Effect of PFAS Molecules for the Human Health

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Per- and polyfluoroalkyl substances (PFAS) are a group of over 4700 heterogeneous compounds with amphipathic properties and exceptional stability to chemical and thermal degradation. The unique properties of PFAS compounds has been exploited for almost 60 years and has largely contributed to their wide applicability over a vast range of industrial, professional and non-professional uses. However, increasing evidence indicate that these compounds represent also a serious concern for both wildlife and human health as a result of their ubiquitous distribution, their extreme persistence and their bioaccumulative potential. In light of the adverse effects that have been already documented in biota and human populations or that might occur in absence of prompt interventions, the competent authorities in matter of health and environment protection, the industries as well as scientists are cooperating to identify the most appropriate regulatory measures, substitution plans and remediation technologies to mitigate PFAS impacts.

PFAS PFOA PFOS human health ecosystem remediation technologies

1. Introduction

Per-and polyfluoroalkyl substances (PFAS) constitute an heterogeneous group of fluorinated synthetic compounds characterized by the presence of at least one perfluorinated methyl group ($-CF_3$) or a perfluorinated methylene group ($-CF_2-$), a variable number of carbon atoms, fluorination degree and presence of other chemical groups. PFAS are almost ubiquitous into the environment, mainly due to their wide dispersive use and applicability in a vast number of industrial sectors and consumer products [1][2]. Increasing concern for human health and wildlife ecology derives from the thermal and chemical stability of PFAS molecules and the multiple routes through which humans and biota can be exposed during their lifetime [3][4][5][6][7]. Of note, while the PFAS family has rapidly expanded into an impressive number of more than 4700 different substances including both the “legacy PFAS” (i.e., PFOS, PFOA) and the “emerging PFAS” (e.g., GenX) [8][9] producers, decision makers as well as researchers try to gain insights on their impact and to find the most appropriate measures to mitigate the potential risks associated with their exposure. Common features of PFAS are represented by their chemical stability which causes environmental persistence [10], their high mobility which confers them a long-range transport potential [11] causing their pervasive spreading even into remote regions (e.g., the Arctic’s or Antarctic’s) [12][13][14] and their tendency to bioaccumulate and biomagnify in biota through the contamination of the food chains [15][16][17][18][19]. The presence of some PFAS has been reported in the blood [20][21], milk [22][23], urine [24] tissues [25][26][27] and organs [28][29][30][31][32] of different human populations living in developed countries and has been associated to a number of adverse health effects. Similarly, relevant concentrations of PFAS have been detected in the air [33][34], groundwater [35][36], freshwater [17][37], marinewater [38][39], drinking water [40][41] and soil [42][43][44] potentially causing ecotoxic effects in the aquatic and terrestrial ecosystems at the trophic levels of primary producers, primary consumers and secondary consumers [45][46]. An additional layer of complexity is given by the coexistence of different mixtures of PFAS substances and other contaminants in the environmental media, for which quantitative risk assessment analysis and toxicologic/ecotoxicologic information is still scarce if not absent [47][48].

2. PFAS Human Exposure and the Potential Effects for Human Health

It is well recognized that multiple exposure pathways can link PFAS emission from the primary or secondary sources to human receptors represented by professional workers as well as general population. Some of the most relevant exposure routes include the inhalation of air and dust particulate, the ingestion of contaminated food and drinking water and the dermal adsorption [49]. Despite the presence of some gaps in the researchers understanding, it is generally accepted that the dietary intake and the consumption of drinking water represent major pathways for the general population [50][51] while the relative contribute of inhalation and dermal contact is far more relevant in case of occupational exposure [52][53][54]. It is also believed that PFOS, PFOA, PFNA and PFHxS are the PFAS species that currently contribute most to human exposure, a reason for which provisional measures limiting their daily intake have been proposed in 2020 by the European Food Safety Authority (EFSA) [55]. Irrespectively to the specific exposure pathway through which they can get in contact with human targets, PFAS substances represent a serious concern for human health potentially inducing alterations in the development, lipid metabolism and endocrine system, cancerogenicity, immunotoxicity, hepatotoxicity and reprotoxicity. In terms of PFAS risk assessment, the EFSA CONTAM Panel was the first international scientific body to include the data from epidemiological studies to derive health-based guidance values for PFOA and PFOS in 2018. The critical effects of PFOS were identified as a rise in blood total cholesterol in adults and a reduction in antibody response to immunization in children. On the other hand, the key consequence of PFOA was a rise in blood total cholesterol. Reduced birth weight (for both chemicals) and an increased incidence of elevated blood levels of the liver enzyme alanine aminotransferase (ALT) (for PFOA) were also taken into account. Tolerable weekly intake (TWI) of 13 ng/kg body weight (b.w.) per week was defined for PFOS and 6 ng/kg b.w. per week for PFOA after benchmark modelling of serum levels of PFOS and PFOA and estimated the associated daily intakes. It was also observed that the exposure to a significant fraction of the population to both chemicals exceeded the proposed TWIs EFSA (2018) Risk to human health related to the presence of PFOS and PFOA in food [56]. The ATSDR (Agency for Toxic Substances and Disease Registry 2018) also included a decreased antibody response to vaccines (PFOA, PFOS, PFHxS, and PFDA) and increased risk of asthma diagnosis (PFOA) among the list of adverse health effects in PFAS-exposed humans (ATSDR Toxicological Profile for Perfluoroalkyls). Furthermore, the International Agency for Research on Cancer (IARC) classed PFOA as “possibly carcinogenic to humans” (Group 2B), based on limited evidence in humans that it might cause testicular and kidney cancer, as well as limited data in animal studies [57].

