Review

Per- and Polyfluoroalkyl Substances (PFAS) as Emerging Obesogens: Mechanisms, Epidemiological Evidence, and Regulatory Challenges

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Per- and Polyfluoroalkyl Substances (PFAS) as Emerging Obesogens: Mechanisms, Epidemiological Evidence, and Regulatory Challenges

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Abstract: The pervasive presence of per- and polyfluoroalkyl substances (PFAS) in the environment and their persistent nature raise significant concerns regarding their impact on human health. This review delves into the obesogenic potential of PFAS, shedding light on their mechanisms of action, epidemiological correlations with obesity and metabolic disorders, and the challenges faced in regulatory frameworks. PFAS, characterized by their carbon-fluorine chains, are ubiquitous in various consumer products, leading to widespread exposure through ingestion of contaminated food and water. Emerging evidence suggests that PFAS may act as endocrine-disrupting chemicals, interfering with lipid metabolism and hormone functions related to obesity. We examine *in vitro*, *in vivo*, human, and *in silico* studies that explore the interaction of PFAS with peroxisome proliferator-activated receptors (PPARs) and other molecular targets, influencing adipogenesis and lipid homeostasis. Furthermore, the review highlights epidemiological studies investigating the association between maternal PFAS exposure and the risk of obesity in offspring, presenting mixed and inconclusive findings that underscore the complexity of PFAS effects on human health. Presently, there are major challenges in studying PFAS toxicity, including their chemical diversity and the limitations of current regulatory guidelines, potential remediation, and detoxification. This review emphasizes the need for a multidisciplinary approach, combining advanced analytical methods, in silico models, and comprehensive epidemiological studies, to unravel the obesogenic effects of PFAS and inform effective public health strategies.

Keywords: Perfluoroalkyl substances; Obesogen; Peroxisome proliferator-activated receptors

1. Introduction

PFAS, per/poly-fluoroalkyl substances, are synthetic compounds used in various commercial and industrial products. All PFAS contain a carbon-fluorine chain of varying lengths and different functional groups at the molecule's terminal end, giving rise to their distinct properties. According to the US Environmental Protection Agency (EPA), there are over 7800 identified PFAS, with many more being formulated and circulated to replace long-chain older-generation compounds [1]. Since these compounds are persistent pollutants both in the environment and the body, older-generation (legacy) PFAS, although replaced, are still detected in the general population. The primary mode of human exposure is ingestion of contaminated food and water, as PFAS is a widely used surfactant on cookware and food packaging and a persistent pollutant of crop-yielding and groundwater [2]. To determine the extent of PFAS exposure through food, total diet studies were conducted by the Food and Drug Administration (FDA), and detectable levels of PFAS were found in seafood in the general food supply. It is important to note that there was no distinction between wild-caught and farmed seafood. Thus, there is no evidence to determine if PFAS contamination was due to agricultural practices or if the pollutant has become a part of the aquatic food chain. A complicating factor in studying PFAS exposure and its effects on human health is that PFAS-based surfactants are technical mixtures comprising extensive chemical and PFAS combinations; these proprietary blends make up nonstick and water-repellant coatings, adhesives, and labels found in food packaging

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materials. The complete chemical profiles of technical mixtures are nearly impossible to determine. To further complicate our understanding, PFAS in food packaging is degraded into secondary toxicants like perfluoroalkyl carboxylic acids, which are also linked to adverse effects on human health [3]. PFAS are a class of synthetic chemicals that have been widely used in industry and consumer products since the mid-twentieth century, and are known to disrupt the thyroid hormone system [4]. Recently, PFAS has been deemed a suspected endocrine-disrupting compound and a potential obesogene [5]. As potential obesogenic compounds, exposure to these compounds has the potential to directly or indirectly promote obesity by dysregulating lipid metabolism or disrupting hormones that mediate hunger signaling, among other things[5].

The aim of this paper is to critically evaluate the burgeoning body of evidence elucidating the obesogenic properties of PFAS compounds. This comprehensive review navigates through the advancements in understanding the obesogenic pathways of PFAS, utilizing modern methodologies and modeling techniques to explain their potential effects on public health and environmental risk management. Central to this discourse is the exploration of the mechanisms by which PFAS may promote obesity and metabolic dysregulation, with an emphasis on their interactions with lipid metabolism and endocrine functions. The review further scrutinizes epidemiological studies that establish a correlation between PFAS exposure and obesity incidence, especially among susceptible demographics such as children and expectant mothers. It endeavors to evaluate the consistency and strength of these epidemiological links, thereby contributing to a nuanced understanding of PFAS-related health risks. Moreover, this paper confronts the complexities inherent in the toxicological evaluation of PFAS, highlighting the vast chemical heterogeneity of these compounds, the constraints posed by current regulatory frameworks, and the challenges associated with remediation and detoxification efforts. By integrating findings from *in vitro*, *in vivo*, and *in silico* research, the review aspires to furnish a holistic perspective on the role of PFAS as putative obesogens. The ultimate aim is to create a basis for the formulation of effective risk management practices and regulatory policies that can curtail the obesogenic impact of PFAS, thereby safeguarding human health.

2. PFAS as Emerging Obesogens

2.1. Integrative Approaches to Understanding PFAS Toxicity and Obesogenicity

The toxicological effects of PFAS on humans present a complex research challenge, as ethical considerations preclude direct experimental exposure [6]. Consequently, our understanding of human toxicity is derived from a combination of methodologies: epidemiological studies, computational *(in silico)* modeling, in vitro assays, and in vivo studies. Computational modeling, in particular, serves as a pivotal tool in elucidating the potential risks and toxicological profiles of these contaminants, thereby reducing the reliance on invasive human sampling techniques [6].

Although the toxicity and bioaccumulation of PFAS have been widely studied, the toxicity of PFAS mixtures as they appear in the environment remains poorly understood [6]. The most widely studied PFAS compounds are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) because they are the most used forms and are stable end products of other PFAS precursors [6]. New studies on the toxicity of PFOA and PFOS ("legacy PFAS"), along with replacement PFAS ("alternative PFAS"), employ animal and cell studies, epidemiological studies in humans, and computational models, continue to display the endocrine disruption and toxicity of PFAS [6,7].

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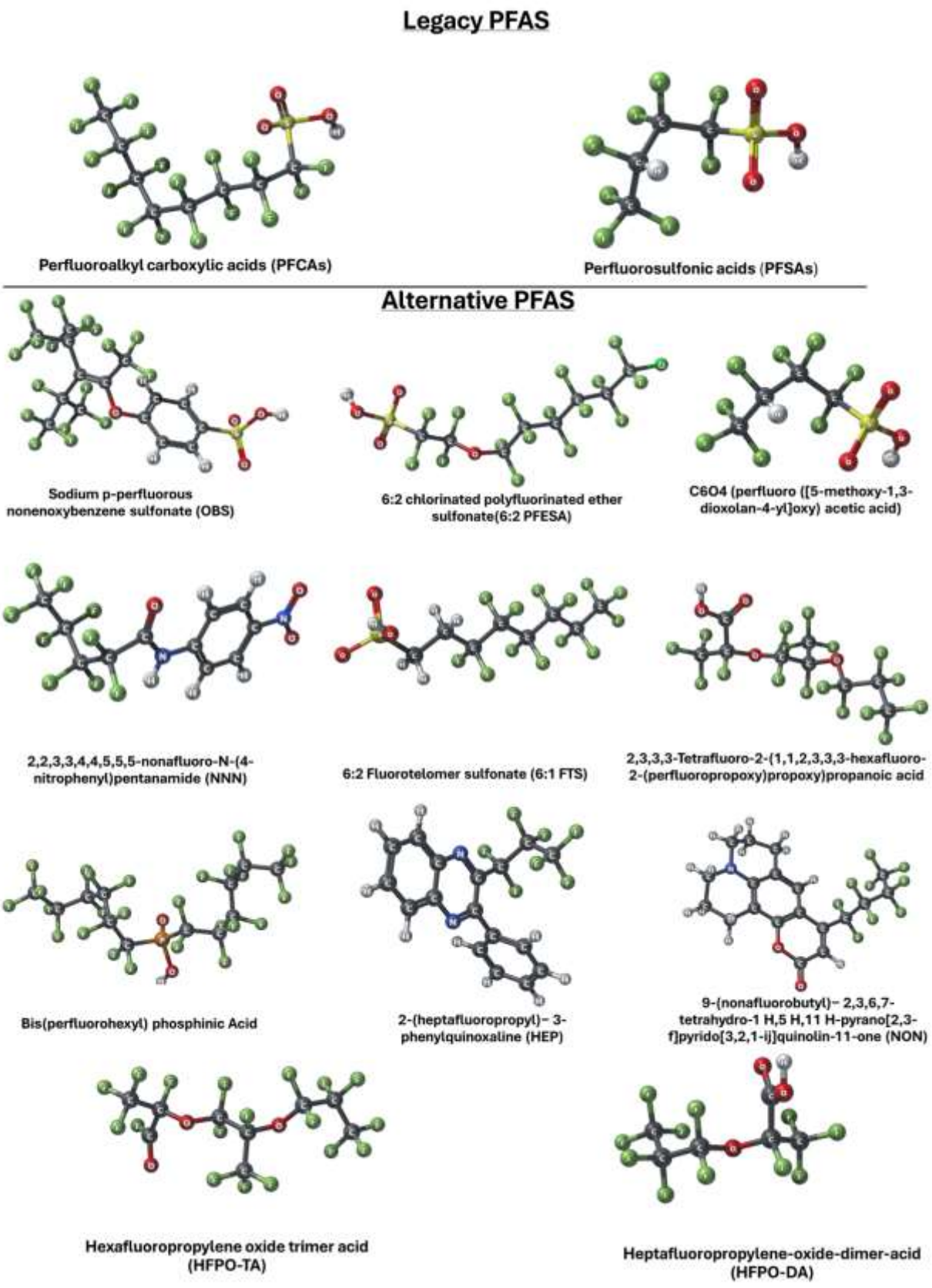

Figure 1. 3D graphical representation of various legacy and alternative PFAS compounds and their chemical structures. Legacy PFAS: Includes perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), which are traditionally monitored PFAS due to their widespread use and persistence in the environment. Alternative PFAS: Depictings a selection of newer, alternative PFAS compounds developed to replace legacy PFAS. These include:

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Hexafluoropropylene oxide trimer acid (HFPO-TA), 2-(Heptafluoropropyl)-3-phenylquinoxaline (HEP), C6O4 (perfluoro[(5-methoxy-1,3-dioxolan-4-yl)oxy] acetic acid), 9-(nonafluorobutyl)-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-11-one (NON), Sodium p-perfluorous nonenoxybenzene sulfonate (OBS), Heptafluoropropylene oxide dimer acid (HFPO-DA), 2,3,3,3-Tetrafluoro-2-(1,2,3,3,3-hexafluoro-2-(perfluoropropoxy)propoxy)propanoic acid, Bis(perfluorohexyl)phosphinic acid, 2,2,3,3,4,4,5,5,5-Nonafluoro-N-(4-nitrophenyl)pentanamide (NNN), 6:2 Fluorotelomer sulfonate (6:1 FTS), Note: Numerous other equally important legacy and alternative PFAS compounds are not shown in the figure.

