Review

A Review on Environmental Occurrence and Toxicity of Perfluorooctanoic Acid and Its Selected Short-Chain Analogs–Perfluorohexanoic Acid and Perfluorobutanoic Acid

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A Review on Environmental Occurrence and Toxicity of Perfluorooctanoic Acid and Its Selected Short-Chain Analogs–Perfluorohexanoic Acid and Perfluorobutanoic Acid

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Abstract

In this review, the occurrence in the environment and human surrounding, as well as toxic action of perfluorooctanoic acid (PFOA) and its selected short-chain analogs – perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA) has been described. These substances belong to a group of polyfluoroalkyl substances (PFASs) widely represented in various compartments of the environment, including air, water and soil. They are also present in dust, drinking water and food, which are main sources of the exposure of humans to these compounds. Due to physico-chemical properties PFASs are strongly resistant to degradation in the biosphere and therefore, effectively accumulate in biota, including humans. PFOA has been produced from decades, but due to its toxicity it has been successively replacing by PFASs of shorter chain, including PFHxA and PFBA, which presence in the environment, as well as risk of human exposure and toxicity has been poorly investigated. It has been proven that PFOA reveals hepatotoxic and endocrine-disrupting activities, as well as exhibits prooxidative, immunotoxic, epigenetic and carcinogenic potential. Hitherto conducted researches have shown that PFHxA and PFBA are less toxic than PFOA, nevertheless additional extensive studies should be conducted in order to determine environmental and toxicological status of these compounds.

Keywords: perfluorooctanoic acid; perfluorohexanoic acid; perfluorobutanoic acid; environment; hepatotoxicity; epigenotoxicity; immunotoxicity

1. Introduction

Industrial activity effects and changes the environment. The example is the introduction of xenobiotics intentionally or as by-products, which is related with the production of other chemical substances. Hazardous substances are those that effect on human health and has been relatively poorly investigated. The example of such chemicals are per- and polyfluoroalkyl compounds (PFASs). First introduced in 1949, PFASs became widely utilized chemicals in the industry and were employed in various food contact materials, including anti-stick kitchen appliances or food wrapping [1].

PFASs are a group of synthetic chemicals containing a carbon chain, in which all (per-) or part (poly-) of the hydrogen atoms were replaced by fluorine atoms. Moreover, these substances have specific functional groups at the end. PFASs can be divided into long-chain (containing at least 7 carbon atoms) and short-chain compounds (containing 4-6 carbon atoms). In respect to their characteristic structure, PFASs reveal amphiphilic properties (simultaneously hydrophobic and hydrophilic). These substances are extremely persistent in the environment, are physically,

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chemically and biologically stable, are strongly resistant to degradation, and reveal accumulation in living organisms, including human [2].

Due to their unusual characteristics, PFASs are utilized in everyday products, such as food packaging, waterproof clothing, non-stick cookware, textiles, cleaners and paints, cosmetics, pesticides and pharmaceuticals [3,4].

Food and drinking water are the most important sources, from which PFASs enter the body; however they also penetrate human organism by dust inhalation or inhalation of small particles, on which they are absorbed. Skin is the least important route of PFASs exposure. PFASs metabolism do not appear in the body, and their removal from human organism is slow.

Numerous research works have studied toxic effects of PFASs on the human body. The analysis of the literature have shown that PFASs contribute to an increased risk of cancer, disrupt function of internal organs, e.g., liver or kidney and cause endocrine and metabolic disorders, including insulin resistance, hypertension and obesity [5].

Taking into account the common occurrence of perfluorooctanoic acid (PFOA) in the environment and its harmful effects on living organisms, this compound was entered into the list of persistent organic pollutants under the Stockholm Convention, and additionally the US Environmental Protection Agency (US EPA) decided to withdraw PFOA from US territory by 2015 [6].

As a result, more and more manufacturers started to replace PFOA with short-chain alternatives (containing up to 6 carbon atoms). Short-chain substitutes show similar properties to long-chain substances as they characterize by strong amphiphilic properties, stability and durability. The examples of alternatives of PFOA are perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA). Research workers have also developed substitutes deprived fluorine atoms, including nanomaterials, nanoparticles and dendrimers, but these substances do not have as many useful properties as short-chain PFASs [7]. Despite the successive elimination and depletion in the use of PFOA, it is still determined at considerable concentrations in human tissues. Moreover, similarly to PFOA, its substitutes of shorter chain have been detected in serum, tissue samples and urine. Despite of a significant reduction of production of PFOA, analytical research works have proven that this compound may exist in the highest amounts among PFASs in serum and urine, which demonstrate its strong ability to bioaccumulate. Moreover, high amounts of PFOA and its substitutes have been determined in human breast milk (PFOA) and human tissues, such as lungs and kidneys (PFBA), and liver and brain (PFHxA).

In respect to the results of studies concerning harmful effects of PFASs on living organisms, the European Food Safety Authority (EFSA) established a weekly tolerable intake of sum of PFASs only for 4.4 ng/kg body weight per person [2].

PFOA substitutes have been introduced into the market as potentially safer compounds [8]. However, there is still too little scientific research to determine their environmental occurrence, human bioaccumulation, toxicity and molecular mechanisms of their action.

This review aims to compare long-chain PFOA with its short-chain analogs (PFHxA, PFBA) in terms of environmental occurrence, bioaccumulation and toxicity, with particular emphasis on whether these substitutes can be considered as safer alternatives.

2. Chemical and Physical Properties

Perfluorooctanoic acid (PFOA) and its short-chain substitutes (i.e. PFHxA, PFBA) are man-made chemicals having a characteristic structure, in which all hydrogen atoms within the carbon chain were replaced by fluorine atoms (Table 1).

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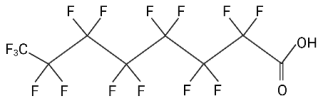
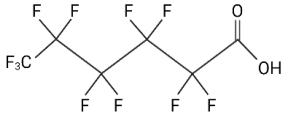
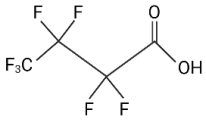
Table 1. Chemical structures of PFOA, PFHxA and PFBA.

PFOA	PFHxA	PFBA
		

PFOA, PFHxA and PFBA have been classified as a perfluoroalkyl acids (PFASs), as their functional residue placed at the end of hydrophobic chain is a hydrophilic carboxyl group. Moreover, bond that links carbon and fluorine is very strong and stable. Physiochemical properties of discussing compounds are presented in Table 2.

Table 2. Physiochemical properties of PFOA, PFHxA and PFBA.

Property	PFOA	PFHxA	PFBA	Reference
CAS number	335-67-1	307-24-4	375-22-4	[9–11]
molecular formula	$C_7F_{15}COOH$	$C_5F_{11}COOH$	C_3F_9COOH	[9–11]
molecular mass [g/mol]	414.07	314	214	[9–11]
physical state at room temperature and atmospheric pressure	white/cream solid	colorless liquid	colorless liquid	[9] [3,10–12]
boiling point [°C]	188 °C	157 °C	121 °C	[10,11,13]
melting point [°C]	54.3 °C	14.0 °C	-17.5 °C	[10,11,13]
density [g/cm³]	1.79 g/cm³	1.69 g/cm³*	1.65 g/cm³	[9–11,14]
vapor pressure [mm Hg] at 25 °C	0.525	0.908	15.8*	[9–11]
pKa	2.5	-0.16	0.08*	[9–11,15]
Log P	2.69	2.51	1.43	[9–11]
water solubility	9.5	9.39×10^{-5}	2.09×10^{-3}	[9–11,16]

 doi:10.20944/preprints202605.0967.v1

[mol/L] at 25 °C	

Abbreviations: CAS - Chemical Abstracts Service number, g/mol – grams per mole; mg/L – milligrams per liter; °C– Celsius degree; mm Hg – millimeters of mercury, *predicted value.

Perfluoroalkyl acids (PFASs) can be created as a result of environmental degradation or metabolic processes from other related per- and polyfluoroalkyl substances (PFASs), as well as precursor compounds. For instance, fluorotelomer carboxylic acids (FTCAs) occurring in landfill are often degraded to PFASs having lower number of fluorine atoms, and mainly PFHxA and PFBA *[17]*.

PFOA, PFHxA and PFBA are weak acids that can partially dissociate in aqueous matrices to perfluorooctanoate ions ($C_7F_{15}COO^-$), perfluorhexanoate ions ($C_5F_{11}COO^-$), perfluorobutanoate ions ($C_3F_9COO^-$) and hydrogen ions (H^+), respectively *[18,19]*. PFOA is comparatively stable in normal environmental conditions, and reveals persistence in the environment, mainly in respect to its resistance to deterioration processes. Nevertheless, PFOA degradation can appear under exposure to high temperatures or UV radiation *[20]*.

3. Usage

In respect to unique properties of PFOA and other PFASs, such as stability, durability and lowering of surface tension, these substances are utilized as ingredients in firefighting foams (aqueous film-forming foams, AFFF), textiles (membranes for waterproof and breathable clothing), electronics devices, semiconductors, cosmetics (body creams), pesticides, automotive waxes and polish, sports articles (ski waxes). They are also used in paints, additives on the surface of non-stick cookware and containers *[21]*, as well as in textile and upholstery (impregnation spray for textiles) *[22]*. Moreover, they are employed in floor covering, including nylon carpets, floor polish and laminated plastic floor covering *[23,24]*.

4. Occurrence in the Environment and Human Surrounding

PFASs are named "perennial chemicals" as they show high accumulation and are chemically stable. PFASs have been determined in river and marine ecosystems, estuaries, and are implicated in hydrological cycles. In respect to their accumulation, when they reach a food chain, their occurrence in food products and water raises *[25,26]*. The prevalent exposure routes of PFASs to general population are consumption of contaminated food and polluted water, while inhalation of contaminated air (PFASs are adsorbed on aerosols and dust particles in indoor and outdoor environments) are less important *[27]* (Table 3.).

Table 3. Presence of PFOA, PFHxA and PFBA in the environment, food, drinking water and dust.

Compound		PFOA	PFHxA	PFBA	References
Presence in the environment	Groundwater (ng/L)	4,470 (Sweden) 2,510 (China) 34.96 (Thailand) 3,260 (Canada) 24,000 (USA)	4000 (USA) 90 (Italy)	70 (Italy)	[28] [29] [30]
	Surface water (ng/L)	522 (Sweden) 223.8 (China) 10.7 (Thailand) 20 (Canada)	1.8–8.1 (Bangladesh) 1.96 (Canada)	1.6–15 (Bangladesh) 2.64 (Canada)	[26] [31] [32]

 doi:10.20944/preprints202605.0967.v1

		11,000 (USA) 1.0–7.5 (Bangladesh)			
	Soil (ng/g)	1290	8000	6200	[33]
	Air (pg/m^3)	1.25–64.28	0.3–1.2	0.1–0.7	[26,34]
	Food (ng/g)	perch 5.22-67.8 eels 5.73-68.8 mussels 8.56-157 eggs 0.05-4.83 meat 8.78-12,1	beef 1.21 oysters 1.2 liver 1.69 chicken 1.02 eggs 0.82	eggs 81.4 meat 0.06-3 pork offal 20 cereals 140	[35–38]
Human surrounding	Drinking water (ng/L)	100 (Sweden) 9.7 (China) 7.6 (Canada) 4,300 (USA)	1400 (USA)	1.3 (Spain) 17.87 (China) 6.9–9.0 (USA)	[28,29,39–41]
	Dust (ng/g)	10-653	3.6-72.5	271 pg/m^3	[42,43]

4.1. Food

Based on literature data, food consists the highest concentrations of PFASs. PFOA has been determined in fish (in perch from 5.22 to 67.8 ng/g; in eels from 5.73 to 68.8 ng/g), crustaceans (from 8.56 to 157 ng/g) *[35]*, eggs (from 0.05 to 4.83 ng/g, while the mean PFBA concentration was 81.4 ng/g), as well as in meat and meat products (from 8.78 to 12.1 ng/g) *[37,44]*. The European Food Safety Authority (EFSA) has established the tolerable daily intake (TDI) of PFOA at 150 ng/kg body weight *[45]*. This value was based on the level of observed adverse effect that was estimated for 0.06 mg/kg b.w./day. For instance, in Taiwan, the exposure to PFOA associated with consumption of contaminated food is 85.1 ng/kg b.w./day *[46]*. Is worth noting that PFASs, including PFOA are widely used in materials that are in contact with food. For instance, PFASs are employed in materials, such as kitchen accessories with non-stick surfaces, fast food packaging, as well as microwaveable popcorn packaging, which may lead to penetration of these substances into food *[46]*. It has been proven that this migration rises along with the increase of temperature, longer contact time with food, and the occurrence of emulsifiers in the food. The degree of migration is compound-specific, nevertheless long-chain compounds, such as PFOA show faster migration than short-chain substitutes *[47]*. Therefore, the European Union has established maximum PFOA levels in selected food products, in which its concentrations have been determined to be the highest (Table 4.).

Table 4. Comparison of permissible levels of PFOA in selected food products. Own elaboration based on the European Union Commission Regulation *[48]*.

Food products	Maximum permissible level of PFOA (µg/kg dry weight)
eggs	0.3
fish meat - Baltic herring, sprat, pike, catfish, tench, whitefish, wild salmon, wild trout	1.0
crustaceans and mussels	0.7
sheep meat	0.2
pork, beef, poultry and mutton offal	0.7

4.2. Water

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PFOA and its short-chain analogs often enter the body through ingestion of contaminated drinking water, and such contamination usually occurs near industrial plants. Based on literature data, PFOA can enter groundwater through the soil, as well as from river or well water *[49]*. Surface and groundwater pollution by PFOA is common, while industrial discharges, firefighting facilities, military operations, factories through leaking pipelines, and transportation have been considered as the main sources. Maximum PFOA concentrations determined in surface water were found to be very different, such as 10.7 ng/L in Thailand, 20 ng/L in Canada, 223.8 ng/L in China, 522 ng/L in Sweden and 11,000 ng/L in USA. In groundwater, PFOA levels have also be found to be variable, for instance low concentration was found in Thailand (34.96 ng/L), high in China (2,510 ng/L), Canada (3,260 ng/L) and Sweden (4,470 ng/L), and as high as 24 μg/L in USA. Generally, in drinking water much lower PFOA concentrations have been determined, such as 7.6 ng/L in Canada, 9.7 ng/L in China, 16.5 ng/L in Thailand and 100 ng/L in Sweden, nevertheless sometimes its level may be very high - 4,3 μg/L in USA [28]. In water of Bangladesh (industrial centers of Dhaka), PFBA, PFHxA and PFOA were detected at low concentrations range from 1.0 to 7.5 ng/L for PFOA, from 1.6 to 15 ng/L for PFBA and from 1.8 to 8.1 ng/L for PFHxA [31]. PFBA, PFHxA and PFOA have been often determined in high concentrations in comparison to another PFASs (e.g., PFOS). For instant, in Canadian freshwater the level of PFBA, PFHxA and PFOA was detected to be 2.64 ng/L, 1.96 ng/L, 1.52 ng/L, respectively [32]. These results show that intensive implementation of short-chain analogs replacing of those PFASs with longer chains occurs.

Similar results were achieved in the study of distribution of 23 PFASs in groundwater and surface water samples taken in urban areas in Brazil (Porto Alegre) [50]. It was revealed that PFOA range in surface water was from 4 to 8 ng/L, PFBA from 0.2 to 2 ng/L, and PFHxA from 1 to 3 ng/L, while PFOA was the most commonly detected. Moreover, the authors observed that concentrations of PFASs were higher in tributary waters than in larger water bodies. For instance, in China, total concentrations of PFASs were up to 260 ng/L in the Huang He River mainstream and at 1526 ng/L in its tributaries, which showed the importance of dilution process and subsequent population exposure [51]. The study conducted in 2019 on surface water and sediments in Melbourne (Australia), showed that among PFASs, PFOA (13 ng/L) was the most commonly determined in urban areas, whereas PFBA (5.7 ng/L) was the most frequently determined in rural areas [52]. The authors excluded contamination from areas near the airport, where the total concentration of 33 PFAS in surface water was as high as 4261 ng/L. They also revealed that median total concentration of 33 types of PFASs in surface water was 63.5 ng/L, while the highest average concentrations were found for PFBA (11.3 ng/L), PFHxA (9.2 ng/L) and PFOA (8.3 ng/L) with the PFOA most frequently detected.

In another study, drinking water contamination by fluoropolymer production plant in Huantai County, China was observed. PFOA was the prevalent reagent in the plant, which discharged it into Xiaoqing River in an amount of 160 kg/day. As a result, high concentration of PFOA were detected in drinking water (5.95–19.3 ng/L), as well as in groundwater (up to 104 ng/L) and soil (4.40 –189 ng/g) [53]. Moreover PFOA was detected in significant concentration in vegetables from local suppliers, including wedge onion pseudostem (64.8 ng/g), wedge onion (825 ng/g), celery (2678 ng/g) and carrots (5303 ng/g) [54]. One of the country realizing monitoring of xenobiotics to assess their human exposure dynamics is Slovenia. Runkel et al. (2023) [27]determined PFOA in all samples tested (0.87-1.16 ng/mL) in Slovenia, whereas the highest concentration of PFOA (1.16 ng/mL) was determined in the population inhabiting of the Ravensko region.

As mentioned above, PFASs are often found in drinking water. These compounds have been frequently determined in drinking water treatment plants that use traditional treatment methods that fail to remove these substances. Worldwide, it has been evaluated that PFOA levels in tap water are in the range from a few ng/L to μg/L. Nevertheless, according to EPA, the concentration of PFOA in prevalent tap waters is very low - about 4 pg/L. Highly advanced technologies may provide better results in removal of PFASs, but this effect also depends on PFASs properties and their concentration in raw water [28].

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In 2018, the Agency for Toxic Substances and Disease Registry (ATSDR) established PFOA admissible concentration in drinking water at 11 ng/L. In 2022, the US Environmental Protection Agency (US EPA) proposed much stricter standards in which the level of PFOA in drinking water should not exceed 4 pg/L [55]. However, in March 2023, the US EPA changed this limit to 4 ng/L [28].