Potential of PFAS to cause a wide range of negative health impacts depends of various factors, such as the conditions of exposure (dose/concentration, duration, route of exposure, etc.) and characteristics associated with the exposed target (e.g., age, sex, ethnicity, health status, and genetic predisposition) [58]. The list of biological functions impacted by PFAS in females and males is rapidly expanding. Endocrine disruptive effects have been reported to affect fertility, body weight control, thyroid and mammary gland function. Developmental effects have been observed in children such as alterations in the behaviour or accelerated puberty but also in the new-borns such as decreased birth weight. Increased risk of kidney, prostate and testicular cancer has been associated with long-term exposure to PFAS in the general population alongside with disturbances in the cholesterol metabolism or reduced efficiency of the immune system against infections.

2.1. In Vitro Studies on PFAS Effects

Selected in vitro studies (published since 2010) exploring toxic effects of PFAS are summarised in **Table 1**. Majority of the presented in vitro studies investigated the impact of PFOA and/or PFOS, while the main observed effects were on thyroid [59][60][61][62] and hepatic cells [63][64][65][66][67]. In vitro exposure of thyroid cells to different PFAS was shown to have varied

thyroid-disrupting effects. Conti et al. (2020) exposed thyroid follicular cells to 1–100 mM PFOS or PFOA, concluding that both substances acutely and reversibly inhibited iodide accumulation by FRTL-5 thyrocytes. Additionally, PFOS prevented sodium iodide symporter-mediated iodide uptake and reduced intracellular iodide concentration in iodide-containing cells. However, this substance did not affect iodide efflux from thyroid cells [59]. Furthermore, Song et al. (2012) documented decreased TPO activity in FTC-238/hrTPO/RSK008 cells after the exposure to different PFOS and PFOA concentrations [60]. At concentration of 10^5 nM PFOA/PFOS, a significant inhibition of cell proliferation was found in rat thyroid cell line-5 (FRTL-5), which mostly occurred due to the increased cell death. The results of this study also suggested that PFOA and PFOS enter thyroid cells by a gradient-based passive diffusion mechanism [61]. Croce et al. (2019) investigated the impact of different long-chain and short-chain PFAS, including PFOS, perfluorobutanesulfonic acid (PFBS), perfluorobutanoic acid (PFBA), perfluorophosphonic acid (PFPA) and perfluoropentanoic acid (PFPeA), on the same cell line (FRTL-5), at various concentrations (up to 100 µM). However, aside from PFOS (100 µM), neither long, nor short-chain PFCs impacted cell survival or interfered with cAMP synthesis. As a result, the authors came to the conclusion that short-chain PFCs had no acute cytotoxic effect on thyroid cells in vitro [62].

Table 1. Selected in vitro studies (published since 2010) exploring the toxicity of polyfluoroalkyl substances (PFAS).