The molecular resemblance of PFAS to fatty acids has sparked concerns regarding their potential to disrupt lipid metabolism, primarily through interactions with fatty acid binding proteins (FABPs) within cells [8,9]. FABPs, prevalent in liver, kidney, and brain tissues, and facilitate the transport of PFAS to the nucleus, impacting peroxisome proliferator-activated receptors (PPARs) [10]. PPARs, crucial for regulating lipid metabolism, cell growth, differentiation, and inflammatory responses, are disrupted upon PFAS binding, leading to lipid homeostasis imbalance. This dysregulation manifests as conditions like dyslipidemia, steatosis, and nonalcoholic fatty liver disease, which are all obesity-related comorbidities [8]. Furthermore, PFAS exposure has been linked to altered PPAR expression in various species, indicating a broad impact across both legacy and emerging PFAS compounds [11]. Additionally, the gut microbiome, known to influence PPARs through short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate, is also affected [12]. Disruption of the gut microbiome can further influence lipid metabolism and contribute to obesity by disrupting SCFA production and PPAR regulation [13]. Thus, PFAS exposure intricately affects lipid metabolism through a cascade of molecular interactions involving FABPs, PPARs, and the gut microbiome [11].

2.2. PFAS Associated Maternal and Childhood Obesity

Obesity is a complex, multi-faceted chronic disease characterized by an excessive accumulation of adipose tissue. Chronic obesity increases one's risk of developing metabolic disorders like dyslipidemia, high blood pressure, heart disease, and certain cancers [14]. Compounding factors like diet/eating patterns, sedentary lifestyle, genetics, socioeconomic status, and mental health influence its progression [15]. Obesity has become a major health concern in the US, with much of the reported healthcare costs being spent on the treatment of ailments related to prolonged obesity [16]. According to the Centers for Disease Control and Prevention (CDC), adult obesity is becoming more prevalent, with nearly forty-two percent of adults in the US considered overweight or obese on the Body Mass Index (BMI) scale. Concerningly, childhood obesity is also on the rise, with overweight and obese children at a much higher risk of poor health outcomes in adulthood when compared to normal-weight children [14]. As of 2020, nearly twenty percent of children in the US were overweight or obese for their age, with a positive correlation between BMI and age [14]. Currently, researchers are studying molecular obesogenic PFAS pathways *in vivo* and *in vitro* models; however, many of the studies have been contradictory or inconclusive [17]. Many epidemiological studies focus on the links of maternal PFAS exposure to childhood obesity. Still, a causal relationship between PFAS exposure and outcomes of obesity for mothers or children has not been determined [18].

Figure 2 below elaborates on the detrimental impacts of maternal PFAS exposure on gestational outcomes and subsequent child development. PFAS are primarily absorbed through the ingestion of contaminated food and water, accumulating in the placenta and umbilical cord, which suggests potential fetal exposure [19,20]. Exposure to some PFAS during pregnancy is correlated with notable increases in gestational weight gain, although this is not universally true for all compounds, such as perfluorooctanesulfonic acid (PFOS), which may exhibit divergent effects [21]. This exposure also tends to result in significant postpartum weight retention, escalating the likelihood of gestational obesity-a known risk factor for cesarean deliveries [21]. The consequences of maternal PFAS exposure extend beyond delivery, potentially leading to preterm births and reduced birth weights [22]. Over time, the children of affected mothers may experience heightened risks of obesity from as early as age five, with girls showing particularly pronounced susceptibility [23–25]. These children may also undergo more rapid increases in BMI during their early childhood years [26].

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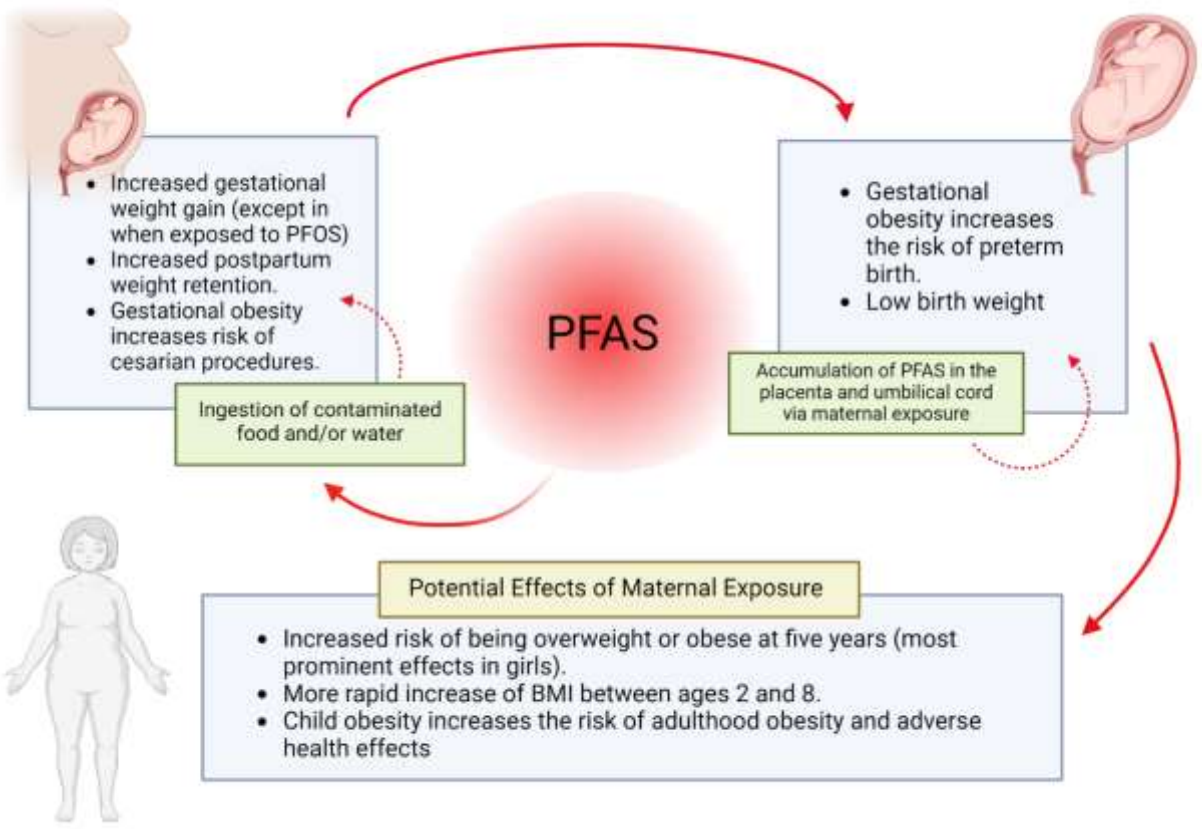

Figure 2. Potential Effects of Maternal Exposure to PFAS on Pregnancy, Fetal Development, and Child Obesity. This figure illustrates the impacts of maternal exposure to PFOA, a type of PFAS, on gestational outcomes, fetal development, and child obesity. Maternal ingestion of contaminated food and water leads to PFOA accumulation in the placenta and umbilical cord, impacting fetal development. Maternal health effects include increased gestational weight gain (except with PFOS), increased postpartum weight retention, and higher cesarean procedure risks due to gestational obesity. For the fetus, gestational obesity increases the risk of preterm birth and low birth weight. In children, maternal PFOA exposure raises the risk of being overweight or obese by age five, particularly in girls, and leads to a more rapid increase in BMI between ages 2 and 8.

2.3. Maternal PFAS Exposure and Offspring Outcomes

Gestational obesity and excessive weight gain can negatively affect both mother and children [27]. These adverse outcomes include preterm births and increased rates of cesarean procedures [28]. Although PFAS toxicity has been linked to PPAR interaction, how PPAR accumulation in the placenta and umbilical cord affects offspring is not yet understood [29]. Studies have shown a positive correlation between family history and PFAS exposure with an increased risk of developing gestational diabetes, and in-utero exposure to PFAS is associated with increased rates of childhood diabetes [30]. At best, PFAS exposure is one of the many confounding factors in the steady increase in obesity, but studies have not been able to pinpoint a specific compound or exposure dose. International concern about PFAS has given rise to numerous epidemiological studies, yielding compelling evidence for some PFAS as obesogenic and others as non-obesogenic for both mother and child [18].

Project Viva, a US study, concluded that although most of the cohort gained excessive weight during pregnancy, higher plasma N-Ethyl-N-[(heptadecafluorooctyl)sulphonyl] glycine (EtFOSSA) was associated with accelerated weight gain as the pregnancy progressed [21]. Additionally, overall higher PFAS plasma concentration was associated with higher instances of postpartum weight retention, with the strongest correlation seen in women considered overweight or obese pre-

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pregnancy [21]. In contrast, PFOS exposure was associated with decreased gestational weight gain [21]. Interestingly, researchers could not conclude a link between pre-pregnancy BMI and PFAS exposure to weight gain during pregnancy [31]. Nevertheless, a study on Ohio mothers showed that mean weight gain among the cohort was highest amongst women whose pre-pregnancy BMIs were normal [28], and increasing gestational weight gain and retention was associated with two-fold increases in serum PFOA, PFOS, and PFNA [28]. Furthermore, the study showed that in utero PFOA exposure was associated with greater adiposity of offspring at eight years old and a more rapid BMI increase from ages two to eight [28].

In agreeance with the Ohio study, a diverse cohort of mothers in a separate US study showed that high gestational plasma perfluoroundecanoic acid (PFUnDA) resulted in higher waist circumference and body fat percentage in children of women who were not obese [32]. However, PFUnDA was associated with less adiposity among obese women [32]. These findings were corroborated by international studies. Analysis of a cohort of mothers from Hamamatsu, Japan, showed that PFOS exposure was associated with lower birth weights [33]. However, high PFOA correlated to low birth weight but higher BMI with age, particularly in female children [33]. A Scandinavian group showed that maternal PFAS exposure was positively associated with increased BMI and skinfold test scores at five years old [30]. These associations, however, do not provide strong evidence of associations between individual PFAS and PFAS plasma concentration and instances of gestational weight gain and childhood obesity [32].

2.4. In Vitro and In Vivo Insights: PFAS's Role in Adipogenesis

The most studied obesogenic pathway of PFAS is the interaction with peroxisome proliferator-activated receptors, PPARs [8]. Of the three isoforms of PPAR (α, β, and γ) [29], PPAR-α and PPAR-γ have been extensively studied as potential targets of PFAS. PPAR-γ, primarily found in adipocytes, regulates adipogenesis, adipocyte differentiation, and lipid metabolism. PPAR-γ interacts with long-chain PFAS and PFAS metabolites; nearly a dozen poly-fluoroalkyl carboxylic acids interact with human PPAR. Studies show that down-regulation is associated with obesity in both rodents and humans [34]. PPAR-α is also a key regulator of lipid metabolism, and both receptors have been shown to interact with PFAS [34]. A study of technical mixtures, commercially available chemicals with unknown PFAS content or concentration, showed measurable effects on estrogenic and PPAR activity [35].

