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> Jane L Espartero, Lore, Yamada, Miko, Ford, Judith, Owens, Gary, Prow, Tarl, Juhasz, Albert 2022 'Health-related toxicity of emerging per- and polyfluoroalkyl substances: comparison to legacy PFOS and PFOA', Environmental Research, vol. 212, article no. 113431, pp. 1-18

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Health-related Toxicity of Emerging Per- and Polyfluoroalkyl Substances: comparison to legacy PFOS and PFOA

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are highly persistent, manufactured chemicals used in various manufacturing processes and found in numerous commercial products. With over 9000 compounds belonging to this chemical class, there is increasing concern regarding human exposure to these compounds due to their persistent, bioaccumulative, and toxic nature. Human exposure to PFAS may occur from a variety of exposure sources, including, air, food, indoor dust, soil, water, from the transfer of PFAS from non-stick wrappers to food, use of cosmetics, and other personal care products. This critical review presents recent research on the health-related impacts of PFAS exposure, highlighting compounds other than Perfluorooctanoic acid (PFOA) and Perfluoroctane sulfonate (PFOS) that cause adverse health effects, updates the current state of knowledge on PFAS toxicity, and, where possible, elucidates cause-and-effect relationships. Recent reviews identified that exposure to PFAS was associated with adverse health impacts on female and male fertility, metabolism in pregnancy, endocrine function including pancreatic dysfunction and risk of developing Type 2 diabetes, lipid metabolism and risk of childhood adiposity, hepatic and renal function, immune function, cardiovascular health (atherosclerosis), bone health including risk for dental cavities, osteoporosis, and vitamin D deficiency, neurological function, and risk of developing breast cancer. However, while cause-and-effect relationships for many of these outcomes were not able to be clearly elucidated, it was identified that 1) the evidence derived from both animal models and humans suggested that PFAS may exert harmful impacts on both animals and humans, however extrapolating data from animal to human studies was complicated due to differences in exposure/elimination kinetics, 2) PFAS precursor kinetics and toxicity mechanism data are still limited despite ongoing exposures, and 3) studies in humans, which provide contrasting results require further investigation of the long-term-exposed population to better evaluate the biological toxicity of chronic exposure to PFAS.

Keywords: Health effects; Human health; Per- and Polyfluoroalkyl substances; PFAS; Toxicity

Funding

This review did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abbreviations: 6:2 Cl-PFESA, 6:2 Chlorinated polyfluorinated ether sulfonic acids; 6:2 diPAPs, 6:2 Fluorotelomer phosphate diester; 6:2 FTAB, 6:2 Fluorotelomer sulfonamide alkyl betaine; 6:2 FTAA, 6:2 Fluorotelomer sulfonamide alkylamine; 6:2 FTOH, Fluorotelomer alcohol; EtFOSAA, N-ethyl-perfluorooctane sulfonamido acetic acid; 6:2 triPAP, 6:2 tri-substituted Polyfluorinated phosphate ester; PFAS, Per- and Polyfluoroalkyl substances; PFBA, Perfluorobutanoic acid; PFBS, Perfluorobutane sulfonate; PFCs, Perfluorochemicals; PFCAs, Perfluorinated carboxylic acids; PFDA, Perfluorodecanoic acid; PFDoDA, Perfluorododecanoic acid; PFHpA, Perfluoroheptanoic acid; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluoroctane sulfonate; PFPeA, Perfluoro-n-pentanoic acid; PFSA, Perfluorosulfonic acid; PFUnDA, Perfluoroundecanoic acid; SAmPAP, Perfluorooctane sulfonamidoethanol based diester; NEtFOSE, N-ethyl perfluorooctane sulfonamido ethanol; NEtFOSA, N-ethyl perfluorooctanesulfonamide; NEtFOSAA, N-ethylperflurooctanesulfonamidoacetic acid; FOSE, Perfluorooctane sulfonamido ethanol; FOSA, Perfluorooctane sulfonamido acetic acid; PFOSA, Perfluorooctanesulfonamide; 6:2 FTMAc, 6:2 Fluorotelomer methacrylate. GenX, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid; ADONA, Ammonium 4,8-dioxa-3H-perflurononanoate; 8-ClPFOS, Sodium 8-chloroperfluoro-1-octanesulfonate; 6:6 PFPiA, Sodium bis(perfluorohexyl) phosphinic acid; PFODA, Perfluoro-n-octadecanoic acid; PFPrS, Sodium Perfluoro-1-propanesulfinate; PFHpS, Sodium Perfluoro-1-hepatanesulfonate; 8:2 DiPAP, Sodium bis(1H,1H,2H,2H-perfluorodecyl)phosphate; DiSAMPAP, Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl]phosphate; PFDoA, Perfluoro-n-dodecanoic acid; PFTeDA, Perfluoro-n-tetradecanoic acid; PFHxA, Perfluoro-n-hexanoic acid; PFTrDA, Perfluoro-n-tridecanoic acid; N-MeFOSAA, N-methylperfluoro-1-octanesulfonamidoacetic acid; PFHxPA, Perfluorohexylphosphonic acid; Cl-PFOPA, 8-Chloroperfluorooctylphosphonic acid; PFECHS, Potassium perfluoro-4-ethylcyclohexanesulfonate; PFUnA, Perfluoroundecanoic acid; PFHxA, Perfluorohexanoic acid; PFMOPrA, Perfluoro-3-methoxypropanoic acid; PFMOBA, Perfluoro (4-Methoxybutanoic) acid; PFECA, Perfluoroalkyl ether carboxylate; 8:2 CLPFESA, 8:2 Chlorinated

polyfluorinated ether sulfonic acids; 6:2 FTSA, 6:2 fluorotelomer sulfonic acid; 6:2 FTSAB, 6:2 fluorotelomer sulfonamide alkyl betaine; Me-PFOSA-AcOH, 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid; PFPpA, 3-Perfluoroheptyl propanoic acid; FHpPA, n-3-perfluoroheptyl propanoic acid.

Highlights

- PFAS toxicity data for humans remains highly variable
- The toxic kinetics and tissue distribution of PFAS are species-specific
- Extrapolating data from animal to human studies is complex and inconclusive
- Emerging PFAS can induce toxicity like legacy PFOS and PFOA
- Adverse response pathways to PFAS differ among species, sexes, and life stages

1. Introduction

Men, women, and children are being exposed to highly persistent per- and polyfluoroalkyl substances (PFAS) that are used in an increasing array of products (cosmetics, soft furnishings, food paper wrappings, paint, and non-stick cookware) despite there is growing evidence of broad toxicity linked to PFAS exposure. Currently, over 9000 compounds belong to this chemical class (Cordner et al., 2021). PFAS consists of a hydrophobic carbon-fluorine (C-F) backbone and a polar functional group. Of the two sub-types, the first, perfluoroalkyl substances are fully fluorinated aliphatic compounds where fluorine replaces all hydrogen atoms attached to the carbon backbone. The second sub-type, polyfluoroalkyl substances, are partially fluorinated compounds where fluorine has replaced all hydrogen atoms attached to at least one carbon (but not all) in the aliphatic backbone (**Figure 1**). Their ubiquitous use in manufacturing and commercial products and subsequent widespread exposure to people has occurred due to their unique physicochemical properties

(amphiphilic nature; see **Table S1** in the Supporting Information) and high chemical / thermal stability resulting from the strength of the C-F bond (O'Hagan, 2008). However, these same properties contribute adversely to the environment. They are distributed widely as they are readily transported in groundwater systems. Perfluoroalkyl substances are persistent (i.e., resistant to chemical and biological oxidation), although polyfluoroalkyl compounds may be oxidized to persistent perfluoroalkyl sulfonic and carboxylic acids. Due to their persistent, bioaccumulative, and toxic nature, some PFAS are listed (perfluorooctane sulfonic acid; PFOS, and perfluorooctanoic acid; PFOA) or proposed for listing (perfluorohexane sulfonic acid; PFHxS) in the Stockholm Convention on Persistent Organic Pollutants while other compounds are already included in regulatory monitoring programs.

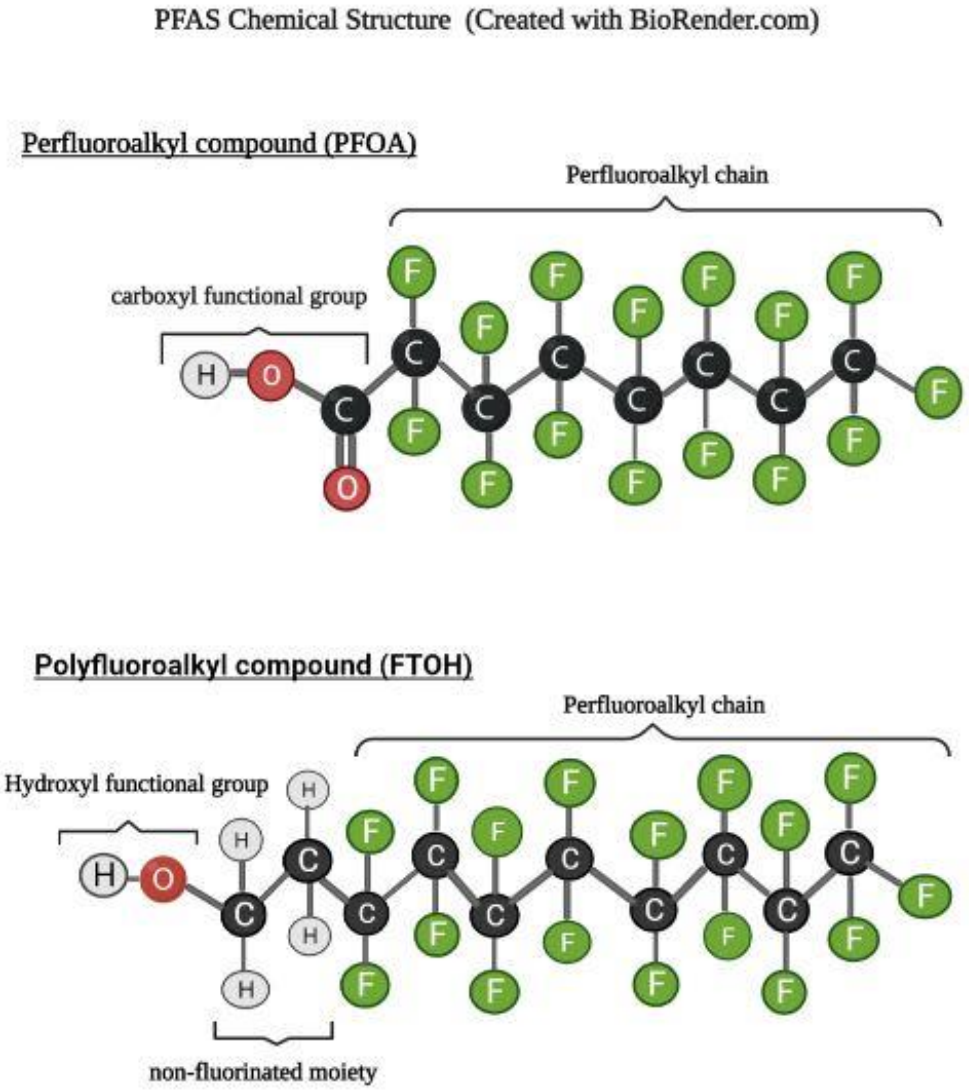


Figure 1. PFAS chemical structure showing carbon-fluorine bond, with hydrophobic tail and hydrophilic functional head. Perfluoroalkyl compound contains a carboxyl functional group (e.g., PFOA). Polyfluoroalkyl compound has a non-fluorinated moiety and hydroxyl functional group (e.g., 8:2 FTOH).

Exposure to PFAS may occur from a variety of sources (**Table 1**; **Figure 2**). This may include entry of PFAS into the environment from manufacturing processes (e.g., emissions, effluent), the use of PFAS-containing firefighting foams and other activities, where exposure to impacted soil, air, and water may occur via oral, inhalation, and dermal routes. Impacted environmental matrices may

contribute to the accumulation of PFAS in livestock, fish, and crops to which people may be exposed once the chemicals have entered the food chain. Exposure may also occur from indoor dust, from the transfer of PFAS from non-stick wrappers to food, and the use of cosmetics and other personal care products. The exposure magnitude may be significantly greater in occupational settings due to increased exposure frequency and elevated PFAS concentrations in exposure matrices (e.g., direct contact with PFAS-containing firefighting foams).