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
thyroid follicular cells	PFOS PFOA	PFOS or PFOA (1–100 mM)	Cytotoxicity: 1 h	• PFOS, but not PFOA, acutely and reversibly inhibited iodide accumulation by FRTL-5 thyrocytes, as well as by HEK-293 cells transiently expressing the Sodium Iodide Symporter (NIS) • PFOS prevented NIS-mediated iodide uptake and reduced intracellular iodide concentration in iodide-containing cells, mimicking the effect of the NIS inhibitor perchlorate • PFOS did not affect iodide efflux from thyroid cells	(Conti, Strazzeri, and Rhoden 2020) [59]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
FTC-238/hrTPO/RSK008 cells	PFOS PFOA	10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M	/	• Decreased TPO activity	(Song et al., 2012) [60]
rat thyroid line-5 (FRTL-5)	PFOS PFOA	1, 10, 10^2, 10^3, 10^4, and 10^5 nM	72 h	• At concentration of 10^5 nM PFOA/PFOS, a significant inhibition of cell proliferation, mainly due to increased cell death, was found • PFOA and PFOS enter thyroid cells by a gradient-based passive diffusion mechanism	(Coperchini et al., 2015) [61]
rat thyroid line-5 (FRTL-5)	FOA, PFOS, perfluorobutanesulfonic acid (PFBS), perfluorobutanoic acid (PFBA), pentafluoropropionic anhydride (PFPA), perfluoropentanoic acid (PFPeA)	0.0001; 0.001; 0.01; 0.1; 1; 100 µM	24 h	• Neither long nor short-chain PFCs affected cell viability (apart from PFOS 100 µM), or interfered with cAMP production • Short-chain PFCs have no acute cytotoxic effect on thyroid cells in vitro	(Croce et al. 2019) [62]
Human hepatoma cell line (HepG2)	perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluoroctanoic acid (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA),	2×10^{-7}, 1×10^{-6}, 2×10^{-6}, 1×10^{-5}, 2×10^{-5} M	24 h	• Except for PFDoA, all the other PFAS increased ROS generation • For PFHxS and PFUnA the	(Wielsøe et al., 2015) [64]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
	perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA).			observed ROS increases were dose-dependent • Cells exposed to PFOA were found to have a significant lower total antioxidant capacity (TAC) compared with the solvent control, whereas a non-significant trend in TAC decrease was observed for PFOS and PFDoA and an increase tendency for PFHxS, PFNA and PFUnA	
Human Embryo Liver L-02 Cells	PFOS	0, 50, 100, 150, 200, or 250 μmol/L	24 or 48 h	• Decreased cell activities, enhanced ROS levels in a concentration-dependent manner • Decreased mitochondrial membrane potential (MMP), and induced autophagy and apoptosis • Enhanced expression of Bax, cleaved-caspase-3, and LC3-II • Induced autophagy; decreased MMP; and lowered Bcl-2,	(Zeng et al., 2021) [65]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
				p62, and Bcl-2/Bax ratio • ROS-triggered autophagy is involved in PFOS-induced apoptosis in L-02 cells	
Human HepaRG liver cells	PFOA, PFOS, and perfluorononanoic acid (PFNA)	6.25, 12.5, 25, 50, 100, 200, 400 µM	6, 24, or 72 h	• All PFAS induced an increase in cellular triglyceride levels, but had no effect on cholesterol levels • PFOA, PFOS, and PFNA increase triglyceride levels and inhibit cholesterogenic gene expression • PFAS induce endoplasmic reticulum stress, which may be an important mechanism underlying some of the toxic effects of these chemicals	(Louisse et al., 2020) [66]
HepaRG cell line	PFOS PFOA	100, 250, 500, 750 µM PFOA 50, 100, 250, 500 µM PFOS	/	• Cholesterol levels in HepaRG cells were not affected by PFOA or PFOS • Both substances strongly decreased	Behr et al., 2021) [67]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
				synthesis of a number of bile acids • The expression of numerous genes whose products are involved in synthesis, metabolism and transport of cholesterol and bile acids was strongly affected by PFOA and PFOS at concentrations above 10 µM • Both substances led to a strong decrease of CYP7A1, the key enzyme catalyzing the rate-limiting step in the synthesis of bile acids from cholesterol, both at the protein level and at the level of gene expression • Both substances led to a dilatation of bile canaliculi	
Neurons	PFOS PFOA	30–300 µM	30 min	• Both PFOS and PFOA can accumulate in cultured neurons and elevate calcium concentrations via release of intracellular calcium stores	(Liu et al., 2011) [68]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
				• 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) were found to take part in PFOS or PFOA inducing calcium release from calcium stores • Calcium release from intracellular stores may partially account for the perturbation of calcium homeostasis caused by PFOS or PFOA	
Primary rat cortical cultures and hiPSC-derived neuronal co-cultures	PFOS PFOA	0.01, 0.1, 1, 10, 100 µM	/	• PFOS and PFOA inhibited the GABA-evoked current and acted as non-competitive human GABAA receptor antagonists • Network activity of rat primary cortical cultures increased following exposure to PFOS (LOEC 100 µM)	(Tukker et al., 2020) [69]
Rat primary hippocampal neurons and astrocytes	PFOS	25, 50, 75, 100, 125 µM for neurons 15, 25, 50, 75, 100 µM for astrocytes	24 h	• Redox imbalance, increased apoptosis and abnormal autophagy in rat primary	(Li et al., 2017) [70]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
				hippocampal neurons. • In astrocytes: altered extracellular glutamate and glutamine concentrations, decreased glutamine synthase activity, as well as decreased gene expression of glutamine synthase, glutamate transporters and glutamine transporters in the glutamate-glutamine cycle	
primary rat embryonic neural stem cells (NSCs)	PFOS	12.5–100 nM	48 h	• Increase in neuronal differentiation • Increased number of CNPase-positive cells, pointing to facilitation of oligodendrocytic differentiation • Upregulation of PPARγ with no changes in PPARα or PPARδ genes • Upregulated mitochondrial uncoupling protein 2 (UCP2)	(Wan Ibrahim et al., 2013) [71]

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
				• Induced Ca^{2+} activity	
rat primary neurons and neural stem cells (NSC) [64][65]	PFOS PFOA	1–250 µM	24 h [65]	• No effects on cell viability or proliferation in primary neurons • PFOS exposure increased the NSC proliferation at the lowest concentration tested (1–100 µM) • PFOS and PFOA caused morphological alterations of NSC-derived neurons. Exposure to 1 and 10 µM PFOA also affected the neurite network and caused an increase in the number of processes and branches per cell NSC, mimicking the immature brain, is clearly more susceptible to PFOS and PFOA exposure than the primary neurons	(Pierozan and Karlsson 2021) [72] [67]
fetal rat testes or seminiferous tubule segments (stage VII-VIII) of adult rats [65]	PFOA	0–100 µg/mL	24 h	• Levels of cAMP, progesterone, testosterone and expression of StAR decreased significantly in	(Eggert et al., 2019) [73]

