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Laboratory assays accepted for biological activity disorder, physiological disability, and tissue damage due to the effect of perflourooctanic acid contamination

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ABSTRACT

Introduction: Polyfluorooctanoic acid (PFOA) is a member of the polyfluoroalkyl substances (PFAS) family that is widely used in various industries due to its unique chemical and physical properties, including high stability in harsh environmental and biological conditions. However, the widespread distribution of PFOA in nature, such as in air, water, food, and farms, has raised concerns about its potential health effects on humans and other organisms. PFOA has been linked to various diseases and organ dysfunction, primarily attributed to its detrimental impact on essential cellular components such as DNA, RNA, and epigenetic factors, which are critical for proper cell function and development. To better understand the dosage levels and exposure durations that can lead to adverse effects, laboratory experiments, as well as in vivo and in vitro studies, are being conducted to ensure that PFOA does not pose any negative impacts on the environment, human health, and organs. Furthermore, efforts are being made to address this issue by exploring alternative chemical materials or degradation methods to mitigate the presence of PFOA. Therefore, the aim of this research is to gather information from various articles that investigate the doses and mechanisms of PFOA's negative impact on health and organ function. This compilation of information will serve as a valuable resource for researchers studying PFOA and provide a comprehensive reference for those seeking general knowledge on PFOA-related topics in the context of health diseases and the medical field.

Keywords: Radiopharmaceuticals, Nuclear Medicine, Neutron Activation, Particle Accelerators.

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1. Introduction

Polyfluorooctanoic acid (PFOA) is a synthetic chemical compound (1, 2) with the molecular formula C8HF15O2. Its molecular structure, depicted in Figure 1, demonstrates modified atom interactions. PFOA exhibits notable chemical properties, particularly in terms of the stable binding between fluorine and carbon atoms. Recent research has focused on degradation techniques and methods for PFOA in various systems and conditions, including catalysis and pH manipulation (1).

Among the degradation methods explored, photo-oxidation has been employed to degrade PFOA. However, it has been found that complete removal and decomposition of PFOA from water through the photochemical process is challenging and inefficient. This is primarily due to the high energy required to break the strong carbon-fluorine (C-F) bond, which is approximately 484 kJ/mol. Consequently, in biological settings such as metabolism (where PFOA shares structural similarities with fatty acids), the enzymes responsible for breakdown, such as ACOT1 acyl-COA esterases, are unable to efficiently decompose the compound present in mitochondria, peroxisomes, and the cytoplasm of various organs (3). Chemical conditions such as high temperature and exposure to light are necessary for the degradation of this strong bond and compound (2).

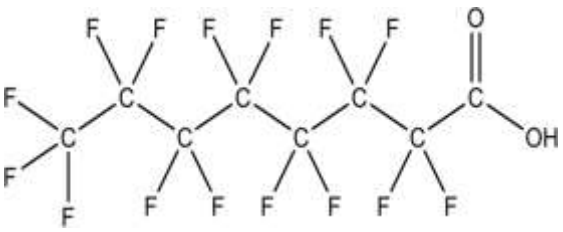

Figure 1: The chemical structure of PFOA (1).

The PFOA and its related chemical compounds have been extensively utilized in various industries (4) such as furniture, household goods, food packaging, firefighting, cleaning products, fireproofing foam (5), automotive, chemical, fabric, leather, paper manufacturing, and cosmetic production since the 1950s (1). Additionally, these compounds are used in fishing equipment to make materials waterproof, oil-proof, and stain-proof, which is why they are also found in garments, carpets, and fabrics. Perfluorooctanoic acid, a chemical compound, has been found in the air, soil, plants, and even in the serum of humans worldwide (2). PFOA is considered a persistent, bioaccumulative, and toxic (PBT) substance (6) due to its unique chemical properties such as hydrophobic and lipophobic nature, as well as its high natural stability (7, 8). It is commonly used in production facilities like Teflon and Scotchgard (9). Studies have shown that the global population is exposed to PFOA on a

daily basis, with concentrations ranging from 0.3 to 150 ng/ml (10). PFOA has the potential to contaminate drinking water (11), making it a significant concern. Various methods such as chromatography, mass spectrometry, and chromatography-mass spectrometry are employed to confirm this claim (1). In terms of food (12), greenhouse experiments have demonstrated contamination in plants like rice and wheat under (13) flooded and non-flooded conditions using high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-GFMAS) instruments (14). Research has also shown that PFOA (15) is present in both drinking and non-drinking water sources, including surface water and groundwater (2).

The notable aspects of PFOA are its inherent stability and persistence in natural and biological environments (1). Recent scientific research has highlighted the significant role of PFOA and PFAS materials in the pollution and toxicity that contribute to various health conditions affecting different organs. Specifically, PFOA has been linked to neurological toxicity and hyperoxidative stress (16-19), as well as immune toxicity (20-22) leading to decreased immunity. Carcinogenic effects have been observed in organs such as the pancreas, prostate, breast, immune system, and kidneys (4, 23, 24). PFOA has also been associated with proximal proliferation, oxidative stress, alterations in membrane potential, genetic expression mutations contributing to genetic disorders, and impacts on epigenetic and DNA methylation processes (26). Furthermore, its interaction with serum albumin (27) has been identified, along with effects on hepatocellular hypertrophy and lipid metabolism (25, 28). The carcinogenic nature of PFOA in humans has been acknowledged by the International Research Agency. PFOA contamination is prevalent in air dust and natural environments (29).