Table 1. Range of PFAS concentrations detected in key exposure sources

Exposure source	Exposure Route	PFAS concentration range	Predominant PFAS	Reference
Water	Oral, dermal	9.0 – 3640 ng/L	PFOA	(Exner and Färber, 2006)
		0.22 – 24.0 ng/L	PFOA	(Ericson et al., 2008)
		0.051 – 501 ng/L	PFPeA	(Boone et al., 2019)
		0.10 – 456 ng/L	PFOA	(Saito et al., 2004)
		20 – 132 ng/L	PFOS	(Nakayama et al., 2007)
Air	Inhalation	7 – 53 pg/m^3	PFOA, PFDA, PFNA	(Ge et al., 2017)
		1.82 – 32.1 pg/m^3	6:2 FTOH, 8:2 FTOH	(Bossi et al., 2016)
		18.0 – 109.9 pg/m^3	FTOH	(Lai et al., 2016)
		3.4 – 34 pg/m^3	PFOA	(Liu et al., 2020)
		2.8 – 68.8 pg/m^3	FTOH	(Wang et al., 2015)
Soil	Incidental ingestion, dermal, inhalation	0.007 – 0.048 µg/kg	PFOA, PFOS	(Rankin et al., 2016)
		0.527 – 34.1 µg/kg	PFOA	(Strynar et al., 2012)
		11 – 2400 µg/kg	PFOS	(Houtz et al., 2013)
		0.665 – 9700 µg/kg	PFOS	(Anderson et al., 2016)
		10 – 48 µg/kg	PFOS	(Zareitalabad et al., 2013)
Indoor dust	Incidental ingestion, dermal, inhalation	27 – 47739 pg/m^3	8:2 FTCA	(Fromme et al., 2015)
		5.93 – 70600 pg/m^3	8:2 FTOH	(Fraser et al., 2012)
		<1.0 – 3100 pg/m^3	MeFOSE	(Goosey and Harrad, 2012)
		890 – 47000 pg/m^3	MeFOSE	(Shoeib et al., 2011)
		200 – 307000 pg/m^3	FTOH	(Langer et al., 2010)
Biosolids	Transfer to fruit / vegetables / livestock	8 – 219 µg/kg	PFOS	(Sepulvado et al., 2011)
		0.05 – 0.33 µg/kg	PFDA	(Gottschall et al., 2017)
		9.7 – 6410 µg/kg	PFOA	(Lindstrom et al., 2011)
		1.4 – 36 µg/kg	PFOS	(Pepper et al., 2021)
		2.0 – 300 µg/kg	6:2 diPAP, 8:2 diPAP	(Moodie et al., 2021)
Fruit / vegetables	Ingestion	0.014 – 50.7 µg/kg	PFBA	(Sznajder-Katarzyńska et al., 2018)
		0.27 – 33 µg/kg	PFBA	(Scher et al., 2018)
		58.8 – 8085 µg/kg	PFBA	(Liu et al., 2019)
		<10.0 – 87 µg/kg	PFBA	(Bao et al., 2020)

		1.28 – 150 µg/kg	PFBA	(Blaine et al., 2014)
Fish / meat	Ingestion	1.38 – 127 µg/kg	PFOS	(Stahl et al., 2014)
		0.47 – 4000 µg/kg	PFOS	(Delinsky et al., 2010)
		0.46 – 640 µg/kg	PFOS	(Gewurtz et al., 2014)
		<0.5 – 406 µg/kg	PFOS	(Becker et al., 2010)
		560 – 9349 µg/kg	PFOS	(Lanza et al., 2017)
Fast food wrappers	Transfer to food - ingestion	6 – 290 µg/kg	PFOA	(Begley et al., 2005)
		5.19 – 341.21 µg/kg	PFHxA	(Zafeiraki et al., 2014)
		<0.40 – 8490 µg/kg	8:2 FTOH	(Yuan et al., 2016)
		15.3 – 28.6 µg/kg	PFOA	(Monge Brenes et al., 2019)
		8.6 – 948.7 µg/kg	PFOA	(Elizalde et al., 2018)
Cosmetics	Dermal	0.01 – 142.8 µg/kg	PFOA	(Keawmanee et al., 2015)
		0.03 – 1440 µg/kg	6:2 FTMAc	(Whitehead et al., 2021)
		60 – 243000 µg/kg	6:2 triPAP, 6:2 diPAP, PFHxA	(Eriksson et al., 2018)
		0.75 – 6500 µg/kg	PFHxA	(Fujii et al., 2013)
		0.11 – 2160 µg/kg	PFOA	(Brinch et al., 2018)

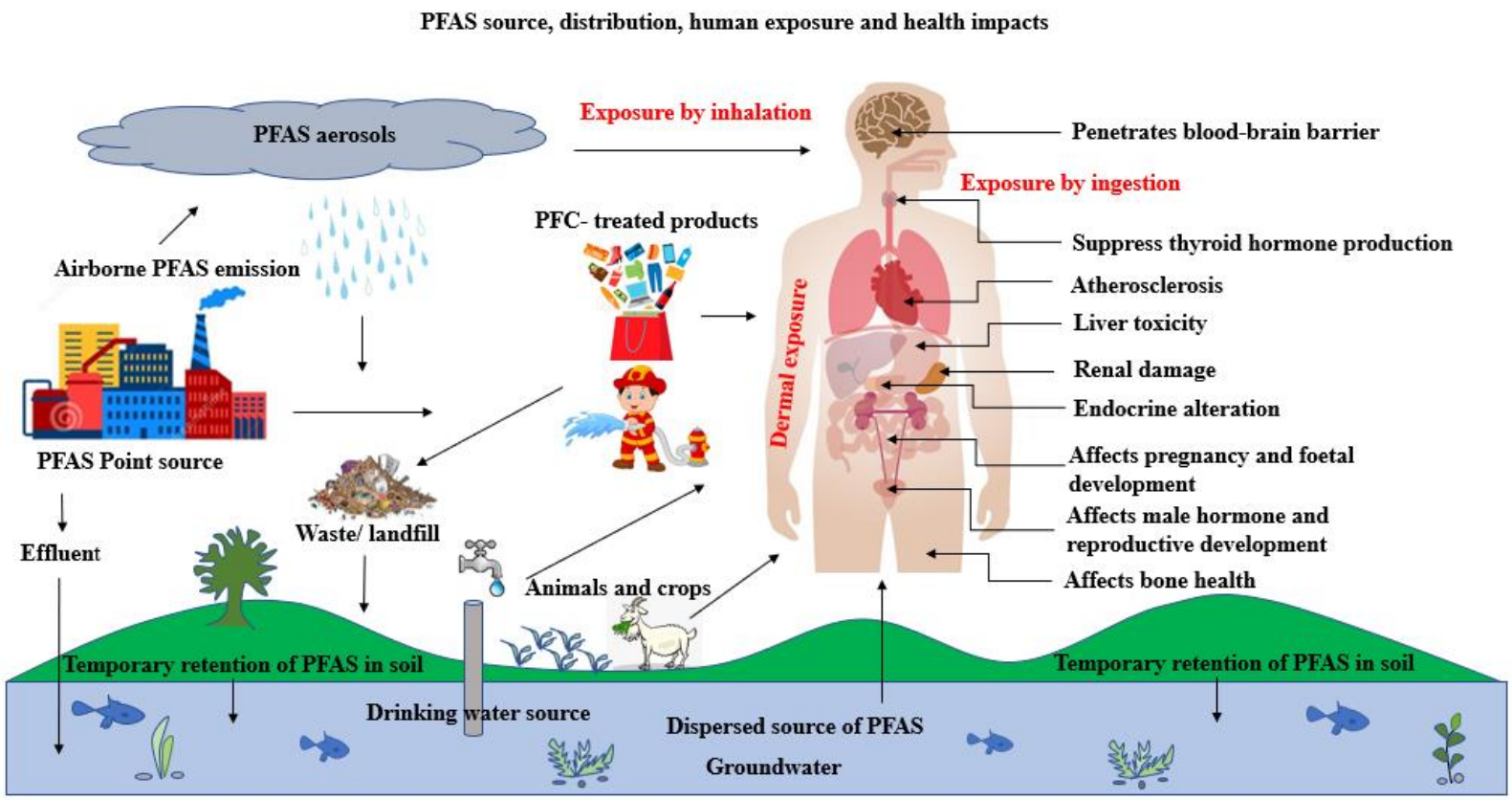


Figure 2. PFAS sources and routes contribute to distribution, human exposure, and health impacts. From PFAS point of source, PFAS may be distributed via the atmospheres, soil, and (drinking) water sources. Humans may be exposed to PFAS via ingestion of contaminated water and food, incidental ingestion of soil and dust, inhalation of contaminated air, and following dermal contact.

As detailed in Kirk et al. (2018) and ATSDR (2021), exposure to PFAS may result in numerous adverse health effects resulting in economic and social burdens globally. In Europe, the health-related cost of PFAS exposure was estimated to be 52-84 billion EUR annually (Goldenman et al., 2019), while equivalent health-related costs for the United States have been estimated to be US$37–59 billion annually (Cordner et al., 2021). However, the full magnitude of health impacts and cost to society from PFAS exposure is still unclear as current figures have relied on limited data from government and public reports.

In a systematic review by (Kirk et al., 2018) which included 221 scientific publications published up until February 7th, 2017, the effect of PFAS exposure on reproduction, pregnant women and their newborn babies, body metabolism, cardiovascular system, immune systems, nervous system, respiratory system, and specific conditions such weight gain, diabetes, cancer, and thyroid gland function was examined. Kirk et al. (2018) reported sufficient evidence for an association between PFOA and PFOS and hypercholesterolemia. Moreover, limited evidence was found between PFOA exposure and kidney and testicular cancer, and limited evidence between PFAS blood concentration and antibody levels following vaccination against preventable infections such as Diphtheria (PFOA, PFOS, PFHxS, PFDA) and Rubella (PFOA, PFOS). However, inadequate evidence was available to assign an association of adverse health outcomes following exposure to other PFAS, frequently detected in the environment. A toxicological profile released subsequentially by the Agency for Toxic Substances and Disease Registry (ATSDR) in May 2021 (ATSDR, 2021) indicated that PFAS exposure (predominantly focused on PFOA and PFOS but also included PFHxS, PFNA, PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, and FOSA) might result in several adverse health impacts on hepatic, cardiovascular, immune, and reproductive systems. Specifically, ATSDR reported the following outcomes in association with PFAS exposure: pre-eclampsia (PFOA, PFOS); increased serum liver enzymes ALT and decreased serum bilirubin (PFOA, PFOS, PFHxS); increase in serum lipids (PFOA, PFOS, PFNA, PFDA); reduced antibody response to vaccines

(PFOA, PFOS, PFHxS, PFDA); and decline in birth weight (PFOA, PFOS). ATSDR also reported adverse effects in laboratory animals such as liver toxicity, developmental toxicity, reproductive toxicity, immune toxicity, and cancer effects. However, cause-and-effect relationships for many of these outcomes could not be elucidated. Given global PFAS contamination, regulatory and community concerns regarding PFAS exposure, and the vast number of compounds in this chemical class, a considerable amount of research into potential adverse health effects associated with PFAS exposure has been undertaken since the reviews of Kirk et al. (2018) and peer-reviewed studies by ATSDR through September 2018. Consequently, this study aimed to review recent research on health-related impacts of PFAS exposure, highlight compounds other than PFOA and PFOS that cause adverse health effects, and, where possible, elucidate cause-and-effect relationships.

2. Review Scope and Methodology

In this critical review, we use Web of Science, PubMed (Medline), and Google scholar to search for publications using the following search terms: ‘per- and polyfluoroalkyl substances’ or ‘PFAS’ and ‘health effects’ or ‘toxicity’ with relevant human and animal studies assessed. Only primary studies in animals and humans investigating the link between one or more PFAS of interest and a health effect or mechanistic biological endpoint that have been published in the last five years were included. This critical review covered numerous cross-sectional analyses, case-control studies, cohort studies, and experimental studies that reported PFAS concentration with lowest observed effect concentration (LOEC). Systematic reviews, Editorial materials, Letters, Meeting Abstract, Corrections paper, and studies published in a language other than English were excluded. Three hundred eighty-three references were imported through to Covidence online software for the screening. Of the 383 studies, forty-five duplicates were removed. Three hundred thirty-eight studies were screened against title and abstract. One hundred ninety-two studies were screened as irrelevant. One hundred forty-six studies were assessed for full-text eligibility. Of the 146 studies, 44 studies reported no health end points, 39 reported no LOEC, 10 were review articles, 1 was a correction paper, and 1 was a report presentation.

This resulted in 51 papers included that were published between 2017 and February 2022 (See Supporting Information Figure S.1. Flow diagram of inclusion and exclusion of literature included in the critical review. Those deemed suitable articles were evaluated for health-related toxicity, route of exposure, and lowest observed effect (LOEC) in the biological matrix. As PFOS and PFOA toxicity has been extensively documented, the primary focus of this review was to detail recent evidence of health-related toxicity of 'lesser known' compounds in addition to renewing previous studies and research on PFOS and PFOA.

3. PFAS Health Effects

Data from selected papers were categorized into three subcategories: PFAS health effects in humans; PFAS toxicity in animals; and PFAS studies that reported nil toxicity to humans. Twenty-three studies have demonstrated PFAS-induced organ toxicity in humans, with adverse effects ranging from reproductive toxicity, endocrine toxicity, cardiovascular damage, respiratory effect, hepatic toxicity, immune effect, skeletal/bone effect, and neurological effect. Nineteen studies have reported PFAS-induced toxicity in laboratory animals, with adverse effects ranging from reproductive, endocrine, neurological, immune, dermal, renal, and hepatic toxicities. Ten studies in humans have demonstrated no adverse impacts to some of the health endpoints due to PFAS exposure. A summary of health effects in humans is shown in **Table 2**, while adverse effects observed in animal models are detailed in **Table 3**. Studies that demonstrated no toxicity to humans due to PFAS exposure are shown in **Table 4**. PFAS concentrations described in these tables are the lowest observed effect concentration (LOEC) expressed as ng/mL.