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cholesterol levels. In HepaRG cells, PFOA, PFOS, and PFNA enhanced triglyceride levels while inhibiting cholesterogenic gene expression. The authors noted that PFAS-induced endoplasmic reticulum stress might be an essential mechanism underpinning some of the harmful effects caused by these compounds [66]. Similarly, Behr et al. (2021) investigated the effects of PFOS/PFAS exposure and found that cholesterol levels in the same cell line (HepaRG) were not affected by neither PFOA nor PFOS. However, in this study, both substances strongly decreased synthesis of a number of bile acids. Moreover, the expression of numerous genes whose products are involved in synthesis, metabolism and transport of

Cell Type	Substance	Treatment Concentration	Incubation Time	Effects	Ref.
[70]				PFOA 50 and 100 µg/mL. PFOA affected cell populations significantly by decreasing the amount of diploid, proliferating, meiotic I and G2/M phase cells in adult rat testis • PFOA did not affect fetal, proliferating or adult rat Sertoli cells but an increased tendency of apoptosis in fetal Leydig cells was observed	[68]
human cell lines such as MCF-7, H295R, LNCaP and MDA-kb2 [72]	PFOA, PFOS, and of six substitutes including perfluorohexanesulfonic acid (PFHxS), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorobutanoic acid (PFBA), ammonium perfluoro(2-methyl-3-oxahexanoate) (PMOH), and 3H-perfluoro-3-[(3-methoxypropoxy) propanoic acid] (PMPP)	various concentrations	24 h when cytotoxicity was assayed in HEK293T, LNCaP or MDA-kb2 cells, for 6 d in MCF-7 cells and for 48 h in H295R cells	• PFOA, PFOS and PMOH enhanced 17β-estradiol-stimulated estrogen receptor β activity, and PFOS, PMOH, PFHxA and PFBA enhanced dihydrotestosterone-stimulated androgen receptor activity • PFOA and PFOS slightly enhanced estrone secretion, and progesterone secretion was marginally increased by PFOA. [74]	(Behr et al.,2018) [74]

PFAS exposure has mostly been linked to adverse outcomes in mice and rats, while the majority of studies explored the toxicity of PFOS, PFOA and PFHxS. Selected in vivo studies (published since 2010) investigating toxic effects of PFAS are presented in **Table 2**.

Table 2. Selected in vivo studies (published since 2010) exploring the toxicity of polyfluoroalkyl substances (PFAS).

Species	Substance	Treatment Dose and Route of Exposure	Incubation Exposure Time	Effects	Ref.
Rats	PFOS	20 or 100 ppm, dietary exposure	7 days	• Changes in liver parameters (increased liver weight; decreased plasma cholesterol, alanine aminotransferase, and triglycerides; decreased liver DNA concentration and increased hepatocellular cytosolic CYP450 concentration; increased liver activity of acyl CoA oxidase, CYP4A, CYP2B, and CYP3A; increased liver proliferative index and decreased liver apoptotic index; decreased hepatocellular glycogen-induced vacuoles; increased centrilobular hepatocellular hypertrophy. • Thyroid parameters (histology, apoptosis, and proliferation) unaffected.	(Elcombe et al., 2012) [75]
Mice	PFOS	10 mg PFOS/kg b.w./day), oral gavage	14 days	• Dysregulated proteins in lipid and xenobiotic metabolism in liver • 16 overexpressed glycoproteins associated with neutrophil degranulation, cellular responses to stress, and protein processing in the endoplasmic reticulum (ER)	(D. Li et al., 2021) [76]
Mice	PFOS	100 µg/kg b.w./day and 1000 µg/kg b.w./day, oral gavage	2 months	• PFOS accumulated in liver, lungs, kidneys, spleen, heart and brain • Its accumulation caused damage in the liver and in the marginal area of the heart • PFOS mainly affected glycerophospholipid metabolism and sphingolipid metabolism in liver • Up-regulated ceramide and • lysophosphatidylcholine (LPC) might lead to liver cell apoptosis	(X. Li et al., 2021) [77]

Species	Substance	Dose and Route of Exposure	Exposure Time	Effects	Ref.
				• Decrease in liver triglyceride (TG) content might result in insufficient energy and cause liver morphological damage	
Rats	PFOA	5 mg/kg b.w./day, oral gavage	28 days	• Increase in hepatic (GGT, ALT, AST and ALP) and renal function (urea and creatinine) biomarkers of toxicities • Decrease in the activity of the enzymatic antioxidants (CAT, GPx, SOD) in liver and kidney tissue • Increase in lipid peroxidation and proinflammatory cytokine IL-1β • Decrease of the antiinflammatory cytokine, IL-10	(Owumi, Bello, and Oyelere 2021) [78]
Mice	PFOA	1, 5, 10, or 20 mg/kg/day, oral gavage	10 days	• Increase in Dnmt1 with decreased Rasal1 expression at higher levels of PFOA exposure. • Rasal1 hypermethylation, followed by the increase in Hdac1, 3 and 4. • Increased mRNA expression levels of TGF-β and α-SMA	(Rashid et al., 2020) [79]
Mice	PFHxS	Up to 3 mg/kg b.w./day, oral gavage	Administered before mating, for at least 42 days in F0 males, and for F0 females, through gestation and lactation. F1 pups-directly for 14 days after weaning	• Adaptive hepatocellular hypertrophy, concomitant decreased serum cholesterol and increased alkaline phosphatase (S. Chang et al., 2018).	(Chang et al., 2018) [80]
Rats	PFHxS	0.05, 5 or 25 mg/kg b.w./day, oral gavage	From gestation day 7 through to postnatal day 22	• PFHxS lowered thyroid hormone levels in both dams and of spring in a dose-dependent manner	(Ramhøj et al., 2020) [81]