The incidence of PFOA contamination is more prevalent in populations residing near industrial areas, as evidenced by research conducted in Japan (12, 30, 31) and the United States (32, 33). In the United States, serum analysis statistics have revealed that 98% of the population is contaminated with PFOA. An important consideration regarding PFOA is its extended excretion duration from the body, which is dependent on the position of PFOA and is characterized by a long half-life of approximately 17-19 days (34). Elimination from the body is a time-consuming process and varies among studies, with estimates ranging from approximately 2.4 years to 3.8 years in humans (26). PFOA undergoes

transformation in the body after ingestion through the gastrointestinal tract (35) and absorption from drinking water. It can also be absorbed through the respiratory system (24) when present in the air, subsequently entering the bloodstream and interacting with serum albumin (27, 36). Fluorescence spectroscopy has demonstrated the interaction of PFOA with hemoglobin proteins in red blood cells (37), but its metabolism is limited or requires specific metabolic conditions within the body (1). Experimental research on rats has shown the presence of PFOA in various bodily locations (38), including the skin, stomach, nervous system, liver, kidney (24), human sperm (4), and blood (3). Previous review articles have described PFOA as having immunotoxic effects, and several articles have addressed the relationship between PFOA and immune toxicity (39).

However, this review article presents contemporary findings from new sources and experiments, with a specific focus on determining the concentration and duration of PFOA exposure in different organs through in vivo and in vitro experiments using various methods and materials.

2. Methods

This article provides a comprehensive review of studies conducted between 2003 and 2023 that examine the impact of PFOA on various organs and tissues, leading to changes in their activities and morphologies. The review includes articles sourced from reputable international journals such as PubMed, International Journal of Environmental Research and Public Health, International Journal of Molecular Sciences, Oxford, Molecular Biology, and Environmental Science and Technology. The analyzed methods employed in these studies yielded descriptive results. The emphasis of this review is on laboratory settings and the experimental methodologies employed, which enable the determination of dosage levels and the duration of effects caused by PFOA.

3. Results

Our findings demonstrate that PFOA exerts detrimental effects on crucial cellular elements such as genes and epigenetic factors, as well as on the physiology of organs and the morphology of tissues. These effects vary depending on factors such as the duration of exposure and the concentrations administered.

3-1. Affect on gene expression

The field of gene expression has gained significant importance in medical research, with scientists striving to uncover the mechanisms underlying cellular activity in various tissues, organs, and diseases, including the brain and different species

(40). Gene expression is influenced by the interplay between DNA, RNA, proteins, and the surrounding environment. In the reviewed article, the changes in gene expression were explored, both cis (through linked polymorphisms) and trans (via diffusible products of other genes), in order to elucidate the regulation of gene expression. Several articles have highlighted the negative impact of PFOA, a chemical substance, on cellular generation and control (41).

These studies have demonstrated the specific effects of PFOA on gene expression levels in various genes and organs. For example, PFOA has been shown to affect follicular growth in the ovaries, induce the unfolded protein response as a response to oxidative stress in genes, cause epigenetic changes in DNA methylation, disrupt hepatic function leading to transcriptional abnormalities in both fetal and maternal contexts, exhibit high activity in hepatic steatosis transcriptome, alter liver size in mice, and influence gene expression in the Hippo pathway. Each of these subjects will be discussed individually in the following sections (10, 14, 25, 26, 34, 42-44).

3-1-1. Effects on epigenetics and DNA methylation

The understanding of gene expression has evolved over time, and a recent article by Holliday proposes a revised definition based on the contributions of various researchers. These researchers include Holliday and Pugh (1975), Riggs (1975), Jones and Taylor (1980), Bird et al. (1985), and Nanney (1958), who have made modifications to the concept of gene expression. Holliday (1994) synthesized the ideas from these groups, and two distinct perspectives emerged. One group believed that gene expression referred to "the study of the changes in gene expression, which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression." The other group defined gene expression as "nuclear inheritance, which is not based on differences in DNA sequence." However, according to the findings of the article, both definitions were deemed insufficient (45).

In a more recent study, Wu and Morris (2001) further refined the definition proposed by Holliday. They suggested that gene expression should be understood as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail changes in DNA sequence." This updated definition takes into account the heritability of gene function and emphasizes that changes in gene expression can occur without alterations in the DNA sequence (45).

In a different article, the modification of epigenetics revealed the involvement of DNA methylation in gene expression. However, the focus of the present study is to investigate the damage and disruption of gene expression caused by PFOA, a specific chemical compound used in production. DNA methylation involves the addition of a methyl group (-CH3) to the C-5 position of the cysteine ring, predominantly occurring in the context of cysteine-guanine dinucleotide (CpG) sites (7).

In recent research, three groups of mice were examined, specifically targeting lung tissues, to determine the impact of PFOA on mRNA activity and the regulation of DNA methylation in the gut, liver, and kidneys. However, no significant positive results were found to indicate the influence of PFOA exposure on changes in physiological function and tissue morphology. Another aspect of the study aimed to investigate the potential negative effects of PFOA on epigenetic components, such as DNMTs, TETs, and histone deacetylase enzymes, which play regulatory roles in epigenetic processes. The analysis of assays was conducted using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) (26).

Furthermore, this article provides insights into the dosage concentration and duration of PFOA exposure in CD-1 mice. PFOA, with a purity of 96%, was administered at two different concentrations (5-20 mg/kg/day) over a period of 10 days. The study also involved the isolation of three isoforms of DNMTs enzymes, which are known to be involved in epigenetic regulation through DNA methylation. However, the results did not indicate any significant impact on the enhancement of epigenetic activity in the lungs associated with high doses of PFOA exposure (26).