4.3. Air and Surface Snow

PFASs levels are not systematically monitored in air or snow. Björnsdotter et al. (2021) [56] investigated seasonal levels and distribution of short-chain PFASs, such as perfluoropropanoic acid (PFPrA) and PFBA in snow cover on the Spitsbergen island (Norwegian Arctic). They detected PFPrA - 0.79–16 ng/m^2 and PFBA - 0.19–170 ng/m^2 in most of the studied samples. It must be noting that the above mentioned compounds were detected in samples collected from the locations, in which their occurrence can only results from long-range processes, such as geographic migration from the cities, in which they are produced and utilized. Moreover, positive association was found between the flux of these substances and solar radiation. In another study conducted near Fayetteville (North Carolina, USA) the presence of PFASs was noted in the air near to a fluoropolymer manufacturing facility [34]. It was found that among short-chain substances, PFBA (0.1–0.7 pg/m^3) and PFHxA (0.3–1.2 pg/m^3) were determined in higher levels than other analyzed PFASs.

4.4. Dust

Many studies have proven that PFASs have an affinity for atmospheric particles, and therefore they can migrate for long distances, for instance, from cities or factories to distant areas without industrial development. Such contamination is associated with both point sources (particular area of pollution), as well the influence of atmospheric factors [26].

Taking into account that 90% of human population's time is spent indoors (homes, schools, workplaces), dust and other kinds of particles are essential sources of human exposure to PFASs [57]. This exposure is linked to processes that release these compounds from everyday products (e.g., clothing, cleaning products, furniture) and leads to their adsorption on dust particles surface. PFOA was determined in 93% of samples of household dust, which were taken in Sweden (median concentration was 13 ng/g) [58]. Differently, a research study realized in China (Tianjin) showed that PFOA and its short-chain analog PFBA were dominant substances in dust samples collected from homes among 23 tested PFASs (PFOA and PFBA were determined in all analyzed samples). It was noted that median PFBA concentrations were 165 pg/m^3 and 271 pg/m^3 in hotels and homes, respectively [43]. The study realized in the Thessaloniki (Greece) showed a range of PFHxA in dust trapped by central air conditioner (i.e. restaurants and electronics shops) in the concentrations ranging from 3.6 to 72.5 ng/g (occurrence in 85% of all analyzed samples), whereas 90-95% of samples consisted of PFOA in significantly higher concentrations range from 10 to 653 ng/g [42].

5. Human Biomonitoring

PFOA has been repeatedly determined in humans environmentally exposed, including serum (0.006-38.5 ng/mL) [59], urine (mean 7.3 ng/mL) [60] and in high concentration in women milk (mean 336 ng/mL) [61]. Due its hydrophobic properties, it has also been detected in kidney (mean 1.5 ng/g), liver (mean 4 ng/g) and lungs (mean 12.1 ng/g) [62]. In humans occupationally exposed, high concentrations of PFOA in full blood (up to 535 ng/mL) were detected [63].

Generally, PFHxA has been found in comparable or higher concentrations than PFOA in human tissues. PFHxA was detected in serum of humans environmentally and occupationally exposed in the concentrations range from 0.07 to 100 ng/mL [64,65]. Moreover, significant concentrations of PFHxA have been determined in liver (mean 68.3 ng/g), brain (mean 141 ng/g) and lung (mean 207 ng/g) [62].

Interestingly, PFBA has been found in the highest concentrations among discussing PFASs in kidney (mean 263 ng/g) and lungs (807 ng/g) of humans environmentally exposed [62]. Moreover

 doi:10.20944/preprints202605.0967.v1

PFBA was determined in brain (mean 1.4 ng/g) and liver (mean 3 ng/g) [62]. In serum of environmentally and occupationally exposed humans, PFBA has been determined in the range from 0.008 to 2.5 ng/mL and from 3.7 to 5.4 ng/mL, respectively [66,67].

Detailed results of human biomonitoring studies concerning PFOA, PFHxA and PFBA are presented in Table 5 and 6.

Table 5a. Presence of PFOA, PFHxA and PFBA in serum of subjects after environmental exposure.

Compound	Material	Concentration (ng/mL)	Subjects	Location	Year of study	Literature
PFOA	serum	0.3-38.5	men, women	Sweden	2008-2020	[59]
PFHxA	serum	0.1-100	men, women	Norway	2013-2014	[64]
PFBA	serum	0.008-2.5	men, women	USA	2020	[67]

Table 5b. Presence of PFOA, PFHxA and PFBA in serum of subjects after environmental exposure.

Compound	Material	Concentration (ng/mL)	Subjects	Location	Year of study	Literature
PFOA	serum	2.9-11	men, women, breastfeeding women	Sweden	2018	[68]
	full of blood	4.8-535	men	Sweden	2007-2010	[63]
PFHxA	serum	0.07-12.2	men	Sweden	2007-2009	[65]
PFBA	serum	3.7-5.4	men	Norway	2008-2009	[69]

 doi:10.20944/preprints202605.0967.v1

Table 6. PFOA, PFHxA and PFBA concentrations in solid tissues.

Compound	Material	Concentration	Subjects	Location	Year of study	Literature
PFOA	urine	7.3 ng/mL	men, women	China	2019	[60]
	human milk	336 ng/L	breastfeeding women	China	2020-2021	[61]
	hair	0.1-6 ng/g	men, women	Spain	2010-2011	[62]
	liver	4 ng/g	men, women	Spain	2008	[62]
	bone	20.9 ng/g	men, women	Spain	2008	[62]
	lung	12.1 ng/g	men, women	Spain	2008	[62]
	kidney	1.5 ng/g	men, women	Spain	2008	[62]
PFHxA	urine	0.3 ng/mL	men, women , children from 7 years of age	USA	2013-2014	[70]
	brain	141 ng/g	men, women	Spain	2008	[62]
	liver	68.3 ng/g	men, women	Spain	2008	[62]
	bone	1.5 ng/g	men, women	Spain	2008	[62]
	lung	207 ng/g	men, women	Spain	2008	[62]
	kidney	2.7 ng/g	men, women	Spain	2008	[62]
PFBA	urine	0.1-0.9 ng/mL	men, women	Spain	2010-2011	[62]
	brain	1.4 ng/g	men, women	Spain	2008	[62]
	liver	3 ng/g	men, women	Spain	2008	[62]
	bone	0.8 ng/g	men, women	Spain	2008	[62]
	lung	807 ng/g	men, women	Spain	2008	[62]
	kidney	263 ng/g	men, women	Spain	2008	[62]

6. Toxicity

The accumulation of PFOA and other PFASs is associated with their ability to bind to circulating serum albumin, through which they are transported to the liver and other organs, where they accumulate and cause damage [71]. Additionally, these substances can disrupt endocrine function by changing estrogen and androgen receptor activity and affecting thyroid hormone homeostasis [72]. Rodent studies have shown that PFASs also impact glucose levels [73], increase leptin and insulin concentrations, and potentially are involved in weight gain [71]. Other adverse effects include liver, pancreatic, and breast cancers, abnormal protein metabolism, and hepatomegaly [44,74–76].

6.1. Hepatotoxicity

PFASs accumulate mainly in the liver, therefore inducing hepatoxicity. PFASs, such as PFOA have been revealed to induce liver damage in animal models, such as mice, rats and monkey showing hepatotoxicity as an important toxicological activity [77–79].

In *in vitro* study, Amstutz and co-workers (2022) [80] assessed the effect of PFASs with different carbon chain-length and functional head-groups (0 – 800 μM) on viability of HepG2 cells and reactive oxygen species (ROS) formation. They noticed that cytotoxic potential of PFASs, as well as ROS

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formation by these substances was dependent on chain-length as stronger effects were observed for PFOA and weaker for PFHxA and PFBA, which exhibited similar toxic potential in tested cells.

The results achieved in animal studies showed that PFASs induce different alterations in the liver, including changes in metabolism, as well as in lipids and protein levels, and caused liver damage. For instance, Das et al. (2017) [81] observed a dose-dependent increase in hepatocellular hypertrophy, and liver weight in response to PFASs, including PFHxA. It must be noted that liver has been considered as one of the main targets of PFHxA toxicity. Sub-chronic toxicity study showed that PFHxA could induce centrilobular hepatocellular hypertrophy in rats [82], while exposure to sub-lethal dosage of PFHxA altered liver cell metabolism in zebrafish [83]. Similarly in an experiment performed on Sprague Dawley rats, it was shown that PFOA significantly changed alanine transferase (ALT) level and induced liver edema and liver toxicity by mechanisms connected with specific protein denaturation [84].

Peroxisome proliferator receptor alpha (PPARα) is a member of nuclear hormone receptor superfamily. The central physiological role of PPARα is as a ligand activated transcription factor whose target genes encode enzymes and proteins implicated in fatty acid transport and catabolism. The activation of PPARα by PFAAs, including PFOA, PFHxS and PFBS has been recognized as the primary mechanism of action in rodent hepatocyte-induced proliferation. For instance, Foreman et al (2009) [85] evaluated the role of PPARα in mediating hepatotoxic effects of PFBA (350 mg/kg/day) in PPAR-a null mice and a mouse line expressing human PPARα in the absence of mouse PPARα. The authors shown that PFBA modulated lipid metabolism, increased liver weight and hepatocyte hypertrophy in wild-type and PPARα humanized mice. They also noticed that PFBA induced hepatocyte focal necrosis with inflammatory cell infiltrate only in wild-type mice. As a result, they concluded that PFBA can activate both the mouse and human PPARα, but a species difference exists in the hepatotoxic response to this chemical. In another study, PFBA induced peroxisome proliferation and increased peroxisomal fatty acid oxidation in rat liver, which are biomarkers of PPARα activity [86]. In addition, when pregnant Kunming mice were exposed to PFOA, it reduced the growth and development of the pups and induced liver damage, which altered the secretion of enzymes implicated in PPARα-induced fatty acid oxidation, which led to hepatic bleeding, local necrosis, enlargement of hepatocytes, and depletion in histone acetylation [87].

PFASs are activators of PPARα, but biological effects of these chemicals are probably also mediated by other factors in addition to PPARα. In order to evaluate this hypothesis, the effects of PFASs, male wild-type and PPARα null mice were administrated by oral gavage with PFHxA (3 or 10 mg/kg/day) for 7 days, and liver expression of genes was assessed by full-genome microarrays. Using data available through a microarray database, PFHxA expression of genes profiles were not found to reveal considerable similarity to profiles from mouse tissues exposed to agonists of the constitutive activated receptor (CAR), estrogen receptor α (ERα), and PPARγ. In conclusion, a negative relationship was found in all treated wild-type mice along with similar but muted effects in PARPα-null mice exposed to PFHxA. In recent study, Robarts et al. (2024) [88] studied human-relevant mechanisms of PFASs action, including PFOA-induced hepatic effects using FRG liver-chimeric humanized mice with livers repopulated with functional human hepatocytes. Male FRG humanized mice were treated with low doses of 0.145 mg/L of PFOA in drinking water for 28 days. They noticed that PFOA induced a decrease in total serum cholesterol and LDL/VLDL fractions, as well as caused significant hepatocyte proliferation. RNA-sequencing with alignment to the human genome showed a total of 162 differentially expressed genes as a result of PFOA exposure. Moreover, upstream regulator analysis showed that PFOA caused activation of p53 and inhibited androgen receptor and nuclear receptor subfamily 1 group D member 1 (NR1D1), which is a transcriptional repressor important in circadian rhythm. Further biochemical studies confirmed NR1D1 inhibition. The authors concluded that new human-relevant molecular mechanism of PFASs exists, including their previously unknown effect on circadian rhythm.

Several studies have shown the effect of PFASs on liver function, metabolism and damage. For instance, PFOA (5 mg and 10 mg/kg) induced steatosis, raised fatty acid translocase and lipoprotein

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lipase expression, as well as depleted oxidation of fatty acids, which led to depletion of energy production in mature 8-week old male mice [89]. In another study, Atemma and co-workers (2022) [90] administrated PFOA at a dose of 0.3 mg/kg to male wild type mice and nuclear PPARα playing essential role in transcriptional regulation of lipid homeostasis [91]. They noted that PFOA disrupted hepatic metabolism in studied animals. PFOA improved glucose and insulin tolerance but reduced body weight and raised liver weight in wildtype and PPARα mice. PFOA in a PPARα-dependent manner also depleted plasma cholesterol and triglycerides. Specifically, 88% of the regulation of gene expression by high-dose of PFOA in mouse liver was dependent on PPARα. In conclusion the authors suggested that the effects caused by PFOA were mediated by multiply nuclear receptors. In another research, exposure to PFOA affected blood cholesterol levels and triglycerides and non-alcoholic fatty liver disease in humanized PPARα mice fed with an American diet [92]. An extended study was conducted by Stoffels et al. (2023) [93] who assessed a global lipidomic analysis on the liver of PFOA-exposed mice. Among all hepatic detected lipids, the levels of more than 350 were statistically raised or depleted. It was also noticed that the levels from many lipid species of various lipid classes, mostly phosphatidylethanolamine, phosphatidylcholine and triacyglycerols were significantly changed. Furthermore, subsequent lipidomic analysis highlighted the pathways substantially affected by PFOA, with the glycerophospholipid metabolism being the most changed and the alterations in the lipidome network, which connects all the lipid species together. In conclusion, this analysis showed heterogeneous distribution of the lipids altered by PFOA, showing different areas of lipid expression connected to PFOA localization.

Schlezinger et al. (2020) [94] tested the hypothesis that PFOA exposure at a human-relevant level dysregulates genes expression that control cholesterol homeostasis in livers of mice that expresses human PPARα (hPPARα). Female and male hPPARα and PPARα null mice were fed with American diet for 6 weeks. The authors noticed that PFOA raised liver mass, which was connected to histologically-evident lipid accumulation. Moreover, PFOA increased serum cholesterol level and caused PPARα activation and constitutive expression of androstane receptor target gene in liver. Moreover, genes expression in four pathways, which regulate cholesterol homeostasis were noted. Furthermore, the authors noticed that PFOA depleted expression of 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr) in a PPARα-dependent manner, as well as reduced expression of low-density lipoprotein receptor (Ldlr) and cholesterol 7 alpha-hydroxylase (Cyp7a1) in a PPARα-independent manner. They concluded that obtained results provided new insight into the effect of PFOA on cholesterol regulation in the liver and the role of hPPARα.

A interesting research was conducted by Jiang et al. (2021) [95] who studied the transcriptomic, proteomic, and metabolomic parameters in mice exposed to PFHxA. Among 24,893 RNAs and 2246 proteins, 566 transcripts and 238 proteins were substantially changed in mice exposed to PFHxA. The authors identified processes, which were implicated in liver damage, such as alterations in biosynthesis of fatty acids, as well as pathways of degradation like purine metabolism (a depletion levels of xanthine and uric acid) and glutathione (GSH) metabolism that might be triggered by PPAR signaling pathway. They also observed that PFHxA caused oxidative stress because an increase in SOD activity and a decrease in GSH level were noticed.

It was shown that high doses of PFOA can induce hepatocellular adenomas in rats [96]. It is known that inhibition of gap-junctional intercellular communication (GJIC) is needed, however insufficient, step of tumorigenesis, and it is therefore a typical response of cells to tumor promoters, oncogenes, growth factors, and nongenotoxic carcinogens, including peroxisome proliferators [97]. PFOA has been recognized as peroxisome proliferators that trigger hepatomegaly and hepatocarcinogenesis in rodents, and have been shown as classic non-genotoxic carcinogens inhibiting GJIC in carcinogenesis.

Upham et al. (2005) [98] noted that PFOA inhibited GJIC and triggered hepatomegaly in rat livers. Although, serum biochemistry of liver enzymes did not revealed any cytotoxic response to this compound, *in vitro* analysis of mitogen-activated protein kinase (MAPK) showed that PFOA activated the extracellular receptor kinase (ERK). The authors also noticed that inhibition of GJIC *in*

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vitro by PFOA depended on ERK and phosphatidylcholine-specific phospholipase C (PC-PLC) activation in the dysregulation of GJIC in an oxidative-dependent mechanism. In conclusion, they stated that *in vitro* analysis of GJIC as an epigenetic marker of tumor promoters, can predict the *in vivo* activity of PFOA, which can dysregulate GJIC *via* ERK and PC-PLC [98].

The gut microbiome functions as a 'metabolic organ' that interacts with the host for a mutually beneficial coexistence. Perturbation in gut microbiome can disrupt gut barrier permeability leading to production of translocated bacteria and leakage of gut-derived products that reach the liver through the portal venous system. This results in inflammation, oxidative stress and liver diseases [99], suggesting a 'liver-gut-axis' existence. In the present study, the impact of subacute (30 mg/kg) and subchronic doses (3 mg/kg) of PFOA (administrated orally for 14 days) on liver and gut microbiota in C57BL/6J mice was evaluated. It was revealed that subchronic and subacute exposure to PFOA induced inflammation of liver, changed antioxidative homeostasis and caused liver histological abnormalities, such as hepatomegaly that led to liver damage. Moreover 16S rRNA sequencing analysis revealed that mice exposed to subacute doses of PFOA had changed amount of intestinal flora, such as Dehalobacterium and Bacteroides genera, which contributed to inflammation of liver and oxidative stress. Moreover, the exposure to subchronic doses mostly caused a reduction in commensal probiotics, such as Lactobacillus and Bifidobacterium genera that are potentially beneficial when liver damage occurs. In conclusion, the authors suggested that liver damage caused by PFOA was partly related to the gut microbiota dysbiosis.

In occupational surveys, the association between human exposure to PFASs and higher risk of liver damage and disfunction have been observed. Choi et al. (2022) [100] in review and meta-analysis evaluated relationship between exposure to PFOA and hepatic diseases by determination of alanine aminotransferase (ALT), which is the standard screening tool for detecting acute hepatic injury. The results showed that people exposed to PFOA had elevated ALT activity in comparison to individuals not exposed to this substance. Nevertheless, the authors of this study concluded that due to limited number of individuals tested in the analysis it is prematurely suggest that PFOA exposure is connected with development of hepatic diseases in humans. Nevertheless, Sen et al. (2022) [101] found a positive association between PFASs, including PFHxA levels in serum and nonalcoholic fatty liver disease (NALFD)-associated lipid changes in human livers. In another research the association between PFOA level in serum and liver enzymes was analyzed using 9523 Americans aged 20 years or older (National Health and Nutrition Examination Survey). The authors suggested that alterations in liver enzymes, including ALT, aspartate aminotransferase and gamma-glutamyl transferase were associated with PFOA exposure [102]. Also, Sen et al. (2021) [101] investigated the effects of PFASs, including PFOA and PFHxA exposure on liver metabolism in human NAFLD cohort of 105 individuals (70 female and 35 male) in order to evaluate metabolism of liver, and in particular metabolism of bile acids. As a result, they noted upregulation of bile acids, triacylglycerols and ceramides, as well as altered glucose level (insulin resistance) and amino acids (alanine, aspartate and glutamate) metabolism. In conclusion, they stated that human exposure to tested PFASs caused metabolic processes connected with NAFLD, and that the observed effect is different and generally stronger in females in comparison to males. To note, the study of Jin et al. (2020) [103] also showed that PFOA caused fatty liver in adults and non-alcoholic fatty liver in children.