SpeciesSubstance	Dose and Route of Exposure	Exposure Time	Effects	Ref.
			• PFHxS did not change TSH levels, weight, histology, or expression of marker genes of the thyroid gland	
Mice	6.1, and 9.1 mg/kg b.w., oral gavage	Neonatal exposure from postnatal day 10	• PFHxS induces persistent developmental neurotoxicity and GAP-43 and CaMKII downregulation via the NMDA receptor-mediated PKCs (α and δ)-ERK/AMPK pathways • Significant memory impairment in adult mice	(Sim and Lee, 2022) [82]

A link between PFOS exposure and hepatic lipid metabolism was found in vivo. After the exposure of C57BL/6 mice to 10 mg PFOS/kg b.w./day by oral gavage for 14 days, 241 proteins involved in lipid and xenobiotic metabolism in liver were found to be dysregulated. 16 overexpressed glycoproteins were associated with neutrophil degranulation, cellular responses to stress, and protein processing in the endoplasmic reticulum (ER) [76]. Li et al. (2021) also documented the ability of PFOS to accumulate in the liver of female BALB/c mice after the exposure to 100 µg/kg b.w./day and 1000 µg/kg b.w./day by oral gavage for 2 months. PFOS accumulated in the lungs, kidneys, spleen, heart and brain as well, causing damage in the liver and in the marginal area of the heart. PFOS mainly affected glycerophospholipid metabolism and sphingolipid metabolism in liver. These authors suggested that the upregulated ceramide and lysophosphatidylcholine (LPC) might lead to liver cell apoptosis, while decrease in liver triglyceride (TG) content might result in insufficient energy and cause liver morphological damage [77]. After the dietary exposure of rats to 20 or 100 ppm PFOS for 7 days, Elcombe et al. (2012) have also noted alterations in various liver parameters (e.g., increased liver weight; decreased plasma cholesterol, alanine aminotransferase, and triglycerides; increased hepatocellular cytosolic CYP450 concentration; increased liver activity of acyl CoA oxidase, CYP4A, CYP2B, and CYP3A; increased liver proliferative index and decreased liver apoptotic index). However, in the aforementioned study, thyroid parameters (histology, apoptosis, and proliferation) were not unaffected [82].

Owumi et al. (2021) investigated hepatic and kidney function of PFOA. After the exposure of rats to PFOA (5 mg/kg b.w./day) for 28 days, the authors noted an increase in hepatic (GGT, ALT, AST and ALP) and renal function (urea and creatinine) as well as biomarkers of toxicity, paralleled by a decrease in the activity of the enzymatic antioxidants (CAT, GPx, SOD) in liver and kidney tissue. In this study, a significant increase in lipid peroxidation and pro-inflammatory cytokine IL-1β in rats' liver and kidney occurred, while a decrease of the anti-inflammatory cytokine, IL-10 was also observed [78]. Similarly, renal damage was found in mice after the exposure to PFOA. Rashid et al. (2020) noted an increase in Dnmt1 with decreased Rasal1 expression at higher levels of PFOA exposure. Rasal1 hypermethylation (an early indicator of fibroblast activation in kidney) was also observed, followed by the increase in Hdac1, 3 and 4, class I & II HDACs which are known to be critically altered in some renal diseases. Furthermore, mRNA levels of TGF-β and α-SMA were significantly increased [79].

Health effects of PFHxS have also been investigated in animal studies. After the oral exposure of F0 and F1 CD-1 mice to 3 mg PFHxS/kg b.w/day, equivocal decrease in live litter size at 1 and 3 mg/kg b.w./day was noted, as well as adaptive hepatocellular hypertrophy, concomitant decreased serum cholesterol and increased alkaline phosphatase [80]. After treating

rat with 0.05, 5 or 25 mg/kg b.w./day PFHxS from gestation day 7 onward to postnatal day 22, Ramhøj et al. (2020) observed a dose-dependent decrease in thyroid hormone levels in both dams and offspring, while TSH levels, weight, histology, or expression of marker genes of the thyroid gland were unaffected [81]. Other neurotoxic effects of PFHxS have been investigated as well. Sim and Lee (2022) have found that PFHxS causes long-term developmental neurotoxicity as well as downregulation of GAP-43 and CaMKII via the NMDA receptor-mediated PKCs (and)-ERK/AMPK pathways in mice after the neonatal exposure, together with significant memory impairment in adult mice [82].