Furthermore, a cohort statistical analysis research study focused on pregnant women residing in the Cincinnati, Ohio, metropolitan area. A total of 199 participants were included for the DNA methylation assay. For this purpose, the Infinium Human Methylation EPIC BeadChip (EPIC array) by Illumina was utilized. The study aimed to investigate the hypermethylation of DNA at CpG sites due to exposure to PFAS and PFOA during pregnancy (3).

The experimental assays involved the collection of cord blood samples (n=266) and peripheral leukocytes at 12 years of age (n=160) to assess the impact of PFOA on epigenetic activities. Serum samples taken at 30.9 weeks of gestation were used for measurement. Gene ontology analysis was

employed to identify molecular pathways associated with the findings. The results of the experiments indicated variations in the number of CpG sites in relation to different PFAS members. A total of 435 cytosine-guanine dinucleotide (CpG) sites were identified, which modified gene regions linked to conditions such as cancers, cognitive health, cardiovascular disease, and kidney function. Furthermore, different numbers of CpG sites were found for different PFAS compounds. Specifically, 12 CpG sites were identified and found to overlap with genes related to PFOA. This article demonstrated a positive association between PFOA and DNA methylation, as evidenced by the modification of CpG sites (3).

3-1-2. The effect of PFOA on the genes of liver and kidney

Acot1, or Acyl-CoA thioesterases, refers to a group of enzymes capable of metabolizing acyl CoA esters associated with non-esterified fatty acids and coenzyme A. The gene encoding this enzyme is expressed in various tissues, including the brain, liver, kidney, adipose tissue, muscle, and testis. In vivo experimental research conducted on rat liver and kidney tissues compared the expression of the Acot1 gene with that in other tissues (3).

The results of the experiment demonstrated that exposure of tissues to PFOA led to an increase in the expression level and transcription of Acot1 proteins. The experimental details involved a 28-day exposure period to PFOA at a dose of 20mg/kg/body weight per day, administered via four daily gavages. PFOA with a purity of 96% was used. Sample collection was conducted at 24, 48, 72, and 96 hours after exposure, with a total of 24 rats divided into two groups (one exposed to PFOA and the other serving as a control) analyzed. The gene activity level showed a sensitive increase in the liver, kidney, muscle, adipose tissue, and testis. Additionally, serum analysis detected the presence of PFOA. However, a decrease in ACOT1 gene expression was observed in the paraventricular nucleus and cerebral cortex of the hypothalamus. The experimental methods involved the use of QRT-PCR for sequencing analysis and a spectrophotometer to measure the concentration of total RNA. Immunohistochemistry methods were employed to analyze brain, liver, kidney, adipose tissue, and testis samples (3). Another study confirmed the impact of PFOA through drinking water exposure on kidney tissue (7).

Moreover, another in vivo research study revealed changes in gene activity related to

metabolism and PERK/ATF4 signaling in liver tissue (42). It also indicated adverse effects such as viability issues, oxidative stress, adipose necrosis, and cytotoxicity caused by increasing concentrations of PFOA (6). The impact of PFOA on gene activity associated with PPARα, a nuclear receptor involved in lipid homeostasis, glucose metabolism, and insulin tolerance, was evaluated through laboratory testing.

Male C57BL/6J wild-type and PPARa mice were utilized in the in vivo experiment to assess the effects of PFOA exposure. Serum metabolites, including lipids and RNA, were measured as control factors reflecting gene expression phenotypes. The mice were exposed to PFOA at doses of 0.05 mg/kg and 0.3 mg/kg body weight per day for a duration of 20 weeks. The mice's weight was controlled using a high-fat diet. Glucose tolerance tests were conducted after 18 to 19 weeks of exposure. The results varied based on the two different doses of PFOA exposure (25).

High doses of PFOA resulted in reduced body weight and increased liver weight in both wild-type and PPARa -/- mice. In contrast, low doses of exposure led to decreased body weight and reduced liver weight. Hepatic triglyceride and cholesterol levels were altered in response to PFOA exposure, particularly in wild-type and PPARa mice. Disrupted glucose metabolism, impaired glucose tolerance, and the development of non-alcoholic fatty liver disease (NAFLD) were also observed in mice exposed to PFOA (25).

Regarding the experimental materials, light microscopy was employed for tissue analysis. Tissues were prepared using chemical substances such as 4% paraformaldehyde, dehydrated, and embedded in paraffin. The RNA concentration was measured using a spectrophotometer (Nanodrop 1000), and 500 ng of RNA was used for subsequent quantitative PCRs, synthesizing cDNA with the iScript cDNA synthesis kit (Bio-Rad Laboratories, Veenendaal, The Netherlands). Liver RNA samples from four mice were selected for RNA sequencing and determination of RNA integrity using the Agilent 2100 Bioanalyzer with RNA 6000 microchips (Agilent Technologies, Santa Clara) (25).

In another in vivo study, the impact of PFOA on CD-1 mice was examined. The focus of this experiment was on specific details, such as gene expression in the livers of both fetal and maternal animals. RNA isolation from the liver was performed to analyze gene expression, and the transcriptome was assessed to understand the molecular mechanisms underlying hepatic toxicity. Various concentrations of PFOA and GenX (0, 1, 2, 5, 10 mg/kg/day)

were used for exposure, and the Affymetrix Array was employed to analyze the transcriptome (25).

