2

Table 2: Carboxylic acids, sulfonic acids and one fluorotelomer sulfonate

	Short Name	Chemical Name	Chemical Formula	CAS Number
1	PFBA	Perfluorobutanoic acid	$C_4HF_7O_2$	375-22-4
2	PFPeA	Perfluoropentanoic acid	$C_5HF_9O_2$	2706-90-3
3	PFHxA	Perfluorohexanoic acid	$C_6HF_{11}O_2$	307-24-4
4	PFHpA	Perfluoroheptanoic acid	$C_7HF_{13}O_2$	375-85-9
5	PFOA	Perfluorooctanoic acid	$C_8HF_{15}O_2$	335-67-1
6	PFNA	Perfluorononanoic acid	$C_9HF_{17}O_2$	375-95-1
7	PFDA	Perfluorodecanoic acid	$C_{10}HF_{19}O_2$	335-76-2
8	PFBS	Perfluorobutane sulfonic acid	$C_4HF_9SO_3$	375-73-5
9	PFHxS	Perfluorohexane sulfonic acid	$C_6HF_{13}SO_3$	355-46-4
10	PFOS	Perfluorooctane sulfonic acid	$C_8HF_{17}SO_3$	1763-23-1
11	6:2 FTS	6:2 fluorotelomer sulfonate	$C_8H_5F_{13}SO_3$	27619-97-2

Letters were sent to participants in April 2018 with tap water results. An example is posted on their web site. The first public report was issued in a press release on November 14, 2018. They found newly identified PFAS including Nafion byproduct 2, PFO4DA, PFO5DoDA and Hydro-EVE in 99, 98, 98 and 76 percent of blood samples, respectively. None of these four substances was found in 20 women living in the Raleigh-Durham area in 2008-9 or from 24 Dayton, Ohio residents with high PFAS exposure between 1992 and 2014. GenX was not detected in blood samples, but elevated levels of other well-known long-chain PFAS were found. The median levels for the newer PFAS chemicals fell from November 2017 to May 2018, but it wasn't clear if the 44 people tested both times continued drinking the area's tap water after learning of GenX's discovery. Community Engagement Events were held in November 2018 to discuss the findings. At this time the study does not address the health impacts. The implications of the new PFAS found so far in this study, all of which had 6 or more carbon atoms, are also unclear.

POPULATION STUDIES

Multiple population studies and reviews have been published in the United States and internationally looking at the health impacts of PFAS. Most of these focus upon long-chain PFAS, but some include PFHxS or the newer 4, 5, and 6-carbon chains. Selected studies are described below. Other studies are referenced in the Toxicological Profile for Perfluoroalkyls described later in this review [24]. Four of these studies are highlighted below.

Grandjean et al looked at a cohort of 656 births in the Faroe Islands during 1997-2000. They determined prenatal levels at 32 weeks gestation and assayed antibody responses to childhood immunizations at ages 5 and 7 [13]. They found that elevated exposures to PFAS in these children were associated with reduced humoral immune response to immunizations.

In 2017 Rappazzo et al published a systematic review of PFAS exposure and childhood health outcomes [21]. The review focused primarily upon PFOA, PFOS and other long-chain PFAS, but some of the studies included PFHxS and PFBS. They observed generally consistent evidence of association with dyslipidemia, impaired immunity including vaccine response, asthma, decreased renal function and higher age at menarche.

Ye et al used U. S. National Health and Nutrition Examination Survey (NHANES) data to look at PFAS levels (PFOS, PFOA, PFHxS and PFNA) in a representative sample of 639 3-11-year-old participants from 2013-2014 [25]. They detected all four chemicals in concentrations similar to those of NHANES 2013-2014 adolescents and adults, suggesting prevalent exposure to these PFAS or their precursors among this age group, most of whom were born after the phase out of PFOS in the United States in 2002. They also noted concentration differences by sex, race/ethnicity and age suggesting lifestyle differences that might impact exposure.