Children are potentially more susceptible to toxicants, including PFASs. Seventy-four children with physician-diagnosed nonalcoholic fatty liver disease were recruited from Children's Healthcare of Atlanta between 2007 and 2015 to evaluate metabolic dysregulation associated with PFASs, including PFOA and PFHxA. An integrative analysis showed a cluster of children with nonalcoholic steatohepatitis (NASH), which was characterized by raised PFASs levels and changed metabolite patterns, such as higher plasma levels of phosphoethanolamine, tyrosine, phenylalanine, aspartate and creatine, and depleted plasma levels of betaine, which proved dysregulation of lipid and amino acid pathways associated with NAFLD pathogenesis. In conclusion, higher PFASs exposure was linked to more severe disease in children with NAFLD, and the authors of the study concluded that tested PFASs may be important toxicants contributing to NAFLD progression [103]. An interesting

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study of Stratakis et al. (2020) [104] showed that prenatal exposure to PFASs, including PFOA was linked to raised susceptibility to liver functions in children. They noted that increased levels of PFOA in maternal blood were connected with higher concentrations of ALAT, aspartate aminotransferase, and gamma-glutamyltransferase in children serum. Moreover, substantial perturbations in amino acids and glycerophospholipid metabolism were noticed.

Stronger associations between exposure to toxicants and adverse effects are usually observed in occupational surveys. In a study, 40 occupational workers from a factory in China and 52 control subjects from the general population were studied in an investigation on the potential health problems of occupational exposure to PFASs using mass spectrometry-based metabolomics analysis. It was noted that the levels of PFASs, including PFOA (0.57 μg/mL), PFHxA (2.15 μg/mL) and PFBS (0.22 μg/mL) were much higher in workers than in control individuals. In the study, 14 potential biomarkers were determined, and they were found to be linked to oxidative stress, fatty acid β-oxidation, and kidney injury. The authors concluded that the health effects of workers were associated with exposure to tested PFASs [105].

6.2. Endocrine-Disrupting Activity

The World Health Organization *[106]* defines endocrine disruptors (EDs) as, „Exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny, or (sub)populations." Some studies have shown that perfluoroalkyl acids behave as endocrine disruptors. Borghoff and co-workers (2018) *[107]* analyzed in their review, data of endocrine potential of PFHxA evaluating estrogen, androgen, thyroid and steroidogenesis pathways as defined by WHO on different models, such as *Japanese medaka*, juvenile rainbow trout, chickens or reproductive parameters in northern bobwhite. They concluded that PFHxA showed no endocrine potential in *J. medaka*, juvenile rainbow trout, chickens or reproductive alterations in northern bobwhite with no significant activity in rodent repeated-dose toxicity, life time cancer, or reproductive and developmental studies. The authors also pointed on that in studies of repeated-dose in mammals PFHxA did not significantly altered organs/tissues (weights or histopathology), such as testes, ovaries, thyroid, prostate, pituitary, mammary gland, or uterine. PFHxA also exhibited negative activity *in vitro* and *in vivo* for disrupting steroidogenesis. Only *in vitro*, weak or negative activity for thyroid (T) transport protein or activation of estrogen (E), androgen (A) or T receptors (T) was noted. Based on this analysis, the authors concluded that PFHxA does not induce alterations in endocrine activity in these models, and therefore it would not be characterized as an endocrine disruptor according to the WHO definition.

Thyroid hormones (THs) play crucial roles in the human endocrine system by regulating protein synthesis, energy metabolism, growth, and development. They also regulate the heart rate and blood pressure and activate hepatic lipolytic enzymes to regulate blood lipids *[108]*. Chengelis et al. (2009) *[82]* conducted 90-day toxicity study, in which male and female Sprague-Dawley rats were treated with PFHxA by oral gavage at dose up to 200 mg/kg/day. As a result of this investigation, no changes in endpoints showing endocrine disrupting potential, such as estrogen, androgen and thyroid receptors, or steroidogenesis pathways were observed. Similarly, a study conducted in Japanese medaka at aqueous concentrations of sodium or ammonium salt of PFHxA of 10 mg/L and 100 mg/L *[109]* did not show any significant differences in fecundity, fertility, survival or estrogen biomarker vitellogenin (Vtg) in comparison to control fish.

Nevertheless, Wasel et al. (2020) *[110]* reported developmental toxicity of PFASs in zebrafish and showed decreasing LC_{50} values with increasing chain-length for PFBA, PFHxA and PFOA. PFOA can result in the endocrine disruption by altering the functions of growth and sex hormones, including activating the estrogen receptor (ER) and inducing ER-mediated transcriptions in cells *[111]*. For instance, *in vitro* studies, PFOA induced expression of estrogen-responsive genes based on different cell lines, such as human CHO-K1 cell line and HepG2 cell line *[112]*. The ER transcriptional activation of PFOA, was also noticed in different species *in vivo*, including rainbow trout (*Oncorhynchus mykiss*), in which induction of Vtg was noticed. In the same study, the authors

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observed a weak interaction of PFHxA with the estrogen pathway, showing a competitive binding affinity in rainbow trout to be < 0.01% of E2; however, they concluded that observed weak affinity did not translate to an *in vivo* response. In other study, Zhang et al. (2014) *[113]*, treated male mice with PFOA (0-20 mg/kg/day) by oral gavage for 28 days. They observed that PFOA considerably caused damage to the seminiferous tubules and decreased progesterone and testosterone levels in the testis in a dose-dependent manner. Moreover, exposure to PFOA caused an increase in sperm quality. Using a quantitative proteomic approach, the authors determined 93 differentially expressed proteins in tested animals treated with PFOA at 5 mg/kg/day. Among determined proteins, insulin like-factor 3 (INSL3) and cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1) as Leydig-cell-specific markers were considerably reduced. The authors also detailly assessed the expression patterns of CYP11A1 and related genes implicated in steroidogenesis in the mouse testis. They found that PFOA in a dose-dependent manner, depleted mRNA and protein levels of CYP11A1, and mRNA levels of 17β-hydroxysteroid dehydrogenase (17β-HSD). Additionally, *in vitro* study revealed a decrease in progesterone level, which was accompanied by reduction in expression of CYP11A1 in cAMP-stimulated mLTC-1 cells. The authors concluded that exposure to PFOA can impair male reproductive function, possibly by altering testosterone levels, and CPY11A1 may be a key steroidogenic enzyme targeted by PFOA.

The mammary gland is particularly vulnerable tissue because of developmental end points, such as functional, milk protein gene expression, and developing neonatal and peripubertal structures. In the study of White et al. (2011) *[114]* one group of dams of mice was treated with 0, 1, or 5 mg of PFOA/kg/day during 17 days of gestation. Additionally, a second group of female mice was treated with PFOA at 1 mg/kg/day during gestation and their F1 and F2 offsprings were continuously treated with low concentration of PFOA (5 ng/mL) in drinking water. The authors found that gestational treatment with PFOA caused delay in mammary gland development or/and lactational differentiation within three tested mice generations. Interestingly, chronic treatment of mice with low-concentrations of PFOA in drinking water (relevant to its level in human water supplies) also changed mammary morphological development in tested animals. Zhao et al. (2012) *[115]* aimed to study the underlying mechanism of mammary gland development in various developmental stages in mice exposed to PFOA. Female 3-week old female wild mice were treated with PFOA at 2.5 mg and 7.5 mg/kg body weight for 2 weeks. The authors observed that PFOA considerably inhibited growth of mammary gland both in Balb/c and C57Bl/6 wild type mice, but not in C57Bl/6 PPARα knockout mice. The authors of the study also observed that PFOA caused a delay or absence of vaginal opening and induced lack of estrous cycling during the experimental period. PFOA also depleted the levels of ovarian steroid hormonal synthetic enzyme and decreased expression of estrogen- or progesterone-induced mammary growth factors. The authors concluded that PFOA effects on ovaries leading to mammary gland development, and that PPARα expression is a contributing factor in tested animals.

Endocrine-disrupting potential of PFOA has also been observed in occupational surveys. Jain et al. (2013) *[116]* determined the effect of PFASs, including PFOA on thyroid stimulating hormone (TSH), free and total thyroxine (FT4, TT4), free and total triiodothyronine (FT3, TT3), and thyroglobulin (TGN) in general population of USA based on data from National Health and Nutrition Examination Survey for the years 2007–2008. It was found that PFOA caused an increase of TSH and TT3 levels. Other epidemiological study assessed the association between PFOA concentrations in the general U.S. adult population and their current thyroid disease (data from the U.S. NHANES from 1999 to 2006) *[117]*. This study found that high concentrations of PFOA in serum were potentially associated with current thyroid disease. In other study, Tan et al. (2024) *[118]* evaluated association between the exposure of the elderly (n=746, aged >60 years) from Taiwan to several PHASs, including PFOA, PFHxA and PFBA and thyroid hormones levels, such as thyroid-stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), free T4 (FT4), and free T3 (FT3) in plasma samples of tested subjects. Interestingly, they observed that the presence of PFBA was negatively correlated with FT4 level, while for PFOA and PFHxA the correlation has not been found.

 doi:10.20944/preprints202605.0967.v1

Generally, the authors observed that PFASs were associated with decreased TSH and FT4 levels and increased T4 and T3 levels, which suggested that PFASs could cause thyroid-disrupting effects in the elderly population. Infants and children are particularly vulnerable to chemical substances. Kim et al. (2016) *[119]* determined exposure levels of 16 PFASs in South Korean infant serum and correlated these levels with thyroid hormones (THs). A case group of infants suffered from congenital hypothyroidism. They observed a weak correlation between the concentration of PFOA and the level of thyroid-stimulating immunoglobulin (TSI) antibodies in serum of tested individuals from case group. In the study of Liu et al. (2020) *[120]* the association between PFASs, including PFOA and glucocorticoids (11-deoxycortisol, cortisol and cortisone) and 2 progestogens [progesterone, 17-hydroxyprogesterone (17OHP)] in the cord sera of 374 neonates (Wuhan, China) was analyzed. They found that PFOA was capable of significantly changing 11-deoxycortisol concentrations in cord sera. In other study, Liu et al. (2021) *[121]* determined the associations between PFASs, including PFOA with estrogens, including estrone, estriol and estradiol levels in cord sera in newborns (n=942, 2013-2014) in Wuhan, China. They found that there was a correlation between PFOA level and estradiol level in neonates. In the review study of Balesteros et al. (2017) *[122]* the associations between PFASs, including PFOA and thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxin (T4) levels or thyroid dysfunctions in pregnant women and children were assessed. The authors found positive associations between PFOS level and TSH level measured in maternal blood.

6.3. Immunotoxicity

Studies have found association between some PFASs and several aspects of immunotoxicity, including immunosuppression, hypersensitivity, and autoimmunity. A number of general population studies have also found significant inverse associations mainly between serum PFOA levels and antibody responses to vaccines.

Recent *in vitro* study assessed the effect of PFBA, PFHxA and PFOA on cytotoxic and oxidative parameters in human peripheral blood mononuclears cells (PBMCs). It was found that PFBA and more strongly PFOA at 100 μg/mL and 200 μg/mL after 24 h of incubation decreased viability of PBMCs, while PFHxA did not change this parameter even at 200 μg/mL. Moreover, PFOA and PFBA after 1 h and particularly after 24 h of incubation significantly depleted ATP level. The authors also noted that all studied PFASs at different concentrations caused oxidative stress and damage in PBMCs (0.1 – 50 μg/mL, 1 h incubation) as they increased ROS and reactive nitrogen species (only PFOA) levels, as well as caused damage to lipids and proteins in studied cells. Generally, most of tested parameters were most strongly altered by PFOA, while PFBA revealed more pronounced alterations that PFHxA [123].

In vivo studies of high, acutely toxic dietary doses of PFOA up to 75 mg/kg/day resulted in suppression of antigen-specific immunoglobulin M (IgM) antibody production, splenic and thymic atrophy, and altered T-cell phenotypic distribution in male C57BL/6 mice [124]. In a TDAR (the T-Dependent Antibody Response assay) study with mice exposed to PFOA, an overall reduction of Th2 cytokines (significant: IL-5 and IL-13; non-significant: IL-4), and response for Th1 cytokines (significant reduction of IL-6, IL-12 and TNF-α) were observed by De Guise and Levin (2007) [125]. This showed a favorable Th1 balance and a general decrease in pro-inflammatory cytokines. The authors postulated a potential role for T helper (Th) cells in the immunotoxicity of PFOA. Significant dose-dependent changes in IgM level (which is involved mainly in early, primary immunity) in response to T-dependent antigens, such as sheep red blood cells (sRBCs) or horse red blood cells were observed in acute and intermediate oral administration in C57BL/6J and C57BL/6N female mice, while the lowest-adverse-effect was observed for a dose of 3.75 mg/kg/day in mice exposed to PFOA in drinking water for 15 days [124,126]. By contrast, no effect on antibody responses was found in Sv/129 rats dosed by PFOA at 30 mg/kg/day in drinking water for 15 days [124], suggesting strain differences in susceptibility to PFOA. Rats appeared to be less sensitive than mice, as no changes in IgM levels were noticed in these animals administered with PFOA (even at acutely toxic oral doses up to 30 mg/kg/day) *via* gavage for 28 days [127]. Similarly, the exposure of male rats to 50 mg/kg/day

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with PFOA by gavage for 14 days did not significantly change the number of T cells, NK cells, or helper T cells [128]. In a developmental mouse study, splenic Treg numbers cells were reduced at the highest dose of PFOA at 2 mg/kg bw/day, whereas isolated $CD4^+$ cells from adult offsprings, exposed *via* the dams to PFOA during gestation and through weaning, secreted lower amounts of the immunosuppressive cytokine IL-10 than cells from controls males only. More recently, Erlich et al. (2023) [2] reported that in mice, a reduced number of thymocytes, splenic lymphocytes and marrow B-lymphoid cells decreased following exposures to PFOA (0.002% w/w in diet). Similarly to the above mentioned studies, Erlich et al. (2023) [2] concluded that PFOA may be recognized as immunosuppressant in rodents, and pointed that rats are more susceptible than mice to antibody immunosuppression induced by PFOA.

Several epidemiological studies have found associations between current serum PFOA levels and diagnosis of asthma in children and adults, while a case-control study found significantly higher serum PFOA levels in asthmatic adolescents as compared to adolescents without asthma [129]. Similarly, positive associations with two immune conditions were detected in two independent studies. The prevalence of self-reported asthma was significantly positively associated with residence in a PFOA-contaminated water district [130] and Taiwanese children [131]. A significant inverse association between eosinophil count among non-asthmatic subjects, C-reactive protein, wheezing, eczema and PFOA level was found by food allergy and otitis media [132]. In the study of Lopez-Espinosa and co-workers (2021) [133], associations were found between peripheral white blood cells counts in a human population in the Mid-Ohio Valley, U.S. and PFOA level in drinking water. In this study, PFOA was positively associated with absolute lymphocyte count and the counts of T-cells, B-cells, and natural killer (NK) cells, whereas, no significant associations were reported for changes in the percentages of B, Th and Tc lymphocyte subsets. The cross-sectional study of osteoarthritis (autoimmune condition) was based on 3731 adults with osteoarthritis and 45,701 without osteoarthritis who lived, worked, or attended school in one of six PFOA-contaminated water districts in the Mid-Ohio Valley and were enrolled in the C8 Health Project. Significant positive associations were found between increasing serum PFOA levels and osteoarthritis prevalence, while the observed association was limited to adults under age of 55 years and those who were not obese [134]. Validated self-reported autoimmune diseases were also analyzed separately among the 3713 polymer plant workers. In this cohort study, more frequently ulcerative colitis and rheumatoid arthritis were reported [135]. Studies evaluating the immunosuppressive effects of PFOA have examined disease resistance and antibody responses. Granum et al. (2013) [136] performed prospective birth cohort study of mother–child pairs in Norway and found associations between a 1-ng/mL increase in maternal perinatal plasma PFOA level and number of episodes of the common cold and other respiratory tract infections, as well as the number of episodes of gastroenteritis with vomiting or diarrhea in the first three years of life and during the third year of children. Also Granum et al. (2013) [136] found significant positive associations between a 1-ng/mL increase in maternal perinatal plasma PFOA and number of episodes of the common cold. In a study of Wang et al. (2019), 21 PFAS were identified in 198 Chinese women of childbearing age. In single PFAS, including PFOA, PFAS were positively associated with Th1 and regulatory T-cell (Treg) cytokines, and negatively associated with Th2 and Th17 cytokines. In this study, the Bayesian Kernel Machine Regression (BKMR) model showed a significantly positive association of PFAS mixture with TGF-β and a negative association with IL-10. A deviation of the immune system from Th2 toward Th1 has been implicated in pregnancy complications, such as recurrent miscarriage, preeclampsia and fetal growth restriction. In another study, Knudsen and co-workers (2018) [137] investigated the association between the sum of 15 PFAS, including PFOA and haematological markers in 189 Greenlandic pregnant women. The markers investigated included white blood cells, lymphocytes, neutrophils and monocytes, which were significantly inversely associated with the sum of PFASs suggesting an immunosuppressive potential of PFASs in pregnancy. Prenatal exposure to PFASs has been inconsistently linked to asthma and allergy, and increased number of infections in early childhood. Impinen et al. (2019) [138] evaluated the association between exposure to PFASs, including PFOA of Norwegian Mothers (1270

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women) and asthma, allergies and common infectious diseases in their children up to 7 years old. As a result, no association between PFOA levels and allergy and asthma were found. The authors also suggested that asthma and allergy in children may be linked to exposure to longer PFASs, as they, and other scientists [139] observed such association for perfluoroundecanoic acid (PFUnDA) and perfluorotriodecanoic acid (PFTrDA).