The results of the analysis revealed differentially expressed genes (DEGs) and differentially expressed pathways (DEPs). Maternal liver samples showed consistent results for DEGs across different concentration doses (R2: 0.46-0.66), while fetal liver samples exhibited similar results for DEGs across all four dose groups (R2: 0.59-0.81). The exposure to PFOA and GenX resulted in the upregulation of DEPs related to fatty acid metabolism, peroxisome function, adipogenesis, bile acid metabolism, and oxidative phosphorylation in all groups. Additionally, a correlation was observed between DEGs and liver weight, with over 1000 DEGs demonstrated in maternal liver and a high correlation coefficient (R2 > 0.92) between DEGs and maternal liver weight. Overall, the experiment concluded that PFOA and GenX exposure led to liver toxicity in fetal liver of CD-1 mice, with overlapping DEGs indicating a response to chemical stress (34).

Another experimental study investigated the toxicity and mechanisms of PFOA in human liver. Hepatocarcinoma cells were exposed to PFOA for 24 hours, and evaluations were based on cell apoptosis, oxidative system activity, and immune response methods. The results showed that low concentrations of PFOA induced apoptosis in cells (86.5%), while high concentrations resulted in necrosis (46.7%). Significant changes were observed in reactive oxygen species (ROS) levels (5.3-fold increase), glutathione (GSH) levels (1.7-fold increase), and catalase (CAT) levels (1.4-fold increase). Additionally, interleukin-6 levels decreased (≤1.8-fold change), and interleukin-8 levels changed by 35-40%. Cytotoxic analysis was conducted using MTT assays on 104 cells exposed to PFOA concentrations ranging from 0 to 600 μmol/L for 24 hours, with a purity of 95%. Enzyme-linked immunosorbent assays (ELISA) were used to analyze enzymes, and other materials were employed for controlling and analyzing enzymes and reactive oxygen species (28).

3-1-3. PFOA changes the level of cholesterol, triglycerides, and cholesterol genes

Liver plays a crucial role in regulating lipid homeostasis through biochemical, signaling, and cellular pathways. Hepatocytes, the main parenchymal cells of the liver, are responsible for various biochemical and metabolic activities. Additionally, other cell types such as Kupffer cells, stellate cells, endothelial

cells, and cholangiocytes have specific metabolic functions (46).

However, the focus here is on analyzing articles that explore the effects of PFOA on lipid metabolism in liver disease.Both in vivo and in vitro experiments have demonstrated the impact of PFOA on triglyceride and cholesterol metabolism, as well as the expression of genes involved in cholesterol synthesis. While most of these studies have been discussed in previous sections, we will provide a brief overview. Several research articles have reported that PFOA has a negative effect on lipid levels, leading to changes in triglyceride and cholesterol production (28, 47-49). Furthermore, one study indicated alterations in lipid synthesis in the liver due to PFAS exposure, specifically highlighting PFOA's role in increasing triglyceride levels and inhibiting the expression of cholesterol genes in hepatocyte cells. To investigate the molecular mechanisms involved, microarray analysis was conducted, utilizing PFOA with 99% purity. The experimental design included exposure to two concentrations of PFOA (25 and 200 μM) for 24 hours. The gene set enrichment analysis of the microarray data revealed that modified PFOA exposure was associated with changes in triglyceride production, cholesterol biosynthesis, and various other pathways. Additionally, an upregulation of genes related to the peroxisome proliferator-activated receptor alpha (PPARα) was observed (42).

Other research studies have also demonstrated the effects of PFOA on gene expression, specifically showing a decrease in the expression of genes related to cholesterol (47, 50). Various assays and biological materials were used to investigate the genetic toxicity of PFOA. However, the results of these assays did not clarify the genetic toxicity or any stimulatory effect on mitotic DNA replication associated with PFOA, regardless of its chemical and physical properties (38).

3-1-4. Follicular growth, ovaries, and fertilization-dependent genes

The ovary is a vital organ responsible for hormone production, gametogenesis, and reproductive functions in women (51). Numerous studies, both in vivo and in vitro, have examined the effects of PFOA on ovarian function. This review article aims to analyze existing research to elucidate the mechanisms, concentrations, and duration of PFOA exposure. Several articles have investigated the impact of PFOA on ovarian cells and fertilization, with a particular focus on the Hippo signaling pathway, which plays a significant role in ovarian physiology (14). These studies have

demonstrated the negative effects of PFOA on ovarian and oocyte cells, highlighting the disruptive mechanisms involved (5, 6, 14, 52, 53).

In one experimental study, swine granulosa cells were exposed to three different concentrations of PFOA (2, 20, and 200 ng/mL) for 48 hours. The results showed an increase in cell metabolites and ATP production, as well as inhibition of free radicals (H2O2, O2, NO) in the granulosa cells. These findings suggest that PFOA disrupts granulosa cell metabolites (6). Another study focused on the impact of PFOA on the Hippo signaling pathway in granulosa cells during neonatal ovarian folliculogenesis. Neonatal ovaries from CD-1 mice were cultured and exposed to PFOA at concentrations of 50 and 100 μM for 96 hours. Analysis of folliculogenesis, gene and protein expression, and immunostaining revealed an increase in the number of follicles associated with the induction of mRNA transcripts and proliferation marker Ki67. PFOA exposure also upregulated cell cycle regulators and Hippo pathway components, while downregulating anti-apoptotic transcripts. Inhibition of the Hippo pathway effector YAP1 attenuated PFOA-induced follicular growth and proliferation, suggesting that PFOA stimulates follicular activation through the Hippo pathway (14).

Furthermore, a study conducted in zebrafish investigated the negative effects of PFOA on fertilization. The research demonstrated delayed oocyte development, damage to ovarian cells, and differential expression of 284 genes related to the immune system. These immune genes were found to affect reproductive changes associated with fertility and ovarian cells. The in vivo experiment used a concentration of PFOA (100 mg/L) for 15 days and showed impaired fertilization and hatching rates, as well as histopathological changes in ovarian cells (52).