Adipogenesis, the formation of adipocytes from fibroblasts, is partially regulated by PPARS. An *in vitro* study investigating the effects of PFAS on adipogenesis showed that PFOS increased cellular lipid content and perfluorohexanesulfonic acid (PFHxS) increased adipogenesis [4,35]. *In vivo* studies on non-obese diabetic mice showed that PFUnDA exposure affected lipid levels in a dose-dependent manner, with the most prominent effects seen at 300 ug/mL [36]. PFUnDA exposure was also associated with inflammation of the islet of Langerhans cells in the pancreas. Conversely, low and medium exposure to the compound yielded a protective effect on lipid levels [4].

Table 1. *In Vitro* and *In Vivo* Studies on PFAS Obesogenic Properties.

Title	Focus	Findings	Methodology	Organism	Experimental techniques	Reference
			***In vitro* Studies**			
In vitro activity of a panel of per- and polyfluoroalkyl substances (PFAS), fatty acids, and pharmaceutic	HFPO-DA and HFPO-DA-AS were the most potent of all PFAS in rat and human PPAR-assays.	- Many PFAS compounds activate both PPARα and PPARγ receptors in human and rat assays, with HFPO-DA,	- In vitro assays with human or rat PPARα or PPARγ ligand binding domains - Evaluation of 16 PFAS, 3 endogenous fatty	humans, rats	- In vitro assays with human or rat PPARα ligand binding domains - In vitro assays with human or rat PPARγ	[37]

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als in peroxisome proliferator-activated receptor (PPAR) alpha, PPAR gamma, and estrogen receptor assays.		HFPO-DA-AS, and NBP2 being the most potent. - A few PFAS compounds (PFHxS, 8:2 FTOH, 6:2 FTOH) also exhibited agonism of the human estrogen receptor. - The activation of PPARα and PPARγ receptors by PFAS may be a molecular initiating event contributing to their in vivo effects.	acids, and 3 pharmaceuticals - Testing for human estrogen receptor (hER) transcriptional activation - Evaluation of receptor activation and relative potencies using EC20, pmaxtop, and AUC	ligand binding domains - Human estrogen receptor (hER) transcriptional activation assays - Evaluation of receptor activation and relative potencies using EC20, pmaxtop, and AUC	
Characterization of Per- and Polyfluorinated Alkyl Substances Present in Commercial Anti-fog Products and Their In vitro Adipogenic Activity	Characterization of PFAS in anti-fog products and their adipogenic activity	PFAS compounds, including FTOHs and FTEOs, were found in anti-fog products. Significant cytotoxicity and adipogenic activity were observed in murine 3T3-L1 cells. FTEOs were identified as a major contributor to adipogenic activity.	GC–HRMS, LC–MS/MS, HPLC–HRMS, in vitro adipogenesis assay	Murine 3T3-L1 preadipocytes	Gas chromatography, liquid chromatography, high-performance liquid chromatography, cell viability and proliferation assays, fluorescence microscopy	[38]
PFAS Environmental Pollution and Antioxidant Responses: An Overview of the Impact	The cellular antioxidant defense system is activated by PFAS.	- PFAS are a group of over 4,600 man-made chemicals that are toxic to both animals and humans, with PFOA and PFOS being the most	The methodology involves summarizing available data from epidemiological studies, in vitro and in vivo	humans	- Measurement of reactive oxygen species (ROS) formation - Lipid peroxidation assays	[39]

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on Human Field		widespread organic pollutants. - PFAS exposure is associated with oxidative stress, which can lead to various adverse health effects in humans such as diabetes, cardiovascular disease, and cancer. - PFAS are endocrine disruptors that can compromise many physiological processes and alter the redox environment.	research on PFAS exposure and its effects on oxidative stress and human health. It includes results from biomonitoring studies and clinical examinations of occupationally exposed workers.		- Enzymatic assays for superoxide dismutase (SOD) - Enzymatic assays for catalase (CAT) - Enzymatic assays for glutathione peroxidase (GPx) - Enzymatic assays for glutathione reductase (GR) - Measurement of glutathione (GSH) levels - Quantitative Real-Time PCR (qRT-PCR) for gene expression analysis	
Thyroid Disrupting Effects of Old and New Generation PFAS	Per- and polyfluoroalkyl substances are persistent pollutants accumulating in waters and soil and recoverable in foods due to their release by food packaging.	- PFAS, including long-chain, short-chain, and newly emerging compounds, can have detrimental effects on thyroid function based on in vitro and animal studies. - Epidemiological studies have shown associations between PFAS exposure and changes in thyroid function parameters in exposed	- Review of recent data on old and new generation PFAS effects on thyroid homeostasis. - Collection of information from in vitro studies, animal models, and in vivo data on exposed workers, general population, and pregnant women. - Laboratory studies on thyroid cell cultures to assess thyroid-disrupting	humans, rats, mice, zebrafish, Xenopus laevis, cats, Atlantic walruses	- Thyroid cell cultures - Luciferase reporter assay - Iodide accumulation assays - cAMP production assays - Oral administration of PFAS - Assaying circulating maternal thyroid hormones - Blood	[40]

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		workers, the general population, and pregnant women/infants, though the results are not entirely consistent. - Further research is needed to fully understand the clinical relevance of PFAS-induced thyroid disruption, especially regarding potential impacts on fetal and child development.	effects. - Review of clinical studies on the relationship between PFAS exposure and thyroid dysfunction, especially during pregnancy. - Use of tables to summarize types of PFAS compounds and recent data from maternal cohorts.		transcriptomic analysis - Transmission electron microscopy	
Prenatal and childhood exposure to per-/polyfluoroalkyl substances (PFASs) and its associations with childhood overweight and/or obesity: a systematic review with meta-analyses	Positive associations were evidenced between prenatal PFNA and BMI in children who were 3 or less years.	- Positive associations were found between prenatal exposure to PFNA and childhood BMI/waist circumference, and between prenatal PFOA exposure and BMI in children over 3 years old. - Negative associations were found between prenatal PFOS exposure and BMI in children 3 years or younger, between prenatal PFHxS	- Conducted a systematic review with meta-analysis. - Searched PubMed and Embase using specific text strings. - Included biomonitoring studies in pregnant women or children up to 18 years assessing BMI, WC, or fat mass. - Conducted meta-analysis when at least three studies reported estimates of associations.	humans	- Search on bibliographic databases (PubMed and Embase) - Biomonitoring studies - Meta-analysis - Stratification by sex and age - Sensitivity analyses	[41]

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		exposure and risk of overweight, and between childhood exposure to PFOA, PFOS, and PFNA and BMI, especially PFOS in boys.	- Developed a method to convert different effect estimates for comparability. - Stratified meta-analyses by sex and age. - Performed sensitivity analyses. - Retrieved 826 articles initially, included 49 in the review, and 30 in the meta-analyses.		
Perfluoroalkyl Substances (PFAS) and Their Effects on the Placenta, Pregnancy, and Child Development: a Potential Mechanistic Role for Placental Peroxisome Proliferator–Activated Receptors (PPARs)	Perfluoroalkyl substances are associated with increased incidence of gestational diabetes, childhood obesity, preeclampsia, and fetal growth restriction.	- PFAS exposure is associated with negative health outcomes during pregnancy, birth, and child development, including gestational diabetes, childhood obesity, preeclampsia, and fetal growth restriction. - The mechanisms involve PFAS interaction with PPARs, which regulate lipid metabolism and placental functions important for healthy pregnancies and child	- Review of existing studies - Includes human population-based associations - Includes in vitro-based experimental data	humans	A review of In vitro-based experimental data and - Human population-based association studies - Molecular biology techniques (e.g., studying interactions with PPARs, lipid homeostasis, inflammation, and invasion)	[42]

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		development. - PFAS interfere with trophoblast lipid homeostasis, inflammation, and invasion, which could be mediated by PFAS-PPAR interactions and other biological mechanisms.				
Evaluation of Per- and Polyfluoroalkyl Substances (PFAS) in vitro toxicity testing for developmental neurotoxicity	Evaluates the developmental neurotoxicity (DNT) of 160 PFAS using in vitro high-throughput screening assays.	42 out of 160 PFAS decreased measures of neural network connectivity and neurite length. PFAS with longer perfluorinated carbon chains (≥8) and higher carbon:fluorine ratios were more likely to be bioactive.	DNT new approach methods (NAMs) battery including microelectrode array neuronal network formation assay (NFA) and high-content imaging (HCI) assays to evaluate proliferation, apoptosis, and neurite outgrowth. Chemical concentration-response data analyzed using the ToxCast Pipeline (tcpl).	In vitro (rat cortical cells, human neural progenitor cells, human glutamatergic enriched neurons)	Microelectrode array (MEA) network formation assay (NFA) High-content imaging (HCI) assays Statistical and bioinformatics analysis using R and ToxCast Pipeline	[43]
In vitro screening of per- and polyfluorinated substances (PFAS) for interference with seven thyroid hormone system targets across nine assays	Screening for interference of PFAS with thyroid hormone system targets	Evaluated activity of 136 PFAS at seven key molecular initiating events (MIE) using nine in vitro assays. Identified 85 PFAS with sufficient activity to produce an EC50 in at least one assay. Several PFAS had strong potency towards	Nine in vitro assays: enzyme inhibition assays (hDIO1, hDIO2, hDIO3, xDIO3, hIYD, xIYD), fluorescence-based assays (hTPO, hTTR, hTBG).	Human, Xenopus	Colorimetric endpoint using Sandell-Kolthoff reaction, fluorescence-based assays	[44]

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		transthyretin binding.				
PFAS and Potential Adverse Effects on Bone and Adipose Tissue Through Interactions With PPARγ	Investigating the effects of PFAS on bone and adipose tissue through interactions with PPARγ	PFAS exposure may lead to several adverse outcomes including altered cell differentiation, bone development issues, increased adipogenesis, metabolic disorders, and bone weakness. PFAS can trigger multiple molecular initiating events through interactions with nuclear receptors like PPARγ.	Literature review, evaluation of epidemiological and toxicological studies on PFAS, PPARγ interaction mechanisms	Human, Mouse, Rat	Review of existing in vitro and in vivo studies. In vitro studies assessing PPARγ activation, bone development anomalies, and adipogenesis, using human mesenchymal stem cells and animal models. Mechanistic exploration of PPARγ's role in MSC differentiation to adipocytes versus osteoblasts.	[45]
			***In vivo* Studies**			
Per- and polyfluoroalkyl substance mixtures and gestational weight gain among mothers in the Health Outcomes and Measures of the Environment study	Investigating the influence of PFAS mixtures on gestational weight gain (GWG) among mothers	Each doubling in serum concentrations of PFOA, PFOS, and PFNA was associated with a small increase in GWG. The association of PFNA with GWG was stronger among women with BMI≥25 kg/m2. There was little association between PFAS and GWG z-scores.	Mass spectrometry, multivariable linear regression, weighted quantile sum regression, restricted cubic splines	Human (pregnant women)	Serum PFAS quantification using mass spectrometry, data analysis using multivariable linear regression, and weighted quantile sum regression	[46]
Environmental toxicants and placental function	The impact of environmental toxicants on placental function and fetal development	Environmental toxicants, such as toxic trace elements, PFAS, and environmental phenols, can	Literature review, meta-analysis, systematic review	Human (pregnant women and fetuses)	Biomonitoring, epidemiological studies, gene expression analysis, epigenetic analysis	[47]