Table 2. Health effects of PFAS exposure in humans

PFAS	*In-vivo* Model (n = x)	Exposure Route	Health effects	LOEC (In biological matrix)	Reference
Reproductive effect					
PFHxS	Human (ovarian follicular fluid) (n = 97)	Undefined exposure	Increased risk to infertility factors (endometriosis, polycystic ovary syndrome, genital tract infections)	1.7 ng/mL	(Kim et al., 2020a); Australia
PFHpA	Human (ovarian follicular fluid) (n = 97)	Undefined exposure	Increased risk to infertility factors (endometriosis, polycystic ovary syndrome, genital tract infections)	0.1 ng/mL	(Kim et al., 2020a); Australia
PFOS	Human (blood) (n = 397)	Undefined exposure	Impact on fetal growth and development (alteration of metabolites such as β-alanine, creatinine, lysine, arginine, pipecolate, and adrenochrome glycine, serine, and threonine	33.9 ng/mL	(Hu et al., 2020b); U.S.A.
EtFOSAA	Human (blood) (n = 397)	Undefined exposure	Impact on fetal growth and development (alteration of metabolites such as β-alanine, creatinine, lysine, arginine, pipecolate, and adrenochrome glycine, serine, and threonine	0.28 ng/mL	(Hu et al., 2020b); U.S.A.
EtFOSAA	Human (blood) (n = 102)	Undefined exposure	Maternal exposure increases the risk of breast cancer in children	0.3 ng/mL	(Cohn et al., 2020); U.S.A.
PFOA	Human (semen) (n = 651)	Undefined exposure	Affects male reproductive health (decreased total testosterone, decreased free testosterone	0.32 ng/mL	(Cui et al., 2020a); China

PFOS	Human (semen) (n = 651)	Undefined exposure	Affects male reproductive health (decreased total testosterone, decreased SHBG)	0.23 ng/mL	(Cui et al., 2020b); China
PFNA	Human (semen) (n = 651)	Undefined exposure	Affects male reproductive health (decreased total testosterone, decreased SHBG)	0.03 ng/mL	(Cui et al., 2020b); China
6:2 Cl PFESA	Human (semen) (n = 651)	Undefined exposure	Affects male reproductive health (decreased total testosterone, decreased SHBG)	0.11 ng/mL	(Cui et al., 2020b); China
PFHxS	Human (blood) (n = 442)	Undefined exposure	Vitamin D deficiency in pregnancy	1.0 ng/mL	(Chang et al., 2021); U.S.A.
PFOS	Human (blood) (n = 442)	Undefined exposure	Vitamin D deficiency in pregnancy	2.03 ng/mL	(Chang et al., 2021); U.S.A.
PFOA	Human (blood) (n = 442)	Undefined exposure	Vitamin D deficiency in pregnancy	0.63 ng/mL	(Chang et al., 2021); U.S.A.
PFNA	Human (blood) (n = 442)	Undefined exposure	Vitamin D deficiency in pregnancy	0.24 ng/mL	(Chang et al., 2021); U.S.A.
PFOS	Human (blood) (n = 433)	Undefined exposure	Elevated insulin and total cholesterol in pregnancy	3.9 ng/mL	(Gardener et al., 2021); U.S.A.
PFNA	Human (blood) (n = 433)	Undefined exposure	Elevated insulin and total cholesterol in pregnancy	0.7 ng/mL	(Gardener et al., 2021); U.S.A.
PFDA	Human (blood) (n = 433)	Undefined exposure	Elevated insulin and total cholesterol in pregnancy	0.2 ng/mL	(Gardener et al., 2021); U.S.A.
Endocrine effect					
PFOA	Human (Progenitor cell) (n = 2)	Cell treatment	Alter pancreatic development (downregulation of PDX1, HNF4α, SOX9 gene)	20.7 ng/mL	(Liu et al., 2018b); China
PFOS	Human (Progenitor cell) (n = 2)	Cell treatment	Alter pancreatic development (downregulation of PDX1, HNF4α, SOX9 gene)	25 ng/mL	(Liu et al., 2018b); China
PFOA	Human (blood) (n = 40)	Undefined exposure	Diabetogenic effect (alteration in glucose homeostasis)	8.8 ng/mL	(Alderete et al., 2019); U.S.A.

PFHxS	Human (blood) (n = 40)	Undefined exposure	Diabetogenic effect (alteration in glucose homeostasis)	2.7 ng/mL	(Alderete et al., 2019); U.S.A.
PFBS	Human (umbilical cord blood) (n = 196)	Undefined exposure	Increased childhood adiposity	0.05 ng/mL	(Chen et al., 2019b); China
PFOA	Human (blood) (n = 103)	Undefined exposure	Impaired glucose metabolism	2.26 ng/mL	(Chen et al., 2020b); U.S.A.
PFOA	Human (blood) (n = 1067)	Undefined exposure	Increased lipid levels (HDL, LDL, total cholesterol)	2.24 ng/mL	(Fan et al., 2020); China
PFOS	Human (blood) (n = 1067)	Undefined exposure	Increased lipid levels (HDL, LDL, total cholesterol)	7.55 ng/mL	(Fan et al., 2020); China
PFHxS	Human (blood) (n = 1067)	Undefined exposure	Increased lipid levels (HDL, LDL, total cholesterol)	1.46 ng/mL	(Fan et al., 2020); China
PFNA	Human (blood) (n = 1067)	Undefined exposure	Increased lipid levels (HDL, LDL, total cholesterol)	1.03 ng/mL	(Fan et al., 2020); China
PFOA	Human (thyroid cells) (n = 7)	Cell treatment	Thyroid toxicity (acute cell death, impaired cell proliferation)	0.1 ng/mL	(Zhang et al., 2021); China
GenX	Human (thyroid cells) (n = 7)	Cell treatment	Thyroid toxicity (acute cell death, impaired cell proliferation)	0.1 ng/mL	(Zhang et al., 2021); China
ADONA	Human (thyroid cells) (n = 7)	Cell treatment	Thyroid toxicity (acute cell death, impaired cell proliferation)	0.1 ng/mL	(Zhang et al., 2021); China
PFOA	Human (blood) (n = 304)	Undefined exposure	Increased in fasting glucose	10.05 ng/mL	(Han et al., 2021); China
PFNA	Human (blood) (n = 304)	Undefined exposure	Increased in LDL cholesterol	1.64 ng/mL	(Han et al., 2021); China
PFUnDA	Human (blood) (n = 304)	Undefined exposure	Increased in LDL cholesterol	0.49 ng/mL	(Han et al., 2021); China
PFOS	Human (blood) (n = 304)	Undefined exposure	Increased in LDL cholesterol	7.60 ng/mL	(Han et al., 2021); China
6:2 Cl-PFESA	Human (blood) (n = 304)	Undefined exposure	Increased in LDL cholesterol	2.21 ng/mL	(Han et al., 2021); China

PFOA	Human (blood) (n = 366)	Undefined exposure	Elevated lipids in childhood (increased total cholesterol, HDL-C, and LDL-C)	1.43 ng/mL	(Blomberg et al., 2021); Denmark
PFHxS	Human (blood) (n = 366)	Undefined exposure	Elevated lipids in childhood (increased total cholesterol, HDL-C, and LDL-C)	0.26 ng/mL	(Blomberg et al., 2021); Denmark
PFNA	Human (blood) (n = 366)	Undefined exposure	Elevated lipids in childhood (increased total cholesterol, HDL-C, and LDL-C)	0.63 ng/mL	(Blomberg et al., 2021); Denmark
PFOS	Human (blood) (n = 366)	Undefined exposure	Elevated lipids in childhood (increased total cholesterol, HDL-C, and LDL-C)	3.08 ng/mL	(Blomberg et al., 2021); Denmark
PFDA	Human (blood) (n = 366)	Undefined exposure	Elevated lipids in childhood (increased total cholesterol, HDL-C, and LDL-C)	0.23 ng/mL	(Blomberg et al., 2021); Denmark
PFOA	Human (blood) (n = 1038)	Undefined exposure	Disruption of glucose homeostasis	4.63 ng/mL	(Zhang et al., 2022); China
PFOS	Human (blood) (n = 1038)	Undefined exposure	Disruption of glucose homeostasis	9.96 ng/mL	(Zhang et al., 2022); China
PFBA	Human (blood) (n = 1038)	Undefined exposure	Disruption of glucose homeostasis	1.86 ng/mL	(Zhang et al., 2022); China
6:2 Cl-PFESA	Human (blood) (n = 1038)	Undefined exposure	Disruption of glucose homeostasis	1.98 ng/mL	(Zhang et al., 2022); China
8:2 Cl-PFESA	Human (blood) (n = 1038)	Undefined exposure	Disruption of glucose homeostasis	0.01 ng/mL	(Zhang et al., 2022); China
Cardiovascular effect					
PFUnDA	Human (carotid plaque) (n = 1016)	Undefined exposure	Atherosclerosis	0.28 ng/mL	(Lind et al., 2017); Sweden
PFOS	Human (blood) (n = 158)	Ingestion	Increase cholesterol and cardiovascular disease	140,036 ng/mL	(Bjorke-Monsen et al., 2020); Norway
PFOS	Human (blood) (n = 158)	Ingestion	Increase cholesterol and cardiovascular disease	155,000 ng/mL	(Bjorke-Monsen et al., 2020); Norway

Respiratory effect					
PFOA	Human (non-small cell lung carcinoma cell line)	Cell treatment	Epigenetic modification, alteration in cell cycle, and apoptosis	4141 ng/mL	(Jabeen et al., 2020); U.S.A.
PFOS	Human (non-small cell lung carcinoma cell line)	Cell treatment	Epigenetic modification, alteration in cell cycle, and apoptosis	5001 ng/mL	(Jabeen et al., 2020); U.S.A.
Hepatic effects					
PFOA	Human (liver cells)	Cell treatment	Decreased viability of liver cells	8281 ng/mL	(Ojo et al., 2021); Australia
PFOS	Human (liver cells)	Cell treatment	Decreased viability of liver cells	8281 ng/mL	(Ojo et al., 2021); Australia
PFNA	Human (liver cells)	Cell treatment	Decreased viability of liver cells	8281 ng/mL	(Ojo et al., 2021); Australia
PFHxS	Human (liver cells)	Cell treatment	Decreased viability of liver cells	8281 ng/mL	(Ojo et al., 2021); Australia
PFDA	Human (liver cells)	Cell treatment	Decreased viability of liver cells	1028 ng/mL	(Ojo et al., 2021); Australia
Immune effect					
PFOA	Human (blood) (n = 1101)	Undefined exposure	Elevated pro-inflammatory cytokine (IL-1 beta production)	2.58 ng/mL	(Papadopoulou et al., 2021b); Norway
Skeletal/Bone effect					
PFDA	Human (blood) (n = 629)	Undefined exposure	Dental caries	0.17 ng/mL	(Wiener and Waters, 2019); U.S.A.
PFOA	Human (blood) (n = 208)	Undefined exposure	Osteoporosis	1.57 ng/mL	(Banjabi et al., 2020); Saudi Arabia
PFNA	Human (blood) (n = 208)	Undefined exposure	Osteoporosis	0.63 ng/mL	(Banjabi et al., 2020); Saudi Arabia
PFUnDA	Human (blood) (n = 208)	Undefined exposure	Osteoporosis	11 ng/mL	(Banjabi et al., 2020); Saudi Arabia

PFBA	Human (blood) (n = 280)	Undefined exposure	Osteoporosis	0.33 ng/mL	(Banjabi et al., 2020); Saudi Arabia
PFPeA	Human (blood) (n = 208)	Undefined exposure	Osteoporosis	0.15 ng/mL	(Banjabi et al., 2020); Saudi Arabia
Neurological effect					
PFOA	Human (cerebrospinal fluid) (n = 223)	Undefined exposure	Penetration of PFASs through a blood-cerebrospinal fluid (CSF) barrier	0.078 ng/mL	(Wang et al., 2018); China
PFOS	Human (cerebrospinal fluid) (n = 223)	Undefined exposure	Penetration of PFASs through a blood-cerebrospinal fluid (CSF) barrier	0.028 ng/mL	(Wang et al., 2018); China
6:2 Cl-PFESA	Human (cerebrospinal fluid) (n = 223)	Undefined exposure	Penetration of PFASs through a blood-cerebrospinal fluid (CSF) barrier	0.051 ng/mL	(Wang et al., 2018); China

Table 3. Toxicity of PFAS exposure in animal models

PFAS	**In-vivo Model (n = x)**	**Exposure Route**	**Adverse effects**	**LOEC (In biological matrix)**	**Reference**
Reproductive effects					
PFOS	Mice (blood, testicular tissue, tissue RNA) (n = 3)	Gavage	Testicular damage (decrease Leydig cells, lower testosterone and decrease expression of DLK1-Dio3 gene)	5000 ng/mL	(Song et al., 2018); China
PFHpA	Rats (Testicular tissue) (n = 18)	Dermal application	Germ cell degeneration (luminal cellular debris, oligospermia)	1×10^6 ng/mL	(Han et al., 2019); Korea
6:2 FTAB; 6:2 FTAA	Zebrafish (gonads) (n = 480)	Flow through exposure	Impaired offspring development (decrease egg	500 ng/mL	(Shi et al., 2019); China

			production; increase malformation and mortality		
PFOS	Bird (eggs) (n = 49)	Oral exposure	Arrest in embryo development	0.596 ng/mL	(Dennis et al., 2022); U.S.A.
PFOS: PFHxS	Bird (eggs) (n = 49)	Oral exposure	Arrest in embryo development	0.375 ng/mL	(Dennis et al., 2022); U.S.A.
Endocrine effect					
PFOS	Dolphins (blood) (n = 19)	Cell treatment	Immune T-cell dysregulation	5000 ng/mL	(Soloff et al., 2017); U.S.A.
6:2 diPAPs	Zebrafish (blood) (n = 60)	Direct exposure	Thyroid toxicity (decrease T4 and T3)	0.05 ng/mL	(Chen et al., 2020a); China
8:2 diPAPs	Zebrafish (blood) (n = 60)	Direct exposure	Thyroid toxicity (decrease T4 and T3)	0.05 ng/mL	(Chen et al., 2020a); China
PFOS	Rats (thyroid tissue) (n = 4)	Cell treatment	Disruption of thyroid function (inhibition of intracellular iodide)	50,000 ng/mL	(Conti et al., 2020); Italy
PFECA	Zebrafish (n = 100)	Direct exposure	Metabolic alteration	50000 ng/mL	(Gebreab et al., 2020); U.S.A.
PFOS	Mice (pancreatic cell)	Cell treatment	Impaired insulin secretion	5000 ng/mL	(Duan et al., 2021); China
PFUnDA	Mice (blood, pancreatic tissue) (n = 32)	Oral treatment	Insulitis	3000 ng/mL	(Hyotylainen et al., 2021); Norway
PFOS	Mice (β-TC-6 cells) (n = 10)	Cell treatment	Reduced insulin release capacity of β-cell	5000 ng/mL	(Qin et al., 2022); China
Neurological effect					
PFOS	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	30,007 ng/mL	(Berntsen et al., 2017); Norway
PFHxS	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	220,063 ng/mL	(Berntsen et al., 2017); Norway
PFOA	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	207,003 ng/mL	(Berntsen et al., 2017); Norway
PFNA	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	46,408 ng/mL	(Berntsen et al., 2017); Norway
PFDA	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	20,563 ng/mL	(Berntsen et al., 2017); Norway