In November 2018 the Journal of Endocrinology & Metabolism published an Advance Article online with research findings by Di Nisio et al [7]. The Veneto region of Italy is heavily polluted with PFAS. The researchers did a cross-sectional study on 212 exposed male high school students from this region compared to 171 non-exposed controls. They looked at anthropometric measurements, seminal parameters, and sex hormones and also performed biochemical studies in established experimental models. They found that increased levels of PFAS in plasma and seminal fluid positively correlated with a reduction in semen quality, testicular volume, penile length and anogenital distance. They noted that PFAS interfere with hormonal pathways, potentially impacting fertility.

LITERATURE REVIEWS AND SCIENTIFIC STUDIES

There is one extensive literature review on short-chain PFAS conducted by the Danish Ministry of the Environment published and posted online in 2015 [15]. The review highlights differences among the short-chain PFAS. Several of these differences were noted in studies performed after publication of Chemours' initial stewardship document. They noted that in general the mean blood elimination half-lives for PFAS depend upon the chemical substance and the animal species and its sex. Blood half-lives are longer for sulfonates than carboxylates, increase with chain length for carboxylates, are shorter for branched isomers, and in animals are often shorter in females due to sex hormone dependent differences in renal clearance. The serum half-lives of PFAS are dose-dependent with longer half-lives for the lower concentrations relative for humans. In general blood elimination half-lives of PFAS in rodents were hours or a few days, in monkeys a little longer and in humans much longer and often years. The blood elimination half-lives of PFAS decrease with shorter chain length. An exception is PFHxS (C6), which has a longer half-life in humans than PFOA and PFOS (C8). An overview of serum half-lives is provided in the Figure 2 below:

Figure 2

TABLE 3-3
OVERVIEW OF SOME SERUM ELIMINATION HALF-LIVES OF SHORT CHAIN PFAS

Species	Substances							
	PFBS		PFHxS		PFBA		PFHxA	
	Male	Female	Male	Female	Male	Female	Male	Female
Rat	<4.5 days	<4 days	29 days	1 day	9 hours	2 hours	1.6 hours	0.6 hours
Mouse			30.5 days	24.8 days	5-16 hours	3 hours	1 hour	
Monkey	95 hours	83 hours	141 days	87 days	40 hours	41 hours	14-47 hours	
Human	24 days	46 days	8.5 years		72 hours	87 hours	32 days	

The primary route of elimination of PFAS is renal. A main reason for the longer half-life in humans compared to experimental animals is that humans have the highest percentage of renal tubular absorption (>99%). This calls into question the animal models used in Chemours' toxicology studies and their extrapolation to humans. Elimination for different PFAS is complex. Fluorotelomers are mainly eliminated through feces. PFBA with a C_3-perfluorocarbon chain is different and has a slower renal clearance than PFHxA. PFBS does not seem be very bioaccumulative ad has a much shorter half-life than PFHxS.

PFAS have a low affinity for lipids but bind to serum proteins, mainly albumin. They are distributed in plasma and in well-perfused tissues such as lung, liver, kidney and spleen, but also bone, testes and brain. The distribution of PFOS and PFOA in humans has not been studied extensively. Maestri et al used mass spectrometry to look at PFOS and PFOA levels in a human post-mortem study [18]. The highest levels of PFOS were in liver, blood, lungs and kidneys, and the highest levels of PFOA were in lungs, kidneys, liver and blood. Highest levels overall were for PFOA in liver – 13.6 ng/g).

There is one published study that includes short-chain PFAS bioaccumulation in humans. Perez et al looked at concentrations of 21 different PFAS in 99 samples of autopsy tissue from brain, liver, lung, bone and kidney [20]. Samples were from subjects who lived in Tarragona in Catalonia, Spain. Tarragona is a port city on the Mediterranean. It is not associated with known industrial exposures, but high levels of PFAS have been reported in soil, water and fish from its river. This study included several short chain PFAS including PFBA, PFBS, PFPeA, PFHxA, and PFHxS. The Danish review summarized the results for these substances and comparable results for PFOA and PFOS.

Figure 3

TABLE 3-1
DISTRIBUTION OF SHORT CHAIN PFAS IN 5 AUTOPSY TISSUES FROM 20 HUMAN INDIVIDUALS OF TARRAGONA, SPAIN (PEREZ ET AL. 2013).