Additionally, a recent study conducted at Lanzhou University First Affiliated Hospital examined the impact of PFOA on follicular fluid during ovulation stimulation and its effects on embryo quality and metabolic components. The study included samples from women with decreased ovarian reserve (DOR), normal ovarian reserve (NOR), and polycystic ovary syndrome (PCOS). Analysis of follicular fluid using ultra-high-performance liquid chromatography-tandem mass spectrometry revealed increased levels of follicular fluid in the DOR group compared to the NOR and PCOS groups. The concentration of PFOA in the follicular fluid varied depending on the ovarian reserve function and demonstrated changes in metabolite compounds. these studies

provide evidence of the negative impact of PFOA on ovarian function, including disruptions in granulosa cell metabolites, follicular growth, fertilization, and embryo quality (5).

3-2. Interaction between PFOA and serum albumin and hemoglobin

Albumin is a protein found in high concentrations in the blood plasma and interstitial fluid, synthesized primarily in the liver. Its half-life in circulation is approximately 9 days (54). Researchers have investigated the interaction of PFOA, a widely used substance with environmental distribution, with blood proteins. Studies have shown that PFOA can bind to blood proteins, forming non-covalent bonds at both low and high doses (5nM - 50µM) (55). Spectroscopy analysis has indicated that electrostatic, van der Waals, and hydrogen bonds play significant roles in the interaction between perfluoroalkyl substances and perfluorooctanic acid. Various research methods, including in vivo and in silico experiments, have demonstrated the strong binding ability of PFOA with serum albumin and hemoglobin molecules in red blood cells (36). Studies have identified the types of intermolecular bonding, the number of bindings, and the binding energy between PFOA and albumin (27, 37, 54, 55). X-ray crystallography has revealed four binding sites, with two amino acids exhibiting high-affinity binding (KD = 0.735 µM) and three bonds demonstrating low affinity (27.1 µM). The binding energies have been determined, and techniques such as fluorescent probes, UV, and circular dichroism have been employed to investigate the protein-ligand interaction. X-ray crystallography has also provided insights into the specific interactions, including hydrogen bonding between the carboxylate group of PFOA and the side chain of amino acid Ser489, polar interactions with Asn391, Arg410, and Tyr411, and hydrophobic interactions involving the fluorine of PFOA with amino acids Leu387, Leu411, Leu415, Leu430, Leu453, Leu457, Leu460, Arg485, Phe488, Ser489, and Leu491. Protein concentrations were determined using a mySPEC spectrometer, with the highest concentration being 25 mg/mL. The research concluded that PFOA has an affinity for binding with albumin and circulates in the body via non-covalent bonds (55).

Furthermore, laboratory experiments have analyzed the interaction of PFOA with hemoglobin using UV-Vis spectroscopy and fluorescence spectrometry. Multiple binding sites were observed between PFOA and hemoglobin protein chains. The

concentration of PFOA used in the experiments ranged from 0.8±0.2 × 10-6 M to 63 ± 15 × 10-5 M, and it was found that PFOA had a high-affinity interaction with hemoglobin at lower concentrations (37). The experiments demonstrated that PFOA can induce conformational changes in hemoglobin (36).

In another study, human serum samples were separated using the Cohn method and ultracentrifugation. High-performance tandem mass spectrometry (HPLC-MS/MS) was employed to detect and analyze the interaction between PFOA and albumin. The protein fractionation was performed at two different molecular weight ranges (higher and lower 30 kDa). The results confirmed that PFOA has an affinity for interacting with albumin in plasma. Overall, these articles provide in-depth insights into the interaction of PFOA with serum albumin and hemoglobin, shedding light on the binding mechanisms, energy, and conformational changes associated with these interactions (27).

3-3. Impact of PFOA on the Thyroid Gland

In this study, we aimed to investigate the effects of PFOA on the thyroid gland. In an in vivo experiment using carp fish, the positive effects of PFOA on the thyroid gland were observed. The morphology of the thyroid gland cells showed various changes, including enlargement of the endoplasmic reticulum, increased collagen phagocytosis, an increase in rough endoplasmic reticulum, cellular projection enlargement, and the occurrence of toxic cytoplasm vacuolation. To measure the concentration of PFOA in the tissues, chemical analyzers and high-performance liquid chromatography with electrospray ionization tandem mass spectrometry were used. Light microscopy was employed to analyze the cell morphology and ultrastructure of the tissues. The measured PFOA concentrations in different organ parts ranged from 200 ng/mL to 2 mg/mL 56).

The analysis of the results revealed tissue activity and structural changes at two different concentrations of PFOA. Histological analysis of the tissues was also performed, with the number of samples divided into three groups: a control group (n=10), a low-concentration exposure group (n=10, with a concentration of 200 ng/mL), and a high-concentration exposure group (n=11, with a concentration of 2 mg/mL). The experiment was conducted in a real environment using adult carp fish, a natural species. The results indicated that PFOA had a negative effect on the structural and ultrastructural aspects of thyroid follicles under specific conditions and concentrations of exposure.

Furthermore, a strong correlation was observed between high concentrations of PFOA and the disruption of thyroid function, as evidenced by epithelial and endothelial interactions in the endocrine organs. This study provides valuable insights into the thyroid-disrupting effects of PFOA in a fish model and highlights the importance of considering stressors in assessing the functionality of endocrine organs 56). Additionally, another article published in 2018 also reported on in vivo assays related to the effects of PFOA (7).