PFUnDA	Rats (neuronal cell) (n = 10)	Cell treatment	Neuronal toxicity	169,188 ng/mL	(Berntsen et al., 2017); Norway
PFOA	Frog larvae (brain) (n = 81)	Direct exposure	Decreased in dopamine levels	1000 ng/mL	(Foguth et al., 2019b); U.S.A.
PFOS	Frog larvae (brain) (n = 81)	Direct exposure	Decreased in dopamine levels	100 ng/mL	(Foguth et al., 2019b); U.S.A.
PFHpA	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	32401 ng/mL	(Menger et al., 2020); Sweden
PFOA	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	4968 ng/mL	(Menger et al., 2020); Sweden
PFNA	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	22740 ng/mL	(Menger et al., 2020); Sweden
PFHxS	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	4968 ng/mL	(Menger et al., 2020); Sweden
PFOS	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	4801 ng/mL	(Menger et al., 2020); Sweden
6:2 FTSA	Zebrafish (embryo-larvae) (n = 40)	Direct exposure	Behavioral alteration	77071 ng/mL	(Menger et al., 2020); Sweden
PFBA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	250 ng/mL	(Rericha et al., 2021); U.S.A.
PFDoA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	25 ng/mL	(Rericha et al., 2021); U.S.A.
PFODA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	247 ng/mL	(Rericha et al., 2021); U.S.A.
PFBS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	222 ng/mL	(Rericha et al., 2021); U.S.A.
PFPeS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	235 ng/mL	(Rericha et al., 2021); U.S.A.
Cl-PFOPA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive	248 ng/mL	(Rericha et al., 2021); U.S.A.

			larval photo motor response)		
8:2 DiPAP	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	253 ng/mL	(Rericha et al., 2021); U.S.A.
DiSAMPAP	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval photo motor response)	245 ng/mL	(Rericha et al., 2021); U.S.A.
PFPeA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	251 ng/mL	(Rericha et al., 2021); U.S.A.
FHpPA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	252 ng/mL	(Rericha et al., 2021); U.S.A.
GenX	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	251 ng/mL	(Rericha et al., 2021); U.S.A.
PFPrS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	250 ng/mL	(Rericha et al., 2021); U.S.A.
PFHpS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	250 ng/mL	(Rericha et al., 2021); U.S.A.
PFOS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	248 ng/mL	(Rericha et al., 2021); U.S.A.
8-Cl PFOS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	248 ng/mL	(Rericha et al., 2021); U.S.A.
PFHxPA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	248 ng/mL	(Rericha et al., 2021); U.S.A.
6:6 PFPi	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval photo motor response)	253 ng/mL	(Rericha et al., 2021); U.S.A.
PFHxA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hyperactive larval startle response)	251 ng/mL	(Rericha et al., 2021); U.S.A.

PFNA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval startle response)	251 ng/mL	(Rericha et al., 2021); U.S.A.
PFTeDA	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval startle response)	250 ng/mL	(Rericha et al., 2021); U.S.A.
PFECHS	Zebrafish (larvae) (n = 400)	Direct exposure	Abnormal behavior response (hypoactive larval startle response)	250 ng/mL	(Rericha et al., 2021); U.S.A.
Immune effect					
PFOS	Fish (blood) (n = 58)	Undefined exposure	Altered immune function (increased lysozyme activity)	122 ng/mL	(Guillette et al., 2020); U.S.A.
PFOA	Mice (blood) (n = 72)	Gavage	Immunotoxicity (reduced TDAR)	1000 ng/mL	(McDonough et al., 2020); U.S.A.
PFOS	Mice (blood) (n = 72)	Gavage	Immunotoxicity (reduced TDAR)	240000 ng/mL	(McDonough et al., 2020); U.S.A.
Dermal effect					
PFOA	Rats (skin) (n = 18)	Dermal treatment	Ulcerative dermatitis	1.2×10^6 ng/mL	(Han et al., 2019); Korea
PFBA	Mice (skin tissue) (n = 5)	Dermal treatment	Change in skin gene expression	620000 ng/mL	(Weatherly et al., 2021); U.S.A.
Renal and Hepatic effect					
PFOS	Chicken (liver) (n = 4)	Direct microinjection on eggs	Altered lipid metabolism in the liver (suppressed transcription of genes relating to lipid catabolism and fatty acid β-oxidation)	100000 ng/mL	(Jacobsen et al., 2018); Sweden
PFCA	Rats (skin) (n = 18)	Dermal treatment	Hepatocellular necrosis and renal tubular necrosis	1000 ng/mL	(Han et al., 2019); Korea
PFOS	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased AST, total protein, and albumin)	122 ng/mL	(Guillette et al., 2020); U.S.A.

PFDA	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased total protein and albumin)	10 ng/mL	(Guillette et al., 2020); U.S.A.
PFHxS	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased albumin)	0.15 ng/mL	(Guillette et al., 2020); U.S.A.
PFNA	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased albumin and uric acid)	0.81 ng/mL	(Guillette et al., 2020); U.S.A.
GenX	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased AST, CK, albumin, and uric acid)	0.81 ng/mL	(Guillette et al., 2020); U.S.A.
PFBS	Fish (blood) (n = 58)	Undefined exposure	Altered liver function (increased AST, CK, albumin, and uric acid)	0.15 ng/mL	(Guillette et al., 2020); U.S.A.
PFBA	Mice (n = 5)	Dermal treatment	Altered liver function (increased AST, CK, albumin, and uric acid)	620000 ng/mL	(Weatherly et al., 2021); U.S.A.

Table 4. PFAS studies show no association with biological endpoints in humans

PFAS	**In-vivo Model (n = x)**	**Exposure Route**	**Biological endpoint**	***p*-value**	**Reference**
Reproductive effect					
PFOA, PFNA, PFUnDA, PFHxS, PFOS, MeFOSAA	Human (blood) (n = 1760)	Undefined exposure	Breast cancer	> 0.05	(Hurley et al., 2018); U.S.A.
PFOS, PFHxS, PFHpS, PFUnA	Human (placenta) (n = 122)	Undefined exposure	Hypertensive disorders of pregnancy	> 0.05	(Bangma et al., 2020); U.S.A.
PFOA, PFOS, PFHxS, PFNA, Me-PFOSA-AcOH	Human (blood) (n = 506)	Undefined exposure	Adverse birth outcomes	> 0.05	(Eick et al., 2020); U.S.A.
PFOA, PFOS, PFNA	Human (blood) (n = 277)	Undefined exposure	Gestational weight gain	> 0.05	(Romano et al., 2021a); U.S.A.

Endocrine effect					
PFDoA	Human (umbilical cord blood) (n = 196)	Undefined exposure	Childhood adiposity	> 0.05	(Chen et al., 2019a); China
PFOA, PFOS, PFHxS	Human (blood) (n = 468)	Undefined exposure	Altered thyroid function	> 0.05	(Lebeaux et al., 2020); U.S.A.
Immune effect					
PFOS, PFNA, PFUnDA, PFDoDA, PFTrDA	Human (blood) (n = 2689**)**	Undefined exposure	Rhino-conjunctivitis, Eczema	> 0.05	(Bamai et al., 2020); Japan
PFOA, PFOS, PFNA, PFHxS	Human (blood) (n = 607)	Undefined exposure	Childhood asthma	> 0.05	(Jackson-Browne et al., 2020b); U.S.A.
PFMOPrA	Mice (blood) (n = 150)	Oral exposure	Altered immune cell function	> 0.05	(Woodlief et al., 2021a); U.S.A.
PFMOBA	Mice (blood) (n = 150)	Oral exposure	Altered immune cell function	> 0.05	(Woodlief et al., 2021a); U.S.A.
Neurological effect					
PFOA, PFOS, PFHxS, PFNA	Human (blood) (n = 193)	Undefined exposure	The neurobehavioral problem in childhood	> 0.05	(Vuong et al., 2021); U.S.A.

3.1. Reproductive Effects

In the study of Kim et al. (2020b), investigations were undertaken to determine whether an association existed between PFAS concentration, fertilization rate, and/or infertility etiology. In this study, follicular fluid was sampled from 97 women who underwent artificial reproduction treatment, and follicular fluid samples were analyzed for PFAS concentration (PFOS, PFOA, PFHxS, and PFNA) using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). PFAS were present in all follicular samples, with PFOS and PFOA detected at concentrations up to 4.9 and 2.4 ng/mL, respectively. A significant association was observed between PFHxS (1.7 ng/mL) and PFHpA (0.1 ng/mL), which were linked to infertility factors such as endometriosis, polycystic ovarian syndrome, genital tract infections, male factors, and idiopathic causes ($p<0.002$; $p<0.04$ respectively). However, follicular fluid PFAS concentrations (e.g., PFOS, PFHxS, PFHpS, PFOA, PFNA, PFDA, PFHpA, and PFUnDA) were not statistically significant with fertilization rate, (*p*-value = 0.114; 0.376; 0.134; 0.633; 0.370; 0.081; 0.677; and 0.061 respectively); only lower fertilization rates were observed with age as a covariate. Moreover, Kim et al. (2020a) indicated that log-transformed PFHxS concentrations were lower in females with endometriosis than women who reported “male factors” as a cause of infertility. At the same time, PFHpA was higher in women with infertility due to female factors (endometriosis, polycystic ovarian syndrome, genital tract infection). One of the mechanistic explanations of the association between PFAS exposure and endometriosis is that PFAS may disrupt the function of nuclear hormone receptors and therefore interfere with steroid hormone synthesis (Du et al., 2013; Henry and Fair, 2013), possibly influencing hormone-dependent diseases such as endometriosis. Endometriosis responds to hormones released from the ovary, causing endometrial-like tissues normally found within the uterine lining to be produced in other body parts such as fallopian tubes, ovaries, and peritoneum. This then causes inflammation, scarring, and even adhesions, resulting in an increased chance of infertility (Macer and Taylor, 2012);(Australian Institute of Health and Welfare 2019). However, this mechanistic explanation regarding PFAS effects

on steroid hormone synthesis needs to be further elucidated and substantiated with more scientific evidence in human studies.

A review of epidemiological studies also suggested that PFAS exposure may have deleterious effects on male reproductive health. Studies have reported that human exposure to PFAS affected sperm morphology (Bach et al., 2016), and exposure to PFOA/PFOS was associated with a lower percentage of viable sperm, a higher percentage of DNA fragmentation, and a decrease in total testosterone and free testosterone (Tarapore and Ouyang, 2021). These adverse impacts were attributed to possible toxic effects of PFOA/PFOS to Leydig, Sertoli, and germ cells, as observed in rodent studies (Tarapore and Ouyang, 2021). In Cui et al. (2020b), serum and semen samples from 651 men were investigated for PFAS concentration against reproductive hormone levels. Results showed a strong association between PFOA (0.32 ng/mL), PFOS (0.23 ng/mL), PFNA (0.03 ng/mL), and 6:2 Cl-PFESA (0.11 ng/mL) and male reproductive hormones characterized by a decrease in total testosterone, decrease in free testosterone, decrease in estradiol, and decrease in sex hormone-binding globulin (SHBG) levels ($p< 0.05$). Kahn et al. (2021), in their systematic review of the evidence of persistent organic pollutants (POPs) affecting fecundability, indicated that at least some PFAS showed an association between decreased couple fecundability, with evidence supporting an association between PFAS and longer time to pregnancy (TTP). In the study of Song et al. (2018), pregnant mice exposed to PFOA (2.5 and 5 mg PFOA/kg BW) had male offspring with testicular damage, where a decrease in the number of Leydig cells was observed ($p<0.01$). These results suggest that PFOA exposure during pregnancy may impact the reproductive capacity of their offspring and disrupt the expression of DLK1 or Dio3 genes, which can impede embryonic development and growth (Ogata et al., 2008). In Han et al. (2019), following dermal exposure of rats to 250 and 1000 mg/kg PFHpA for two weeks, germ cell degeneration, luminal cellular debris, and oligospermia were observed at both doses consistent with testicular toxicity ($p<0.05$). Studies undertaken to date have explored extreme dermal exposure scenarios that are not likely to be relevant to human exposure

levels and subsequently may show that unrealistic exposure levels are needed to demonstrate subsequent reproductive toxicity. In this way, these studies counterintuitively support a low-risk profile for these compounds and dermal exposure routes at environmental exposure levels profiled to date.

In a metabolome-wide association study conducted by Hu et al. (2020a), metabolic changes in women during pregnancy and early postpartum were linked with PFOS and EtFOSAA exposure in order to determine the association between prenatal exposure and the health and development of children. Blood samples were analyzed from 397 subjects, although it was not identified how individuals were exposed to PFAS. PFOS and EtFOSAA showed different impacts on the urea cycle's metabolites amino acid pathways. For example, metabolites such as β-alanine, creatinine, lysine, arginine, pipecolate, and adrenochrome decreased as the ratio of EtFOSAA to PFOS increased. Metabolites were positively associated with PFOS concentrations (33.9 ng/mL; $p<0.05$) but were negatively associated ($p>0.05$) with its precursor EtFOSAA. In contrast, glycine, serine, and threonine were positively associated with EtFOSAA concentration (0.28 ng/mL; $p<0.05$), but not with PFOS. (Hu et al., 2020a) identified that exposure to PFOS and EtFOSAA may lead to maternal metabolic changes that impact fetal development by disrupting the intrauterine environment and nutrition.