Abraham et al. (2020) [140] observed considerable associations between PFOA serum level and levels of vaccine antibodies against *Haemophilus infuenza* type b, tetanus and diphtheria in 101 healthy 1-year-old children. PFOA levels were negatively associated with production of interferon gamma (IFNɣ) in *ex-vivo* lymphocytes after stimulation with tetanus and diphtheria toxoid. The study showed no effect of PFOA on infections during the first year of life, but obtained results that confirmed negative associations between PFOA level and parameters of the immune response, which had been observed in other epidemiological studies. The results connected with PFASs, including PFOA immunotoxicity concerning a study of 99 Norwegian children at 3 years old revealed that maternal serum concentration of PFOA was linked to depleted vaccine responses in children, and in particular toward rubella vaccine, as well as raised cases of common cold and gastroenteritis [136]. Similarly, the research of Mogensen et al. (2015) [141] showed that prenatal exposure to PFOA was particularly associated with the pre-booster antibody concentration at 5 years of age, and concomitant exposure was linked to response to 5-year booster, while concentrations of antibody at 7 year depended mostly on the current exposure to PFOA level. The prospective study was also conducted in adolescents (516 subjects) to assess PFOA-associated alterations in antibody responses to childhood vaccines at 7 years of age. The results revealed that diphtheria but not tetanus antibody concentration was depleted by 25% at increased serum level of PFOA at the age of 13. In adults, a decrease in antibody response against influenza A H3N2 virus were associated with increasing serum PFOA levels; however, there were no associations with two other strains of influenza virus (influenza A H1N1 and influenza B) [142]. Interesting study was conducted by Grandjean et al. (2020) [143] who used (from Danish biobanks) samples of plasma from 323 non-pregnant subjects (aged 30-70 years) with confirmed SARS-CoV-2 infection and determined concentrations of five PFAS, including PFOA and PFBA. They observed that only increased level of PFBA in plasma was linked to an elevated risk of more severe course of COVID-19. The authors concluded that it may be due to accumulation of PFBA in the lungs.

In order to elucidate immunotoxic effect of PFASs, the EFSA panel (2020) [144] proposed transactivation of several nuclear receptors, as observed from *in vivo* and *in vitro* studies, including PPARs, NF-κB (nuclear factor kappa B), CAR (constitutive activated receptor), Nrf2 (nuclear factor erythroid 2-related factor 2), PXR (pregnane X receptor) and RXR (retinoid X receptor). Although some of these nuclear receptors may have an indirect effect on immune health, the following sections were focused on the modulation of NF-κB and PPARs. This selection was made because of the observed interactions of NF-κB and PPARs with the immune system, while data on other nuclear receptors were less conclusive.

6.4. Epigenotoxicity

Epigenetic modifications play a considerable role in regulation of various functions of organisms during its development. These modifications, include methylation of DNA, modifications of histones, as well as small non-coding RNAs expression of regulating genes [145].

Liu and Irudayaraj (2020) [146] studied epigenetic changes and inhibitory mechanisms of PFOA in human breast epithelial cells (MCF7). They observed that exposure to PFOA at 100 μM and 200 μM (24 h or 48 h) changed mobility of DNA (cytosine-5)-methyltransferase 3A (DNMT3A) and caused inhibition of enzymatic activity of DNA methyl transferases (DNMTs), which resulted in global DNA demethylation. Based on distribution profile of histone 3 lysine 9 tri-methylation (H3K9me3), which is considered to be a hallmark for constitutive organization of heterochromatin, the authors deduced that PFOA (200 μM and 400 μM) significantly altered organization of heterochromatin, increasing its redistribution around periphery of the nucleus. The authors of this

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study proposed detailed explanation for their findings. They suggested that PFOA caused dissociation of DNMTs from the nucleosome, which resulted in loss of its function to methylate CpG sites even at presence of increased expression of DNMT3a mRNA, which led to a reduced level of global methylation of DNA. At this level, the global 5 mC loss induced changes in chromatin packaging at the single nucleosome level. The obtained results showed that PFOA caused epigenetic changes and alterations in heterochromatin packaging by direct effected on DNMTs, which may enhance susceptibility to diseases, such as cancer.

DNA methylation is one of the crucial epigenetic mechanism mainly regulated by ten-eleven translocation (TET) methylcytosine dioxygenases and DNMTs controlling gene expression by adding or removing methyl groups on cytosine at specific Cytosine – Guanine (CpG) sites in those genes [147].

Wen et al. (2020) [148] observed that PFOA caused global epigenetic changes, particularly DNA methylation, in mouse liver (1 mg or 10 mg/kg/day; 10 days), as well as induced tissue-specific changes in RNA binding proteins affecting alternative splicing factors. Moreover PFOA caused gene expression in mammalian target of rapamycin (mTOR) pathway, as well as decreased phosphatase and tensing homolog (Pten) expression, which is primary inhibitor of mTOR pathway. In another study, Rashid et al. (2020) observed that PFOA decreased the expression of DNA methyltransferases (Dnmt1, Dnmt3a, Dnmt3b) in CD1 mice (1-20 mg/kg/day; 10 days), in the colon and small intestine. Moreover, they observed that expression of ten-eleven translocation genes (Tet2 and Tet3) was dysregulated in the small intestine, while in the colon Tets remained unaffected. The tight junction genes Claudins (Cldn), Occludin (Ocln), and Tight Junction Protein (Tjp) were also strongly changed in the small intestine. The authors concluded that PFOA induces DNA methylation and changes genes expression crucial for maintaining the physical barrier of the intestine, with stronger effects observed in small intestine in comparison to the colon. Similarly, PFOA has been shown to induce epigenetic changes in kidney of CD-1 mice (1-5 mg/kg/day; 10 days). The authors noted that PFOA caused variable methylation yielding 879 differentially methylated regions. The mRNA expression showed significant rise in Dnmt1 and depleted RAS protein activator like 1 (Rasal1) expression, which is an early indicator of fibroblast activation in kidney. They also noticed considerable increase in histone deacetylase (Hdac) 1, 3 and 4, which are critically altered in some renal diseases. Furthermore substantial increase in mRNA expression levels of beta tumor growth factor (TGF-β) and alpha smooth muscle actin (α-SMA) was noted. Finally, the KEGG and Go enrichment pathways analysis of decreased representation bisulfite data also showed pathways involved in renal fibrosis. The author concluded that obtained results suggested that epigenetic alterations in kidney trigger the expression of early markers of fibroblast activation.

An interesting study was conducted by Ahmad et al. (2021) [147] who assessed accumulation of PFOA in lungs, and studied alterations in mRNA expression of methylation of DNA regulator genes DNA methyltransferases (Dnmts), as well as ten-eleven translocation (Tets) along with membrane proteins angiotensin transforming enzyme 2 (Ace2) and transmembrane Serine Protease 2 (Tmprss2) genes implicated in the infection of SARS-CoV-2 virus. They used CD1 mice that were orally exposed to 5 mg and 20 mg/kg/day PFOA for 10 days. The authors observed that PFOA accumulated in lungs in a dose-dependent manner. They also observed that Dnmts and Tets were considerably downregulated, while expression level of angiotensin-converting enzyme 2 (Ace2) and transmembrane serine protease 2 (Tmprss2) significantly increased. Moreover, they noted considerable hypomethylation in CpG islands in the promotor region of Tmprss2. In conclusion, it was stated that PFOA by increased expression of tested membrane receptors possibly cause higher susceptibility of SARS-CoV-2 infections. The confirmation of the above findings may be the results of Grandjean et al. (2020) [149] who assessed samples from 323 subjects (30–70 years old) from Denmark with known SARS-CoV-2 infection on the presence PFASs. As a result, they noticed that increased PFBA plasma concentrations (PFAS that accumulates in the lungs) were associated with an elevated risk of a more severe course of COVID-19.

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6.5. Genotoxicity and Cancerogenicity

Genotoxicity refers to the capability of a substance to damage the genetic material of a cell leading to mutations that may finally cause cancer or hereditary genetic disorders. The substances that reveal genotoxic influence can act directly or indirectly on DNA, for example, by ROS formation that cause oxidative deterioration of genetic material.

PFOA, PFHxA and PFBA reveal characteristics, which are typical for the group of peroxisome proliferators. PPARα activation in *in vivo* models, including mice and rats is believed to be connected with ROS induction and damage to DNA that may be implicated in peroxisome proliferator-mediated carcinogenesis. Nevertheless, this mode of activity has been revealed to be highly species-specific and the most probably has small importance in humans [150,151].

Eriksen et al. (2010) [152] studied the effect of perfluorinated compounds (PFCs), including PFOA and PFHxA on intracellular ROS formation and DNA damage in HepG2 cells. The authors of the study noticed that PFOA raised ROS production by 1.52-fold, but did not caused DNA damage, which was measured by determination of DNA strand-breaks, alkali-labile sites and purine oxidation. They also noticed that PFHxA did not increase ROS level nor caused DNA damage. Summing up, achieved results proved that only PFOA was capable of inducing ROS level, while both investigated compounds did not exhibit genotoxic potential in a cell line representing the human liver. In *in vivo* study, Crebelli et al. (2019) [153] evaluated genotoxic potential of PFOA (0.1, 1 and 5 mg/kg body weight) and PFBA (5 mg/kg body weight) that were given in drinking water for mice for 5 weeks. They noted that PFOA administration at its highest dose revealed considerable liver hypertrophy with signs of cell injury. The authors also observed that ALT and AST levels were raised, while no lipid peroxidation and oxidative stress occurred. After PFBA administration, only mild liver hypertrophy was noticed. Moreover, tested substances did not induce genotoxic alterations. The authors concluded that PFOA caused significantly stronger liver damage than PFBA, which was not due to oxidative stress, whereas none of tested compounds induced genotoxic effects in liver, as well as in the other tested tissues, such as bone marrow (micronucleated reticulocytes), spleen (splenocytes) and testis.

According to scientific literature focused on the study and analysis of carcinogenic substances, several features of PFASs, such as PFOA can be recognized that assist in carcinogenic risk or the likelihood of cancer development. These characteristics embrace: metabolic activation, induction of oxidative stress, inflammation, immunosuppressive influence, and the ability to transform cells, making them “immortal” [151,154].

Among perfluorocarboxylic acids (PFCAs), PFOA has been the most thoroughly investigated. It has been shown that exposure to PFOA is positively related with development of liver, pancreas, colon, breast, and testicles cancers [155]. Literature analyses have proven that PFOA is carcinogenic in mice [156], and large doses of PFOA induced hepatocellular adenomas in rats [96]. The mechanism of PFOA action connected with potential tumorigenic activity in breast cancer was studied by Pierozan et al. (2018) [157]. They used human breast epithelial cells (MCF-10A) that after exposure to PFOA at 50 μM and 100 μM revealed a higher rate of proliferation by accelerating G0/G1 to S phase transition of the cell cycle. Moreover, it was observed that PFOA raised levels of cyclin-dependent kinases, including D1, CDK4 and CDK6, along with a depletion of the level of kinase inhibitor p27. It is worth noting that estrogen receptor antagonist did not reveal any effect on promotion of cell proliferation by PFOA, whereas PPARα antagonist was capable of preventing of proliferation of tested cells. Achieved results revealed that the underlying mechanisms of action of PFOA implicates PPARα-dependent pathways. Interestingly, the authors noted that PFOA was able to stimulate of cell migration and invasion, what may suggest its potential to provoke neoplastic transformation of human breast epithelial cells.

In epidemiological studies, it has been proven that elevated serum concentrations of PFOA was connected with a higher risk of various cancer types [158].

Barry et al. (2013) [159] studied the correlation between residents in Mid-Ohio Valley (Washington, West Virginia, U.S) who were exposed to PFOA in drinking water polluted with

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chemical plant and cancer incidence. The cohort consisted of 32,254 adults who were interviewed in 2008–2011 to achieve medical history, whereas retrospective PFOA serum levels were determined for each participant from 1952 to 2011. The authors evaluated the correlation between cumulative PFOA exposure and 21 different cancer types, and found positive associations for kidney, thyroid and testicular cancers. In other epidemiological study conducted by Girardi and Merler (2019) [160], the association between PFASs exposure and mortality (1970–2018) of 462 male employees who had worked at production of PFOA since 1968 was evaluated. They determined very high PFOA concentrations in serum of 120 workers (2000–2013), which were from 19 to 91,900 ng/mL. They showed association between cumulative PFOA level and increased mortality related to liver cancer, as well as malignant neoplasm of lymphatic and hematopoietic tissue. Studies concerning highly exposed humans to PFOA revealed an association between exposure to this chemical and elevated incidence of kidney cancer, whereas it was unclear if PFOA or other PFASs behave as renal carcinogens, and thus, may increase risk of renal cell carcinoma (RCC) in humans environmentally exposed. In other study, Shearer et al. (2021) evaluated the association between the concentrations of PFOA in serum of the general population of U.S (with 324 RCC cases) and incidence of RCC risk. The experiment revealed that there was a positive association between exposure to PFOA and RCC risk. The authors of the study concluded that PFOA is a renal carcinogen and may pose a significant threat to people. Recent study that used serum samples from individuals occupationally exposed to PFOA, such as workers in chemical plants producing PFASs, as well as residents living near such facilities, revealed a correlation between elevated PFOA levels and the incidence of breast cancer in women, particularly tumors lacking hormone receptors [161].

Since PFOA was previously classified as “possibly carcinogenic to humans” many new research have evaluated the relationship between exposure to this substance and cancer risk in both animals and humans.

In the light of these findings, in 2024, the International Agency for Research on Cancer (IARC) decided to reclassify PFOA as carcinogenic to humans (Group 1), taking into consideration sufficient evidence archived from both human and animal studies, as well as mechanistic data.

A panel of 30 scientists reached this conclusion based on:

- sufficient evidence in animals, showing elevated incidences of benign and malignant tumors in both sexes;
- strong evidence in humans, revealing that PFOA causes epigenetic alterations (e.g., changes in methylation of CpG, modifications of histone and miRNA expression), oxidative stress induction (e.g., elevated ROS formation);
- immunosuppressive effects, including depletion of immune responses to vaccines and elevated susceptibility to infectious diseases, as PFOA has been observed to suppress production of cytokine and decrease of lymphocyte level [162].

The IARC expert group also point out that PFOA elevated cellular proliferation by promotion of the activity of glycolytic pathway, and caused cell proliferation and migration. They also underlined that PFOA was able to influence pathways connected to nutrient and energy supply, which can lead to cell death [156].

As for PFBA, the available data are insufficient to assess its carcinogenic potential. Animal studies have not shown satisfactory evidence concerning cancer induction by this substance [163]. For example, a 2-year toxicology and carcinogenicity research was conducted in male and female Sprague-Dawley rats, which were orally treated with PFBA (7 days per week) at doses ranging from 2.5 to 100 mg/kg/day (males) or from 5 to 200 mg/kg/day (females) [164]. As a result, there was no evidence that PFBA is capable of inducing of tumors. Regarding PFHxA, no carcinogenic effects have been noticed in rats [164]. An overview of *in vivo*, *ex vivo*, *in vitro*, clinical and epidemiological studies on PFAS toxicity is presented in Table 7.

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Table 7. Overview of *in vitro*, *ex vivo*, *in vivo*, clinical and epidemiological studies on PFASs toxicity.

	Study type	Sample/model	Sample size	Dose/exposure	Conclusion	References
Hepatotoxicity	*In vivo*	Sprague-Dawley rats	10 rats/sex/group	Up to 200 mg/kg/day PFHxA by gavage for 90 days	PFHxA exposure was associated with lower red blood cell parameters, higher reticulocyte counts and lower globulin level, higher liver enzymes, centrilobular hepatocellular hypertrophy	[82]
	In vivo	Pregnant Kunming mice and female offspring	50 pregnant mice (5 groups of 10)	0, 1, 2.5, 5, 10 mg/kg/day PFOA (gestational days 0-17)	Prenatal PFOA exposure impairs pup growth and development, induces liver damage, disrupts PPAR-α–mediated fatty acid oxidation, causes oxidative stress, and reduces histone acetylation	[87]
	Ex vivo	FRG liver-chimeric humanized mice (human hepatocytes)	n=3 for each experiment	0.067 mg/L PFOA, 0.145 mg/L PFOS, 1 mg/L GenX in drinking water for 28 days	PFOS decreased total cholesterol and LDL/VLDL, GenX increased LDL/VLDL, and PFOA had no significant effect	[88]
	Clinical	Children with nonalcoholic fatty liver disease (NAFLD)	74	Serum PFASs concentrations (median): PFOA 3.42 ng/mL, PFOS 3.59 ng/mL, and PFHxS 1.53 ng/mL	Higher PFOS and PFHxS levels were linked to increased risk of nonalcoholic steatohepatitis (NASH) and liver fibrosis in predominantly obese children, with PFHxS also	[103]

 doi:10.20944/preprints202605.0967.v1

					associated with inflammation and higher NAFLD activity. Elevated PFAS exposure correlated with distinct metabolic alterations.	
	Epidemiology	US adult participants (NHANES)	9,523	Environmental exposures measured in serum (geometric mean): PFOA - nonobese: 2.2 ng/mL, obese: 2.0 ng/mL, PFOS – nonobese: 6.3 ng/mL, obese: 5.5ng/mL, PFDA – nonobese :0.21 ng/mL, obese: 0.18ng/mL	In obese participants, ALT was positively associated with PFOA, PFHxS, and PFNA serum concentrations, while PFOA and PFNA were also linked to increased gamma-glutamyl transpherase (GGT)	[102]
Immunotoxicity	*In vivo*	Mice	A total of 40 mice per endpoint	PFOA in drinking water: 0-30 mg/kg/day	PFOA exposure suppressed IgM antibody production	[126]
	Prospective cohort	Infants/children cohort (Norway)	99 mother-child pairs	Maternal serum level (geometric mean) of PFOA: 1.1 ng/mL, PFOS; PFOS: 5.6 ng/mL	Prenatal PFASs exposure was linked to immunosuppression in early childhood, evidenced by lower anti-rubella antibody levels and increased episodes	[136]

 doi:10.20944/preprints202605.0967.v1

					of common cold and gastroenteritis	
	Ex vivo	1-year-old children	101	PFOA plasma level (geometric mean): 3.8 ng/mL	PFOA levels were inversely associated with IFNɣ production in stimulated lymphocytes following tetanus and diphtheria toxoid exposure	[140]
	In vitro	Human Namalwa B lymphocyte and human Jurkat T lymphocyte cells	cell culture experiments	concentrations up to 100 μM PFASs (PFOA, PFBA, PFNA, PFDA and other)	PFASs reduce RAG1 and RAG2 expression and suppress IL-2 promoter activity, potentially impairing antibody responses	[165]
	In vitro	Human mammary epithelial cell line MCF-10A	cell culture experiments	50 -1000 μM of PFOA	PFOA promotes migration and invasion of human breast epithelial cells, indicating its potential to induce neoplastic transformation	[157]
Cancerogenity	Epidemiology (C8 cohort)	Residents in contaminated water areas (C8 cohort cancer study), adults	32,254	PFOA-contaminated drinking water exposures (median): community 24.2 ng/mL, worker 112.7 ng/mL	PFOA exposure was associated with an increased risk of kidney and testicular cancer; however, interpretation is limited by the survivor cohort design and the high lethality of certain malignancies, such as pancreatic and lung cancer	[159]

 doi:10.20944/preprints202605.0967.v1

	Epidemiology (occupational cohort)	Factory workers exposed occupationally	462 males	Occupational exposure to PFOA (geometric mean): 4048 ng/mL	Workers with the highest cumulative PFOA exposure had a significantly increased risk of liver cancer, liver cirrhosis, diabetes, and hematologic malignancies	[160]
	Epidemiology (case-control of renal carcinoma, RCC)	Renal cell carcinoma cases and controls	324 cases	Serum PFOA measurements (geometric mean): 3.6-4.8 ng/mL (depending on the study group)	A statistically significant positive exposure-response relationship was observed between prediagnostic serum PFOA levels and subsequent RCC risk	[166]
Reproductive disorder	*In vivo*	Male mice	80 (5 groups of 16)	0-20 mg/kg/day PFOA by gavage for 28 days	PFOA impaired seminiferous tubules, decreased testicular testosterone and progesterone in a dose-dependent manner, and reduced sperm quality	[113]
	In vivo	CD-1 mice, gestational exposure studies	50 dams	Various gestational exposure regimes (i.e. 0, 1, or 5 mg PFOA/kg/day)	Gestational and chronic low-dose PFOA exposure impaired mammary gland development and lactational differentiation across generations in mice	[114]
Epigenotoxici	*In vitro*	Human breast epithelial cells (MCF7)	cell culture experiments	100 μM and 200 μM (24 h or 48 h)	PFOA altered the mobility of DNA (cytosine-5)-methyltransferase 3A (DNMT3A) and	[146]

 doi:10.20944/preprints202605.0967.v1

					inhibited DNA methyltransferase (DNMT) activity, leading to global DNA demethylation	
Endocrine disruption	*In vivo*	Cynomolgus monkeys (male and female)	6-8 monkeys/ sex/dose	0, 0.03, 0.15, 0.75 mg/kg/day orally for 182 days	Adverse effects were seen at 0.75 mg/kg/day (PFOS) and included mortality in 2 of 6 males, decreased body weight, increased liver weight, and reduced levels of cholesterol, triiodothyronine (without hypothyroidism), and estradiol	[77]
	In vitro	Human osteosarcoma Saos-2 cells and epithelial colorectal adenocarcinoma Caco-2 cells	cell culture experiments	PFOA 400 ng/mL	PFOA disrupts vitamin D receptor signaling through endocrine interference, altering gene responses and reducing osteoblast mineralization	[167]
	In vitro	Primary bovine granulosa cells	cell culture experiments	0, 4, and 40 μM PFOA for 48 and 96 h	PFOA may impair granulosa cell steroidogenesis by inducing mitochondrial dysfunction	[168]