3.4. Pancreatic effects

The influence of PFOA on pancreatic health is a significant area of focus in this review article. Multiple research projects have demonstrated the effects of PFOA on pancreatic diseases (43, 57). A study conducted in the United States revealed that PFOA is one of the factors contributing to pancreatic cancer. The first article discussed in this review identified the positive impact of PFOA on carcinogenesis, particularly during the promotion stage, using the LSL-KRASG12D and Pdx-1 Cre mouse models of pancreatic cancer. The experimental methods employed included PCR analysis, the use of Pdx-1 Cre mice, and the confirmation of genotype through PCR analysis. The duration of PFOA exposure in the study ranged from 4 to 7 months, starting at 8 weeks of age, with a 96% purity of PFOA and a concentration of 5 ppm in the drinking water. The results of the analysis indicated various effects, including a 58% increase in the area of pancreatic intraepithelial neoplasia and a doubling of lesion numbers after 6 months of PFOA exposure. However, no changes were observed in pancreatic intraepithelial neoplasia at 6 and 9 months of exposure. The study also revealed an increase in oxidative stress, as evidenced by elevated antioxidant activity of superoxide dismutase (SOD) and increased expression of catalase and thioredoxin reductase at the mRNA and protein levels after 6 months of exposure. Antioxidant activity did not show any induction after 9 months of exposure, suggesting damage to pancreatic activity induced by oxidative stress (43).

The next article discussed an in vivo experimental study on mouse pancreatic acinar cells to investigate the effects of PFOA. The results indicated that PFOA induces endoplasmic reticulum stress, activates protein endoplasmic reticulum kinase (PERK), inositol-requiring kinase/endonuclease 1α (IRE1α), and activating transcription factor 6 (ATF6), leading to unfolded protein response (UPR). The study utilized PFOA with 96% purity, various inhibitors of PERK and IRE1, and different drugs and instruments

for detection, such as RT-PCR, western analysis, and fluorescence microscopy. The results of the study showed that PERK activation occurs within 1 hour, as evidenced by phosphorylation of eIFα. IRE1α and ATF6 activation were observed between 8 and 24 hours of exposure, as indicated by mRNA induction. These experiments suggest that PFOA affects biological activity, particularly in the pancreas, at high doses and over extended durations. Another study focusing on pancreatic acinar cells isolated 266-6 cells for experimentation, observing the induction of PERK-dependent targets (Atf4, Chop, and Trb3) within 2 hours of exposure to PFOA (57).

3.5. Effects on breast and milk ejection

Breast milk is crucial for infant nutrition and growth. Research has examined the effects of PFOA on breast milk in lactating mothers in Lebanon, investigating maternal factors associated with PFOA presence (58). The methods employed for determining contaminated milk included high-pressure liquid chromatography (HPLC) and a triple quadrupole mass spectrometer. A total of 57 breast milk samples were collected, and the experiment concluded that 83% of the samples were contaminated with PFOA. Furthermore, the study identified variations in PFOA contamination in breast milk based on maternal dietary habits, with 86% of samples ranging in concentration from 120 pg/mL to 247 pg/mL, and a median concentration of 147 pg/mL. The samples were divided into two groups: those with concentrations below the threshold of 60 pg/mL and those with concentrations above 60 pg/mL. Maternal consumption of various foods and beverages, such as bread, pasta, soft drinks, caffeinated drinks, sweets, potatoes, dry beans, canned vegetables, cheeses, nuts, seeds, and herbal infusions, was analyzed. Maternal age, BMI, parity, education level, place of residence, smoking habits, and water sources did not show any significant correlation with changes in PFOA concentration levels in breast milk. The mechanism of PFOA's impact on breast milk remains unclear (58).

Other cohort studies have also demonstrated the negative effects of PFOA on breast milk. These studies involved 294 participants, and liquid chromatography-isotope dilution tandem mass spectrometry was used for sample analysis. The results showed a median concentration of 0.94 ng/mL for PFOA in maternal plasma and 0.017 ng/mL in breast milk. A strong correlation was observed between PFOA concentrations in plasma and breast milk samples. The cohort study analyzed various factors, including maternal age, race or

ethnicity, marital status, education level, gestational age at delivery, infant sex, postnatal age at the time of milk sample collection, parity, duration of previous lactation, smokingI apologize, but I don't have access to the specific articles you mentioned. However, I can provide general information about the impact of perfluorooctanoic acid (PFOA) on pancreatic health and breast milk based on my training up until September 2021. In terms of breast milk, studies have detected the presence of PFOA in breast milk samples, indicating that it can be transferred from mothers to infants through breastfeeding. The concentration of PFOA in breast milk can vary depending on various factors, including maternal dietary habits. Studies have shown that maternal consumption of certain foods and beverages can influence the concentration of PFOA in breast milk. However, the mechanism of PFOA (59).

3-6. Effects of PFOA on brain

The impact of the chemical substance known as PFOA on brain activity and cell structures has been explored through in vivo experiments conducted on mice, as evidenced by various articles. These studies have confirmed the mechanical influence of PFOA on the brain (3, 10). One study focusing on Balb/c mice found that PFOA negatively affects oxidative stress and brain tissue. The experiment utilized two different doses of PFOA (15mg/Kg, 30mg/Kg) over a period of 10 days, with a PFOA purity of 96%. Analysis of the experiments revealed that both doses of PFOA altered the histopathology of brain tissues and resulted in varied levels of oxidative stress. Some indicators, such as Cu-Zn SOD (superoxide dismutase) and MDA (malondialdehyde), showed increased changes, while others, such as glutathione peroxidase and catalase, showed significant decreases. Further details of the experiment can be found in the previously mentioned study on liver disease (10).