Cohn et al. (2020) conducted a nested case-control study wherein daughters of mothers who had previously been exposed to PFAS and were diagnosed with breast cancer were identified. Serum samples from 102 subjects were analyzed for PFAS using SPE-HPLC-MS/MS, and cholesterol levels were measured enzymatically. Results showed that daughters had an increased risk of developing breast cancer when their mothers had both high concentrations of plasma EtFOSAA (0.3 ng/mL; $p<0.05$), a PFOS precursor (Buck et al., 2011), and high levels of cholesterol (2.8×10^6 ng/mL; $p<0.05$). Exposure to EtFOSAA and high cholesterol levels may occur during vulnerable windows in early life through breastfeeding. The link between EtFOSAA and cholesterol is due to EtFOSAA

having a high affinity for lipids. In most human populations, the optimum level for total cholesterol is <1.50 x 10^6 ng/mL in adults (Grundy et al., 2019) and <1.7 x 10^6 ng/mL in children (McNeal et al., 2013). Studies into breast cancer indicate that hypercholesterolemia is one of the risk factors and that the cholesterol metabolite 27-hydroxycholesterol (27 HC) induces the estrogen receptor-positive breast cancer cells to proliferate. A potential explanation linking maternal EtFOSAA exposure and increased breast cancer risk in children is altered placental-fetal transfer or possible transfer of high levels of 27 HC in the presence of high levels of EtFOSAA via breast milk. Since 27 HC is an estrogen receptor agonist, it affects the developing mammary gland during the perinatal period, influencing breast cancer development (Nelson et al., 2013). Animal studies have confirmed that mammary glands are vulnerable to epigenetic changes, endocrine disruption, and carcinogenesis following exposure to environmental chemicals (Fenton and Birnbaum, 2015). To date, studies on the effects of PFAS in developing mammary glands have been limited to PFOA exposure in animal models. They have reported reduced development of ductal epithelia after maternal dosing (Tucker et al., 2015). However, the mechanism linking EtFOSAA with breast cancer is unclear.

In a prospective birth cohort study by (Chang et al., 2021) that included 442 pregnant women, blood samples were collected for PFAS and 25(OH)D measurement. Vitamin D deficiency was observed in >95 % of the study participants (total 25(OH)D < 20 ng/mL) at various PFAS concentrations (PFHxS, 1.0 ng/mL; PFOS, 2.03 ng/mL, PFOA, 0.63 ng/mL; PFNA, 0.24 ng/mL; p<0.05).; but negatively associated with PFPeA (0.66 ng/mL, p>0.05). These results suggested that Vitamin D metabolism may be altered in pregnancy when individuals are exposed to PFAS. However, results also showed that some associations were modified by fetal sex.

In a correlation study published by Gardener et al. (2021), 433 pregnant women were assessed for plasma PFAS concentration along with fasting insulin, total cholesterol, and triglyceride levels [mean (SD) values of 97.23 (131.96); 6.15 (1.19); and 2.43 (0.97) mmol/L respectively]. In over 98% of the study population, there were detectable concentrations of PFAS (PFNA (max [6.8 ng/mL]);

PFOS (max [208 ng/mL]); PFOA (max [7.2 ng/mL]); PFHxS (max [12.6 ng/ml]), which correlated with both maternal insulin and total cholesterol. Specifically, PFNA and PFOS concentrations were associated with high levels of maternal insulin (0.19 ng/mL, 0.15 ng/mL; $p<0.01$, $p<0.04$ respectively) while PFNA, PFOS and PFDA concentrations were associated with high levels of total cholesterol (0.11 ng/mL, 0.13 ng/mL, 0.11 ng/mL; $p<0.02$, $p<0.01$, $p<0.03$ respectively). Although a large sample size was used in this study, the correlation between PFAS concentration and insulin and total cholesterol may be limited by confounding factors such as diet, obesity, and other metabolic health factors. However, the association between PFAS exposure and insulin and cholesterol levels were supported by other studies (Christensen et al., 2016; He et al., 2018; Seo et al., 2018). Erinc et al. (2021) reviewed the epidemiological and mechanistic evidence for the link between PFAS and hypertensive disorders of pregnancy (HDP), including preeclampsia and gestational hypertension. For some PFAS (e.g., PFOA, PFOS, PFHxS, PFHpA, PFBS, PFNA), disruption in the placenta and immune function caused oxidative stress and lipid metabolism, which lead to HDP, although epidemiological evidence and the plausible mechanism that support PFAS as risk factors were limited. Animal studies have also shown that PFOA has reproductive toxicity and can lead to early abortion in mice (Abbott et al., 2012; Lau et al., 2006). However, doses used in animal experiments were high and did not reflect human exposure levels. PFOA is a developmental toxicant, significantly affecting body weight, serum insulin, and leptin levels (Hines et al., 2009). Fragki et al. (2021) reviewed the modulation of lipid homeostasis by the two most common PFAS, PFOA, and PFOS, emphasizing the underlying mechanisms for humans. Fragki et al. (2021) also indicated that the contrast between human and animal data might be an artifact of dose, with different molecular pathways involved at very low versus high levels. Moreover, activation or suppression of nuclear receptors (e.g., PPARα and HNF4α) signaling pathway plays an essential role in cellular events requiring further investigation.

In Shi et al. (2019) other PFAS such as 6:2 FTAB and 6:2 FTAA exhibited reproductive effects in animal experiments. These surfactants have been used as alternatives to PFOS in aqueous film-forming foams (Shi et al., 2018). The toxic effects of 6:2 FTAB and 6:2 FTAA were observed during an assessment of survival and developmental impacts on zebrafish embryos using a flow-through exposure system. Since zebrafish share approximately 70 percent genetic homology with humans, it is a widely used animal model to study embryonic development and genetic causes of human disease (Bradford et al., 2017). These compounds may be transformed *in vivo* leading to short-chain PFCA, which may increase testosterone and estrogen levels in female zebrafish and decrease egg production in offspring when exposed to 50 and 500 ng/mL, respectively ($p<0.05$). In contrast, in male zebrafish, testosterone levels were found to decrease at exposure concentrations of 500 ng/mL. While altered estrogen and testosterone levels were found to affect ovarian development and spermatogenesis, thyroid hormone levels (triiodothyronine; T3 and thyroxine; T4) decreased in female zebrafish at 50 and 500 µg/L but increased in male zebrafish. These findings suggest that 6:2 FTAB is quickly metabolized in zebrafish and that co-exposure to both 6:2 FTAB and 6:2 FTAA may have an endocrine-disrupting effect. The physiological results suggest that 6:2 FTAB undergoes biotransformation and is metabolized into 6:2 FTAA, which subsequently accumulates and affects zebrafish reproductive capability. In Dennis et al. (2020), 49 bobwhite quails were exposed to PFOS and PFOS: PFHxS mixture via drinking water for up to 90 days. Quail eggs (n = 1529) were examined for growth and survival over a 90-day exposure with 21-day hatchling observations. An arrest in the embryonic development of eggs was observed when exposed to PFOS (0.596 ng/mL, $p<0.001$) and PFOS: PFHxS mixture (0.375 ng/mL, $p<0.001$). Moreover, their findings suggest that adverse effects resulting from exposure to PFOS and PFHxS mixtures may occur at drinking water when chronically exposed.

Other studies have shown that 6:2 FTAB can induce oxidative stress, immune toxicity, and apoptosis, increasing the chance of malformations in zebrafish during embryonic development (Shi

et al., 2018). An increase in vitellogenin, an egg yolk precursor found in all oviparous species that provides protein, lipid, and nutrients for the developing egg, was also observed in the liver of exposed male zebrafish (Shi et al., 2019). Vitellogenin is synthesized and secreted by the liver under the stimulation of estrogen. Therefore, an increase in vitellogenin in male zebrafish suggests that the FTAB and FTAA have estrogenic activity. Earlier studies have also demonstrated the estrogen-like activity of some PFAS, including PFOA, PFOS, and FTOH (Liu et al., 2007; Maras et al., 2006). Blake and Fenton (2020) synthesized the evidence for the role of some PFAS in thyroid function disruption, kidney disease, and metabolic syndrome and the role of the placenta as the target of PFAS and a programming agent of adult disease. Blake and Fenton (2020) indicated that early life PFAS insults might disrupt placental growth and function, thereby increasing susceptibility for later life chronic health conditions, which may be further exacerbated by lifelong PFAS exposure. However, further experimental toxicology studies designed to assess the placenta are needed to determine the sensitivity of the placenta to PFAS and identify the molecular mechanism of placental toxicity.

3.2. Endocrine Effects

Previous studies have reported that PFAS, particularly PFOA and PFOS, interrupt differentiation of pancreatic progenitor cells, causing downregulation of pancreatic and duodenal homeobox 1 (PDX1) (Liu et al., 2018a), a transcription factor necessary for pancreatic development (El-Gohary et al., 2017). Deficiency in PDX1 can cause diabetes through pancreatic agenesis and β-cell dysfunction (Fujimoto and Polonsky, 2009). Furthermore, in a recent study by Liu et al. (2020), using a human pluripotent stem cell pancreatic induction model and human pancreatic progenitor cell endocrine induction model, PFAS were investigated for their potential to induce pancreatic toxicity. An alteration in pancreatic development was observed with PFOA and PFOS blood concentrations of 20.7 and 25 ng/mL, respectively, resulting in the downregulation of the endodermal marker genes PDX1, HNF4α and SOX9. HNF4α directly regulates glycolysis and glucose transport genes, affecting insulin secretion. In contrast, the SOX9 gene is critical in providing instructions to make an essential

protein in embryonic development (Stanger, 2008). Suppression of these genes thus interferes with pancreatic development and function. It is now necessary to explore the hypothesis that exposure to PFAS can result in dose-dependent pancreatic dysfunction in order to develop improved regulations with appropriately defined exposure levels.

Alderete et al. (2019) conducted a prospective study involving forty obese children where PFOA, PFOS, PFHxS, and glucose concentrations were measured at baseline and longitudinally 1 – 3 years after enrolment. A pathway enrichment analysis was also performed to assess metabolic alterations associated with PFAS exposure. Alderete et al. (2019) identified that every 1 ng/mL increase in blood PFOA and PFHxS concentration was associated with a 30.6 and 10.2 mg/dL increase in 2-hour glucose level, respectively. Additionally, for a 1 ng/mL increase in blood PFHxS concentration, there was a 17.8 mg/dL increase in glucose area under the curve. Data showed that baseline PFOA (8.8 ng/mL) and PFHxS (2.7 ng/mL) concentrations, and every 1 ng/mL increase thereafter was associated with an alteration in metabolic pathways as well as glucose homeostasis (p<0.05). Han et al. (2021) evaluated the association of PFAS exposure, the risk of Type 2 diabetes, and the levels of glycemic biomarkers and lipid fractions in participants (n = 304) from Shandong Province, East China. Their results showed an inverse association with the risk of Type 2 diabetes after adjusting for age, sex, and body mass index. However, PFOA (10.05 ng/mL) was positively associated with an increase in fasting plasma glucose (p<0.05), which is known to promote the development of type 2 diabetes. PFNA, PFUnDA, PFOS, and 6:2 Ci-PFESA were positively associated with LDL cholesterol (1.64 ng/mL, 0.49 ng/mL, 7.60 ng/mL, and 2.21 ng/mL respectively; p<0.05). In Zhang et al. (2022), cross-sectional data (n = 1038) from Isomers of C8 Health Project in China indicated that PFAS concentrations (PFOA, 4.63 ng/mL; PFOS, 9.96 ng/mL; PFBA, 1.86 ng/mL; 6:2 Cl-PFESA, 1.98 ng/mL; and 8:2 Cl-PFESA, 0.01 ng/mL) were positively associated with glucose homeostasis markers (fasting glucose, fasting insulin, homeostasis model assessment for insulin resistance, and homeostasis model assessment of β cell function after adjusting for

confounders), $p<0.05$. Birru et al. (2021) identified a novel pathway between PFAS exposure and the development of gestational diabetes mellitus (GDM). Birru et al. (2021) found that biomarkers of thyroid hormones and glucose metabolism were associated with GDM, with biological plausibility that PFAS dysregulates thyrotropin (TSH) that may disrupt glucose homeostasis, thus increasing the risk of GDM. However, their data also showed that PFAS was negatively associated with triiodothyronine (T3) and thyroxine (T4). Qi et al. (2020) summarized the epidemiologic evidence for associations between exposure to PFAS and the development of obesity, diabetes, and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis. About two-thirds of 54 studies reported a positive association between PFAS exposure and obesity and Type 2 diabetes prevalence. However, further studies are required in extensive cohort studies and mechanistic laboratory studies to support this possible association. In studies conducted by Hyotylainen et al. (2021), it was demonstrated that when non-obese diabetic mice (n = 32) were exposed to PFUnDA (3000 ng/mL) in drinking water at mating, during gestation and lactation, and first weeks of life of female offspring, pancreatic insulitis developed, which was associated with altered lipid metabolism. Moreover, impaired insulin secretion and reduced insulin release capacity of β cells were demonstrated *in vitro* when mice pancreatic cells were treated with PFOS at 5000 ng/mL ($p<0.05$) (Duan et al., 2021; Qin et al., 2022). These reports highlight a strong association between PFAS exposure and the development of type 2 diabetes, which is a critical topic for further research.