2.3. Human Studies on PFAS Effects

Selected human studies (published since 2010) investigating toxic effects of PFAS are presented in **Table 3**. Majority of the human studies explored the linkage between PFAS concentration and lipid status, mainly cholesterol level [83][84], while a study was also conducted to assess the connection between PFAS and cholesterol at the gene expression level [85]. Eriksen et al. (2013) discovered substantial positive relationships between PFOS, PFAS, and total cholesterol in 753 individuals, while sex and prevalence of diabetes were suggested to influence the connection between these two substances and cholesterol [83]. Fletcher et al. (2013) observed an inverse relationship between serum PFOA levels and the expression level of genes involved in cholesterol transport in whole blood (NR1H2, NPC1 and ABCG1). A positive correlation was found between PFOS and a transcript involved in cholesterol mobilization (NCEH1), while a negative relationship was seen between PFOS and a transcript involved in cholesterol transport (NCEH2). Sex-specific effects were also noticed in this study [85]. On the other hand, in a study involving 815 participants ≤18 years of age, Geiger et al. (2014) found that serum PFOA and PFOS were related with high total cholesterol and LDL-C levels, regardless of age, gender, race-ethnicity, body mass index, yearly family income, physical activity, or serum cotinine levels. PFOA and PFOS were not shown to be substantially linked with aberrant HDL-C and triglyceride levels [84].

Table 3. Selected human studies (published since 2010) exploring the toxicity of polyfluoroalkyl substances (PFAS).

Substance	Population	Measured Parameters	Results	Ref.
PFOS PFOA	middle-aged Danish population; 753 individuals (663 men and 90 women), 50–65 years of age, nested within a Danish cohort of 57,053 participants	serum levels of total cholesterol	• Statistically significant positive associations between PFOS, PFAS and total cholesterol level • Sex and prevalent diabetes modified the association between PFOA and PFOS and cholesterol	(Eriksen et al., 2013) [83]
PFOS PFOA	815 participants ≤18 years of age from the National Health and Nutrition Examination Survey 1999–2008	dyslipidemia: total cholesterol >170 mg/dL, low-density lipoprotein cholesterol (LDL-C) >110 mg/dL, high-density lipoprotein cholesterol (HDL-C) <40 mg/dL or triglycerides >150 mg/dL.	• Serum PFOA and PFOS-positively associated with high total cholesterol and LDL-C, independent of age, sex, race-ethnicity, body mass index, annual household income, physical activity and serum cotinine levels	(Geiger et al., 2014) [84]

Substance	Population	Measured Parameters	Results	Ref.
			• PFOA and PFOS-not significantly associated with abnormal HDL-C and triglyceride levels.	
PFOS PFOA	290 individuals (144 men + 146 women) exposed to background levels of PFOS and elevated concentrations of PFOA through drinking water, aged between 20 and 60 years	expression of genes involved in cholesterol metabolism	• Inverse associations between serum PFOA levels and the whole blood expression level of genes involved in cholesterol transport (NR1H2, NPC1 and ABCG1) • A positive association between PFOS and a transcript involved in cholesterol mobilisation (NCEH1), and a negative relationship with a transcript involved in cholesterol transport (NR1H3) • Reductions in the levels of mRNAs involved in cholesterol transport were seen with PFOA in men (NPC1, ABCG1, and PPARA) and in women (NR1H2 expression) • Increase in the levels of a cholesterol mobilisation transcript (NCEH1) in women. • PFOS was positively associated with expression of genes involved in both cholesterol mobilisation and transport in women (NCEH1 and PPARA)	(Fletcher et al., 2013) [85]
PFOA PFOS PFHxS PFNA PFDA	2883 participants, (1801 non-obese and 1082 obese), aged more than or equal to 20 years old	liver function parameters: AST, ALT, GGT, ALP, and total bilirubin (TB)	• Among obese participants only, alanine aminotransferase (ALT)-positively associated with PFOA, PFHxS, and PFNA • PFOA and PFNA were associated with gamma GGT in obese participants	(Jain and Ducatman 2019) [86]

Substance	Population	Measured Parameters	Results	Ref.
14 PFCs	Healthy men from the general population, median age of 19 years	total testosterone (T), estradiol (E), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and inhibin-B and Semen samples analysis	• PFOS levels-negatively associated with testosterone, calculated free testosterone (FT), free androgen index (FAI) and ratios of T/LH, FAI/LH and FT/LH • Other PFCs were found at lower levels than PFOS and did not exhibit the same associations. • PFC levels were not significantly associated with semen quality	(Joensen et al., 2013) [87]
PFOA PFOS PFHxS PFNA	1682 males and females 12 to 80 years of age	testosterone (T), thyroid stimulating hormone (TSH), and free and total triiodothyronine (FT3, TT3) and thyroxine (FT4, TT4)	• Exposure to PFAS may be associated with increases in FT3, TT3, and FT4 among adult females • During adolescence, PFAS may be related to increases in TSH among males and decreases in TSH among females • No significant relationships were observed between PFAS and T in any of the models	(Lewis, Johns, and Meeker 2015) [88]
PFOS PFOA	3076 boys and 2931 girls aged 8–18 years	subjects were classified as having reached puberty based on either hormone levels (total >50 ng/dL and free >5 pg/mL testosterone in boys and estradiol >20 pg/mL in girls) or onset of menarche	• For boys, there was a relationship of reduced odds of reached puberty (raised testosterone) with increasing PFOS (delay of 190 days between the highest and lowest quartile) • For girls, higher concentrations of PFOA or PFOS were associated with reduced odds of postmenarche (130 and 138 days of delay, respectively)	(Lopez-Espinosa et al., 2011) [89]