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		cross the placenta and impact fetal development through endocrine disruption, oxidative stress, and epigenetic changes. These toxicants may lead to adverse outcomes such as preterm birth, low birth weight, and pregnancy loss.				
Umbilical cord serum concentrations of perfluorooctane sulfonate, perfluorooctanoic acid, and the body mass index changes from birth to 5 1/2 years of age	Investigating the impact of prenatal exposure to PFAS on the BMI trajectory of children from birth to 5 1/2 years	Prenatal exposure to PFOS and PFOA was associated with lower BMI SDS during infancy but an increase in BMI SDS in later childhood, particularly among girls.	Growth curve modeling, high-performance liquid chromatography (HPLC), tandem mass spectrometry (MS/MS)	Human (children from the Hamamatsu Birth Cohort)	BMI measurements, log10-transformed PFAS concentrations, statistical analysis using STATA and Mplus	[33]
Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian study	The associations between prenatal exposure to endocrine disruptive chemicals (EDCs) and fetal growth	Prenatal exposure to PFOA, PCB 153, and HCB was associated with higher odds for SGA birth among Swedish women, with stronger associations in male offspring. No significant associations were found in the Norwegian cohort.	Case-cohort study, linear and logistic regression with 95% confidence intervals (CIs)	Human (mother-child pairs)	Measurement of PFASs and OCs in maternal serum, statistical analysis using linear and logistic regression	[33]
Pregnancy Per- and Polyfluoroalkyl Substance Concentratio	Associations between PFAS plasma concentrations during	Pregnancy concentrations of certain PFAS were associated with greater	Prospective cohort study, multivariable regression analysis	Human (pregnant women and	Plasma PFAS quantification using online solid-phase extraction HPLC-	[21]

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ns and Postpartum Health in Project Viva: A Prospective Cohort	pregnancy and postpartum anthropometry, blood pressure, and blood biomarkers	adiposity, higher systolic blood pressure, and adverse changes in blood biomarkers at 3 years postpartum.		postpartum women)	MS/MS, measurement of anthropometric data, blood pressure, and blood biomarkers	
Exposure to Per- and Polyfluoroalkyl Substances and Adiposity at Age 12 Years: Evaluating Periods of Susceptibility	Assessing the associations of repeated pre- and postnatal serum PFAS concentrations with adolescent adiposity and risk of overweight/obesity	Serum PFOA and PFHxS concentrations during pregnancy were associated with modest increases in central adiposity and risk of overweight/obesity, with no consistent pattern for postnatal concentrations.	Longitudinal cohort study, multiple informant models, generalized estimating equations	Human (mother-offspring pairs)	Serum PFAS quantification using online solid-phase extraction HPLC-MS/MS, anthropometry, dual-energy X-ray absorptiometry	[18]
Early-life exposure to perfluoroalkyl substances in relation to serum adipokines in a longitudinal birth cohort	Assessing the relationship between early-life PFAS exposure and serum adipokine concentrations in children	Significant associations between PFAS exposure at 18 months and 5 and 9 years with changes in leptin, leptin receptor, and resistin levels at age 9. No significant association for PFAS exposure at birth.	Longitudinal cohort study, multivariable linear regression models, Bayesian kernel machine regression (BKMR)	Human (mother-child pairs)	Serum PFAS quantification using online solid-phase extraction HPLC-MS/MS, serum adipokine measurements using ELISA kits	[48]
Prenatal exposure to perfluoroalkyl substances modulates neonatal serum phospholipids, increasing risk of type 1 diabetes	The study examines the impact of prenatal exposure to perfluoroalkyl substances (PFAS) on neonatal serum phospholipids and the subsequent risk of developing	- High PFAS exposure during pregnancy is associated with decreased cord serum phospholipids. PFAS exposure correlates with progression to T1D-associated islet	- PFAS levels and metabolomic profiles were determined from pregnant mothers and newborn infants' cord serum. A combination of cohort studies (EDIA and DIABIMMUNE) and mouse	Human (mother-infant cohorts) and Non-obese diabetic (NOD) mice	- Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) Lipidomics and bile acid profiling Clustering and correlation analysis using R	[49]

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	type 1 diabetes (T1D).	autoantibodies in offspring. Similar lipid profile changes were observed in both human and non-obese diabetic (NOD) mice models.	models were used to validate findings. Techniques included ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for PFAS analysis, and lipidomic and bile acid profiling.		statistical programming	
Exposure to Perfluoroalkyl Substances and Glucose Homeostasis in Youth	Examines the associations between exposure to per- and polyfluoroalkyl substances (PFAS) and glucose metabolism in overweight/obese youth.	- High PFHxS levels in females associated with dysregulated glucose metabolism beginning in late puberty. PFHxS exposure associated with 25-mg/dL higher 60-min glucose and 25% lower b-cell function postpuberty in females. No consistent associations observed in males or with other PFAS.	- Longitudinal cohort study with annual visits. OGTT performed to estimate glucose metabolism and b-cell function. PFAS measured using liquid chromatography-high-resolution mass spectrometry (LC-HRMS).	Overweight/obese adolescents and young adults	- Oral Glucose Tolerance Test (OGTT) Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) Linear mixed effects models and linear regression models Sensitivity analysis	[50]
A Review of the Pathways of Human Exposure to Poly- and Perfluoroalkyl Substances (PFASs) and Present Understanding of Health Effects	Serum concentrations of legacy PFASs in humans are declining globally.	- More than 4,000 PFAS chemicals have been manufactured, with hundreds detected in the environment. - Serum levels of legacy PFAS are declining globally, but exposures to newer PFAS	The study is a review of existing research on sources, trends, and health effects of PFAS exposure, including epidemiologic evidence from multiple studies.	humans		[51]

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		compounds are not well characterized. - Significant associations have been found between PFAS exposure and adverse immune outcomes in children, as well as dyslipidemia. - Evidence for cancer and neurodevelopmental impacts is limited, but preliminary evidence suggests significant health effects from emerging PFAS chemicals.				
Invited Perspective: PFAS and the Childhood Obesity Phenotype—Challenges and Opportunities	Per- and polyfluoroalkyl substances are a group of manmade chemicals.	- Higher prenatal exposure to PFAS was associated with a slightly higher risk of overweight or obesity in children aged 2-5 years. - The association was not sex-specific, meaning it was similar in boys and girls. - The prevalence of overweight/obesity in the study population was around 20%, which is	- Data source: Environmental influences on Child Health Outcomes (ECHO) consortium - Exposure assessment: Maternal serum or plasma concentrations of PFAS - Study design: Examination of associations between prenatal PFAS exposure and childhood obesity - Data pooling: Eight prospective	humans	- Maternal serum or plasma concentrations to assess prenatal exposure to PFAS - Body Mass Index (BMI) to define overweight/obesity	[52]

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		worryingly high and consistent with previous estimates in US and European children.	cohorts from various U.S. locations - Outcome measurement: Body mass index (BMI) defined as ≥85th percentile for age and sex - Specific chemicals assessed: Seven long-chain PFAS, including PFOS and PFOA			
Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research	The absence of toxicity data for PFAS is a concern.	- Epidemiological studies have found associations between PFAS exposure and various health effects, including immune, thyroid, liver, metabolic, reproductive, and developmental issues, as well as cancer. - These findings are supported by concordant data from experimental animal studies. - More advanced approaches are needed to accelerate the development of toxicity information for the many PFAS lacking data.	- Review of existing literature on toxicological effects of PFAS - Assessment of epidemiological studies revealing associations between PFAS exposure and health effects - Concordance with experimental animal data - Proposal of contemporary and high-throughput approaches (read-across, molecular dynamics, protein modeling) to accelerate toxicity information development	humans, various animals	- Epidemiological studies - Experimental animal studies - Read-across - Molecular dynamics - Protein modeling	[53] [17]

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		- An appropriate degree of precaution may be needed to protect human health given the known health effects of some PFAS.				
PFAS exposure and overweight/obesity among children in a nationally representative sample.	Perfluoroalkyl substances are associated with intermediate cardiovascular disease outcomes among children.	- There is an association between higher levels of PFOA exposure and increased risk of overweight/obesity in children. - Higher quartiles of PFOA exposure were associated with higher odds ratios for overweight/obese BMI z-score.	- Aim: Explore the relationship between PFASs and overweight/obesity and abdominal obesity among children. - Sample: 2473 US children aged 12-18 years from NHANES 1999-2012. - Measures: PFOA and PFOS levels, BMI, and waist circumference. - Definitions: Overweight/obesity (BMI z-score ≥ 85th percentile), abdominal obesity (waist circumference ≥90th percentile). - Analysis: Dose-response relationships and multivariable adjustments to determine associations.	humans	- Statistical analysis of associations - Anthropometric measurements (BMI, waist circumference) - Use of standardized growth charts or reference data - Multivariable adjustment techniques - Data from the National Health and Nutrition Examination Survey (NHANES)	[54]
Exposure to perfluoroalkyl and polyfluoroalky	Prenatal exposures to four different types of PFAS were not	- There was no evidence of a positive association	- Systematic review to synthesize literature and	humans	- Systematic review - Database search	[55]

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l substances and pediatric obesity: a systematic review and meta-analysis	statistically associated with changes in body mass index or waist circumference.	between prenatal PFAS exposure and pediatric obesity. - Postnatal exposure to certain PFAS chemicals was inversely associated with changes in BMI in children. - The findings should be interpreted cautiously due to the small number of studies.	explore heterogeneity - Searched six databases for relevant studies - Included studies with individual-level PFAS and anthropometric data from children up to 12 years old - Excluded studies evaluating obesity measures at birth - Full-text review and quality assessment using OHAT criteria - Created forest plots to summarize measures of association and assess heterogeneity - Used funnel plots to assess small-study effects - Identified 24 studies, 19 with cohort design, and included 13 in the meta-analysis		- Full-text review - Quality assessment using OHAT criteria - Forest plots - Funnel plots - Trim and Fill method	
Assessing the human health risks of perfluorooctane sulfonate by in vivo and	Exposure to PFOS has caused hepatotoxicity, neurotoxicity, reproductive toxicity,	- Exposure to PFOS has been shown to cause a variety of toxic effects in laboratory	- Systematic review of in vivo and in vitro studies from 2008 to 2018	laboratory animals, human cell systems	- In vivo studies - In vitro studies	[56]

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in vitro studies.	immunotoxicity, thyroid disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity in laboratory animals and many in vitro human systems.	animals and human cell systems, including hepatotoxicity, neurotoxicity, reproductive toxicity, immunotoxicity, thyroid disruption, cardiovascular toxicity, pulmonary toxicity, and renal toxicity. - These findings, along with related epidemiological studies, confirm the human health risks of PFOS, especially from exposure through food and drinking water. - The main mechanisms of PFOS toxicity that have been widely studied are oxidative stress and disruption of physiological processes due to the similarity of PFOS to fatty acids.	- Analysis of epidemiological studies		
Prenatal Exposure to Perfluorooctanoate and Risk of Overweight at 20 Years of Age: A	Low-dose developmental exposure to PFOA was positively associated with anthropometry at 20 years in female offspring.	- In utero exposure to PFOA was positively associated with overweight and high waist circumference in female offspring	- Prospective cohort study with 665 pregnant women recruited in 1988-1989. - PFOA measured in	humans	- Measurement of PFOA in serum samples - Recording of BMI and waist circumference - Collection and	[57]