Chen et al. (2019a), conducted a prospective birth cohort study involving 1,140 women wherein children at five years of age (n = 196) were investigated for an association between PFAS, specifically PFBS, and childhood adiposity. Chen et al. (2019a) found that in these children PFBS (0.05 ng/mL) had a significant positive association with waist circumference and waist to height ratio ($p<0.05$), suggestive of a positive association with prenatal exposure to PFBS. Fan et al. (2020) investigated and compared the effects of PFAS, particularly the effects of single-PFAS exposure and the combined effects of multiple PFAS exposures, on the adult serum lipid profile (n = 1067). Fan et

al. (2020) reported that increases in the serum lipid profile were positively associated with PFOA, PFOS, PFHxS, and PFNA (2.24 ng/mL, 7.55 ng/mL, 1.46 ng/mL, and 1.03 ng/mL respectively; $p<0.008$), with albumin mediating the toxic effect of PFAS exposure on lipid levels. Fan et al. (2020) further suggested that exposure to multiple PFAS may trigger different dose-response functions, suggesting a need for a more comprehensive epidemiological and toxicological investigation of multiple PFAS exposures than single-PFAS exposure. (Blomberg et al., 2021) estimated the associations between PFAS and lipid concentration at birth and several points in childhood, at 18 months, 5 years, and 9 years from a prospective cohort in the Faroe Islands (n = 490). Their study showed that PFOA (1.43 ng/mL), PFHxS (0.26 ng/mL), PFNA (0.63 ng/mL), PFOS (3.08 ng/mL), and PFDA (0.23 ng/mL) were measured at 5 years and 9 years old were positively associated with increased lipid concentration at age nine. This result indicated that the exposure period was a significant modifier of PFAS effects. Increased lipid profile in childhood is a risk factor for the later development of cardiovascular disease. Published literature suggests possible effects of PFAS, particularly long-chain PFAS exposure, on prenatal and postnatal growth, adiposity, and puberty in children and adolescents. However, outcomes vary due to their association with different stages of life. Moreover, the plausible mechanism through which PFAS may affect early-life growth -and puberty includes activation of peroxisome proliferator-activated receptor, alterations of thyroid or steroid hormone synthesis and metabolism, and their weak estrogenic or anti-androgenic properties.

In Gebreab et al. (2020), acute lethal effects along with impaired development, impacts on locomotor behavior, and alteration in metabolic profiles were observed when zebrafish (n = 100) were exposed to PFECA (50000 ng/mL), a PFOA alternative, consistent with mitochondrial disruption and peroxisome proliferator-activated receptor activation characterized by dysfunctions of carbohydrate, lipid, and amino acid metabolism.

Epidemiological studies have reported the detrimental effects of PFAS compounds (both long and short-chain and PFAS alternatives) on thyroid function (Coperchini et al., 2021). Although the

role of PFAS as thyroid disruptors in humans remains controversial, several *in vitro* studies have demonstrated the adverse effects of PFAS on thyroid cells. It has been reported that PFAS can accumulate in thyroid cells, interfere with thyroid hormone synthesis, modify thyroid peroxidase activity and iodine uptake, resulting in cytotoxicity and genotoxicity (Coperchini et al., 2021). *In vivo* animal studies have confirmed a thyroid disruptive effect following exposure to parent PFAS and their alternatives. Chen et al. (2020a) observed a decrease in triiodothyronine (T3) and thyroxine (T4) levels of zebrafish following exposure to 6:2 and 8:2 polyfluoroalkyl phosphate diesters (diPAPS) (0.05 ng/mL; $p<0.001$). DiPAPs are the PFAS that are most commonly used in food contact materials and detected in household dust (3.6 – 692 ng/g) (Eriksson and Kärrman, 2015). Both diPAPs can bind with thyroid hormone receptors (TRα) and transthyretin (TTR), which compete with T3 and T4 for thyroid hormone receptors and transthyretin, respectively. The binding affinities of PFAS to TTR are contributed by the steric and hydrophobic nature of PFAS. Like PFOA and PFOS, diPAPs display thyroid-disrupting effects, and this suggests that at an environmentally relevant concentration, both diPAPs may behave as thyroid disruptors. In Zhang et al. (2021), PFOA alternatives (GenX and ADONA) were investigated for their thyroid disrupting effects *in vitro* using both rat thyroid cell lines and primary normal human thyroid cells (n = 7). Zhang et al. (2021) reported that PFOA and GenX reduced thyroid viability and impaired cell proliferation in a dose and time-dependent manner, GenX being more toxic than PFOA at the same condition and concentration (0.1 ng/mL). In comparison, ADONA (0.1 ng/mL) showed no apparent harmful effects on the viability and proliferation of the thyroid cells. However, PFOA, GenX, and ADONA altered gene expression in the thyroid cells.

The thyroid function disruption associated with PFOS was characterized by inhibition of iodide transport by thyroid follicular cells using a rat model (50,000 ng/mL; $p<0.05$) (Conti et al., 2020). Data from the National Health and Nutrition Examination Survey (NHANES) for the years 2007-2008 (Wen et al., 2013) suggested that stimulating thyroid hormone (TSH) levels increased

with increasing PFOA concentration (p<0.01). The levels of total triiodothyronine (TT3) increased with increased concentration of PFOA (p<0.01) while total thyroxine (TT4) increased with increased concentration of PFHxS (p<0.01) (Jain, 2013). Translating these observations to a human context is problematic due to variability in associated specific combinations of age, race, gender, iodine intake, and thyroid status.

PFAS have also been associated with immunotoxicity. In Soloff et al. (2017), a significant correlation between PFOS and $CD8^+$ and $CD4^+$ T cell proliferation was observed in environmentally exposed dolphins (PFOS 5000 ng/mL vs. Peripheral Blood Leukocyte *in vitro*; p<0.001). Furthermore, their observations showed that exposure to ecologically relevant levels of PFOS promoted the production and proliferation of proinflammatory cytokines. This indicates that environmentally relevant doses of PFOS dysregulate the cellular immune compartment, leading to chronic immune activation and subsequent disease.

3.3. Cardiovascular Effects

In a prospective investigation of vasculature in Swedish seniors, Lind et al. (2017) assessed carotid artery atherosclerosis in 1016 participants aged 70 years by measuring intima-media thickness (IMT) using ultrasound. Results showed that PFUnDA exposure was associated with carotid plaque formation, (a parameter that is often used to predict future cerebrovascular or coronary events). Moreover, the relationship between PFUnDA and carotid plaque formation was more pronounced in women (PFUnDA 0.28 ng/mL; p<0.03); the reason for this relationship was unclear. However, it may be attributable to the age of study participants (70 years old) who were approximately twenty years post-menopause. Menopause is an important cardiovascular risk factor in women, in which ovarian hormone deprivation impacts cardiovascular function (Vitale et al., 2007).

The impact of PFAS exposure has also been investigated in nulliparous women of fertile age (Bjorke-Monsen et al. (2020). PFOS concentrations were positively associated with total cholesterol (TC) (140,036 ng/mL; p<0.01) and low-density lipoprotein (LDL) (155,000 ng/mL; p<0.004) while

fish intake was positively correlated with TC and LDL (p<0.01; p<0.06 respectively). Although the source of PFAS exposure was not experimentally defined, this finding suggests that PFOS accumulation in fish and its subsequent consumption is a significant pathway for vascular PFAS exposure.

3.4. Respiratory Effects

Evidence of carcinogenic potential of PFAS was demonstrated in a few studies (Caverly Rae et al., 2015; Steenland et al., 2015) and also in ATSDR (2021) and NIOSH (2021). Jabeen et al. (2020) reported that epigenetic modification, alteration in cell cycle, and apoptosis were evident when non-small cell lung carcinoma cells were exposed to PFOA and PFOS (4140.7 ng/mL and 5001.3 ng/mL, respectively), suggestive of a deleterious effect in patients with pre-existing lung disease or potential induction of lung carcinogenesis. Moreover, Jabeen et al. (2020) indicated that lower doses of PFAS (10 µM) might cause the activation of cell proliferation, whereas higher doses (400 µM) may lead to apoptosis, following the CASP9-CASP3 axis. This imbalance in cell activity could be a precursor to cancer development.

3.5. Hepatic and Renal Effects

Ojo et al. (2021) investigated the individual and combined toxicity of PFAS compounds in human liver cells (HepG2). Their results showed that both individual (PFOA, 8281 ng/mL; PFOS, 10003 ng/mL; PFNA, 9282 ng/mL; PFHxS, 8002 ng/mL; and PFDA, 10282 ng/mL) and combined PFAS (20 µM of each compound) induced concentration-dependent cytotoxicity and depletion of glutathione (GSH) levels in HepG2 cells. At a similar PFOS exposure concentration (10000 ng/mL), where the chemical was directly injected into chicken eggs, altered lipid metabolism in the liver was observed of the hatched chickens (Jacobsen et al., 2018). In fish (Striped Bass) (n = 58), altered liver function characterized by increased aspartate aminotransferase, total protein, albumin, uric acid, and creatine kinase was observed at different concentration of PFAS (PFOS, 122 ng/mL; PFDA, 10 ng/mL; PFHxS, 0.15 ng/mL; PFNA, 0.81 ng/mL; GenX, 0.81 ng/mL; and PFBS, 0.15 ng/mL)

(Guillette et al., 2020). In Weatherly et al. (2021), sustained dermal treatment in mice with PFBA (6.2×10^5 ng/mL) for 28 days induced systemic toxicity in the liver and kidneys and altered serum chemistries. Immune cells, including B-cells, CD11b-cells, and skin T-cells, were significantly increased ($p>0.05$) in the draining lymph nodes. These findings indicated an alteration of the peroxisome proliferator-activated receptor (PPAR) target gene, suggesting a role of a PPARα pathway in generating toxicity through the skin. A growing body of evidence showed that PFAS threatens kidney health. However, gaps still exist, especially since the production of novel replacement PFAS compounds is emerging (Stanifer et al., 2018).

Dermal exposure to PFOA and short-chain PFCA were investigated in v*itro* and *in vivo* models by Han et al. (2019). In the *in vitro* model, human skin equivalents were topically treated with 0.25 mM and 2.5 mM PFOA and short-chain PFCAs (PFPeA, PFHxA, and PFHpA) for six days, cell viability, interleukin, and oxidative stress markers were measured. For the *in vivo* model, systemic effects were investigated in rats wherein rats were dermally treated with 250 and 1000 mg/kg PFHpA (treatment range was 3-22 mg/cm^2) for two weeks, after which clinical and anatomic pathology was observed. Results showed that exposure to PFHpA caused histopathologic lesions including renal tubular necrosis, hepatocellular necrosis, and germ cell degeneration at 250 and 1000 mg/kg/day. There was also a statistically significant increase in liver weight and a decrease in prostate weight observed at 250 mg/kg body weight/day ($p<0.05$). Furthermore, PFOA dermal exposure was associated with ulcerative dermatitis (PFOA 1.2 x10^6 ng/mL; $p<0.001$), and 83% of dermally exposed rats died. These results suggest that more frequent exposure to PFOA through dermal exposure may negatively impact the skin, resulting in chemical-related systemic effects, However, concentrations tested in animal models are unrealistically high compared to human exposure levels.

3.6. Immune Effect

PFAS have been identified as immune hazards and have caused immunotoxicity in mice. In McDonough et al. (2020), mice (n = 72) were exposed to a commercial AFFF formulation for ten

days at a dose of 1.88 to 10 mg/kg/day via gavage. Their results showed that exposure to AFFF components (PFOA, 1000 ng/mL; and PFOS, 240000 ng/mL) reduced the T-cell-dependent antibody response (TDAR), a sensitive measure of immunotoxicity. In a sub cohort study by Papadopoulou et al. (2021a) involving 1101 mother-child pairs, prenatal PFOA (2.58 ng/mL) was positively associated with pro-inflammatory cytokines, IL-beta, suggestive of increased inflammation.

3.7. Skeletal / Bone Effects

Wiener and Waters (2019) identified that PFDA was associated with dental cavities in children (PFDA 0.17 ng/mL; $p<0.002$), possibly due to PFAS occurrence in drinking water (Dimensions of Dental Hygiene 2021; Dentistry Today 2020). These findings contrasted with outcomes from the NHANES cross-sectional study among U.S adolescents (1999-2012), where there was no association between serum perfluorinated alkyl acid concentration and dental cavities (Puttige Ramesh et al., 2019). Factors such as age, economic status, race, teeth brushing, and frequency of dental visits may each have contributed to the likelihood of dental cavities and not PFAS exposure. Still, pathways could occur in which PFDA influences the growth of teeth in a similar way to its effects on other cells. In particular, PFAS's estrogen-disrupting effect may lead to the disruption of carbonic anhydrase, which is necessary for enamel development (Yin and Paine, 2017). Carbonic anhydrase, and carbonate transporters, are involved in pH regulation in the maturation stage of amelogenesis (Lacruz et al., 2017). This controversy supports the need for more intensive research to determine if there are any adverse effects of PFAS exposure on dental cavities or development.