The key molecular mechanisms underlying the toxicity of PFOA and its short-chain analogs are summarized in Table 8.

 doi:10.20944/preprints202605.0967.v1

Table 8. Summary of molecular mechanisms of action of PFOA and its selected short-chain analogs (PFHxA, PFBA) highlighting both shared and compound-specific pathways based on selected studies discussed in the toxicity section.

Mechanism	PFOA	PFHxA	PFBA	Molecular targets/pathway	References
PPARα activation	Strong activation	Strong activation	Strong activation	PPARα, lipid metabolism genes, β-oxidation	[85,88,94]
Oxidative stress (ROS/RNS generation)	Strong induction	Strong induction	Strong induction	ROS, antioxidant enzymes (SOD, GSH), mitochondrial dysfunction	[80,95,123]
Endocrine disruption	Well-documented (ER activation, steroidogenesis disruption)	Weak or negligible	Limited evidence	Estrogen receptor (ER), androgen pathways, thyroid hormones	[107,111–113]
Immunotoxicity	Well-documented (reduced antibody response, cytokine modulation)	Weak to moderate	Moderately supported	Cytokines (IL-6, TNF-α), T/B cells, IgM response	[124,126,136]
Epigenetic modifications	Well-documented (DNA methylation, DNMT inhibition, histone changes)	Poorly studied	Limited evidence	DNMTs, TET enzymes, histone modifications (H3K9me3)	[146–148,169]
Lipid metabolism disruption	Well-documented	Moderately supported	limited evidence	PPARα, lipid transport, cholesterol homeostasis	[89,92,93]
Mitochondrial dysfunction /	Well-documented	Limited evidence	Moderately supported	ATP production,	[123]

 doi:10.20944/preprints202605.0967.v1

energy metabolism	(reduced ATP levels)			mitochondrial respiration	
Inflammatory pathways activation	Well-documented	Limited evidence	Moderately supported	NF-κB, cytokines, immune signaling	[45,99]
Carcinogenic-related pathways	Well-documented (non-genotoxic mechanisms)	Not well established	Not established	Cell proliferation, ERK/MAPK, PPARα	[151,157,159]

7. Conclusions

Perfluorooctanoic acid (PFOA) and its selected short-chain analogs – perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA) belong to a group of perfluoroalkyl acids (PFASs). Due to their unique properties, i.e. stability, durability and lowering of surface tension, they have found many applications in different branches of industry and are used in various everyday products. PFASs are named "perennial chemicals" because they exhibit strong resistance to degradation in the environment and exhibit high accumulation in biota, including humans. It has been proven that the main exposure routes of these substances to general population is consumption of contaminated food and water, as well as inhalation of dust. These substances have been determined in surface, and in higher concentrations, in ground waters, in which PFBA, PFHxA and PFOA are quite often determined with higher frequency than another PFASs. PFOA and its substitutes have also been repeatedly detected in drinking water that is one of the most important sources of exposure of general population to these compounds. It has also been proven that food, and particularly fish, meat and meat products (PFOA) and cereals (PFBA), are the most significant sources of the exposure of humans to PFASs. Dust on which PFASs are adsorbed is also essential source of exposure to these toxicants to humans, as it contains high concentrations of PFBA, PFHxA and PFOA. PFASs concentrations are not systematically measured in the air; however discussed compounds and PFBA in the highest concentrations have been detected in the atmosphere.

PFOA has been produced from decades, but due to its toxicity it was successively replaced by PFASs of shorter chain, including PFHxA and PFBA. Generally PFASs, and particularly PFOA and PFHxA accumulate mainly in the liver, therefore inducing hepatoxicity. Nevertheless, PFBA accumulates more effectively in lungs in comparison to PFHxA and PFOA. Several studies have shown the effect of discussed PFASs on liver function, metabolism and damage.

The activation of PPARα by PFASs, including PFOA, PFHxS and PFBS has been recognized as the primary mechanism of action in rodent hepatocyte-induced proliferation. It was also observed that PFOA caused activation of p53 and inhibited androgen receptor and nuclear receptor subfamily 1 group D member 1, which showed that new human-relevant molecular mechanism of PFASs exists, including its previously unknown effect on circadian rhythm. PFOA was also revealed to be partly responsible for the gut microbiota dysbiosis. Moreover, occupational surveys, have found association between human exposure to PFBA, PFHxA and PFOA and higher risk of liver damage and disfunction. Some PFASs are known as endocrine-disrupting compounds. PFHxA have not been shown to induce alterations in endocrine activity in various animal models. In contrast, PFOA can induce endocrine disruption by altering the functions of growth and sex hormones, including activating the estrogen receptor (ER), The authors also suggested that exposure to PFOA can impair reproductive function, possibly by altering testosterone levels, and CPY11A1. Endocrine-disrupting potential of PFOA has also been observed in occupational surveys, which revealed that it increased thyroid stimulating hormone and triiodothyronine levels. Generally, the obtained results may point that high concentrations of PFOA in serum may be potentially associated with thyroid disease.

 doi:10.20944/preprints202605.0967.v1

Another study showed that the presence of PFBA was negatively correlated with thyroxine level, while for PFOA and PFHxA such correlation has not been found. Immunotoxic potential of PFASs has been shown both *in vivo* and in epidemiological studies. In mice PFOA caused suppression of antigen-specific immunoglobulin M antibody production, splenic and thymic atrophy, and altered T-cell phenotypic distribution. It also altered Th1 cytokine balance and decreased pro-inflammatory cytokines. Therefore, the author concluded that PFOA may be recognized as immunosuppressant in rodents. Several epidemiological studies have found associations between serum PFOA levels and diagnosis of asthma in children and adults, as well as positive correlation has been found between PFOA levels and osteoarthritis, ulcerative colitis and rheumatoid arthritis. Moreover maternal serum concentration of PFOA was linked to deplete various vaccine responses in children. Interestingly, increased PFBA, but not PFOA level was positively associated with elevated risk of more severe course of COVID-19, which might be due to higher accumulation of PFBA in the lungs. EFSA panel suggested that transactivation of several nuclear receptors, particularly PARPs and NF-κB (nuclear factor kappa B) may be responsible for immunotoxic potential of PFASs, such as PFOA and PFBA. The studies have also shown that PFOA exhibited epigenetic potential as it caused global DNA demethylation and significantly altered organization of heterochromatin, which may contribute to development of diseases, such as cancer. It was also observed that PFOA caused DNA global methylation in liver and changes in genes expression crucial for maintaining the physical barrier of the intestine. Moreover, PFOA induced considerable increase in histone deacetylases, which function is critically important in some renal diseases. Epigenetic changes induced by PFOA seem to be the most probably involved in development of various cancer types and other diseases. The results concerning epigenetic activity of PFHxA and PBMA are scarce. It was noticed that increased PFBA plasma concentrations were associated with an elevated risk of a more severe course of COVID-19. PFASs, including PFOA, PFHxA and PFBA have shown properties, which are characteristic for the group of peroxisome proliferators that are recognized as non-genotoxic chemical substances. The result of the studies showed that although PFOA, PFHxA and PFBA induced ROS formation, they did not caused damage to DNA. Among perfluorocarboxylic acids (PFCAs), carcinogenic potential of PFOA has been the most thoroughly studied. It has been proven that exposure of rodents to PFOA were positively associated with liver, pancreas, colon, breast, and testicles cancers. Obtained results showed that the underlying mechanisms of carcinogenic PFOA action involves PPARα-dependent and kinases-dependent pathways. In epidemiological studies, it has been proven that increased serum concentrations of PFOA have been associated with a higher risk of different cancer types, including liver, kidney, thyroid and testicular cancers, as well as malignant neoplasm of lymphatic and hematopoietic tissue. In the light of these findings, in 2024, the International Agency for Research on Cancer (IARC) classified PFOA as carcinogenic to humans (Group 1). As for PFBA, the available data are insufficient to evaluate its carcinogenic potential, animal studies have not provided conclusive evidence of its carcinogenicity. Regarding PFHxA, no carcinogenic effects have been observed in rats.

Hitherto conducted researches have shown that PFHxA and PFBA are less toxic than PFOA, nevertheless additional extensive studies should be conducted in order to determine environmental and particularly toxicological status of these compounds.

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Abbreviations

17OHP	17-hydroxyprogesterone
17β-HSD	17β-hydroxysteroid dehydrogenase
A	Androgen
Ace2	Membrane proteins angiotensin transforming enzyme 2
AFFF	Aqueous film-forming foams
ALT	Alanine aminotransferas
ATSDR	Agency for Toxic Substances and Disease Registry
BKMR	Bayesian Kernel Machine Regression
CAR	Constitutive activated receptor
CERI	Centre for Educational Research and Innovation
Cldn	Genes Claudins
CpG	Cytosine at specific Cytosine – Guanine sites
CYP11A1	Cytochrome P450 cholesterol side-chain cleavage enzyme
Cyp7a1	Cholesterol 7 alpha-hydroxylase
DNA	Deoxyribonucleic acid
DNMT3A	DNA (cytosine-5)-methyltransferase 3A
DNMTs	DNA methyl transferases
Dnmts	DNA regulator genes DNA methyltransferases
EFSA	European Food Safety Authority
ER	Estrogen receptor
ERK	Extracellular receptor kinase
ERα	Estrogen receptor α
FT3	Free triiodothyronine
FT4	Free thyroxine
GJIC	Gap-junctional intercellular communication
GSH	Glutathione
H	Estrogen
H3K9me3	Histone 3 lysine 9 tri-methylation
Hdac	Histone deacetylase
Hmgcr	3-hydroxy-3-methylglutaryl-CoA reductase
hPPARα	Human peroxisome proliferator receptor alpha
IARC	International Agency for Research on Cancer
IFNγ	Interferon gamma
IgM	Immunoglobulin M
INSL3	Insulin like-factor 3
Ldlr	Low-density lipoprotein receptor
MAPK	Mitogen-activated protein kinase
MCF7	Human breast epithelial cells
mRNA	messenger RNA
mTOR	Mammalian target of rapamycin
NALFD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-κB	Nuclear factor kappa B
NHANES	National Health and Nutrition Examination Survey
NR1D1	Nuclear receptor subfamily 1 group D member 1
Nrf2	Nuclear factor erythroid 2-related factor 2
Ocln	Genes Occludin
PBMCs	Human peripheral blood mononuclears cells
PC-PLC	Phosphatidylcholine-specific phospholipase C
PFAAs	Perfluoroalkyl acids
PFASs	Polyfluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFCs	Perfluorinated compounds
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid

 doi:10.20944/preprints202605.0967.v1

PFPrA	Perfluoropropanoic acid
PFTrDA	Perfluorotriodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PPARα	Peroxisome proliferator receptor alpha
Pten	Phosphatase and tensing homolog
PXR	Pregnane X receptor
Rasal1	RAS protein activator like 1
RCC	Renal cell carcinoma
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
RXR	Retinoid X receptor
SOD	Superoxide Dismutase
sRBCs	Sheep red blood cells
T	Thyroid
T3	Triiodothyronine
T4	Thyroxin
TDAR	T-Dependent Antibody Response assay
TDI	Tolerable daily intake
TET	Ten-eleven translocation
TGF-β	Beta tumor growth factor
TGN	Thyroglobulin
Th	T helper cells
THs	Thyroid hormones
Tjp	Genes Tight Junction Protein
Tmprss2	Transmembrane Serine Protease 2
Treg	Regulatory T-cell
TSH	Thyroid stimulating hormon
TSI	Thyroid-stimulating immunoglobulin
TT3	Total triiodothyronine
TT4	Total thyroxine
US EPA	US Environmental Protection Agency
WHO	World Health Organization
α-SMA	Alpha smooth muscle actin

References

1. Groh, K.J.; Geueke, B.; Martin, O.; Maffini, M.; Muncke, J. Overview of Intentionally Used Food Contact Chemicals and Their Hazards. *Environ Int* **2021**, *150*, 106225, doi:10.1016/j.envint.2020.106225.
2. Ehrlich, V.; Bil, W.; Vandebriel, R.; Granum, B.; Luijten, M.; Lindeman, B.; Grandjean, P.; Kaiser, A.-M.; Hauzenberger, I.; Hartmann, C.; et al. Consideration of Pathways for Immunotoxicity of Per- and Polyfluoroalkyl Substances (PFAS). *Environ Health* **2023**, *22*, 19, doi:10.1186/s12940-022-00958-5.
3. Spyrakis, F.; Dragani, T.A. The EU's Per- and Polyfluoroalkyl Substances (PFAS) Ban: A Case of Policy over Science. *Toxics* **2023**, *11*, 721, doi:10.3390/toxics11090721.
4. Hofmann, A.; Mishra, J.S.; Yadav, P.; Dangudubiyyam, S.V.; Blesson, C.S.; Kumar, S. PFOS Impairs Mitochondrial Biogenesis and Dynamics and Reduces Oxygen Consumption in Human Trophoblasts. *J Environ Sci Public Health* **2023**, *7*, 164–175.
5. India-Aldana, S.; Yao, M.; Midya, V.; Colicino, E.; Chatzi, L.; Chu, J.; Gennings, C.; Jones, D.P.; Loos, R.J.F.; Setiawan, V.W.; et al. PFAS Exposures and the Human Metabolome: A Systematic Review of Epidemiological Studies. *Curr Pollut Rep* **2023**, *9*, 510–568, doi:10.1007/s40726-023-00269-4.
6. Podder, A.; Sadmani, A.H.M.A.; Reinhart, D.; Chang, N.-B.; Goel, R. Per and Poly-Fluoroalkyl Substances (PFAS) as a Contaminant of Emerging Concern in Surface Water: A Transboundary Review of Their Occurrences and Toxicity Effects. *J Hazard Mater* **2021**, *419*, 126361, doi:10.1016/j.jhazmat.2021.126361.
7. Danish Environmental Protection Agency *Alternatives to Perfluoroalkyl and Polyfluoro-Alkyl Substances (PFAS) in Textiles*; LOUS Survey of Chemical Substances in Consumer Products; Danish EPA: Copenhagen, 2015;
8. Hull, S.D.; Deen, L.; Petersen, K.U.; Jensen, T.K.; Hammer, P.; Wils, R.S.; Frankel, H.N.; Ostrowski, S.R.; Tøttenborg, S.S. Time Trends in Per- and Polyfluoroalkyl Substances (PFAS) Concentrations in the Danish

 doi:10.20944/preprints202605.0967.v1

Population: A Review Based on Published and Newly Analyzed Data. *Environmental Research* **2023**, *237*, 117036, doi:10.1016/j.envres.2023.117036.