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Prospective Cohort Study		at 20 years of age. - Maternal PFOA concentrations were positively associated with biomarkers of adiposity (insulin, leptin, leptin-adiponectin ratio) in female offspring. - The findings support the hypothesis that early-life exposure to endocrine disruptors, even at low concentrations, may contribute to the obesity epidemic.	maternal serum at gestational week 30. - Offspring follow-up at 20 years for BMI, waist circumference, and adiposity biomarkers. - Data collection included interviews, blood samples, and health records. - Follow-up involved web-based questionnaires and clinical exams. - Statistical analyses: linear regression for continuous outcomes, log-Poisson regression for dichotomous outcomes. - Adjustments for maternal age, education, smoking status, pre-pregnancy BMI, parity, infant birth weight, and offspring age. - Log-transformation of adiposity biomarkers due to skewed distributions.	processing of blood samples (separation into serum, plasma, erythrocytes; freezing) - Time-resolved immunofluorometric assay for adiponectin and leptin - Commercial Insulin ELISA kit for plasma insulin - Linear regression for continuous outcomes - Log-Poisson regression for dichotomous outcomes - Division of maternal PFOA concentrations into quartiles for trend analysis

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Prenatal Perfluoroalkyl Substance Exposure and Child Adiposity at 8 Years of Age: The HOME Study	Prenatal perfluoroalkyl substance exposure and adiposity in children born to women who lived downstream from a fluoropolymer manufacturing plant.			humans	[58]
Perfluoroalkyl and Polyfluoroalkyl Substances and Body Size and Composition Trajectories in Midlife Women: The Study of Women's Health Across the Nation 1999–2018	Certain PFAS were positively associated with greater body size and body fat.	- Higher concentrations of certain PFAS (PFOS, linear PFOA, EtFOSAA, MeFOSAA, PFHxS) were associated with greater body size and body fat at baseline and faster increases in body size and body fat over time in midlife women. - No significant associations were found between PFNA and body size or composition.	- Examined associations of serum PFAS concentrations with body size and composition trajectories. - Included 1,381 midlife women with 15,000 repeated measures. - Follow-up period averaged 14.9 years (range: 0-18.6 years). - Body size and composition assessed using objective measurements and dual-energy X-ray absorptiometry. - Near-annual visits for assessments. - Used linear mixed models with piecewise linear splines to model non-linear trajectories.	humans	- Measurement of serum PFAS concentrations - Objective measurement of weight - Objective measurement of waist circumference (WC) - Dual-energy X-ray absorptiometry (DXA) for body composition - Linear mixed models with piecewise linear splines for data analysis	[59]

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			- Multivariable adjustments made for potential confounders.			
Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: A systematic review	Higher PFOS and PFOA concentrations were associated with decreased average birth weight in most studies.	- Higher PFOS and PFOA concentrations were associated with decreased average birth weight in most studies, but only some results were statistically significant. - The impact on public health is unclear, but the global exposure to PFASs warrants further investigation.	- Systematic literature searches in MEDLINE and EMBASE - Inclusion of original studies on pregnant women with measurements of PFOA or PFOS in maternal blood or umbilical cord - Investigation of citations and references from included articles to find more relevant studies - Extraction of study characteristics and results into structured tables - Assessment of completeness of reporting, risk of bias, and confounding	humans	- Systematic literature searches in MEDLINE and EMBASE - Measurement of PFOA or PFOS in maternal blood or umbilical cord - Investigation of citations and references from included articles - Extraction of study characteristics and results to structured tables - Assessment of completeness of reporting, risk of bias, and confounding	[59,60]
The Role of Persistent Organic Pollutants in Obesity: A Review of Laboratory and Epidemiological Studies	Persistent organic pollutants are potential obesogens that may affect adipose tissue development and functioning, thus promoting obesity.	- Laboratory data demonstrate that POPs can contribute to obesity through mechanisms like dysregulation of adipogenesis regulators, affinity for nuclear receptors, epigenetic	- Review of existing laboratory data - Review of in vivo studies - Review of epidemiological data - Discussion of mechanisms linking POPs to	humans	- In vitro assays for dysregulation of adipogenesis regulators (PPARγ and C/EBPα) - Receptor binding assays - Epigenetic profiling techniques	[61]

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		effects, and proinflammatory activity. - In vivo studies show the impact of POPs on adipogenesis is affected by factors like sex, age, and exposure duration. - Epidemiological data show a significant association between POP exposure and obesity, as well as obesity-related metabolic disturbances, though more research is needed.	adipose tissue dysfunction and obesity	- Inflammation assays - In vivo studies in living organisms - Epidemiological studies	
Association of Perfluoroalkyl and Polyfluoroalkyl Substances With Adiposity	A higher plasma PFAS concentration was associated with increases in weight and hip girth over time.	- Higher plasma PFAS concentrations were associated with increases in weight and hip girth over time, but this association was attenuated in the group that received a lifestyle intervention of diet and exercise. - The authors suggest that a lifestyle intervention of diet and exercise can mitigate the	- Prospective cohort study with 957 participants from the Diabetes Prevention Program (DPP) and its follow-up study (DPPOS). - Participants randomized into pharmacologic intervention (metformin), placebo, or lifestyle intervention groups. - Lifestyle intervention included training	humans	- Online solid-phase extraction-high-performance liquid chromatography-isotope dilution-tandem mass spectrometry - Calibrated balance scale for weight measurement - Tape measure for waist circumference and hip girth - Lange skinfold calipers for skinfold	[62]

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		obesogenic effects of environmental chemicals like PFASs.	in diet, physical activity, and behavior modification. - Plasma concentrations of six PFASs measured at baseline and two years after randomization. - Weight, waist circumference, and hip girth measured at baseline and scheduled visits. - Blood samples analyzed using high-performance liquid chromatography-isotope dilution-tandem mass spectrometry. - Statistical analyses included adjusted linear regression models for cross-sectional associations and longitudinal mixed-effects regression models for prospective associations.	thickness - Computed tomography for visceral and subcutaneous fat - Adjusted linear regression models - Longitudinal mixed-effects regression models		
Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A	Exposures to some PFAS in utero are associated with adverse outcomes for both mother and offspring.	- PFAS exposure is associated with adverse health outcomes, including reduced kidney function, metabolic	- Review of existing literature - Synthesis of evidence on PFAS effects on thyroid function,	humans	a review	[63]

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review including the placenta as a target tissue and possible driver of peri- and postnatal effects.		syndrome, thyroid disruption, and adverse pregnancy outcomes. - Exposure to PFAS during pregnancy is linked to hypertensive disorders of pregnancy (HDP), preeclampsia, and low birth weight in offspring. - The placenta is an understudied target of PFAS exposure, and placental dysfunction may contribute to the relationship between PFAS exposure and increased risk of chronic diseases in adulthood.	kidney disease, and metabolic syndrome - Emphasis on the placenta as a target tissue and programming agent of adult disease		
Early-life perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) exposure cause obesity by disrupting fatty acids metabolism and enhancing triglyceride synthesis in Caenorhabditis elegans.	Low concentrations of PFOA and PFOS induced obesity in Caenorhabditis elegans.	- Low concentrations of PFOA and PFOS (0.1 and 1 μM) induced obesity in C. elegans, which was not due to increased feeding rate. - PFOA and PFOS exposure altered the fatty acid composition, decreasing saturated fatty acids and	- Used Caenorhabditis elegans as an in vivo model. - Investigated lipid accumulation, feeding behaviors, fatty acids composition, and genetic regulation. - Exposed C. elegans to low concentrations of PFOA and PFOS	nematode (Caenorhabditis elegans)	- Use of Caenorhabditis elegans as an in vivo model - Chemical exposure experiments with PFOA and PFOS - Analysis of fatty acid composition - Mutant assays - Gene expression analysis (e.g.,	[64]

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		increasing polyunsaturated fatty acids. - Genes related to fatty acid desaturation (mdt-15, nhr-49, fat-6) and fatty acid/triglyceride synthesis (fasn-1, dgat-2) were associated with the increased body fat, triglycerides, and lipid droplet content in C. elegans exposed to PFOA and PFOS.	(0.1 and 1 μM). - Conducted mutant assay and mRNA levels analysis to study genetic regulation.		quantitative PCR)	
Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood	Prenatal exposure to perfluoroalkyl substances was associated with small increases in adiposity measurements in mid-childhood.	- Prenatal exposure to perfluoroalkyl substances (PFASs) was associated with small increases in adiposity measurements in mid-childhood, but only among girls. - No associations were found between prenatal PFAS exposure and early-childhood adiposity measures, or for boys.	- Measured plasma PFAS concentrations in 1,645 pregnant women at median 9.6 weeks gestation. - Assessed overall and central adiposity in children at median ages 3.2 years (early childhood) and 7.7 years (mid-childhood) using anthropometric and DXA measurements. - Fitted multivariable linear regression models to estimate exposure-outcome associations and evaluated effect	humans	- Plasma analysis for PFAS concentrations - Anthropometric measurements - Dual X-ray absorptiometry (DXA) - Multivariable linear regression models	[65]

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			modification by child sex.			
Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: Low doses induce elevated serum leptin and insulin, and overweight in mid-life	Low-dose effects of PFOA on body weight gain, as well as leptin and insulin concentrations in mid-life are important to explore.	- Low doses of PFOA (0.01-0.3 mg/kg) during development increased body weight, serum insulin, and serum leptin in mid-life in female CD-1 mice. - The effects of in utero PFOA exposure on body weight were no longer detected at 18 months of age. - High doses of PFOA decreased white adipose tissue and spleen weights, but increased brown adipose tissue weight, in both intact and ovariectomized mice.	- Study subjects: CD-1 mice - Exposure scenarios: (1) in utero exposure, (2) in utero exposure followed by ovariectomy (ovx), (3) adult exposure - Exposure duration: 17 days during pregnancy or as young adults - PFOA doses: 0, 0.01, 0.1, 0.3, 1, 3, or 5mg PFOA/kg BW - Measurements: body weight (postnatal day 1, weaning, mid-life, late life), serum insulin and leptin levels, weights of white adipose tissue, spleen, brown adipose tissue, and liver	mice	- Exposure to various doses of PFOA - Measurement of body weight at specific time points (postnatal day 1, weaning, mid-life) - Measurement of serum insulin and leptin levels - Ovariectomy (ovx) - Measurement of white adipose tissue weight - Measurement of spleen weight - Measurement of brown adipose tissue weight - Measurement of liver weight	[66]
Diet as an Exposure Source and Mediator of Per- and Polyfluoroalkyl Substance (PFAS) Toxicity	Western diets enriched in high fat and high cholesterol containing foods may be an important human exposure route of PFAS.	- PFAS exposure is associated with a range of health effects in both animals and humans, including hyperlipidemia and fatty liver disease. - There are inconsistencies between animal	The methodology involves reviewing existing literature to outline dietary exposure sources of PFAS, describe associated metabolic health effects, and examine studies	mice, rats, monkeys	- Oral gavage - Dietary exposure - Serum concentration measurement - Plasma lipid analysis - Hepatic histology	[8]