Banjabi et al. (2020) observed a high prevalence of osteoporosis in a case-control study undertaken on 208 individuals in Saudi Arabia. Plasma PFAS analysis in addition to bone mineral density (BMD) showed an association between PFAS concentration and osteoporosis [PFOA (1.57 ng/mL; $p<0.01$); PFBA (0.33 ng/mL; $p<0.01$); PFNA (0.63 ng/mL; $p<0.05$); PFPeA (0.15 ng/mL; $p<0.01$) and PFUnDA (11 ng/mL; $p<0.01$)]. Higher incidence of lower lumbar spine density in premenopausal women exposed to PFAS have been reported and believed to be due to competition

of calcitriol on the binding site of vitamin D receptors, which resulted in changes in osteoblast and decreased bone mineralization (Vemula and Olajide, 2021). Although lifestyle factors such as lack of physical activity, bone fracture, low calcium intake, estrogen deficiency, and vitamin D deficiency have been identified as common risk factors for osteoporosis (RACGP 2021), recent evidence suggests that environmental chemicals may also contribute to bone pathology. Bone metabolism is highly hormone-dependent, and since environmental chemicals have endocrine-disrupting effects, exposure to these chemicals may alter both estrogen and androgen activities. Estrogen plays a role in normal bone turnover, while androgens are involved in bone resorption activity (Manolagas et al., 2013). Other hormones involved in bone health include parathyroid hormones, calcitriol (from vitamin D), and calcitonin (Bhattarai et al., 2020). PFOA has been shown to disrupt vitamin D activity by competition on vitamin D receptors inhibiting vitamin D-responsive genes in target cells (Di Nisio et al., 2020). Moreover, PFOA and PFOS have been reported to alter the expression of the parathyroid hormone receptor 2 (PTH2R) gene, the known factor for osteoporosis risk (Galloway et al., 2015). Taken together, these studies show that PFAS exposure has multiple mechanisms that can adversely affect the bone.

3.8. Neurological Effects

In Wang et al. (2018), 223 matched-pairs of human serum and cerebrospinal fluid (CSF) samples were assessed for both PFAS concentration and PFAS penetration across the blood-CSF barrier. The concentration of exogenous chemicals such as PFAS in CSF is often used as a surrogate for evaluating the potential penetration of these chemicals into the central nervous system. It can predict the concentration of the chemicals in the brain interstitial fluid (Liu et al., 2009). Results showed that the penetration of PFAS was positively correlated with the barrier permeability index (BPI). Further results showed that serum PFOA (7.4 ng/mL); PFOS (6.4 ng/mL); 6:2 Cl-PFESA (6.2 ng/mL) concentrations were positively associated with BPI (p<0.001). CSF concentration of PFOA (0.078 ng/mL); PFOS (0.028 ng/mL); 6:2 Cl-PFESA (0.051 ng/mL) were also positively associated

with BPI (p<0.001). These results suggest that barrier integrity was an indicator of PFAS' ability to penetrate across the blood-CSF barrier.

Neurological effects of PFAS exposure were previously reported in animal studies that focused on the effects of PFAS carbon chain length and functional group. In a study undertaken by Berntsen et al. (2017), the cytotoxicity of PFAS of various chain lengths (C6 – C11) was investigated using cerebellar granule neurons from rats, wherein PFAS toxicity potencies (based on LC_{50} values) were observed using cell viability trypan blue and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays after 24 hours of exposure to PFAS. Outcomes highlighted that PFUnDA (12,971 ng/mL) was the most toxic PFAS causing both mitochondrial and cell membrane damage (p<0.001) while other PFAS also exhibited toxicity affecting cerebellar granule neuron's viability (PFDA 16,964 ng/mL; p<0.001); (PFOS 19,004 ng/mL; p<0.001); (PFNA 33,877 ng/mL; p<0.05); (PFOA 176,812 ng/mL; p<0.001); and (PFHxS 200,057 ng/mL; p<0.001). These concentrations are more likely to be relevant in occupational settings where high blood serum PFAS concentrations may be observed. An *in vitro* study using Xenopus oocytes that express the human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor has also shown that PFOS and PFOA are potent, non-competitive $GABA_A$ receptor antagonists and, as such, antagonize the inhibitory function of the central nervous system (Tukker et al., 2020). This potential mechanism needs to be studied in more relevant models to ensure relevance to humans but provides an informed starting point. In Foguth et al. (2019a), frog larvae at the Gosner stage 25, exposed to different concentrations of PFOS and PFOA for 30 days, were assessed for changes in neurotransmitter levels. A significant decrease (p<0.001) in dopamine concentration in the brains of frogs treated with PFOA and PFOS at 1000 ng/mL and 100 ng/mL, respectively was observed. In Menger et al. (2020), significant behavioral alterations were observed in zebrafish embryo-larvae (n = 40) when directly exposed to PFHpA, PFOA, PFNA, PFHxS, PFOS, and 6:2 FTSA (32401 ng/mL, 4968 ng/mL, 22740 ng/mL, 4968 ng/mL, 4801 ng/mL, and 77071 ng/mL, respectively. Menger et al. (2020) indicated that significant alteration in the behavior of the

zebrafish was detected in PFAS with a perfluorocarbon chain length of more than five carbons. In Rericha et al. (2021) where zebrafish (n = 400) were exposed to lower PFAS concentrations compared to Menger et al., zebrafish still exhibited abnormal behavior response (hyperactive larval photo motor response) when exposed directly to PFBA (250 ng/mL); PFDoA (25 ng/mL); PFODA (247 ng/mL); PFBS (222 ng/mL); PFPeS (235 ng/mL); Cl-PFOPA (248 ng/mL); 8:2 DiPAP (253 ng/mL); and DiSAMPAP (245 ng/mL); and hypoactive larval photo motor response with PFPeA (251 ng/mL); FHpPA (252 ng/mL); GenX (251 ng/mL); PFPrS (250 ng/mL); PFHpS (250 ng/mL); PFOS (248 ng/mL); 8-CL PFOS (248 ng/mL); PFHxPA (248 ng/mL); 6:6 PFpi (253 ng/mL); PFHxA (251 ng/mL); PFNA (251 ng/mL); PFTeDA (250 ng/mL); and PFECHS (250 ng/mL).

Dassuncao et al. (2019) hypothesized that PFAS accumulate in phospholipids and protein-rich tissues. Their study investigated the phospholipid content in whale tissue to predict PFAS accumulation. The authors hypothesized that the association between phospholipid content and PFAS concentration is related to the PFAS functional group and the carbon chain length of PFAS. Results showed that total PFAS were highest in the liver; phospholipids were highest in the brain, and total lipids were most elevated in fat. The phospholipids in the brain were significantly associated with PFAS (86 ng/mL; $p<0.001$); longer chain PFAS were correlated considerably with phospholipid content, whereas PFAS with <10 carbons were not associated. This suggests that longer chain PFAS penetrates the brain more efficiently than shorter chain PFAS. Other studies have also observed that long-chain PFAS are elevated in the brain of mammals (Ahrens et al., 2009; Greaves et al., 2012). The accumulation and tissue distribution of PFAS are attributed to the partitioning mechanism of phospholipids and binding to specific proteins (Armitage et al., 2012), as both PFAS and phospholipids contain a hydrophilic head and a hydrophobic tail, and both structural proteins and storage lipids are sorption compartments for some PFAS.

From a functional perspective, PFAS is reported to cause an alteration in the neurochemical signaling in the brain, neuronal toxicity, and accumulation in the brain, causing changes in animal

behaviors. The enzymatic activity of monoamine oxidase (MAO), acetylcholinesterase (AChE), glutamine synthase (GS), dopamine-2 (D2), muscarinic cholinergic (mAChR), and gamma-butyric acid type A (GABA-A) were observed to be associated with PFAS exposure. Eggers Pedersen et al. (2015) suggested that PFAS may elicit changes in neurotransmitters in the brain of polar bears affecting neuro-behavior. MAO is an enzyme that breaks down adrenaline, dopamine, norepinephrine, and serotonin (Yeung et al., 2019), which are involved in the regulation of neuronal activity, while GABA-A is essential to the function of the entire basal ganglia, which is responsible for both motor and behavioral functions (Goetz et al., 2007). In a previous study undertaken by Greaves et al. (2013), PFAS were also reported to have accumulated in the brain of polar bears. Higher PFAS concentrations were noted in the inner brain (pons/medulla, thalamus, and hypothalamus) than the outer brain. In contrast, the concentration of PFOS was reported to be highly correlated with brain lipid content. Other studies with mice have also reported that disturbances in these enzymes and neurochemical receptors could lead to behavioral aggressiveness (Shih, 2004) and cognitive deficits (Wess, 2004).

While data from different epidemiological and animal studies provide evidence for an association with, several studies in humans demonstrate no evidence for an association with the following endpoints: breast cancer (Hurley et al., 2018), hypertensive disorders in pregnancy (Bangma et al., 2020), adverse birth outcomes (Eick et al., 2020), gestational weight gain (Romano et al., 2021b), childhood adiposity (Chen et al., 2019a), altered thyroid function (Lebeaux et al., 2020), rhino-conjunctivitis and eczema (Bamai et al., 2020), childhood asthma (Jackson-Browne et al., 2020a), and altered immune cell function (Woodlief et al., 2021b) (**Table 4**).

4. Impact of PFAS Exposure on Peroxisome Proliferator-Activated Receptors

Several animal and human studies have indicated that PFAS may mimic endogenous ligands of target receptors such as PPARs and therefore may interfere in critical cell signaling pathways (Jiang et al., 2015). PPARs are ligand-activated transcription factors essential in cell regulation and

various metabolic processes. They are expressed in different tissue and cells, including adipocytes, hepatocytes, endothelial cells, and muscles, with different clinical outcomes depending on isoform and distribution (Grygiel-Górniak, 2014). PPARs also have diverse functions in humans, including essential skin barrier functions and permeability, inhibition of epidermal cell growth, differentiation of the epidermis, and skin inflammatory response regulation. PPARs and their ligands modulate different peroxisomal processes and are found mainly in tissue associated with high fatty acid metabolism and thus are expressed mainly in the liver. They are, however, also found in the adrenal and pituitary glands, kidney, muscle, heart, fats, B and T lymphocytes, vascular smooth muscle, and keratinocytes. Thus, human exposures to PFAS may potentially affect several PPAR activities and cause a cascade of pathophysiology.

Research dating back to 2006 has shown that PFOA is a known activator of PPARα in mice. In a study undertaken by Rosen et al. (2008), gene expression was evaluated in both wild-type and PPARα-null mice after oral treatment with PFOA (3000 ng/mL) and the PPARα agonist Wy14,643 (50,000 ng/mL). In wild-type mice, PFOA and Wy14,643 induced activation of PPARα, whereas, in PFOA-treated null mice, changes were detected in gene expression related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle regulation. In other experiments undertaken by Minata et al. (2010), PFOA was found to cause hepatocyte and bile duct injuries in PPARα treated mice eliciting dose-dependent hepatic and bile duct damage in wild-type mice. Furthermore, in chicken embryos exposed to the PPARα agonist or PFOS or PFOA, genetic transcription was observed in fat metabolism (Strömqvist et al., 2012). Moreover, in a study undertaken by Jiang et al. (2013), cardiac morphological and functional changes were observed in chicken embryos and hatchlings after injection with PPARα agonist. PFOA and PPARα agonists were also involved in developmental cardiotoxicity in chickens, and it was also reported that PFOA was more potent than PFOS in activating PPARα in mice (Takacs and Abbott, 2006).

More recently, PFAS have been associated with an increased incidence of gestational diabetes, potentially due to placental PPAR dysfunction driven in part by altered DNA methylation and miRNA expression (Gao et al., 2017). *In vitro* data also support the mechanistic connection between PFAS exposure and PPAR in metabolic disorders during pregnancy, which may result in increased mRNA fatty acid uptake expression leading to increased cholesterol, total lipids, and triglyceride concentration in cord blood (Spratlen et al., 2020). Studies have also demonstrated that PFAS interferes with trophoblast lipid homeostasis, inflammation and invasion, and which interaction mediates these in part between PFAS and PPARs (Szilagyi et al., 2020). Furthermore, interspecies variation in sensitivity to PFAS can be immense. Species traits causing differences related to toxicokinetic (uptake, distribution, biotransformation, elimination) (Nyman et al., 2014) are critical to predict compounds' behavior and safety predicting in humans.

5. Conclusions

PFAS toxicity has been reported in both animals and humans. Recent evidence reported PFAS exposure to humans affects female and male fertility, metabolism in pregnancy, endocrine function including pancreatic dysfunction and risk of developing Type 2 diabetes, lipid metabolism and risk of childhood adiposity, hepatic and renal function, immune function, cardiovascular health (atherosclerosis), bone health including risk for dental cavities, osteoporosis, and vitamin D deficiency, neurological function, and risk of developing breast cancer. In laboratory animals, PFAS exposure has been reported to affect reproduction, endocrine function, immune function, and neurological function. These findings were consistent with the findings published by Kirk et al. (2018) and ATSDR (2021). However, cause-and-effect relationships are still emerging for many of these adverse health outcomes. Due to the complexity of PFAS chemistry and differences in PFAS toxicokinetic between humans and experimental animals, interpreting animal data is problematic. For example, the elimination half-life of PFOA in Sprague Dawley rats (2 h-6 d), *Cynomolgus* monkeys (21-30 d), and humans (3.8 years) vary considerably (Kim et al., 2016), presumably due to differential

expression of organic anion transporters between species, which may influence kinetic profiles of different PFAS (Sundström et al., 2012). Differences in elimination kinetics have also been observed between male and female rats, although this phenomenon has not been reported for other species (Kim et al., 2016). The slower elimination kinetics in male rats (2-4 h versus 6 days for PFOA) has been attributed to sex hormone modulated renal tubular transport that results in lower renal clearance of PFOA (Kim et al., 2016). Elimination kinetic models need to be established with robust human data to ensure relevant interpretation of animal model data, but more importantly, to sparingly use animal models only where toxicity is a genuine concern.

PFAS bioaccumulation and distribution *in vivo* is dependent on the PFAS functional group, length of the perfluoroalkyl chain, and exposure dose (Fujii et al., 2013). These parameters will influence plasma and tissue binding, renal and biliary clearance, tissue concentrations, maternal-fetal transfer, and lactation transfer. While dose-related effects have been observed in some animal studies (**Table 3**), experiments were often conducted at exposure concentrations 3-4 orders of magnitude higher than current tolerable daily intake values (Department of Agriculture, Water, and the Environment, NEMP, 2018). A greater understanding of PFAS toxicokinetic is required to facilitate the translation of toxicity data from animal models to human scenarios.