PFAS substance	Mean concentrations ng/g w. w.				
	Liver	Bone	Brain	Lung	Kidney
PFBA	12.9	<LOD	13.5	304	464
PFBS	0.9	3.2	<LOD	17.8	8
PFPeA	1.4	0.8	<LOD	44.5	<LOD
PFHxA	11.5	35.6	18.0	50.1	5.6
PFHxS	4.6	1.8	3.2	8.1	20.8
Perfluorohexyl ethanoic acid (FHEA); metabolite of 6:2 FTOH	92.6	42.5	18.6	2.4	23.7
PFOA	13.6	60.2	<LOD	29.2	2.0
PFOS	102	<LOD	4.9	29.1	75.6

LOD = Limit of detection

This study reveals high concentrations of short-chain PFAS in human tissues. PFBA was present in strikingly high concentrations in lung and kidney, and three of the short-chain PFAS were present in higher concentrations than PFOA or PFOS in brain. These findings raise concerns that these chemicals behave differently in humans than in laboratory animals and challenge the toxicology studies used by Chemours to obtain approvals of the compounds.

The Danish review provides summaries of several studies looking at levels in human blood. Results vary widely, and it is difficult to draw general conclusions.

Studies looking at maternal to fetal transfer demonstrated significant correlation. The proportion of PFHxS was higher than PFOS in cord blood compared to maternal blood, indicating that shorter chain PFAS were transferred relatively more efficiently. This finding was confirmed in a later study. Lactational transfer does not appear to be significant.

Detailed information on individual short-chain PFAS is included in the review and will not be summarized in this document.

REGULATORY AGENCIES

PFAS in the United States are regulated by the EPA, the Agency for Toxic Substances and Disease Registry (ATSDR) in the Department of Health and Human Services, and by state Departments of Environmental Quality (DEQ). DEQs have been active in states impacted by the manufacture of PFAS or by site contamination.

In Europe PFAS are regulated under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). REACH was enacted in June 2007 with a mandate to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the assessment of substances in order to reduce the number of tests on animals. PFAS are a subject of concern internationally, and many countries have environmental and regulatory agencies actively involved in studying the health impacts of these chemicals.

US Government Regulatory Agencies

ATSDR has developed the Toxicological Profile for Perfluoroalkyls [24]. Drafts for public comment were published in May 2009, August 2015 and June 2018. The profile is 852 pages long and provides a very comprehensive review of the available data.

In its summary of health effects, ATSDR notes that comparison of the toxicity of PFAS across species is problematic due to differences in elimination half-lives, lack of adequate mechanistic data, species differences in the mechanism of toxicity for some endpoints, and differences in measurement of exposure levels between epidemiologic and experimental studies. They also note substantial differences in the rate of elimination of PFAS across species and cite data similar to the Danish review as summarized in Figure 4:

Figure 4

Table 1-1. Summary of Estimated Elimination Half-lives for Select Perfluoroalkyls

	Humans	Nonhuman primates	Rats[a]	Mice[a]
PFOA	8 years (Olsen et al. 2007a)	20.1–32.6 days (Butenhoff et al. 2004c)	Males: 44–322 hours Females: 1.9–16.2 hours	
PFOS	5.4 years (Olsen et al. 2007a)	110–170 days (Chang et al. 2012; Seacat et al. 2002)	179–1,968 hours	731–1,027 hours
PFHxS	8.5 years (Olsen et al. 2007a)	87–141 days (Sundström et al. 2012)	Males: 382–688 hours Females: 1.03–41.28 hours	597–643 hours
PFBuS	665 hours (Olsen et al. 2009)	8.0–95.2 hours (Chengelis et al. 2009; Olsen et al. 2009)	2.1–7.42 hours	
PFBA	72 hours (Chang et al. 2008b)	40.3–41.0 hours (Chang et al. 2008b)	1.03–9.22 hours	2.79–13.34 hours