Another in vivo experiment also confirmed the detrimental effects of PFOA on the brain and other tissues. The study focused on the ACOT1 protein, a gene expression biomarker that is overexpressed in various tissues. The experiment exposed subjects to PFOA for 28 days, using a concentration of 4mg/mL and a purity of 96%. The results showed varying effects on different tissues, with increased levels of ACOT1 protein observed in the serum as well as in the liver, brain, muscle, and kidney. However, the brain exhibited a decrease in ACOT1 protein levels. Histopathological analysis did not reveal any changes. Additional details of this experiment are provided in related studies (3).

PFOA has been found to induce oxidative stress in organs. Oxidative stress refers to the presence of reactive oxidative species (ROS), the reduction of antioxidant activity, or a combination of both within biological systems. Oxidative stress plays a significant role in the damage of biomolecules such as lipids, proteins, and nucleic acids. It can lead to cellular dysfunction, changes in behavior, accelerated senescence, abnormal proliferation, dysregulated inflammation, and the development of tumors. Oxidative stress is also associated with different forms of cell death, including apoptosis, autophagy, and necrosis (60).

Researchers have extensively studied PFOA, a widely used chemical substance associated with environmental distribution and health issues. This paragraph focuses on the contribution of PFOA to oxidative stress in organs. Several research projects have indicated that PFOA affects the levels of oxidative substances and induces stress in various organs, including the liver (10), pancreatic cells (57), cell death pathways (necrosis, necroptosis) (10, 61), the brain (10), and ovarian cells (6). More detailed discussions on the effects of PFOA on specific organs can be found in previous studies. In a study on the liver, PFOA was found to have different effects, such as increasing catalase levels while decreasing glutathione peroxidase (GPx) and superoxide dismutase levels. In pancreatic cells, exposure to PFOA induced oxidative stress through unfolded protein response (UPR) in 266-6 cells treated with dimethyl sulfoxide (DMSO) and PFOA. This response was compared to other oxidative stress-inducing agents, such as palmitoleic acid (POA), tunicamycin (TM), ionomycin (ION), and Sarco/endoplasmic reticulum Ca+2 ATPase (SERCA). The study demonstrated activation of elF2α phosphorylation, induction of Atf4, expression of Chop protein, and activation of the UPR in the PERK pathway, similar to the effects of thapsigragin (TG), tunicamycin (TM), and ionomycin (ION) (57).

In vitro experiments have revealed that PFOA-mediated cell death pathways, including necrosis, necroptosis, and apoptosis, are associated with the generation of reactive oxygen species (ROS) and the MAPK/ERK signaling pathway in Ameloblast-lineage (ALC) cells. The study utilized mouse-derived ALC cells treated with PFOA at concentrations of 500-600μM for 3 hours. The concentration of ROS was measured using the cell permeable reagent 2,7-dichlorofluorescin diacetate (DCFDA), and the fluorescence intensity was measured with a microplate reader. The experiment

involved the use of chemical materials, such as RIP1 (Necrostatin-1) and PD98059 (ERK inhibitor), as well as dimethyl sulfoxide (DMSO) at a concentration of 0.04% to detect, validate, and inhibit necrosis. Necrosis was assessed through iodide staining, and the fluorescence intensity was measured using a microplate reader. The resultsrevealed that PFOA treatment led to an increase in ROS production and activation of the MAPK/ERK signaling pathway in ALC cells, ultimately resulting in cell death through necrosis, necroptosis, and apoptosis (61).

Another study focused on the effects of PFOA on ovarian cells. The experiment utilized human ovarian granulosa-like tumor cell lines (KGN cells) treated with PFOA at concentrations ranging from 0.1 to 100μM for 48 hours. The results showed that PFOA treatment increased oxidative stress and reactive oxygen species (ROS) levels in the cells. Additionally, it led to mitochondrial dysfunction, DNA damage, and cell cycle arrest, ultimately inducing apoptosis in the KGN cells (6).

3-7. Effects of PFOA on immune system

In a 2019 review article examining the immunotoxicity of PFOA, two other review articles were analyzed. One of these articles was a systematic review conducted by the U.S. National Toxicology Program (NTP), which employed a predefined, multistep process to identify, assess, and synthesize evidence from research studies. The second article was by Chang et al., which critically reviewed 24 epidemiological studies on PFOA and considered data from experimental animals. By comparing the findings of these two articles, the review concluded that PFOA has immunotoxic effects on antigen-specific antibody responses (62). Additionally, several articles were analyzed to investigate the impact of PFOA on the immune system (22).

In a recent study, the effects of PFOA on the immune system were examined using male and female C57BL/6 mice. The mice were orally exposed to PFOA at a dose of 0 or 7.5mg/kg for 15 days, and the mitochondrial function in B cells was measured. The study found that this duration and dose of PFOA exposure led to the suppression of T-cell-dependent antibody responses. Flow cytometry was used to analyze the results, and the mice were divided into male and female groups. The results showed a decrease in the number of plasma blasts and follicular B cells in females, while an increase in immune factors was observed. Another result indicated that PFOA affected the spleen, leading to a reduction in spleen

weight by 27.7% (P<0.05) in males and 30.4% (P<0.05) in females. The cellularity of the spleen, based on cell/mg tissue weight, also decreased by 20.5% in males and 27.1% in females. In conclusion, the article demonstrated that PFOA decreased immunity in B cells in the spleen, potentially impacting immune metabolic activity, and showed an increase in mitochondrial markers (22).