An additional research priority is to elucidate the impact of precursor compounds on PFAS exposure and toxicological effects. Perfluoroalkyl sulfonic acids and perfluoroalkyl carboxylic acids are recalcitrant to biotransformation. However, other polyfluorinated compounds may undergo *in vivo* transformation and contribute to bioaccumulation effects in target organs (**Figure 3**).

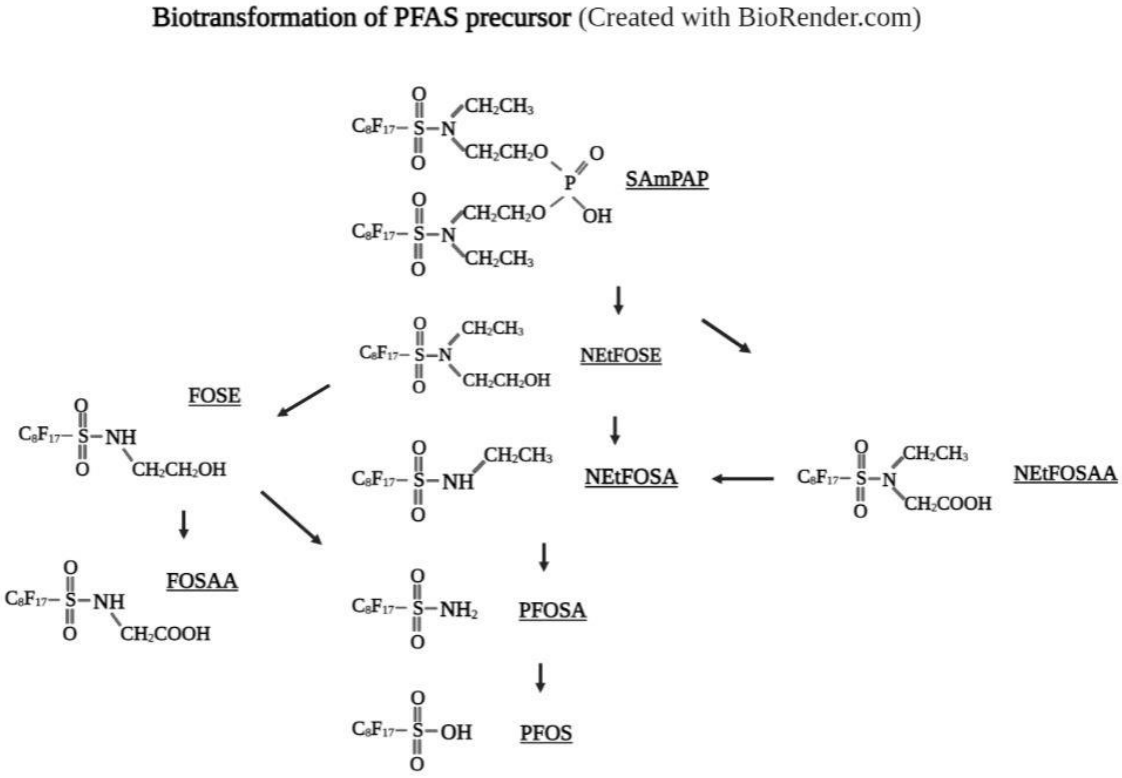


Figure 3. Biotransformation of PFAS precursor in fish tissue. Perfluorooctane sulfonamidoethanol based diester (SAmPAP) can potentially transform into PFOS via multiple intermediates (Glaser et al., 2021). SAmPAP has been manufactured historically in the U.S and has been used in food contact paper and packaging.

This scenario is particularly pertinent when assessing exposure to complex PFAS mixtures (e.g., environmental matrices impacted with PFAS-containing firefighting foams) where both measurable and non-measurable precursor compounds may contribute to total PFAS exposure (Hees, 2017). Conceivably, hepatic precursor transformation in environmental media may increase exposure to priority PFAS, although this would not be identified in the administered dose when using standard analytical approaches. Quantifying the concentration of individual precursors is analytically challenging. However, the total oxidizable precursor (TOP) assay may be utilized to overcome analytical limitations (Robel et al., 2017). The TOP assay pre-treats sample extracts (60 mM potassium persulfate at 90°C for 6 h) to expose underlying PFAS not amenable to formal analysis. In this way, 'unseen' precursor compounds in exposure media will be oxidized to produce perfluoroalkyl

sulfonic acids or perfluoroalkyl carboxylic acids, which contribute to the toxicological response. This information would be valuable for dose calculations for exposure and toxicity studies.

Evidence derived from both animal models and humans suggests that PFAS may have a deleterious impact on both animals and humans. However, extrapolating animal studies and comparing them to human studies does not draw firm conclusions. Moreover, studies in humans provided contrasting results. Investigation of PFAS exposure in a chronically exposed population is needed to evaluate the biological toxicity of chronic exposure to PFAS. Investigating the mechanisms of actions, toxicokinetic and adverse outcome pathways must be extended to elucidate differences in responses among species, between the sexes, and life stages. Moreover, the major gaps in the current literature identified are the lack or limited studies that investigate the route of exposure as most of them were undefined, the critical time window of exposure, a mixture of PFAS, and various dose-response relationships between PFAS and target endpoint..

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgment

Lore Jane L. Espartero: Conceptualization, Investigation, Writing- original draft, writing – reviewing and editing. **Miko Yamada**: Writing – reviewing and editing. **Judith Ford**: Writing – reviewing and editing; **Gary Owens**: Supervision, writing – reviewing and editing. **Tarl Prow**: Conceptualization, Supervision, Writing- reviewing and editing. **Albert Juhasz**: Conceptualization, writing – reviewing and editing.

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Supporting Information Table S.1. Physicochemical Properties of PFAS compounds (PubChem 2021; SGS 2021).

Name	Acronym	CAS #	Molecular Formula	Molecular weight	Boiling point [° C]	Melting point [° C]	Solubility in water 20-25 °C (g/L)	Density 20 °C (g/mL)	Vapor pressure [Pa]	Dissociation constant [pKa]
Perfluorooctanoic acid	PFOA	335-67-1	$C_8HF_{15}O_2$	414.07	188-192	37-60 °C	3.4-9.5	1.8	4-1300	0.16-3.8
Perfluorooctane sulfonate	PFOS	1763-23-1	$C_8HF_{17}O_3S$	500.13	249	54	1.52-1.57	-	6.7	-6-2.6
Perfuorobutanoic acid	PFBA	375-22-4	$C_4HF_7O_2$	214.04	121	-17.5 °C	Miscible	1.65	1307	-0.2-0.7
Perfluorohexanoic acid	PFHxA	307-24-4	$C_6\ HF_{11}O_2$	314.05	143	14	21.7	1.72	457	-0.13
Perfluoroheptanoic acid	PFHpA	375-85-9	$C_7HF_{13}O_2$	364.06	175	30	4.2	1.79	158	-0.15
Perfluorononanoic acic	PFNA	375-95-1	$C_9\ HF_{17}O_2$	464.08	218	56-59	9.5	1.75	1.3	0.21
Perfluorodecanoic acid	PFDA	335-76-2	$C_{10}\ HF_{19}O_2$	514.09	218	77-88	9.5	1.76	0.2	-0.17
Perfluoroundecanoic acid	PFUnDA	2058-94-8	$C_{11}\ HF_{21}O_2$	564.09	160-230	83-101	0.004	1.76	-1.65	-0.17
Perfluorododecanoic acid	PFDoDA	307-55-1	$C_{12}\ HF_{23}O_2$	614.1	245	107-109	0.0007	1.77	0.01	-0.17-0.8
Perfluorobutane Sulfonate	PFBS	375-73-5	$C_4\ HF_9O_3S$	300.1	211	76-84	46.2-56.6	1.81	631	-6-5.0
Perfluorohexane Sulfonate	PFHxS	432-50-8	$C_6\ HF_{13}O_3S$	400.12	238-239	-	2.3	1.84	58.9	0.14
Perfluoropentanoic acid	PFPeA	2706-90-3	$C_5\ HF_9O_2$	264.05	124.4	-17.5	112.6	1.7	1057	-0.06

Perfluorooctane Sulfonamide	PFOSA	754-91-6	$C_8H_2F_{17}NO_2S$	499.15	-	154-155	8.04	-	33.33	-
N-ethyl-perfluorooctane sulfonamido acetic acid	N-EtFOSAA	2991-50-6	$C_{12}H_8F_{17}NO_4S$	585.24	-	-	-	-	-	-
6:2 Fluorotelomer sulfonamide alkyl betaine	6:2 FTSAB	34455-29-3	$C_{15}H_{19}F_{13}N_2O_4S$	570.37	-	≥150	-	-	0.017	-
6:2 Fluorotelomer phophatediester	6:2 diPAPs	57677-95-9	$C_{18}H_9F_{30}O_4P$	790.2	-	-	$8x10^{-7}$	-	0	-
-8:2 Fluorotelomer phophatediester	8:2 diPAPs	678-41-1	$C_{20}H_9F_{34}O_4P$	990.2	-	-	$5x10^{-12}$	-	0	-
6:2 Chlorinated polyfluorinated ether sulfonic acid	6:2 Cl-PFESA	73606-19-6	$C_8F_{16}ClSO_4K$	-	-	-	-	-	-	-
Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamido) ethyl] phosphate	DiSAMPAP	2965-52-8	$C_{24}H_{19}F_{34}N_2O_8PS_2$	1204.5	-	-	-	-	-	-
N-ethyl perfluorooctane sulfonamido ethanol	NetFOSE	1691-99-2	$C_{12}H_{10}F_{17}NO_3S$	571.25	-	-	-	-	-	-
N-ethyl perfluorooctanesulfonamide	NetFOSA	4151-50-2	$C1_0H_6F_{17}NO_2S$	527.20	-	-	-	-	-	-
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	$C_{11}H_6F_{17}NO_4S$	571.20	-	-	-	-	-	-
2- (N-Methyl-perfluorooctane sulfonamido) acetic acid	Me-PFOSA-AcOH	2355-31-9	$C_{11}H_6F_{17}NO_4S$	571.21	-	-	-	-	-	-
8:2 Chlorinated polyfluorinated ether sulfonic acid	8:2 Cl-PFESA	83329-89-9	$C_{10}F_{20}ClSO_4K$	-	-	-	-	-	-	-
6:2 fluorotelomer sulfonic acid	6:2 FTSA	27619-97-2	$C_6H_5F_9O_3S$	428.17	-	-	-	-	-	-

Sodium 8-chloroperfluoro-1-octanesulfonate	8- Cl PFOS	-	$C_8ClF_{16}NaO_3S$	538.57	-	-	-	-	-	-
8-Chloroperfluorooctylphosphonic acid	Cl-PFOPA	2252239-09-9	$C_8H_2ClF_{16}O_3P$	516.50	-	-	-	-	-	-
Sodium bis(perfluorohexyl) phosphinic acid	6:6 PFPiA	70609-44-8	$C_{12}F_{26}NaO_2P$	724.05	-	-	-	-	-	-
2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid	GenX	62037-80-3	$C_6HF_{11}O_3$	347.08	-	-	-	-	298.15	-
Ammonium 4,8-dioxa-3H-perflurononanoate	ADONA	919005-14-4	$C_7H_2F_{12}O_4$	378.07	-	-	-	-	-	-
6:2 Fluorotelomer sulfonamide alkyl betaine	6:2 FTAB	34455-29-3	$C_{15}H_{19}F_{13}N_2O_4S$	570.4	-	-	-	-	-	-
6:2 Fluorotelomer alcohol	6:2 FTOH	ChemSpider ID: 62741	$C_8F_{13}OH_5$	364.1	-	-	-	-	-	-
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1	$C_{12}HF_{23}O_2$	614.10	249	108	-	-	-	-
Perfluoro-n-octadecanoic acid	PFODA	16517-11-6	$C_{18}HF_{35}O_2$	914.14	-	-	-	-	-	-
n-3-perfluoroheptyl propanoic acid	FHpPA	812-70-4	$C_{10}H_5F_{15}O_2$	442.12	-	83-85	-	-	-	-
Sodium Perfluoro-1-propanesulfinate	PFPrS	423-41-6	$C_3HF_7O_3S$	250.09	-	-	-	-	-	-
Perfluorohexylphosphonic acid	PFHxPA	40143-76-8	$C_6H_2F_{13}PO_3$	400.03	-	>79	-	-	-	-
Perfluoro-n-hexanoic acid	PFHxA	307-24-4	$C_6HF_{11}O_2$	314.05	157	-	15.7	-	39730.1	-0.16
Perfluoro-n-tetradecanoic acid	PFTeDA	376-06-7	$C_{13}F_{27}COOH$	714.11	-	-	-	-	-	-

Potassium perfluoro-4-ethylcyclohexanesulfonate	PFECHS	80988-54-1	$C_8F_{15}O_3S^-$	461.13	-	-	-	-	-	-
Sodium Perfluoro-1-hepatanesulfonate	PFHpS	375-92-8	$C_7HF_{15}O_3S$	450.12	-	-	-	-	-	-
Perfluoroundecanoic acid	PFUnDA	2058-94-8	$C_{11}HF_{21}O_2$	564.09	-	-	-	-	-	-
Perfluoro-3-methoxypropanoic acid	PFMOPrA	377-73-1	$C_4HF_7O_3$	230.04	-	-	-	-	-	-
Perfluoro-n-tridecanoic acid	PFTriDA	72629-94-8	$C_{13}HF_{25}O_2$	664.10	-	-	-	-	-	-
Perfluoro (4-Methoxybutanoic) acid	PFMOBA	863090-89-5	$C_5HF_9O_3$	280.04	-	-	-	-	-	-

Supporting Information Figure S.1. Flow diagram of inclusion and exclusion of literature included in the critical review.