This profile also points out as previously noted that the mechanism of toxicity of the PFAS have not been fully elucidated. Rodent studies suggest that activation of peroxisome proliferator-activated receptor-α (PPARα) may contribute to hepatotoxicity, immunotoxicity and developmental toxicity. However, humans and non-human primates are less responsive to

PPARα agonists. Epidemiologic studies use serum PFAS levels as a biomarker of exposure whereas experimental studies utilize concentrations. Evidence to date suggests that serum levels in humans might not accurately reflect bioaccumulation for some PFAS. They note that although physiologically based pharmacokinetic models have been developed for rodents and humans, these models are not sufficient to allow for comparisons between administered doses in laboratory animals and serum concentrations in humans.

The profile also provides additional data regarding the Washington Works facility in West Virginia. That study looked primarily at PFOA and PFOS. The average PFOA level for workers at the facility between 2001-2004 was 1,000 ng/ml. The mean PFOA level in highly-exposed residents without occupational exposure near the facility was 423 ng/ml in 2004-2005. The geometric mean concentration in the US population in 2005-2006 was 3.92ng/ml. They noted the same list of health outcomes including pregnancy-induced hypertension/pre-eclampsia, liver damage, increased serum lipids, increased risk of thyroid disease, decreased antibody response to vaccines, kidney and testicular cancer. In addition, they noted an increased risk of asthma, increased risk of decreased fertility and small decreases in birth weight. They sub-categorized the risk according to specific PFAS. PFHxS was implicated in liver damage and decreased antibody response to vaccines.

The profile lists 13 ongoing studies on PFAS. Some of these include PFHxS, but none focus upon short-chain PFAS.

Between June 25, 2018 and September 5, 2018, the EPA held a series of community engagement events in areas impacted by PFAS. These include Exeter, NH; Horsham, PA; Colorado Springs, CO; Fayetteville, NC; Leavenworth, KS; and the Tribal Lands and Environment Forum in Spokane, WA. A link to a representative presentation in North Carolina is included in the bibliography [8]. These forums included state DEQs and the ATSDR. Although they focused on PFOS and PFOA, the EPA indicated that a toxicity assessment is underway for GenX and PFBS.

The presentations provide information on the National Defense Authorization Act & 2018 Omnibus Appropriations. Ten million dollars has been allocated for fiscal year 2018 to conduct short term (<2 years) statistically-based PFAS biomonitoring exposure assessments (EAs) at no less than 8 current or former Department of Defense sites. EAs will include measurement of PFAS in serum and urine, as well as limited environmental sampling. An additional 10 million dollars has been allocated for long-term (5-7 year) studies. Details of the study have not yet been published, although it seems likely that it will include GenX and other short-chain PFAS since they were the major contaminants in the North Carolina area. The proposed study design is posted in slide presentations available online.

The June 2018 Toxicological Profile was delayed because of concerns about the discrepancies between EPA and ATSDR recommendations, which set lower limits for PFAS. The public comment period ended August 20, 2018. The EPA published a fact sheet on November 18, 2018 [9]. It focuses upon GenX chemicals and PFBS. They note that for GenX animal studies have

shown health effects in the kidney, blood, immune system, developing fetus, and especially in the liver following oral exposure. The data are suggestive of cancer. For PFBS animal studies have shown health effects on the thyroid, reproductive organs and tissues, developing fetus, and kidney following oral exposure. Overall, the thyroid and kidney are particularly sensitive to PFBS. The data are inadequate to evaluate cancer.

The EPA indicates that their studies thus far complete two of the four steps required to complete the toxicity assessment – hazard identification and dose-response. A management plan will not be finalized until an exposure assessment and risk characterization have been completed. The EPA has developed draft reference doses (RfDs) for PFBS and GenX chemicals based upon assumptions that PFBS is less toxic than GenX chemicals, PFOA, and PFOS; and that GenX chemicals are less toxic that PFOA and PFOS. The draft chronic doses of PFBS and GenX compared to PFOA and PFOS are indicated in Figure 5.