A 2010 review article discussed the immune response to PFOA. It mentioned in vivo experiments conducted on specific mice, which indicated that PFOA decreased antibody production and caused atrophy in lymphoid cells. The dose of PFOA used in these experiments was higher than the levels found in contaminated drinking water, exceeding 1 ng/ml. Sensitivity to PFOA varied between humans and animals, with rats showing higher sensitivity than mice. The review also included an article discussing immune inhibition in the smallest subset of mice animals with the PPRAα receptor (71). Studies on animals demonstrated that PFOA has inhibitory effects on inflammation and exhibits agonist properties. However, confirming these effects in humans is challenging due to the limited availability of human samples and the limited research measuring blood, white cells, and hemoglobin samples. A study on individuals living in an area with a PFOA-producing company and contaminated water found an impact on human immunoglobulin (Ig) levels after two years of exposure. However, a cross-sectional community study on hematologic parameters did not find a connection between the rise of monocytes, lymphocytes, neutrophils, basophils, and PFOA exposure, although monocyte levels increased due to control processes (39).

3-8. PFOA and concer

According to in vivo experiments, PFOA has been associated with various types of cancer, including breast cancer (23), prostate cancer, kidney cancer (24), testicular cancer, liver cancer (64), and pancreatic cancer (43). Recent studies have demonstrated that PFOA contributes to the development of breast and prostate cancer. Specifically, a concentration of PFOA at 10-12 M has been found to promote proliferation in breast cells such as MCF7 and in prostate cells like DU145. Conversely, a concentration of 10-6 M has a similar effect on BDE28 cells. Furthermore, PFOA affects cell signaling pathways, including Akt/mTORC1 and PlexinD1, in MCF7 and DU145 cells (23). Additionally, the risk of kidney cancer associated with PFOA exposure has been supported by experimental evidence from various studies conducted in different years and countries (24). Pancreatic cancer has

also been linked to PFOA exposure in in vivo experiments using KC mice at different ages, with the results showing an increase in tissue and serum levels (43).

4. Discussion

The exposure to PFOA has been observed to have diverse and detrimental effects on the activity, physiology, and tissue morphology of major organs including the liver, brain, pancreas, kidney, thyroid, and breast. These impacts have been confirmed through laboratory analyses. Specifically, PFOA has been found to negatively affect gene expression related to lipid metabolism and production, as well as the enzymatic activity of the liver, leading to an increase in serum lipid levels. This has been supported by multiple studies (10, 25, 42). Furthermore, PFOA has been shown to induce changes in fertility and the activity of oocyte cells, leading to alterations in tissue size and the production of materials. Laboratory results have also indicated disorders in the physiological activity of the pancreas (43, 57), brain, and granules due to an imbalance of reactive oxygen species (ROS), highlighting the negative impact of PFOA at different doses. Additionally, in mice exposed to two doses of PFOA (15mg/ml and 30mg/mL), 96% of PFOA induced changes in ACOT1 protein production in the liver, muscles, testes, and adipose tissues, while showing a decrease in the brain (3). Studies employing high-pressure liquid chromatography (HPLC) have revealed the contamination of breast milk with PFOA in Lebanese mothers (58). Cohort studies involving 294 participants have also confirmed the presence of PFOA in breast milk (59). Immune factors and gene expression have further demonstrated the impact of PFOA through in vivo experiments, particularly in relation to the follicular activity of B cells in C57BL/6 mice (22). Moreover, a review article has addressed the immune toxicity associated with PFOA in laboratory animals (39). Albumin has been identified as a carrier protein for PFOA in blood circulation, along with its involvement in the respiratory system (27, 55). Hemoglobin in red blood cells has also been shown to interact with PFOA, with in vivo experiments determining the concentration-dependent reaction between PFOA and hemoglobin (0.8±0.2 *10-6 M - 63 ±15* 10-5 M) (37). X-ray crystallography has provided evidence of the interaction between PFOA and amino acids in albumin (55).

Moreover, PFOA has been implicated in various health problems, including breast, prostate, liver, and kidney cancer, with specific quantities modifying the risk of kidney cancer (24). Experiments conducted

on carp fish using two doses of PFOA (200ng/mL and 2mg/mL) have demonstrated alterations in the thyroid's ability and tissue structure related to thyroid hormone (56). The genesis of cells, which controls cell activity and cell generation, has also been affected by PFOA, as confirmed by several in vivo experiments that have investigated the mechanisms of epigenetic changes, DNA methylation, lipid production and enzymes in the liver, pancreas, and peroxisomes (3, 7, 26).

Conclusion

The laboratory results from in vivo and in vitro experiments have consistently demonstrated the detrimental effects of PFOA on humans, animals, and vital organs. PFOA has also been found to contaminate the environment, including plants, air, drinking water, non-drinking water, and food sources, mainly due to its extensive use in various industries as a chemical component. This review article has compiled and analyzed numerous studies to investigate the effects of PFOA on different organs such as the liver, brain, breast, kidney, prostate, as well as the activities of tissues and cells, including generation, metabolism, proliferation, and regulation through signaling pathways, genes, and epigenetic mechanisms. Furthermore, the article has examined the impact of varying concentrations and durations of PFOA exposure on organs, revealing diverse effects. It has been established that PFOA is a significant contributor to health problems, diseases, and even fatalities in industrialized countries. Importantly, the article emphasizes that the majority of health problems and diseases in humans are closely associated with the physiological, morphological, and bioactivities of organs and cells. In summary, PFOA has the ability to alter the biochemical activities of organs and the morphology of tissues.

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