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		and human studies on the effects of PFAS on lipid metabolism and cardiometabolic profiles. - More research is needed using human-relevant animal models and on the toxicity of emerging PFAS, as well as the dietary modulation of PFAS toxicity.	on dietary interactions with PFAS exposure. The review includes data from epidemiological studies, animal studies, and regulatory agencies.		- Gene expression analysis - Use of genetically engineered animal models	
Effect of Per and Poly-Fluoroalkyl Substances on Pregnancy and Child Development.	PFAS exposure occurs through the Peroxisome Proliferator-Activated Receptor, leading to increased fat deposition and profound health effects in child growth and development.	- PFAS exposure during pregnancy disrupts placental health and breastfeeding, leading to impaired child growth and development. - PFAS exposure increases adipocyte number, alters lipid metabolism, and leads to increased adiposity and weight gain through activation of PPAR-γ and ER-α. - PFAS concentrations are positively correlated in maternal serum.	- Detailed literature survey using online databases (Science Direct, Google Scholar, Scopus, Cochrane, PubMed) - Focus on effects of PFAS on maternal and child health, particularly neurological complications - Neurotoxicity testing using SH-SY5Y human-derived cell line (in vitro model) - In vivo studies in mice and human cell lines to investigate PPAR-γ and ER-α activation - Analysis of PFAS	humans, mice	- SH-SY5Y human-derived cell line (in vitro model) - In vivo studies in mice - Human cell lines - Liquid chromatography/ quadrupole mass spectrometry	[67]

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			concentrations in maternal sera using liquid chromatography/ quadrupole mass spectrometry			
Halogenated bisphenol-A analogs act as obesogens in zebrafish larvae (Danio rerio).	Halogenated bisphenol-A analogs induced lipid accumulation in zebrafish larvae.	- Halogenated BPA analogs like TBBPA and TCBPA are rapidly absorbed and metabolized by zebrafish, primarily through sulfation. - TBBPA and TCBPA act as agonists for both human and zebrafish PPAR-gamma, a key regulator of adipogenesis. - Exposure to TBBPA, TCBPA, and TBT during early zebrafish development leads to increased body mass index (BMI) in juvenile zebrafish at 1 month of age.	- Zebrafish larvae were used as an in vivo model. - Embryonic exposure to TBBPA and TCBPA was analyzed for lipid accumulation using Oil Red-O staining. - Activation of human and zebrafish PPARγ was assessed in zebrafish and reporter cell lines. - Metabolic fate of TBBPA and TCBPA was analyzed using high-performance liquid chromatography (HPLC). - Zebrafish larvae were housed under controlled conditions and exposed to chemicals dissolved in DMSO. - GFP expression was quantified in transgenic	zebrafish (Danio rerio)	- Oil Red-O staining - High-performance liquid chromatography (HPLC) - Use of transgenic zebrafish (Tg(hPPARγ-eGFP)) - Reporter cell lines stably transfected with PPARγ-LBD - Luminescence measurement using a plate reader - GFP quantification using a plate reader - 3D microscopy live imaging using Nikon AZ100M microscope - Solid-phase extraction (SPE) - Washing and staining of fixed larvae with Oil Red-O solution - Calculation of	[68]

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			zebrafish embryos to assess PPARγ activation. - Larvae were fed an egg yolk diet and treated with chemicals daily until 11 days post-fertilization (dpf). - Lipid accumulation was assessed by Oil Red-O staining, and larvae were imaged using microscopy. - Weight and length of juvenile zebrafish were recorded at 30 days post-fertilization (dpf) to calculate BMI.	BMI as weight/(length)²		
Per- and polyfluoroalkyl substances and obesity, type 2 diabetes and non-alcoholic fatty liver disease: a review of epidemiologic findings	Causal links between per- and polyfluoroalkyl substances and obesity, diabetes, and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis require further large-scale prospective cohort studies combined with mechanistic laboratory studies to better assess these associations.	- There is a growing body of literature linking per- and polyfluoroalkyl substances (PFAS) exposure to obesity, type 2 diabetes, and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis. - Approximately two-thirds of studies found positive associations between PFAS exposure and the prevalence of	- Review of existing literature - Search of PubMed for human studies on obesity, diabetes, and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis - Summary of historical use, chemistry, routes of exposure, and epidemiologic evidence	humans		[69]

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		obesity and/or type 2 diabetes. - More research is needed to establish causal links between PFAS and these health outcomes.			
A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens	Microplastic exposure in laboratory animals is linked to various forms of inflammation, immunological response, endocrine disruption, alteration of lipid and energy metabolism, and other disorders.	- Microplastics are ubiquitous in the environment and human food chain, leading to widespread human exposure. - The increase in global obesity over the past 5 decades coincides with the rise in plastics production and use. - The authors hypothesize that exposure to microplastics and plastic additives (obesogens) may be contributing to the global obesity pandemic.	- Compilation of data from various studies on MP concentrations in air, dust, drinking water, food, and beverages. - Use of spectroscopy-based methods (FTIR, Raman, X-ray photoelectron spectroscopy, energy dispersive x-ray spectroscopy, scanning electron microscopy) for identification and quantification. - Biomonitoring studies to provide direct evidence of MP exposure in humans. - Analysis of human and pet animal stool specimens for MP content. - Measurement of MP concentrations in human tissues	humans, dogs, cats	- FTIR (Fourier-transform infrared spectroscopy) - Raman spectroscopy - X-ray photoelectron spectroscopy - Energy dispersive X-ray spectroscopy - Scanning electron microscopy - Biomonitoring studies (analysis of human tissues and stool)	[70]

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			such as lungs and placenta.			
Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study	Higher baseline plasma perfluoroalkyl substance concentrations were associated with a greater weight regain, especially in women.	- Higher baseline plasma PFAS concentrations were significantly associated with greater weight regain, especially in women. - Higher baseline plasma PFAS concentrations, particularly PFOS and PFNA, were significantly associated with a greater decline in resting metabolic rate during weight loss and a smaller increase in resting metabolic rate during weight regain.	- Prospective analysis within the POUNDS Lost randomized clinical trial. - Participants: 621 overweight and obese individuals aged 30-70 years. - Intervention: Four energy-reduced diets designed to induce weight loss. - Measurements: Baseline plasma concentrations of major PFASs; body weight at baseline, 6, 12, 18, and 24 months; RMR and other metabolic parameters at baseline, 6 months, and 24 months. - Statistical analysis: Linear regression to examine associations between baseline PFAS levels and changes in body weight and RMR.	humans	- Body weight measurement - Resting metabolic rate (RMR) assessment using Deltatrac II Metabolic Monitor - Dual energy X-ray absorptiometry (DXA) for body fat mass and lean mass - Computed tomography (CT) scanner for visceral and subcutaneous abdominal fat - Online solid phase extraction and liquid chromatography coupled to a triple quadropole mass spectrometer for PFAS concentrations - Synchron CX7 and CX5 systems for glucose, insulin, cholesterol, and HbA1c - Ultrasensitive immunoassay for plasma leptin and soluble leptin receptor - Competitive	[71]

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					electrochemilumi nescence immunoassay for thyroid hormones - Direct hybridization using Illumina HumanHT-12 v3 Expression BeadChip for gene expression - Baecke physical activity questionnaire for physical activity assessment	
Perfluoroocta nesulfonic acid (PFOS) and perfluorohexa nesulfonic acid (PFHxS) alter the blood lipidome and the hepatic proteome in a murine model of diet-induced obesity.	Perfluorooctanes ulfonic acid and perfluorohexane sulfonic acid increase the risk of metabolic and inflammatory disease induced by diet.	- PFOS and PFHxS increased the expression of genes involved in lipid metabolism and oxidative stress in the liver of mice fed a high-fat, high-carbohydrate diet. - PFOS and PFHxS altered the blood lipidome, changing the levels of various lipid species, including phosphatidylcho lines, phosphatidyleth anolamines, plasmogens, sphingomyelins, and triglycerides. - PFOS and PFHxS led to an increase in	- Male C57BL/6J mice were used. - Mice were fed either a low-fat diet or a high fat high carbohydrate (HFHC) diet. - PFOS or PFHxS were included in the feed at 0.0003% w/w for 29 weeks. - Lipidomic, proteomic, and gene expression profiles were determined. - Effects on lipid metabolism and oxidative stress were measured in the liver and blood.	mice	- Lipidomic profiling - Proteomic profiling - Gene expression profiling	[72]

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		oxidized lipid species in the blood lipidome of mice fed a high-fat, high-carbohydrate diet.				
Associations of Prenatal Per- and Polyfluoroalkyl Substance (PFAS) Exposures with Offspring Adiposity and Body Composition at 16–20 Years of Age: Project Viva	Higher prenatal PFAS concentrations were associated with higher obesity risk in late adolescence.	- Higher prenatal PFAS exposures, particularly PFOS, PFOA, and PFNA, were associated with increased risk of obesity in late adolescence. - There was an interaction between PFOA and PFOS, where the positive association between PFOS and obesity was stronger when PFOA levels were lower. - The PFAS mixture as a whole was associated with increased obesity risk and higher BMI. - Children with higher prenatal PFOS, EtFOSAA, and MeFOSAA had higher rates of BMI increase starting from 9-11 years of age.	- Studied 545 mother–child pairs from Project Viva cohort. - Measured six PFAS in maternal early pregnancy plasma samples. - Assessed anthropometric measures and body composition in late adolescence. - Used bioelectrical impedance analysis and dual-energy X-ray absorptiometry for body composition. - Analyzed associations with obesity/adiposity using multivariable Poisson and linear regression models. - Evaluated PFAS mixture effects using Bayesian kernel machine regression and quantile g-	humans	- Measurement of PFAS in maternal plasma samples - Bioelectrical impedance analysis - Dual-energy X-ray absorptiometry - Multivariable Poisson regression models - Linear regression models - Bayesian kernel machine regression (BKMR) - Quantile g-computation - Fractional-polynomial models	[73]