Figure 5

Chemical	Chronic RfD (mg/kg-day)
PFBS	0.01*
GENX chemicals	0.00008*
PFOA	0.00002
PFOS	0.00002
	*indicates draft value

It is unclear how the EPA developed these standards based upon information in the fact sheet. There is a 60-day public comment period during which the EPA does not plan to issue any regulations. They state that "EPA is making the draft toxicity assessments available to provide states, tribes and local governments with the tools they need to better understand PFBS and GenX chemicals. Once the assessments are issued, state, tribal, and local partners can use this information to help inform whether local actions are needed to protect public health."

REACH

Brendel et al published a review focusing upon environmental concerns of short-chain PFAS and a strategy for regulation under REACH [4]. They note that long-chain (C8-C14) PFAS are either regulated under REACH or currently being evaluated for regulation. Short-chain PFAS (including PFBA, PFBS and PFHxA) are not regulated and therefore being used more extensively. They write that short-chain PFAS have been assumed to have a lower bioaccumulation potential and improved environmental properties compared to long chain PFAS. However, short-chain PFAS are as persistent as long chain PFAS, have different but not less alarming properties of concern

and are already widely distributed in the environment. The concerns regarding short-chain PFAS include:

- Persistence in the environment
- Low adsorption potential and mobility
- Inability to remove from water by current technologies
- Potential for long-range transport
- Permanent exposure results in continuous and poorly reversible concentrations in organisms with a risk of adverse health effects on human and the environment, which will increase with increasing exposure
- Unknown distribution in terrestrial systems and along food chains, especially since they are known to enrich in edible parts of plants

The authors recommend including short-chain PFAS in regulation under REACH.

The Netherlands

Dordrecht in The Netherlands is the site of a Chemours plant that currently uses Gen X technology to produce PFAS. The Ministry of Health, Welfare and Sport (RIVM) commissioned a report to determine to what extent the three substances are harmful to people living near the factory [2]. They looked at three perfluorinated substances used or formed during the production of PFAS – FRD-903 (4-carbon), FRD-902 (4-carbon) and E1 (5-carbon). They concluded that it is not possible to reach a conclusion on the human bioaccumulation potential of FRD-902 or FRD-903 in the absence of data on the human clearance time. There are insufficient data for E1, but it does not contain a hydrophilic group and the human clearance time and bioaccumulation potential are expected to be higher than for PFOA. They concluded that classification as carcinogenic category 2 (suspected human carcinogen) is justified for FRD-903 and FRD-902. They did conclude that based on available data, inhalation of FRD-903 and FRD-902 by populations near the plant did not pose a health risk; however, there were insufficient data to evaluate risks related to E1. They did not have sufficient information to determine whether there was a possibility of exposure by contaminated drinking water in the vicinity of the plant.

SUMMARY AND DISCUSSION

There are extensive data indicating that as a group PFAS pose a serious risk to human health. Regulatory agencies have recognized the environmental and health risks of the long-chain polyfluoroalkyl substances and have taken measures to eliminate them. However, despite the halt in the manufacture and use of these chemicals, they will remain in the environment indefinitely and pose significant threats to humans and wildlife with persistent exposure.

Substitution with GenX and other short-chain PFAS poses similar, and possibly greater, threats due to their mobility and persistence in the environment and their pharmacokinetics in

humans. The health impacts are poorly defined. Current models are inadequate for evaluating health risks in humans, and initial safety studies done by Chemours have been widely discredited by researchers. Scientists and advocacy groups are actively petitioning to include the short-chain PFAS in overall PFAS regulations, both to phase them out of manufacturing and industrial use and to lower acceptable levels in the environment. The Madrid Statement, published in May 2015 and signed by over 200 scientists from around the globe, puts forth many of the concerns cited in this review [3]. They note in particular that little information is publicly available on the chemical structures, properties, uses and toxicological profiles of short -chain PFAS. They also raise the concern that increasing use of these fluorinated alternatives will lead to increasing levels of stable perfluorinated degradation products in the environment, and possibly also in the biota and humans, and that this would increase the risks of adverse effects on human health and the environment. Additional health studies are currently being conducted worldwide, but the extent of penetration and their persistence lend urgency to efforts to discontinue their production and use.

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