Substance	Population	Measured Parameters	Results	Ref.
PFOS PFOA PFNA	2292 children (6–9 years of age)	estradiol, total testosterone, and IGF-1	• In boys, PFOA concentrations were significantly associated with testosterone levels; PFOS with estradiol, testosterone, and IGF-1; and PFNA with IGF-1 • In girls, significant associations were found between PFOS and testosterone and IGF-1; and PFNA and IGF-1	(Lopez-Espinosa et al., 2016) [90]
PFOS PFOA	424 mother-infant pairs	estrone (E1), b-estradiol (E2), and estriol (E3), infants: head circumference, body weight, body length	• PFOS was positively related to E1 and E3, but negatively related to E2 • Serum PFOA was positively related to serum E1 and negatively related to head circumference at birth • Serum E2 was negatively related to head circumference, body weight, and body length at birth and serum E3 was positively related to body weight • Serum E3 mediated the relationship between serum PFOS and body weight • PFAS could affect estrogen homeostasis and fetal growth during pregnancy and estrogens might mediate the association between exposure to PFAS and fetal growth	(Wang et al., 2019) [91]
PFOS PFOA	47,092 adults	alanine transaminase (ALT), γ-glutamyltransferase (GGT), direct bilirubin	• Positive association between PFOA and PFOS concentrations and serum ALT level, a marker of hepatocellular damage.	(Gallo et al., 2012) [92]

Substance	Population	Measured Parameters	Results	Ref.
			• The relationship with bilirubin appears to rise at low levels of PFOA and to fall again at higher levels.	
PFHpA PFOA PFNA PFDA PFUnDA PFDoDA PFHxS PFOSA	1002 individuals from Sweden (50% women) at ages 70, 75 and 80	bilirubin and hepatic enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT)	• Positive associations of PFHpA, PFOA, PFNA, PFDA, and PFUnDA with ALP • Concentrations of PFHpA, PFOA, PFNA, and PFOS were positively associated with the activity of ALT • The changes in PFAS concentrations were positively associated with GGT and inversely associated with the changes in circulating bilirubin	(Salihovic et al., 2018) [30]
PFOS PFOA PFHxS	3297 participants from Ronneby, a municipality with drinking water highly contaminated by PFAS (exposed group)	thyroid hormone levels, with adjustments for age, sex and BMI	• No associations between PFAS and thyroid hormones in adults and seniors except for a positive association between PFAS and fT4 in males over 50 • Higher thyroid hormone levels in the preteen children from Ronneby compared to the reference group • Weak evidence of associations between increased PFAS levels and decreased fT3 in preteen boys, and decreased TSH in teenage males	(Y. Li et al., 2021) [93]
PFOA PFOS	101 healthy 1-year-old children	Antibodies against haemophilus infuenza type b, tetanus and diphtheria, interferon gamma, cholesterol	• Significant associations between PFOA, but not PFOS concentrations, and adjusted levels of vaccine antibodies	(Abraham et al., 2020) [94]

Substance	Population	Measured Parameters	Results	Ref.
			against haemophilus influenza type b, tetanus and diphtheria • PFOA levels inversely related to the interferon gamma (IFN) production of ex-vivo lymphocytes after stimulation with tetanus and diphtheria toxoid • No infuence of PFOA and PFOS on infections and cholesterol level during the frst year of life	
PFOA PFOS	1146 children	serum concentrations of specific IgG antibodies against tetanus and diphtheria at ages 5 and 7	• Approximate BMDL of 1 ng/mL serum for both PFOS and PFOA for the serum concentrations of specific IgG antibodies against tetanus and diphtheria at ages 5 and 7 • Proposed reference concentration of about 0.1 ng/mL as the serum-based target	(Budtz-Jørgensenet al., 2018) [95]
PFHxS, PFOS, PFOA, PFDA, PFNA	275 males and 349 females participated in clinical examinations and provided blood samples at ages 18 months and 5 years	serum concentrations of antibodies against tetanus and diphtheria vaccines determined at age 5	• Pre-natal exposure showed inverse associations with the antibody concentrations five years later, with decreases by up to about 20% for each two-fold higher exposure • Associations for serum concentrations at 18 months and 5 years were weaker • Concentrations estimated for ages 3 and 6 months showed the strongest inverse associations with antibody concentrations at age 5 years, particularly for tetanus	(Grandjean et al., 2017) [96]

Substance	Population	Measured Parameters	Results	Ref.
			• Joint analyses showed statistically significant decreases in tetanus antibody concentrations by 19–29% at age 5 for each doubling of the PFAS exposure in early infancy	
PFHxS, PFOA, PFOS, PFNA, PFDA.	516 subjects	PFAS serum concentrations and concentration of antibodies against diphtheria and tetanus	• Diphtheria antibody concentrations decreased at elevated PFAS concentrations at 13 y and 7 y; the associations were statistically significant for perfluorodecanoate (PFDA) at 7 y and for perfluorooctanoate (PFOA) at 13 y, both suggesting a decrease by ~25% for each doubling of exposure • Structural equation models showed that a doubling in PFAS exposure at 7 y was associated with losses in diphtheria antibody concentrations at 13 y of 10–30% for the five PFAS	(Grandjean et al., 2017) [97]