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			computation. - Assessed BMI trajectories using fractional-polynomial models.			
Exposure to Perfluoroalkyl Chemicals and Cardiovascular Disease: Experimental and Epidemiological Evidence	Legacy and new PFAS can be incorporated in platelet cell membranes giving a solid rationale to the observed increase risk of cardiovascular events in the populations exposed to PFAS by directly promoting thrombus formation.	- Exposure to PFAS may increase the risk of cardiovascular disease through worsening of cardiovascular risk factors and a direct prothrombotic effect on platelets. - Mechanistic studies suggest PFAS can accumulate in platelet membranes and alter their function, leading to increased platelet activation and thrombus formation. - These platelet-mediated effects may help explain the observed increase in cardiovascular events in PFAS-exposed populations.	- Review of epidemiological studies on PFAS exposure and cardiovascular disease. - Selection criteria for studies: sample size, study design (longitudinal preferred), intensity of exposure. - Analysis of mechanistic studies on PFAS incorporation in platelet membranes and thrombus formation. - Summarized data in tables on clinical, epidemiological, and experimental studies.	humans	- Liquid chromatography/ mass-mass spectrometry (LC-MS/MS) - Thrombin receptor activator peptide 6 (TRAP-6) stimulation - Microfluidic biochip pre-coated with collagen - Measurement of large microvesicles expressing C41 and binding annexin V - Bilayer fluidity-sensitive probe Merocyanin 540 - Platelet aggregation under flow conditions with/without acetylsalicylic acid	[74]
Per/poly fluoroalkyl substances induce lipid accumulation via the serotonergic	Perfluorononanoic acid, perfluorooctanesulfonamide, and perfluorooctane sulfonate promote fat	- Exposure to PFNA, PFOSA, and PFOS significantly increased lipid accumulation in C. elegans, with	- Model organism: Caenorhabditis elegans - Exposure concentration: 1	Caenorhabditis elegans (C. elegans)	- Use of Caenorhabditis elegans as a model organism - Bodipy 493/503 staining	[75]

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signaling pathway in	accumulation in Caenorhabditis elegans.	PFNA showing the highest level of lipid accumulation. - PFNA, PFOSA, and PFOS downregulated the expression of genes involved in serotonin production and beta-oxidation, and upregulated the expression of a gene involved in triacylglycerol synthesis. - The study demonstrates that PFNA, PFOSA, and PFOS promote fat accumulation through the serotonin-involved pathway and lipogenesis, leading to an obesogenic effect.	μM PFNA, PFOSA, and PFOS - Lipid accumulation measurement: bodipy 493/503 and nile red staining methods - Food intake measurement: pharyngeal pumping rate - Gene expression evaluation: tph-1, mod-1, nhr-76, atgl-1, and dgat-2		- Nile red staining - Measurement of pharyngeal pumping rate - Gene expression analysis	
Do perfluoroalkyl substances aggravate the occurrence of obesity-associated glucolipid metabolic disease?	Perfluoroalkyl substances are aggravating the occurrence of obesity-associated glucolipid metabolic disease.	- Both obesity and PFASs exposure can independently cause disruptions in glucose and lipid metabolism. - Obesity is a crucial factor that increases the incidence of GLMD induced by PFASs. - PFASs are exacerbating the	- Summarized epidemiological studies on PFASs and obesity-related GLMD - Reviewed relevant experimental evidence - Proposed three research programs to explore the synergistic mechanism of PFASs and obesity	humans	- Epidemiological surveys Experimental studies on animal models Statistical analysis of literature data	[76]

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		development of obesity-associated GLMD, such as diabetes, cardiovascular disease, and liver disease.	- Recommended three suggestions to mitigate the harm of PFASs pollutants to humans			
Reduced Birth Weight and Exposure to Per- and Polyfluoroalkyl Substances: A Review of Possible Underlying Mechanisms Using the AOP-HelpFinder	Prenatal exposure to per- and polyfluorinated substances may impair fetal growth.	- PFAS are associated with oxidative stress, which triggers increased PPARγ expression and activation of growth signaling pathways, leading to hyperdifferentiation of pre-adipocytes and reduced adipose tissue weight, which may reduce birth weight. - PFAS may also impair fetal growth through endocrine effects, including estrogenic effects and thyroid-damaging effects that are associated with decreased body and organ weight in animal studies.	- Used the Adverse Outcome Pathway (AOP)-helpFinder tool to search PubMed - Focused on studies examining PFAS exposure in relation to birth weight, oxidative stress, hormones/hormone receptors, or growth signaling pathways - Initial search yielded 1880 articles - Screened down to 106 experimental studies after abstract screening	animals	- In vivo animal studies - In vitro studies - Measurement of reactive-oxygen species (ROS) generation - Measurement of peroxisome proliferator-activated receptor (PPAR)γ expression - Assays for hormone levels - Gene expression analysis related to thyroid function	[77]
Association between gestational PFAS exposure and Children's adiposity in a diverse population.	Perfluoroundecanoic acid was associated with their children having higher waist circumference z-score.	- There were more non-Hispanic Black and Hispanic children with overweight/obesity compared to non-Hispanic white and	- Estimated associations between gestational PFAS concentrations and childhood adiposity. - Measured six	humans	- Ultra-high-performance liquid chromatography with tandem mass spectrometry - Body mass	[5,78]

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		Asian/Pacific Islander children. - Among women without obesity, higher levels of perfluoroundecanoic acid (PFUnDA) were associated with their children having higher waist circumference, fat mass, and body fat percentage. - The associations between PFAS and children's adiposity varied significantly by maternal race-ethnicity, although the direction of the associations was inconsistent. - Among children of women with obesity, higher levels of PFOS, perfluorononanoic acid, and perfluorodecanoic acid were associated with less adiposity.	PFAS in first trimester blood plasma using ultra-high-performance liquid chromatography with tandem mass spectrometry. - Sample: non-smoking women with low-risk singleton pregnancies (n = 803). - Adiposity measures in children aged 4-8 years: BMI, waist circumference, fat mass, fat-free mass, % body fat. - Adjusted for confounders.		index (BMI) - Waist circumference (WC) - Fat mass - Fat-free mass - % body fat	
Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General	Polyfluoroalkyl chemicals are used commonly in commercial applications and are detected in humans and the environment worldwide.	- Serum concentrations of PFOS, PFOA, and PFNA were positively associated with total cholesterol and non-HDL cholesterol levels	- Data source: 2003–2004 NHANES - Participants: 12–80 years old - Sampling design: Complex	humans	- Linear regression - Automated solid-phase extraction coupled to isotope dilution/high-	[79]

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U.S. Population	in the general U.S. population. - Serum concentrations of PFHxS were negatively associated with total cholesterol and non-HDL cholesterol levels, in contrast to the other PFCs studied. - The association between PFNA and cholesterol levels was the strongest and most consistent, despite lower serum concentrations of PFNA compared to PFOS and PFOA.	multistage probability sampling - Measurements: Blood and urine samples at a mobile examination center - PFC measurement: Automated solid-phase extraction coupled to isotope dilution/high-performance liquid chromatography/ tandem mass spectrometry - Analysis: Linear regression controlling for covariates - Outcomes: Cholesterol, body size, insulin resistance - Exposure modeling: Quartiles of PFC concentration - Statistical software: SAS version 9.1 Proc SURVEYREG - Adjustments: Relevant covariates instead of NHANES sampling weights	performance liquid chromatography/ tandem mass spectrometry - Enzymatic measurement of total cholesterol (TC) and high-density lipoprotein (HDL) - Calculation of non-HDL cholesterol - Estimation of low-density lipoprotein (LDL) using the Friedewald formula - Homeostatic model assessment (HOMA) method - Enzymatic measurement of plasma insulin and glucose - SAS version 9.1 Proc SURVEYREG procedure for statistical analysis - Identification and exclusion of influential points and outliers using studentized residuals, predicted values, and scatter plots

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Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts Scientific Opinion of the Panel on Contaminants in the Food chain.	Perfluorochemicals in residents of the United States in 2001 through 2002.			mice, rats, cynomolgus monkeys	- High-Performance Liquid Chromatography (HPLC) Electrospray Mass Spectrometry - Liquid Chromatography coupled to High-Resolution Mass Spectrometry	[80]
PFAS health effects database: Protocol for a systematic evidence map.	Regulators, scientists, and citizens need to stay informed on the growing health and toxicology literature related to PFAS.	- The goal of this study is to identify and organize the available literature on the health and toxicological effects of 29 PFAS of emerging concern. - The study will search the PubMed database for primary research studies investigating the link between PFAS and health effects, toxicology, or biological mechanisms. - The extracted and coded information from the included studies will be visualized in a publicly available, interactive database, and	- Search PubMed for health or toxicological studies on 29 PFAS of emerging concern. - Include studies with primary research linking PFAS to health, toxicological, or biological endpoints. - Title and abstract screening by a single reviewer for inclusion; two independent reviewers for exclusion. - No study quality assessment. - Extract and code study characteristics, checked by a second reviewer. - Visualize data in a publicly available,	humans	- Literature search in PubMed - Title and abstract screening - Full text review - Data extraction and coding - Data visualization using Tableau Public	[81]

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		the results will be published in a narrative summary.	interactive database on Tableau Public. - Publish results in a narrative summary.			
Prenatal Per- and Polyfluoroalkyl Substance (PFAS) Exposures, Individually and as a Mixture, Are Associated With Obesity Risk at 16-20 Years in the Project Viva Prospective Cohort: Implications for PFAS as Hazardous Substances for Developmental Health	Prenatal PFAS exposures may have long-lasting, intergenerational obesogenic effects.	- Prenatal exposure to higher levels of PFOS and PFNA was associated with a greater risk of obesity in adolescence. - There was an interaction between PFOS and PFOA, where the positive association between PFOS and obesity was stronger when PFOA levels were lower, and PFOA had a negative association with obesity when PFOS levels were higher. - Exposure to a mixture of higher concentrations of PFAS was associated with a greater risk of obesity in a dose-dependent manner.	- Prospective pre-birth cohort study (Project Viva) - Measured PFAS in maternal plasma samples collected in the first trimester - Measured child BMI at mid-adolescent visit (median: 17.4 years) - Defined obesity as BMI ≥ 95th percentile for age and sex based on CDC Growth Charts - Used Poisson regression with robust variance estimates for individual PFAS associations - Used Bayesian kernel machine regression (BKMR) for PFAS mixtures associations - Adjusted for maternal age, education, pre-pregnancy BMI, race/ethnicity, parity, and smoking status	humans	- Measurement of PFAS in maternal plasma samples - Measurement of child BMI - Poisson regression with robust variance estimates - Bayesian kernel machine regression (BKMR)	[82]

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			during pregnancy			
Effects of triphenyl phosphate exposure during fetal development on obesity and metabolic dysfunctions in adult mice: Impaired lipid metabolism and intestinal dysbiosis.	Fetal exposure to triphenyl phosphate can promote the development of obesity and metabolic dysfunctions in adult mice.	- Fetal exposure to triphenyl phosphate (TPHP) led to increased obesity, metabolic dysfunction, and altered lipid metabolism and gut microbiome in adult mice. - TPHP exposure during fetal development promoted the development of obesity and related metabolic disorders in adult mice. - Fetal TPHP exposure modulated gut microbiome composition and host-gut co-metabolism, which may contribute to the observed metabolic dysfunctions.	- Exposure to TPHP during fetal development and lactation at three doses (10, 100, and 1000 μg/kg BW) - Evaluation in adult male mice fed a low-fat diet (LFD) or high-fat diet (HFD) - Examination of body weight, liver weight, histopathology, blood biochemistry, gene expression, and gut microbiota compositions and metabolic functions - Gas chromatography-mass spectrometry (GC-MS) for fatty acid composition analysis - 16S rRNA gene sequencing and 1H NMR based fecal metabolomics for gut microbiome composition and host-gut co-metabolism	mice	- Body weight measurement - Liver weight measurement - Histopathology - Blood biochemistry assays - Gene expression analysis - Gut microbiota analysis - Gas chromatography-mass spectrometry (GC-MS) - 16S rRNA gene sequencing - 1H NMR based fecal metabolomics	[83]
Health-related toxicity of emerging per- and polyfluoroalky	Evidence derived from both animal models and humans	- Exposure to PFAS has been associated with a wide range of adverse health	- Critical review of recent research on PFAS exposure	humans, animals		[84]