9. National Library of Medicine Perfluorohexanoic Acid Available online: https://toxnet.nlm.nih.gov/cgi bin/sis/search/a?dbs+hsdb:@term+@DOCNO+8299.
10. U.S. Environmental Protection Agency CompTox Chemicals Dashboard Available online: https://comptox.epa.gov/dashboard (accessed on 22 April 2026).
11. Agency for Toxic Substances and Disease Registry *Toxicological Profile for Perfluoroalkyls*; ATSDR Toxicological Profiles; Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2018;
12. Royal Society of Chemistry Perfluorohexanoic Acid (CAS 307-24-4) Available online: https://www.chemspider.com/Search.aspx.
13. Lide, D.R. *CRC Handbook of Chemistry and Physics*; 84th edition.; CRC Press, 2003;
14. Savu, P.M. Fluorinated Higher Carboxylic Acids. *Kirk-Othmer Encyclopedia of Chemical Technology* 2000.
15. Ylinen, M.; Kojo, A.; Hanhijärvi, H.; Peura, P. Disposition of Perfluorooctanoic Acid in the Rat after Single and Subchronic Administration. *Bull Environ Contam Toxicol* **1990**, *44*, 46–53, doi:10.1007/BF01702360.
16. Kauck, E.A.; Diesslin, A.R. Some Properties of Perfluorocarboxylic Acids. *Ind. Eng. Chem.* **1951**, *43*, 2332–2334, doi:10.1021/ie50502a044.
17. Chen, Y.-F.; Liu, T.; Hu, L.-X.; Chen, C.-E.; Yang, B.; Ying, G.-G. Unveiling Per- and Polyfluoroalkyl Substance Contamination in Chinese Paper Products and Assessing Their Exposure Risk. *Environment International* **2024**, *185*, 108540, doi:10.1016/j.envint.2024.108540.
18. Prevedouros, K.; Cousins, I.T.; Buck, R.C.; Korzeniowski, S.H. Sources, Fate and Transport of Perfluorocarboxylates. *Environ Sci Technol* **2006**, *40*, 32–44, doi:10.1021/es0512475.
19. Strynar, M.J.; Lindstrom, A.B. Perfluorinated Compounds in House Dust from Ohio and North Carolina, USA. *Environ Sci Technol* **2008**, *42*, 3751–3756, doi:10.1021/es7032058.
20. Deng, Y.; Liang, Z.; Lu, X.; Chen, D.; Li, Z.; Wang, F. The Degradation Mechanisms of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) by Different Chemical Methods: A Critical Review. *Chemosphere* **2021**, *283*, 131168, doi:10.1016/j.chemosphere.2021.131168.
21. Gebbink, W.A.; Ullah, S.; Sandblom, O.; Berger, U. Polyfluoroalkyl Phosphate Esters and Perfluoroalkyl Carboxylic Acids in Target Food Samples and Packaging--Method Development and Screening. *Environ Sci Pollut Res Int* **2013**, *20*, 7949–7958, doi:10.1007/s11356-013-1596-y.
22. Kotthoff, M.; Müller, J.; Jürling, H.; Schlummer, M.; Fiedler, D. Perfluoroalkyl and Polyfluoroalkyl Substances in Consumer Products. *Environ Sci Pollut Res Int* **2015**, *22*, 14546–14559, doi:10.1007/s11356-015-4202-7.
23. Buck, R.C.; Franklin, J.; Berger, U.; Conder, J.M.; Cousins, I.T.; de Voogt, P.; Jensen, A.A.; Kannan, K.; Mabury, S.A.; van Leeuwen, S.P.J. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr Environ Assess Manag* **2011**, *7*, 513–541, doi:10.1002/ieam.258.
24. Agency for Toxic Substances and Disease Registry *Toxicological Profile for Perfluoroalkyls*; ATSDR Toxicological Profiles; Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2015;
25. Leung, S.C.E.; Shukla, P.; Chen, D.; Eftekhari, E.; An, H.; Zare, F.; Ghasemi, N.; Zhang, D.; Nguyen, N.-T.; Li, Q. Emerging Technologies for PFOS/PFOA Degradation and Removal: A Review. *Sci Total Environ* **2022**, *827*, 153669, doi:10.1016/j.scitotenv.2022.153669.
26. Wee, S.Y.; Aris, A.Z. Environmental Impacts, Exposure Pathways, and Health Effects of PFOA and PFOS. *Ecotoxicol Environ Saf* **2023**, *267*, 115663, doi:10.1016/j.ecoenv.2023.115663.
27. Runkel, A.A.; Stajnko, A.; Snoj Tratnik, J.; Mazej, D.; Horvat, M.; Přibylová, P.; Kosjek, T. Exposure of Children and Adolescents from Northeastern Slovenia to Per- and Polyfluoroalkyl Substances. *Chemosphere* **2023**, *321*, 138096, doi:10.1016/j.chemosphere.2023.138096.
28. Wee, S.Y.; Aris, A.Z. Revisiting the "Forever Chemicals", PFOA and PFOS Exposure in Drinking Water. *npj Clean Water* **2023**, *6*, 57, doi:10.1038/s41545-023-00274-6.
29. Anderson, J.K.; Luz, A.L.; Goodrum, P.; Durda, J. Perfluorohexanoic Acid Toxicity, Part II: Application of Human Health Toxicity Value for Risk Characterization. *Regul Toxicol Pharmacol* **2019**, *103*, 10–20, doi:10.1016/j.yrtph.2019.01.020.

 doi:10.20944/preprints202605.0967.v1

30. Randazzo, A.; Pavan, F.; Gea, M.; Maffiotti, A. Perfluoroalkyl Substances (PFASs) in Groundwater and Surface Water in the Turin Metropolitan Area (Italy): An Attempt to Unravel Potential Point Sources and Compliance with Environmental/Drinking Water Quality Standards. *Sci Total Environ* **2025**, *958*, 177973, doi:10.1016/j.scitotenv.2024.177973.
31. Morales-McDevitt, M.E.; Dunn, M.; Habib, A.; Vojta, S.; Becanova, J.; Lohmann, R. Poly- and Perfluorinated Alkyl Substances in Air and Water from Dhaka, Bangladesh. *Environ Toxicol Chem* **2022**, *41*, 334–342, doi:10.1002/etc.5255.
32. Lalonde, B.; Garron, C. Perfluoroalkyl Substances (PFASs) in the Canadian Freshwater Environment. *Arch Environ Contam Toxicol* **2022**, *82*, 581–591, doi:10.1007/s00244-022-00922-x.
33. Khair Biek, S.; Khudur, L.S.; Rigby, L.; Singh, N.; Askeland, M.; Ball, A.S. Assessing the Impact of Immobilisation on the Bioavailability of PFAS to Plants in Contaminated Australian Soils. *Environ Sci Pollut Res Int* **2024**, *31*, 20330–20342, doi:10.1007/s11356-024-32496-7.
34. Zhou, J.; Baumann, K.; Surratt, J.D.; Turpin, B.J. Legacy and Emerging Airborne Per- and Polyfluoroalkyl Substances (PFAS) Collected on PM2.5 Filters in Close Proximity to a Fluoropolymer Manufacturing Facility. *Environ Sci Process Impacts* **2022**, *24*, 2272–2283, doi:10.1039/d2em00358a.
35. Teunen, L.; Bervoets, L.; Belpaire, C.; De Jonge, M.; Groffen, T. PFAS Accumulation in Indigenous and Translocated Aquatic Organisms from Belgium, with Translation to Human and Ecological Health Risk. *Environ Sci Eur* **2021**, *33*, 39, doi:10.1186/s12302-021-00477-z.
36. Wang, F.; Zhao, C.; Gao, Y.; Fu, J.; Gao, K.; Lv, K.; Wang, K.; Yue, H.; Lan, X.; Liang, Y.; et al. Protein-Specific Distribution Patterns of Perfluoroalkyl Acids in Egg Yolk and Albumen Samples around a Fluorochemical Facility. *Sci Total Environ* **2019**, *650*, 2697–2704, doi:10.1016/j.scitotenv.2018.10.006.
37. Chen, W.-L.; Bai, F.-Y.; Chang, Y.-C.; Chen, P.-C.; Chen, C.-Y. Concentrations of Perfluoroalkyl Substances in Foods and the Dietary Exposure among Taiwan General Population and Pregnant Women. *J Food Drug Anal* **2018**, *26*, 994–1004, doi:10.1016/j.jfda.2017.12.011.
38. Bonato, T.; Pal, T.; Benna, C.; Di Maria, F. Contamination of the Terrestrial Food Chain by Per- and Polyfluoroalkyl Substances (PFAS) and Related Human Health Risks: A Systematic Review. *Sci Total Environ* **2025**, *961*, 178337, doi:10.1016/j.scitotenv.2024.178337.
39. Martínez, J.; Picardo, M.; Peñalver, A.; Fabregas, J.; Aguilar, C.; Borrull, F. Occurrence and Health Risk Assessment of PFAS and Possible Precursors: A Case Study in a Drinking Water Treatment Plant and Bottled Water (South Catalonia, Spain). *Environ Sci Pollut Res Int* **2024**, *31*, 56536–56549, doi:10.1007/s11356-024-34805-6.
40. Li, Y.; Li, J.; Zhang, L.; Huang, Z.; Liu, Y.; Wu, N.; He, J.; Zhang, Z.; Zhang, Y.; Niu, Z. Perfluoroalkyl Acids in Drinking Water of China in 2017: Distribution Characteristics, Influencing Factors and Potential Risks. *Environ Int* **2019**, *123*, 87–95, doi:10.1016/j.envint.2018.11.036.
41. Mroczko, O.; Preisendanz, H.E.; Wilson, C.; Mashtare, M.L.; Elliott, H.A.; Veith, T.L.; Soder, K.J.; Watson, J.E. Spatiotemporal Patterns of PFAS in Water and Crop Tissue at a Beneficial Wastewater Reuse Site in Central Pennsylvania. *J Environ Qual* **2022**, *51*, 1282–1297, doi:10.1002/jeq2.20408.
42. Besis, A.; Botsaropoulou, E.; Samara, C.; Katsoyiannis, A.; Hanssen, L.; Huber, S. Perfluoroalkyl Substances (PFASs) in Air-Conditioner Filter Dust of Indoor Microenvironments in Greece: Implications for Exposure. *Ecotoxicol Environ Saf* **2019**, *183*, 109559, doi:10.1016/j.ecoenv.2019.109559.
43. Yao, Y.; Zhao, Y.; Sun, H.; Chang, S.; Zhu, L.; Alder, A.C.; Kannan, K. Per- and Polyfluoroalkyl Substances (PFASs) in Indoor Air and Dust from Homes and Various Microenvironments in China: Implications for Human Exposure. *Environ Sci Technol* **2018**, *52*, 3156–3166, doi:10.1021/acs.est.7b04971.
44. Wang, W.; Zhou, W.; Wu, S.; Liang, F.; Li, Y.; Zhang, J.; Cui, L.; Feng, Y.; Wang, Y. Perfluoroalkyl Substances Exposure and Risk of Polycystic Ovarian Syndrome Related Infertility in Chinese Women. *Environ Pollut* **2019**, *247*, 824–831, doi:10.1016/j.envpol.2019.01.039.
45. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel); Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; Del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.-C.; et al. Risk to Human Health Related to the Presence of Perfluoroalkyl Substances in Food. *EFSA J* **2020**, *18*, e06223, doi:10.2903/j.efsa.2020.6223.

 doi:10.20944/preprints202605.0967.v1

46. Ramírez Carnero, A.; Lestido-Cardama, A.; Vazquez Loureiro, P.; Barbosa-Pereira, L.; Rodríguez Bernaldo de Quirós, A.; Sendón, R. Presence of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials (FCM) and Its Migration to Food. *Foods* **2021**, *10*, 1443, doi:10.3390/foods10071443.
47. Susmann, H.P.; Schaider, L.A.; Rodgers, K.M.; Rudel, R.A. Dietary Habits Related to Food Packaging and Population Exposure to PFASs. *Environ Health Perspect* **2019**, *127*, 107003, doi:10.1289/EHP4092.
48. European Commission *Commission Regulation (EU) 2022/2388 of 7 December 2022*; 2022;
49. Davis, K.L.; Aucoin, M.D.; Larsen, B.S.; Kaiser, M.A.; Hartten, A.S. Transport of Ammonium Perfluorooctanoate in Environmental Media near a Fluoropolymer Manufacturing Facility. *Chemosphere* **2007**, *67*, 2011–2019, doi:10.1016/j.chemosphere.2006.11.049.
50. Stefano, P.H.P.; Roisenberg, A.; D'Anna Acayaba, R.; Roque, A.P.; Bandoria, D.R.; Soares, A.; Montagner, C.C. Occurrence and Distribution of Per-and Polyfluoroalkyl Substances (PFAS) in Surface and Groundwaters in an Urbanized and Agricultural Area, Southern Brazil. *Environ Sci Pollut Res Int* **2023**, *30*, 6159–6169, doi:10.1007/s11356-022-22603-x.
51. Zhao, P.; Xia, X.; Dong, J.; Xia, N.; Jiang, X.; Li, Y.; Zhu, Y. Short- and Long-Chain Perfluoroalkyl Substances in the Water, Suspended Particulate Matter, and Surface Sediment of a Turbid River. *Science of The Total Environment* **2016**, *568*, 57–65, doi:10.1016/j.scitotenv.2016.05.221.
52. Paige, T.; De Silva, T.; Buddhadasa, S.; Prasad, S.; Nugegoda, D.; Pettigrove, V. Background Concentrations and Spatial Distribution of PFAS in Surface Waters and Sediments of the Greater Melbourne Area, Australia. *Chemosphere* **2024**, *349*, 140791, doi:10.1016/j.chemosphere.2023.140791.
53. Xie, L.-N.; Wang, X.-C.; Dong, X.-J.; Su, L.-Q.; Zhu, H.-J.; Wang, C.; Zhang, D.-P.; Liu, F.-Y.; Hou, S.-S.; Dong, B.; et al. Concentration, Spatial Distribution, and Health Risk Assessment of PFASs in Serum of Teenagers, Tap Water and Soil near a Chinese Fluorochemical Industrial Plant. *Environ Int* **2021**, *146*, 106166, doi:10.1016/j.envint.2020.106166.
54. Liu, Z.; Lu, Y.; Song, X.; Jones, K.; Sweetman, A.J.; Johnson, A.C.; Zhang, M.; Lu, X.; Su, C. Multiple Crop Bioaccumulation and Human Exposure of Perfluoroalkyl Substances around a Mega Fluorochemical Industrial Park, China: Implication for Planting Optimization and Food Safety. *Environment International* **2019**, *127*, 671–684, doi:10.1016/j.envint.2019.04.008.
55. U.S. Environmental Protection Agency *Drinking Water Health Advisories for Perfluorooctanoic Acid (PFOA)*; US EPA, 2022;
56. Björnsdotter, M.K.; Hartz, W.F.; Kallenborn, R.; Ericson Jogsten, I.; Humby, J.D.; Kärrman, A.; Yeung, L.W.Y. Levels and Seasonal Trends of C1-C4 Perfluoroalkyl Acids and the Discovery of Trifluoromethane Sulfonic Acid in Surface Snow in the Arctic. *Environ Sci Technol* **2021**, *55*, 15853–15861, doi:10.1021/acs.est.1c04776.
57. Schweizer, C.; Edwards, R.D.; Bayer-Oglesby, L.; Gauderman, W.J.; Ilacqua, V.; Jantunen, M.J.; Lai, H.K.; Nieuwenhuijsen, M.; Künzli, N. Indoor Time-Microenvironment-Activity Patterns in Seven Regions of Europe. *J Expo Sci Environ Epidemiol* **2007**, *17*, 170–181, doi:10.1038/sj.jes.7500490.
58. Weiss, J.M.; Jones, B.; Koekkoek, J.; Bignert, A.; Lamoree, M.H. Per- and Polyfluoroalkyl Substances (PFASs) in Swedish Household Dust and Exposure of Pet Cats. *Environ Sci Pollut Res Int* **2021**, *28*, 39001–39013, doi:10.1007/s11356-021-13343-5.
59. Nyström-Kandola, J.; Ahrens, L.; Glynn, A.; Johanson, G.; Benskin, J.P.; Gyllenhammar, I.; Lignell, S.; Vogs, C. Low Concentrations of Perfluoroalkyl Acids (PFAAs) in Municipal Drinking Water Associated with Serum PFAA Concentrations in Swedish Adolescents. *Environ Int* **2023**, *180*, 108166, doi:10.1016/j.envint.2023.108166.
60. Feng, X.; Chen, X.; Yang, Y.; Yang, L.; Zhu, Y.; Shan, G.; Zhu, L.; Zhang, S. External and Internal Human Exposure to PFOA and HFPOs around a Mega Fluorochemical Industrial Park, China: Differences and Implications. *Environ Int* **2021**, *157*, 106824, doi:10.1016/j.envint.2021.106824.
61. Yao, J.; Dong, Z.; Jiang, L.; Pan, Y.; Zhao, M.; Bai, X.; Dai, J. Emerging and Legacy Perfluoroalkyl Substances in Breastfed Chinese Infants: Renal Clearance, Body Burden, and Implications. *Environ Health Perspect* **2023**, *131*, 37003, doi:10.1289/EHP11403.