Other studies explored the link between PFAS concentration and different hormones, such as thyroid [88][93] and sex hormones [91][93][94], as well as development [89][91]. By assessing the connection between the levels of 14 PFAS in healthy men from the general population and different sex hormones and semen sample quality, Joensen et al. (2013) found that only PFOS levels were negatively associated with testosterone, calculated free testosterone (FT), free androgen index (FAI) and ratios of T/LH, FAI/LH and FT/LH. Other PFAS were found at lower levels than PFOS and did not exhibit the same associations [87]. Also, after measuring PFAS levels in 1682 males and females 12 to 80 years of age, Lewis et al. (2015)

found no significant relationships between any of the PFAS and testosterone. PFAS were suggested to be associated with increases in FT3, TT3, and FT4 among adult females. The authors concluded that, during the adolescence, PFAS may be related to increases in TSH among males and decreases in TSH among females [88], suggesting sex-specific effects. In contrast, Li et al. (2021) discovered no associations between PFAS and thyroid hormones in adults and seniors in 3297 participants from Ronneby, a municipality with highly contaminated drinking water by PFAS (exposed group), with the exception of a positive association between PFAS and fT4 in males over 50. Thyroid hormone levels were observed to be higher in Ronneby preteen children compared to the control group. Weak evidence of a link between increasing PFAS levels and lower fT3 in preteen boys and lower TSH in adolescent men was found [93].

Lopez-Espinosa et al. (2011) aimed to investigate whether PFOS and PFOA were linked to the markers of sexual development. Their study included 3076 boys and 2931 girls aged 8–18 years. For boys, there was a link between increased PFOS and a lower chance of reaching puberty. Higher PFOA or PFOS concentrations in girls were related to a lower risk of post menarche [89]. The same group of researchers examined the link between PFAS levels and estradiol, total testosterone, and IGF-1 in 2292 children. In boys, PFOA concentrations were substantially related to testosterone levels; PFOS concentrations were related to estradiol, testosterone, and IGF-1, while PFNA concentrations were linked to IGF-1. Significant linkage was discovered in girls between PFOS and testosterone and IGF-1, as well as PFNA and IGF-1 [90]. Furthermore, Wang et al. (2019) concluded that PFAS may affect estrogen homeostasis and foetal growth during pregnancy, and that estrogens may mediate the relationship between PFAS exposure and foetal growth after examining 424 mother-infant pairs [91].

Some of the studies also explored the linkage between the PFAS exposure and liver function [31][97][98]. In 47,092 adult participants, Gallo et al. (2012) found a positive association between PFOA and PFOS concentrations and serum ALT level. On the other hand, the relationship with bilirubin appeared to increase at low levels of PFOA and decrease at higher levels [92]. In 1002 individuals from Sweden, Salihovic et al. (2018) have also found a positive association of PFHpA, PFOA, PFNA, and PFOS concentrations and ALT activity, but also positive associations of PFHpA, PFOA, PFNA, PFDA, and PFUnDA with ALP. These authors noted that the changes of investigated PFAS concentrations were positively associated with gamma glutamyl transferase (GGT) levels and inversely associated with the changes in circulating bilirubin [30]. On the other hand, in 2883 participants, (1801 non-obese and 1082 obese), Jain and Ducatman investigated the connection between liver function alterations and various PFAS. They concluded that connections might only be observed in the obese participants: alanine aminotransferase (ALT) was positively associated with PFOA, PFHxS, and PFNA. On the other hand, PFOA and PFNA were associated with GGT [86]. Epidemiological studies revealed a connection between PFAS and decrease in vaccination antibody production in early infants and children, especially having in mind that, if breastfed, they have a relatively high exposure and may be more susceptible as their immune system develops. Abraham et al. (2020) found significant associations between the concentration of PFOA, but not PFOS, and adjusted levels of vaccine antibodies against Haemophilus influenza type b, tetanus and diphtheria for which no observed adverse effect concentrations (NOAECs) were 12.2, 16.9 and 16.2 µg/L, respectively. Furthermore, PFOA levels were shown to be inversely related to the interferon gamma (IFN-γ) production of ex-vivo lymphocytes after stimulation with tetanus and diphtheria toxoid [94]. Furthermore, Budtz-Jorgensen E and Grandjean P (2018) found an approximate BMDL of 1 ng/mL serum for both PFOS and PFOA for the serum concentrations of specific IgG antibodies against tetanus and diphtheria at ages 5 and 7 as outcome parameters. These authors proposed the reference concentration of about 0.1 ng/mL as the serum-based target, a level which is below the most reported human serum-PFAS concentrations [95]. Grandjean et al. (2017) discovered that prenatal exposure to PFAS had an inverse relationship with antibody concentrations five years later, while concentrations measured at 3 and 6 months of age had the highest inverse relationships with antibody concentrations at 5 years of age, especially for tetanus [96]. The same

authors have found that diphtheria antibody concentrations dropped at higher PFAS concentrations at 13 and 7 years after booster vaccinations at 5 years of age; the correlations were statistically significant for PFDA at 7 years and PFOA at 13 years, implying a 25% decrease for each doubling of exposure [97].

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