 doi:10.20944/preprints202605.0967.v1

62. Pérez, F.; Nadal, M.; Navarro-Ortega, A.; Fàbrega, F.; Domingo, J.L.; Barceló, D.; Farré, M. Accumulation of Perfluoroalkyl Substances in Human Tissues. *Environ Int* **2013**, *59*, 354–362, doi:10.1016/j.envint.2013.06.004.
63. Nilsson, H.; Kärrman, A.; Rotander, A.; Van Bavel, B.; Lindström, G.; Westberg, H. Professional Ski Waxers' Exposure to PFAS and Aerosol Concentrations in Gas Phase and Different Particle Size Fractions. *Environ. Sci.: Processes Impacts* **2013**, *15*, 814, doi:10.1039/c3em30739e.
64. Poothong, S.; Thomsen, C.; Padilla-Sanchez, J.A.; Papadopoulou, E.; Haug, L.S. Distribution of Novel and Well-Known Poly- and Perfluoroalkyl Substances (PFASs) in Human Serum, Plasma, and Whole Blood. *Environ Sci Technol* **2017**, *51*, 13388–13396, doi:10.1021/acs.est.7b03299.
65. Lucas, K.; Gaines, L.G.T.; Paris-Davila, T.; Nylander-French, L.A. Occupational Exposure and Serum Levels of Per- and Polyfluoroalkyl Substances (PFAS): A Review. *Am J Ind Med* **2023**, *66*, 379–392, doi:10.1002/ajim.23454.
66. Ferber, R.; Noehren, B.; Hamill, J.; Davis, I.S. Competitive Female Runners with a History of Iliotibial Band Syndrome Demonstrate Atypical Hip and Knee Kinematics. *J Orthop Sports Phys Ther* **2010**, *40*, 52–58, doi:10.2519/jospt.2010.3028.
67. Zheng, G.; Eick, S.M.; Salamova, A. Elevated Levels of Ultrashort- and Short-Chain Perfluoroalkyl Acids in US Homes and People. *Environ. Sci. Technol.* **2023**, *57*, 15782–15793, doi:10.1021/acs.est.2c06715.
68. Xu, Y.; Fletcher, T.; Pineda, D.; Lindh, C.H.; Nilsson, C.; Glynn, A.; Vogs, C.; Norström, K.; Lilja, K.; Jakobsson, K.; et al. Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. *Environ Health Perspect* **2020**, *128*, 77004, doi:10.1289/EHP6785.
69. Freberg, B.I.; Haug, L.S.; Olsen, R.; Daae, H.L.; Hersson, M.; Thomsen, C.; Thorud, S.; Becher, G.; Molander, P.; Ellingsen, D.G. Occupational Exposure to Airborne Perfluorinated Compounds during Professional Ski Waxing. *Environ. Sci. Technol.* **2010**, *44*, 7723–7728, doi:10.1021/es102033k.
70. Calafat, A.M.; Kato, K.; Hubbard, K.; Jia, T.; Botelho, J.C.; Wong, L.-Y. Legacy and Alternative Per- and Polyfluoroalkyl Substances in the U.S. General Population: Paired Serum-Urine Data from the 2013-2014 National Health and Nutrition Examination Survey. *Environ Int* **2019**, *131*, 105048, doi:10.1016/j.envint.2019.105048.
71. Halldorsson, T.I.; Rytter, D.; Haug, L.S.; Bech, B.H.; Danielsen, I.; Becher, G.; Henriksen, T.B.; Olsen, S.F. Prenatal Exposure to Perfluorooctanoate and Risk of Overweight at 20 Years of Age: A Prospective Cohort Study. *Environ Health Perspect* **2012**, *120*, 668–673, doi:10.1289/ehp.1104034.
72. Mora, A.M.; Oken, E.; Rifas-Shiman, S.L.; Webster, T.F.; Gillman, M.W.; Calafat, A.M.; Ye, X.; Sagiv, S.K. Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. *Environ Health Perspect* **2017**, *125*, 467–473, doi:10.1289/EHP246.
73. Braun, J.M.; Chen, A.; Romano, M.E.; Calafat, A.M.; Webster, G.M.; Yolton, K.; Lanphear, B.P. Prenatal Perfluoroalkyl Substance Exposure and Child Adiposity at 8 Years of Age: The HOME Study. *Obesity (Silver Spring)* **2016**, *24*, 231–237, doi:10.1002/oby.21258.
74. Lum, K.J.; Sundaram, R.; Barr, D.B.; Louis, T.A.; Buck Louis, G.M. Perfluoroalkyl Chemicals, Menstrual Cycle Length, and Fecundity: Findings from a Prospective Pregnancy Study. *Epidemiology* **2017**, *28*, 90–98, doi:10.1097/EDE.0000000000000552.
75. Zhang, N.; Wang, W.; Li, W.; Liu, C.; Chen, Y.; Yang, Q.; Wang, Y.; Sun, K. Inhibition of 11β-HSD2 Expression by Triclosan via Induction of Apoptosis in Human Placental Syncytiotrophoblasts. *J Clin Endocrinol Metab* **2015**, *100*, E542-549, doi:10.1210/jc.2014-4376.
76. Karnes, C.; Winquist, A.; Steenland, K. Incidence of Type II Diabetes in a Cohort with Substantial Exposure to Perfluorooctanoic Acid. *Environ Res* **2014**, *128*, 78–83, doi:10.1016/j.envres.2013.11.003.
77. Seacat, A.M.; Thomford, P.J.; Hansen, K.J.; Olsen, G.W.; Case, M.T.; Butenhoff, J.L. Subchronic Toxicity Studies on Perfluorooctanesulfonate Potassium Salt in Cynomolgus Monkeys. *Toxicol Sci* **2002**, *68*, 249–264, doi:10.1093/toxsci/68.1.249.
78. Elcombe, C.R.; Elcombe, B.M.; Foster, J.R.; Chang, S.-C.; Ehresman, D.J.; Butenhoff, J.L. Hepatocellular Hypertrophy and Cell Proliferation in Sprague-Dawley Rats from Dietary Exposure to Potassium

 doi:10.20944/preprints202605.0967.v1

Perfluorooctanesulfonate Results from Increased Expression of Xenosensor Nuclear Receptors PPARα and CAR/PXR. *Toxicology* **2012**, *293*, 16–29, doi:10.1016/j.tox.2011.12.014.

79. Qazi, M.R.; Hassan, M.; Nelson, B.D.; DePierre, J.W.; Abedi-Valugerdi, M. Both Sub-Acute, Moderate-Dose and Short-Term, Low-Dose Dietary Exposure of Mice to Perfluorooctane Sulfonate Exacerbates Concanavalin A-Induced Hepatitis. *Toxicol Lett* **2013**, *217*, 67–74, doi:10.1016/j.toxlet.2012.12.001.
80. Amstutz, V.H.; Cengo, A.; Gehres, F.; Sijm, D.T.H.M.; Vrolijk, M.F. Investigating the Cytotoxicity of Per- and Polyfluoroalkyl Substances in HepG2 Cells: A Structure-Activity Relationship Approach. *Toxicology* **2022**, *480*, 153312, doi:10.1016/j.tox.2022.153312.
81. Das, K.P.; Wood, C.R.; Lin, M.T.; Starkov, A.A.; Lau, C.; Wallace, K.B.; Corton, J.C.; Abbott, B.D. Perfluoroalkyl Acids-Induced Liver Steatosis: Effects on Genes Controlling Lipid Homeostasis. *Toxicology* **2017**, *378*, 37–52, doi:10.1016/j.tox.2016.12.007.
82. Chengelis, C.P.; Kirkpatrick, J.B.; Radovsky, A.; Shinohara, M. A 90-Day Repeated Dose Oral (Gavage) Toxicity Study of Perfluorohexanoic Acid (PFHxA) in Rats (with Functional Observational Battery and Motor Activity Determinations). *Reprod Toxicol* **2009**, *27*, 342–351, doi:10.1016/j.reprotox.2009.01.006.
83. Mahapatra, C.T.; Damayanti, N.P.; Guffey, S.C.; Serafin, J.S.; Irudayaraj, J.; Sepúlveda, M.S. Comparative in Vitro Toxicity Assessment of Perfluorinated Carboxylic Acids. *J Appl Toxicol* **2017**, *37*, 699–708, doi:10.1002/jat.3418.
84. Liu, H.; Sun, W.; Zhou, Y.; Griffin, N.; Faulkner, S.; Wang, L. iTRAQ-Based Quantitative Proteomics Analysis of Sprague-Dawley Rats Liver Reveals Perfluorooctanoic Acid-Induced Lipid Metabolism and Urea Cycle Dysfunction. *Toxicol Lett* **2022**, *357*, 20–32, doi:10.1016/j.toxlet.2021.12.016.
85. Foreman, J.E.; Chang, S.-C.; Ehresman, D.J.; Butenhoff, J.L.; Anderson, C.R.; Palkar, P.S.; Kang, B.-H.; Gonzalez, F.J.; Peters, J.M. Differential Hepatic Effects of Perfluorobutyrate Mediated by Mouse and Human PPAR-Alpha. *Toxicol Sci* **2009**, *110*, 204–211, doi:10.1093/toxsci/kfp077.
86. Ikeda, T.; Aiba, K.; Fukuda, K.; Tanaka, M. The Induction of Peroxisome Proliferation in Rat Liver by Perfluorinated Fatty Acids, Metabolically Inert Derivatives of Fatty Acids. *J Biochem* **1985**, *98*, 475–482, doi:10.1093/oxfordjournals.jbchem.a135302.
87. Li, D.; Zhang, L.; Zhang, Y.; Guan, S.; Gong, X.; Wang, X. Maternal Exposure to Perfluorooctanoic Acid (PFOA) Causes Liver Toxicity through PPAR-α Pathway and Lowered Histone Acetylation in Female Offspring Mice. *Environ Sci Pollut Res Int* **2019**, *26*, 18866–18875, doi:10.1007/s11356-019-05258-z.
88. Robarts, D.R.; Paine-Cabrera, D.; Kotulkar, M.; Venneman, K.K.; Gunewardena, S.; Foquet, L.; Bial, G.; Apte, U. Identifying Novel Mechanisms of Per- and Polyfluoroalkyl Substance-Induced Hepatotoxicity Using FRG Humanized Mice. *Arch Toxicol* **2024**, *98*, 3063–3075, doi:10.1007/s00204-024-03789-0.
89. Wan, H.T.; Zhao, Y.G.; Wei, X.; Hui, K.Y.; Giesy, J.P.; Wong, C.K.C. PFOS-Induced Hepatic Steatosis, the Mechanistic Actions on β-Oxidation and Lipid Transport. *Biochim Biophys Acta* **2012**, *1820*, 1092–1101, doi:10.1016/j.bbagen.2012.03.010.
90. Attema, B.; Janssen, A.W.F.; Rijkers, D.; van Schothorst, E.M.; Hooiveld, G.J.E.J.; Kersten, S. Exposure to Low-Dose Perfluorooctanoic Acid Promotes Hepatic Steatosis and Disrupts the Hepatic Transcriptome in Mice. *Mol Metab* **2022**, *66*, 101602, doi:10.1016/j.molmet.2022.101602.
91. Darbre, P.D. Endocrine Disruptors and Obesity. *Curr Obes Rep* **2017**, *6*, 18–27, doi:10.1007/s13679-017-0240-4.
92. Schlezinger, J.J.; Hyötyläinen, T.; Sinioja, T.; Boston, C.; Puckett, H.; Oliver, J.; Heiger-Bernays, W.; Webster, T.F. Perfluorooctanoic Acid Induces Liver and Serum Dyslipidemia in Humanized PPARα Mice Fed an American Diet. *Toxicol Appl Pharmacol* **2021**, *426*, 115644, doi:10.1016/j.taap.2021.115644.
93. Stoffels, C.B.A.; Angerer, T.B.; Robert, H.; Poupin, N.; Lakhal, L.; Frache, G.; Mercier-Bonin, M.; Audinot, J.-N. Lipidomic Profiling of PFOA-Exposed Mouse Liver by Multi-Modal Mass Spectrometry Analysis. *Anal Chem* **2023**, *95*, 6568–6576, doi:10.1021/acs.analchem.2c05470.
94. Schlezinger, J.J.; Puckett, H.; Oliver, J.; Nielsen, G.; Heiger-Bernays, W.; Webster, T.F. Perfluorooctanoic Acid Activates Multiple Nuclear Receptor Pathways and Skews Expression of Genes Regulating Cholesterol Homeostasis in Liver of Humanized PPARα Mice Fed an American Diet. *Toxicol Appl Pharmacol* **2020**, *405*, 115204, doi:10.1016/j.taap.2020.115204.

 doi:10.20944/preprints202605.0967.v1

95. Jiang, L.; Hong, Y.; Xie, G.; Zhang, J.; Zhang, H.; Cai, Z. Comprehensive Multi-Omics Approaches Reveal the Hepatotoxic Mechanism of Perfluorohexanoic Acid (PFHxA) in Mice. *Sci Total Environ* **2021**, *790*, 148160, doi:10.1016/j.scitotenv.2021.148160.
96. Abdellatif, A.; Al-Tonsy, A.H.; Awad, M.E.; Roberfroid, M.; Khan, M.N.U. Peroxisomal Enzymes and 8-Hydroxydeoxyguanosine in Rat Liver Treated with Perfluorooctanoic Acid. *Dis Markers* **2003**, *19*, 19–25, doi:10.1155/2003/135859.
97. Trosko, J.E.; Upham, B.L. The Emperor Wears No Clothes in the Field of Carcinogen Risk Assessment: Ignored Concepts in Cancer Risk Assessment. *Mutagenesis* **2005**, *20*, 81–92, doi:10.1093/mutage/gei017.
98. Upham, B.L.; Park, J.-S.; Babica, P.; Sovadinova, I.; Rummel, A.M.; Trosko, J.E.; Hirose, A.; Hasegawa, R.; Kanno, J.; Sai, K. Structure-Activity-Dependent Regulation of Cell Communication by Perfluorinated Fatty Acids Using in Vivo and in Vitro Model Systems. *Environ Health Perspect* **2009**, *117*, 545–551, doi:10.1289/ehp.11728.
99. Meng, X.; Li, S.; Li, Y.; Gan, R.-Y.; Li, H.-B. Gut Microbiota's Relationship with Liver Disease and Role in Hepatoprotection by Dietary Natural Products and Probiotics. *Nutrients* **2018**, *10*, 1457, doi:10.3390/nu10101457.
100. Choi, J.; Kim, J.-Y.; Lee, H.-J. Human Evidence of Perfluorooctanoic Acid (PFOA) Exposure on Hepatic Disease: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* **2022**, *19*, 11318, doi:10.3390/ijerph191811318.
101. Sen, P.; Qadri, S.; Luukkonen, P.K.; Ragnarsdottir, O.; McGlinchey, A.; Jäntti, S.; Juuti, A.; Arola, J.; Schlezinger, J.J.; Webster, T.F.; et al. Exposure to Environmental Contaminants Is Associated with Altered Hepatic Lipid Metabolism in Non-Alcoholic Fatty Liver Disease. *Journal of Hepatology* **2022**, *76*, 283–293, doi:10.1016/j.jhep.2021.09.039.
102. Jain, R.B. Concentration of Selected Liver Enzymes across the Stages of Glomerular Function: The Associations with PFOA and PFOS. *Heliyon* **2019**, *5*, e02168, doi:10.1016/j.heliyon.2019.e02168.
103. Jin, R.; McConnell, R.; Catherine, C.; Xu, S.; Walker, D.I.; Stratakis, N.; Jones, D.P.; Miller, G.W.; Peng, C.; Conti, D.V.; et al. Perfluoroalkyl Substances and Severity of Nonalcoholic Fatty Liver in Children: An Untargeted Metabolomics Approach. *Environ Int* **2020**, *134*, 105220, doi:10.1016/j.envint.2019.105220.
104. Stratakis, N.; V Conti, D.; Jin, R.; Margetaki, K.; Valvi, D.; Siskos, A.P.; Maitre, L.; Garcia, E.; Varo, N.; Zhao, Y.; et al. Prenatal Exposure to Perfluoroalkyl Substances Associated With Increased Susceptibility to Liver Injury in Children. *Hepatology* **2020**, *72*, 1758–1770, doi:10.1002/hep.31483.
105. Lu, Y.; Gao, K.; Li, X.; Tang, Z.; Xiang, L.; Zhao, H.; Fu, J.; Wang, L.; Zhu, N.; Cai, Z.; et al. Mass Spectrometry-Based Metabolomics Reveals Occupational Exposure to Per- and Polyfluoroalkyl Substances Relates to Oxidative Stress, Fatty Acid β-Oxidation Disorder, and Kidney Injury in a Manufactory in China. *Environ Sci Technol* **2019**, *53*, 9800–9809, doi:10.1021/acs.est.9b01608.
106. World Health Organization *Global Assessment of the State-of-the-Science of Endocrine Disruptors*; International Programme on Chemical Safety: Geneva, 2002;
107. Borghoff, S.J.; Fitch, S.; Rager, J.E.; Huggett, D. A Hypothesis-Driven Weight-of-Evidence Analysis to Evaluate Potential Endocrine Activity of Perfluorohexanoic Acid. *Regul Toxicol Pharmacol* **2018**, *99*, 168–181, doi:10.1016/j.yrtph.2018.09.001.
108. Razvi, S.; Jabbar, A.; Pingitore, A.; Danzi, S.; Biondi, B.; Klein, I.; Peeters, R.; Zaman, A.; Iervasi, G. Thyroid Hormones and Cardiovascular Function and Diseases. *J Am Coll Cardiol* **2018**, *71*, 1781–1796, doi:10.1016/j.jacc.2018.02.045.
109. Chemical Evaluation and Research Institute (CERI) *Fish Short Term Reproduction Assay of Perfluorohexanoic Acid, Ammonium Salt or Sodium Salt in Medaka*; Chemical Evaluation and Research Institute (CERI), 2015;
110. Wasel, O.; Thompson, K.M.; Gao, Y.; Godfrey, A.E.; Gao, J.; Mahapatra, C.T.; Lee, L.S.; Sepúlveda, M.S.; Freeman, J.L. Comparison of Zebrafish in Vitro and in Vivo Developmental Toxicity Assessments of Perfluoroalkyl Acids (PFAAs). *J Toxicol Environ Health A* **2021**, *84*, 125–136, doi:10.1080/15287394.2020.1842272.
111. Kranthi Kumar, K.; Uma Devi, B.; Neeraja, P. Integration of in Silico Approaches to Determination of Endocrine-Disrupting Perfluorinated Chemicals Binding Potency with Steroidogenic Acute Regulatory Protein. *Biochem Biophys Res Commun* **2017**, *491*, 1007–1014, doi:10.1016/j.bbrc.2017.07.168.

 doi:10.20944/preprints202605.0967.v1

112. Qiu, Z.; Qu, K.; Luan, F.; Liu, Y.; Zhu, Y.; Yuan, Y.; Li, H.; Zhang, H.; Hai, Y.; Zhao, C. Binding Specificities of Estrogen Receptor with Perfluorinated Compounds: A Cross Species Comparison. *Environment International* **2020**, *134*, 105284, doi:10.1016/j.envint.2019.105284.
113. Zhang, H.; Lu, Y.; Luo, B.; Yan, S.; Guo, X.; Dai, J. Proteomic Analysis of Mouse Testis Reveals Perfluorooctanoic Acid-Induced Reproductive Dysfunction via Direct Disturbance of Testicular Steroidogenic Machinery. *J Proteome Res* **2014**, *13*, 3370–3385, doi:10.1021/pr500228d.
114. White, S.S.; Stanko, J.P.; Kato, K.; Calafat, A.M.; Hines, E.P.; Fenton, S.E. Gestational and Chronic Low-Dose PFOA Exposures and Mammary Gland Growth and Differentiation in Three Generations of CD-1 Mice. *Environ Health Perspect* **2011**, *119*, 1070–1076, doi:10.1289/ehp.1002741.
115. Zhao, Y.; Tan, Y.S.; Strynar, M.J.; Perez, G.; Haslam, S.Z.; Yang, C. Perfluorooctanoic Acid Effects on Ovaries Mediate Its Inhibition of Peripubertal Mammary Gland Development in Balb/c and C57Bl/6 Mice. *Reproductive Toxicology* **2012**, *33*, 563–576, doi:10.1016/j.reprotox.2012.02.004.
116. Jain, R.B. Association between Thyroid Profile and Perfluoroalkyl Acids: Data from NHNAES 2007-2008. *Environ Res* **2013**, *126*, 51–59, doi:10.1016/j.envres.2013.08.006.
117. Melzer, D.; Rice, N.; Depledge, M.H.; Henley, W.E.; Galloway, T.S. Association between Serum Perfluorooctanoic Acid (PFOA) and Thyroid Disease in the U.S. National Health and Nutrition Examination Survey. *Environ Health Perspect* **2010**, *118*, 686–692, doi:10.1289/ehp.0901584.
118. Tan, K.; Zhang, Q.; Wang, Y.; Wang, C.; Hu, C.; Wang, L.; Liu, H.; Tian, Z. Associations between Per- and Polyfluoroalkyl Substances Exposure and Thyroid Hormone Levels in the Elderly. *Sci Total Environ* **2024**, *920*, 170761, doi:10.1016/j.scitotenv.2024.170761.
119. Kim, D.-H.; Kim, U.-J.; Kim, H.-Y.; Choi, S.-D.; Oh, J.-E. Perfluoroalkyl Substances in Serum from South Korean Infants with Congenital Hypothyroidism and Healthy Infants--Its Relationship with Thyroid Hormones. *Environ Res* **2016**, *147*, 399–404, doi:10.1016/j.envres.2016.02.037.
120. Liu, H.; Pan, Y.; Jin, S.; Li, Y.; Zhao, L.; Sun, X.; Cui, Q.; Zhang, B.; Zheng, T.; Xia, W.; et al. Associations of Per-/Polyfluoroalkyl Substances with Glucocorticoids and Progestogens in Newborns. *Environ Int* **2020**, *140*, 105636, doi:10.1016/j.envint.2020.105636.
121. Liu, H.; Pan, Y.; Jin, S.; Sun, X.; Jiang, Y.; Wang, Y.; Ghassabian, A.; Li, Y.; Xia, W.; Cui, Q.; et al. Associations between Six Common Per- and Polyfluoroalkyl Substances and Estrogens in Neonates of China. *Journal of Hazardous Materials* **2021**, *407*, 124378, doi:10.1016/j.jhazmat.2020.124378.
122. Ballesteros, V.; Costa, O.; Iñiguez, C.; Fletcher, T.; Ballester, F.; Lopez-Espinosa, M.-J. Exposure to Perfluoroalkyl Substances and Thyroid Function in Pregnant Women and Children: A Systematic Review of Epidemiologic Studies. *Environ Int* **2017**, *99*, 15–28, doi:10.1016/j.envint.2016.10.015.
123. Kaczmarska, I.; Mokra, K.; Michałowicz, J. Perfluorooctanoic Acid and Its Short-Chain Substitutes Induce Cytotoxic and Prooxidative Changes in Human Peripheral Blood Mononuclear Cells: A Comparative Study. *Int J Mol Sci* **2025**, *26*, 5408, doi:10.3390/ijms26115408.
124. Agency for Toxic Substances and Disease Registry *Toxicological Profile for Perfluoroalkyls*; Toxicological Profiles; Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2021;
125. De Guise, S.; Levin, M. Suppression of Th2 Cytokines as a Potential Mechanism for Reduced Antibody Response Following PFOA Exposure in Female B6C3F1 Mice. *Toxicol Lett* **2021**, *351*, 155–162, doi:10.1016/j.toxlet.2021.09.002.
126. Dewitt, J.C.; Copeland, C.B.; Strynar, M.J.; Luebke, R.W. Perfluorooctanoic Acid-Induced Immunomodulation in Adult C57BL/6J or C57BL/6N Female Mice. *Environ Health Perspect* **2008**, *116*, 644–650, doi:10.1289/ehp.10896.
127. Loveless, S.E.; Hoban, D.; Sykes, G.; Frame, S.R.; Everds, N.E. Evaluation of the Immune System in Rats and Mice Administered Linear Ammonium Perfluorooctanoate. *Toxicol Sci* **2008**, *105*, 86–96, doi:10.1093/toxsci/kfn113.
128. Iwai, H.; Yamashita, K. A Fourteen-Day Repeated Dose Oral Toxicity Study of APFO in Rats. *Drug Chem Toxicol* **2006**, *29*, 323–332, doi:10.1080/01480540600653143.
129. Zhou, Y.; Hu, L.-W.; Qian, Z.; Geiger, S.D.; Parrish, K.L.; Dharmage, S.C.; Campbell, B.; Roponen, M.; Jalava, P.; Hirvonen, M.-R.; et al. Interaction Effects of Polyfluoroalkyl Substances and Sex Steroid Hormones on Asthma among Children. *Sci Rep* **2017**, *7*, 899, doi:10.1038/s41598-017-01140-5.

 doi:10.20944/preprints202605.0967.v1

130. Anderson-Mahoney, P.; Kotlerman, J.; Takhar, H.; Gray, D.; Dahlgren, J. Self-Reported Health Effects among Community Residents Exposed to Perfluorooctanoate. *New Solut* **2008**, *18*, 129–143, doi:10.2190/NS.18.2.d.
131. Dong, G.-H.; Tung, K.-Y.; Tsai, C.-H.; Liu, M.-M.; Wang, D.; Liu, W.; Jin, Y.-H.; Hsieh, W.-S.; Lee, Y.L.; Chen, P.-C. Serum Polyfluoroalkyl Concentrations, Asthma Outcomes, and Immunological Markers in a Case-Control Study of Taiwanese Children. *Environ Health Perspect* **2013**, *121*, 507–513, doi:10.1289/ehp.1205351.
132. Chang, E.T.; Adami, H.-O.; Boffetta, P.; Wedner, H.J.; Mandel, J.S. A Critical Review of Perfluorooctanoate and Perfluorooctanesulfonate Exposure and Immunological Health Conditions in Humans. *Crit Rev Toxicol* **2016**, *46*, 279–331, doi:10.3109/10408444.2015.1122573.
133. Lopez-Espinosa, M.-J.; Carrizosa, C.; Luster, M.I.; Margolick, J.B.; Costa, O.; Leonardi, G.S.; Fletcher, T. Perfluoroalkyl Substances and Immune Cell Counts in Adults from the Mid-Ohio Valley (USA). *Environ Int* **2021**, *156*, 106599, doi:10.1016/j.envint.2021.106599.
134. Innes, K.E.; Ducatman, A.M.; Luster, M.I.; Shankar, A. Association of Osteoarthritis with Serum Levels of the Environmental Contaminants Perfluorooctanoate and Perfluorooctane Sulfonate in a Large Appalachian Population. *Am J Epidemiol* **2011**, *174*, 440–450, doi:10.1093/aje/kwr107.
135. Steenland, K.; Zhao, L.; Winquist, A. A Cohort Incidence Study of Workers Exposed to Perfluorooctanoic Acid (PFOA). *Occup Environ Med* **2015**, *72*, 373–380, doi:10.1136/oemed-2014-102364.
136. Granum, B.; Haug, L.S.; Namork, E.; Stølevik, S.B.; Thomsen, C.; Aaberge, I.S.; van Loveren, H.; Løvik, M.; Nygaard, U.C. Pre-Natal Exposure to Perfluoroalkyl Substances May Be Associated with Altered Vaccine Antibody Levels and Immune-Related Health Outcomes in Early Childhood. *J Immunotoxicol* **2013**, *10*, 373–379, doi:10.3109/1547691X.2012.755580.
137. Knudsen, A.-K.S.; Long, M.; Pedersen, H.S.; Bonefeld-Jørgensen, E.C. Persistent Organic Pollutants and Haematological Markers in Greenlandic Pregnant Women: The ACCEPT Sub-Study. *Int J Circumpolar Health* **2018**, *77*, 1456303, doi:10.1080/22423982.2018.1456303.
138. Impinen, A.; Longnecker, M.P.; Nygaard, U.C.; London, S.J.; Ferguson, K.K.; Haug, L.S.; Granum, B. Maternal Levels of Perfluoroalkyl Substances (PFASs) during Pregnancy and Childhood Allergy and Asthma Related Outcomes and Infections in the Norwegian Mother and Child (MoBa) Cohort. *Environ Int* **2019**, *124*, 462–472, doi:10.1016/j.envint.2018.12.041.
139. Goudarzi, H.; Miyashita, C.; Okada, E.; Kashino, I.; Kobayashi, S.; Chen, C.-J.; Ito, S.; Araki, A.; Matsuura, H.; Ito, Y.M.; et al. Effects of Prenatal Exposure to Perfluoroalkyl Acids on Prevalence Ofallergic Diseases among 4-Year-Old Children. *Environ Int* **2016**, *94*, 124–132, doi:10.1016/j.envint.2016.05.020.
140. Abraham, K.; El-Khatib, A.H.; Schwerdtle, T.; Monien, B.H. Perfluorobutanoic Acid (PFBA): No High-Level Accumulation in Human Lung and Kidney Tissue. *Int J Hyg Environ Health* **2021**, *237*, 113830, doi:10.1016/j.ijheh.2021.113830.
141. Mogensen, U.B.; Grandjean, P.; Heilmann, C.; Nielsen, F.; Weihe, P.; Budtz-Jørgensen, E. Structural Equation Modeling of Immunotoxicity Associated with Exposure to Perfluorinated Alkylates. *Environ Health* **2015**, *14*, 47, doi:10.1186/s12940-015-0032-9.
142. Looker, C.; Luster, M.I.; Calafat, A.M.; Johnson, V.J.; Burleson, G.R.; Burleson, F.G.; Fletcher, T. Influenza Vaccine Response in Adults Exposed to Perfluorooctanoate and Perfluorooctanesulfonate. *Toxicol Sci* **2014**, *138*, 76–88, doi:10.1093/toxsci/kft269.
143. Grandjean, P.; Timmermann, C.A.G.; Kruse, M.; Nielsen, F.; Vinholt, P.J.; Boding, L.; Heilmann, C.; Mølbak, K. Severity of COVID-19 at Elevated Exposure to Perfluorinated Alkylates. *PLoS One* **2020**, *15*, e0244815, doi:10.1371/journal.pone.0244815.
144. European Food Safety Authority *Risk to Human Health Related to the Presence of Perfluoroalkyl Substances in Food*; EFSA Panel on Contaminants in the Food Chain (CONTAM): Parma, Italy, 2020;
145. Farsetti, A.; Illi, B.; Gaetano, C. How Epigenetics Impacts on Human Diseases. *Eur J Intern Med* **2023**, *114*, 15–22, doi:10.1016/j.ejim.2023.05.036.
146. Liu, W.; Irudayaraj, J. Perfluorooctanoic Acid (PFOA) Exposure Inhibits DNA Methyltransferase Activities and Alters Constitutive Heterochromatin Organization. *Food Chem Toxicol* **2020**, *141*, 111358, doi:10.1016/j.fct.2020.111358.

 doi:10.20944/preprints202605.0967.v1

147. Ahmad, S.; Wen, Y.; Irudayaraj, J.M.K. PFOA Induces Alteration in DNA Methylation Regulators and SARS-CoV-2 Targets Ace2 and Tmprss2 in Mouse Lung Tissues. *Toxicol Rep* **2021**, *8*, 1892–1898, doi:10.1016/j.toxrep.2021.11.014.
148. Wen, Y.; Chen, J.; Li, J.; Arif, W.; Kalsotra, A.; Irudayaraj, J. Effect of PFOA on DNA Methylation and Alternative Splicing in Mouse Liver. *Toxicol Lett* **2020**, *329*, 38–46, doi:10.1016/j.toxlet.2020.04.012.
149. Grandjean, P.; Timmermann, C.A.G.; Kruse, M.; Nielsen, F.; Vinholt, P.J.; Boding, L.; Heilmann, C.; Mølbak, K. Severity of COVID-19 at Elevated Exposure to Perfluorinated Alkylates 2020.
150. O'Brien, M.L.; Spear, B.T.; Glauert, H.P. Role of Oxidative Stress in Peroxisome Proliferator-Mediated Carcinogenesis. *Crit Rev Toxicol* **2005**, *35*, 61–88, doi:10.1080/10408440590905957.
151. Temkin, A.M.; Hocevar, B.A.; Andrews, D.Q.; Naidenko, O.V.; Kamendulis, L.M. Application of the Key Characteristics of Carcinogens to Per and Polyfluoroalkyl Substances. *Int J Environ Res Public Health* **2020**, *17*, 1668, doi:10.3390/ijerph17051668.
152. Eriksen, K.T.; Raaschou-Nielsen, O.; Sørensen, M.; Roursgaard, M.; Loft, S.; Møller, P. Genotoxic Potential of the Perfluorinated Chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in Human HepG2 Cells. *Mutat Res* **2010**, *700*, 39–43, doi:10.1016/j.mrgentox.2010.04.024.
153. Crebelli, R.; Caiola, S.; Conti, L.; Cordelli, E.; De Luca, G.; Dellatte, E.; Eleuteri, P.; Iacovella, N.; Leopardi, P.; Marcon, F.; et al. Can Sustained Exposure to PFAS Trigger a Genotoxic Response? A Comprehensive Genotoxicity Assessment in Mice after Subacute Oral Administration of PFOA and PFBA. *Regul Toxicol Pharmacol* **2019**, *106*, 169–177, doi:10.1016/j.yrtph.2019.05.005.
154. Li, L.-Y.; Guan, Y.; Chen, X.-S.; Yang, J.-M.; Cheng, Y. DNA Repair Pathways in Cancer Therapy and Resistance. *Front Pharmacol* **2020**, *11*, 629266, doi:10.3389/fphar.2020.629266.
155. Qu, Y.; Sheng, N.; Ji, S.; Li, Z.; Wang, J.; Pan, Y.; Hu, X.; Zheng, X.; Li, Y.; Song, H.; et al. Dietary Seafood as a Potential Modifier in the Relationship between Per- and Polyfluoroalkyl Substances (PFASs) Burden and Prediabetes/Diabetes: Insights from a Nationally Representative Cross-Sectional Study. *Journal of Hazardous Materials* **2024**, *473*, 134645, doi:10.1016/j.jhazmat.2024.134645.
156. IARC Working Group on the Identification of Carcinogenic Hazards to Humans *Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonic Acid (PFOS)*; IARC Monographs on the Identification of Carcinogenic Hazards to Humans; International Agency for Research on Cancer: Lyon (FR), 2025; ISBN 978-92-832-4532-2.
157. Pierozan, P.; Jerneren, F.; Karlsson, O. Perfluorooctanoic Acid (PFOA) Exposure Promotes Proliferation, Migration and Invasion Potential in Human Breast Epithelial Cells. *Arch Toxicol* **2018**, *92*, 1729–1739, doi:10.1007/s00204-018-2181-4.
158. Bartell, S.M.; Vieira, V.M. Critical Review on PFOA, Kidney Cancer, and Testicular Cancer. *J Air Waste Manag Assoc* **2021**, *71*, 663–679, doi:10.1080/10962247.2021.1909668.
159. Barry, V.; Winquist, A.; Steenland, K. Perfluorooctanoic Acid (PFOA) Exposures and Incident Cancers among Adults Living near a Chemical Plant. *Environ Health Perspect* **2013**, *121*, 1313–1318, doi:10.1289/ehp.1306615.
160. Girardi, P.; Merler, E. A Mortality Study on Male Subjects Exposed to Polyfluoroalkyl Acids with High Internal Dose of Perfluorooctanoic Acid. *Environ Res* **2019**, *179*, 108743, doi:10.1016/j.envres.2019.108743.
161. Chang, V.C.; Rhee, J.; Berndt, S.I.; Moore, S.C.; Freedman, N.D.; Jones, R.R.; Silverman, D.T.; Gierach, G.L.; Hofmann, J.N.; Purdue, M.P. Serum Perfluorooctane Sulfonate and Perfluorooctanoate and Risk of Postmenopausal Breast Cancer According to Hormone Receptor Status: An Analysis in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. *Int J Cancer* **2023**, *153*, 775–782, doi:10.1002/ijc.34487.
162. Zahm, S.; Bonde, J.P.; Chiu, W.A.; Hoppin, J.; Kanno, J.; Abdallah, M.; Blystone, C.R.; Calkins, M.M.; Dong, G.-H.; Dorman, D.C.; et al. Carcinogenicity of Perfluorooctanoic Acid and Perfluorooctanesulfonic Acid. *Lancet Oncol* **2024**, *25*, 16–17, doi:10.1016/S1470-2045(23)00622-8.
163. Davis, J.A.; Taylor, M.M.; Kraft, A.; Lambert, J.C.; Radke, E.; Schlosser, P.; Angrish, M.; Arzuaga, X.; Congleton, J.; Druwe, I.; et al. *IRIS Toxicological Review of Perfluorobutanoic Acid (PFBA, CASRN 375-22-4) and Related Salts*; EPA IRIS Assessments; U.S. Environmental Protection Agency: Washington (DC), 2022;

 doi:10.20944/preprints202605.0967.v1

164. Klaunig, J.E.; Shinohara, M.; Iwai, H.; Chengelis, C.P.; Kirkpatrick, J.B.; Wang, Z.; Bruner, R.H. Evaluation of the Chronic Toxicity and Carcinogenicity of Perfluorohexanoic Acid (PFHxA) in Sprague-Dawley Rats. *Toxicol Pathol* **2015**, *43*, 209–220, doi:10.1177/0192623314530532.
165. Janssen, A.W.F.; Jansen Holleboom, W.; Rijkers, D.; Louisse, J.; Hoekstra, S.A.; Schild, S.; Vrolijk, M.F.; Hoogenboom, R.L.A.P.; Beekmann, K. Determination of in Vitro Immunotoxic Potencies of a Series of Perfluoralkylsubstances (PFASs) in Human Namalwa B Lymphocyte and Human Jurkat T Lymphocyte Cells. *Front Toxicol* **2024**, *6*, 1347965, doi:10.3389/ftox.2024.1347965.
166. Shearer, J.J.; Callahan, C.L.; Calafat, A.M.; Huang, W.-Y.; Jones, R.R.; Sabbisetti, V.S.; Freedman, N.D.; Sampson, J.N.; Silverman, D.T.; Purdue, M.P.; et al. Serum Concentrations of Per- and Polyfluoroalkyl Substances and Risk of Renal Cell Carcinoma. *J Natl Cancer Inst* **2021**, *113*, 580–587, doi:10.1093/jnci/djaa143.
167. Di Nisio, A.; Rocca, M.S.; De Toni, L.; Sabovic, I.; Guidolin, D.; Dall'Acqua, S.; Acquasaliente, L.; De Filippis, V.; Plebani, M.; Foresta, C. Endocrine Disruption of Vitamin D Activity by Perfluoro-Octanoic Acid (PFOA). *Sci Rep* **2020**, *10*, 16789, doi:10.1038/s41598-020-74026-8.
168. Kabakci, R.; Clark, K.L.; Plewes, M.R.; Monaco, C.F.; Davis, J.S. Perfluorooctanoic Acid (PFOA) Inhibits Steroidogenesis and Mitochondrial Function in Bovine Granulosa Cells in Vitro. *Environ Pollut* **2023**, *338*, 122698, doi:10.1016/j.envpol.2023.122698.
169. Rashid, F.; Ahmad, S.; Irudayaraj, J.M.K. Effect of Perfluorooctanoic Acid on the Epigenetic and Tight Junction Genes of the Mouse Intestine. *Toxics* **2020**, *8*, 64, doi:10.3390/toxics8030064.