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l substances: Comparison to legacy PFOS and PFOA.	suggested PFAS may exert harmful impacts on both animals and humans.	impacts, including effects on fertility, metabolism, endocrine function, lipid metabolism, hepatic and renal function, immune function, cardiovascular health, bone health, neurological function, and cancer risk. - However, the cause-and-effect relationships for many of these outcomes have not been clearly elucidated, and there are still limitations in our understanding of PFAS precursor kinetics, toxicity mechanisms, and the long-term effects of chronic PFAS exposure in humans. - Further investigation of the long-term-exposed population is required to better evaluate the biological toxicity of chronic PFAS exposure.	- Compilation and analysis of findings from multiple recent studies - Comparison of evidence from animal models and human studies - Evaluation of cause-and-effect relationships - Identification of gaps in current knowledge and need for further investigation			
Verification of In Vivo	Exposure to FC8-diol, FC10-diol,	- Exposure to FC8-diol, FC10-	- Tiered testing strategy with	Fathead minnows	- Exposure of fathead minnows	[85]

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Estrogenic Activity for Four Per- and Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists via New Approach Methodologies.	and HFPO-DA caused concentration-dependent increases in the expression of transcript coding for vitellogenin and estrogen receptor alpha in fish exposed *in vivo.*	diol, and FC8-DOD caused concentration-dependent increases in the expression of vitellogenin and estrogen receptor alpha, and reduced expression of insulin-like growth factor and apolipoprotein eb, indicating estrogenic activity in vivo. - The rank order of estrogenic potency in vivo matched the previous in vitro screening results, after accounting for differences in bioconcentration. - These findings provide a screening-level benchmark for estimating the potential estrogenic hazards of PFAS and a basis for identifying structurally similar PFAS that may also have estrogenic activity.	high-throughput in vitro screening as the initial tier. - Evaluation of in vitro screening effectiveness by exposing fathead minnows to five PFAS for 96 hours. - Measurement of transcript expression for vitellogenin, estrogen receptor alpha, insulin-like growth factor, and apolipoprotein eb. - Comparison of in vivo results with in vitro findings to validate the screening method and establish potency rank order.	(Pimephales promelas)	to PFAS - Measurement of transcript expression (vitellogenin, estrogen receptor alpha, insulin-like growth factor, apolipoprotein eb) - Bioconcentration analysis	
Early-Life Exposure to Perfluoroalkyl Substances and Childhood	Children with higher PFAS concentrations had lower insulin resistance	- Early-life exposure to PFASs was not associated with adverse metabolic effects	- Studied 665 mother-child pairs from Project Viva cohort (1999-2002)	humans	- Quantification of PFAS concentrations in plasma - Biochemical	[48]

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Metabolic Function	in mid-childhood.	in mid-childhood. - In fact, children with higher PFAS concentrations had lower insulin resistance.	- Quantified PFAS concentrations in maternal plasma at first prenatal visit (median 9.6 weeks gestation) and in child plasma at mid-childhood (median 7.7 years) - Assessed leptin, adiponectin, and HOMA-IR in mid-childhood - Used covariate-adjusted linear regression models and stratified analyses by child sex		assays for leptin, adiponectin, and HOMA-IR - Covariate-adjusted linear regression models - Stratified analyses by child sex	
Perfluoroalkyl and Polyfluoroalkyl Substances and Body Size and Composition Trajectories in Midlife Women: The Study of Women's Health Across the Nation 1999–2018	Certain PFAS were positively associated with large body size and body fat.	- Certain PFAS (PFOS, linear PFOA, EtFOSAA, MeFOSAA, PFHxS) were positively associated with larger body size and higher body fat at baseline and over time in midlife women. - Women with the highest PFAS levels had significantly higher weight, waist circumference, fat mass, and proportion of fat compared to those with the lowest levels.	- Examined associations of serum PFAS concentrations with body size and composition trajectories. - Included 1,381 midlife women with 15,000 repeated measures. - Follow-up period averaged 14.9 years. - Body size and composition assessed using objective measurements and dual-energy X-ray absorptiometry.	humans	- Measurement of serum PFAS concentrations - Objective measurement of weight - Objective measurement of waist circumference (WC) - Dual-energy X-ray absorptiometry (DXA) for body composition - Linear mixed models with piecewise linear splines for data analysis	[59]

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		- Higher PFAS levels were also associated with faster annual increases in weight, waist circumference, and fat mass over the 14.9 year follow-up period.	- Near-annual visits for assessments. - Used linear mixed models with piecewise linear splines to model non-linear trajectories. - Multivariable adjustments made for confounders.		
Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary	PFAS exposures target the ovary and represent major risks for women's health.	- PFAS are present in follicular fluid and can pass through the blood-follicle barrier. - Epidemiological studies have found associations between higher PFAS exposure and disruptions in ovarian function, such as later menarche, irregular menstrual cycles, earlier menopause, and reduced sex hormone levels. - Experimental studies have confirmed adverse effects of PFAS on ovarian folliculogenesis and steroidogenesis, potentially	- The study is a review of human population and toxicological studies. - A comprehensive review was performed by searching PubMed. - Extensive search terms were used, including both general and specific keywords related to PFAS and ovarian function.	humans	- Activation of peroxisome proliferator-activated receptors - Disruption of gap junction intercellular communication - Induction of thyroid hormone deficiency - Antagonism of ovarian enzyme activities - Inhibition of kisspeptin signalling	[87]

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		through various mechanisms.				
Exposure to perfluoroalkyl substances (PFAS) and liver injury: a systematic review and meta-analysis	Perfluoroalkyl substances are synthetic chemicals widely used in industry and consumer products that persist in the environment and bioaccumulate in food webs and human tissues.	- There is consistent evidence from human and animal studies that exposure to certain PFAS (PFOA, PFOS, PFNA) is associated with liver injury, as indicated by increased levels of liver enzymes and liver steatosis. - PFOA exposure was specifically associated with increased levels of the liver enzymes AST and GGT in humans. - PFAS-exposed rodents showed increased ALT levels, liver steatosis, and liver weight compared to non-exposed rodents.	- Systematic review of literature on PFAS exposure and liver injury. - Searched PubMed and Embase through January 27, 2021, using relevant keywords. - Data synthesis focused on two primary outcomes: serum alanine aminotransferase (ALT) and steatosis. - Included other measures of liver injury as secondary outcomes. - Synthesized evidence from at least three observational studies per PFAS using a weighted z-score approach for human studies. - Summarized direction and significance of exposure effects on hepatic enzyme abundance and activity for animal studies.	humans, rodents	- Literature search in PubMed and Embase - Measurement of serum alanine aminotransferase (ALT) - Measurement of steatosis - Weighted z-score approach for synthesizing observational study data - Synthesis of data on hepatic enzyme abundance and activity in animal studies	[88]
Per- and Polyfluoroalkyl Substance	Investigates the association between PFAS	- Doubling of EtFOSAA associated with	- Longitudinal cohort study with follow-ups	Human (pregnant women	- High-performance liquid	[89]

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Exposure, Gestational Weight Gain, and Postpartum Weight Changes in Project Viva	exposure during pregnancy and subsequent gestational weight gain and postpartum weight changes.	0.37 kg more weight gain during pregnancy. Doubling of PFOA associated with 0.55 kg more weight retention at 1-year postpartum and 0.91 kg more weight gain at 3 years postpartum. Higher PFOS associated with more weight gain at 3 years postpartum. Stronger postpartum weight change associations in women with higher pre-pregnancy BMI.	at 1 and 3 years postpartum. PFAS levels measured in plasma using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Analysis included multivariable linear regression and Bayesian Kernel Machine Regression (BKMR) for mixture analysis.	and postpartum mothers)	chromatography-tandem mass spectrometry (HPLC-MS/MS) Multivariable linear regression Bayesian Kernel Machine Regression (BKMR)

2.5. Epidemiological Evidence linking PFAS exposure to obesity and metabolic dysfunction

Epidemiological investigations have consistently demonstrated associations between PFAS exposure and the incidence of obesity and metabolic dysregulation Canova Li [74,90–92]. Within the realm of obesogenic research, a substantial body of evidence underscores the link between PFAS exposure and increased risk of adverse health outcomes. These synthetic compounds have the potential to interfere with endocrine functions [93], and potentially disrupt lipid and glucose homeostasis, which can lead to hyperlipidemia, diabetes, and obesity, each a recognized precursor to cardiovascular morbidity. Notably, PFAS exposure is associated with alterations in lipid profiles, including elevations in total cholesterol, LDL cholesterol, and triglycerides, as well as diminished glucose tolerance and insulin sensitivity [90–92,94,95]. Exposure can contribute to obesity-related comorbidities, as these compounds may adversely affect the resting metabolic rate, thereby complicating weight management efforts [62,71].

Epidemiological investigations have established correlations between PFAS exposure and various health complications, with the depth of understanding evolving in tandem with the emergence of new data [17]. The National Toxicology Program (NTP), among other research institutions, is at the forefront of exploring the health ramifications of PFAS, underscoring the dynamic nature of this research domain [96].

Further complicating the cardiovascular risk profile, PFAS exposure has been implicated in the elevation of blood pressure and a heightened risk of hypertension, directly contributing to cardiovascular pathology [97]. The interaction of PFAS with platelet membranes, altering their fluid dynamics and permeability, can lead to increased platelet activation and aggregation, as well as microvesicle release, potentially exacerbating thrombotic events [98,99]. More direct evidence derives from a study conducted by De Toni et al., which showed that PFAS can alter the functionality of platelets using liquid chromatography/mass-mass spectrometry (LC-MS/MS) to analyze the incorporation of PFAS into cell membranes. Researchers demonstrated that platelets are the major

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target of PFOA accumulation (about 10% of total blood PFOA) and C6O4 [98,99]. Furthermore, computational docking analysis and bilayer fluidity measurements further suggested a possible interaction of PFAS with phospholipids, altering the membrane structure and properties [100]. Such alterations may facilitate the formation of thrombi and arterial blockages, potentially leading to severe cardiovascular events like myocardial infarction and stroke.

The metabolic implications of PFAS exposure extend to diabetes, hyperglycemia, and insulin resistance, with multiple epidemiological studies reporting positive correlations between PFAS exposure and these metabolic derangements, as well as dyslipidemia, hypertension, and obesity, particularly in adolescent populations [101,102]. Mechanistic insights provided by Tumova (2016) and Roth (2020) elucidate the role of PFAS in exacerbating metabolic dysfunction, highlighting the contribution of contaminated diets and the dysregulation of free fatty acid metabolism in skeletal muscle [8,103].

Prenatal and early-life exposures to PFAS, even at low doses, have been linked to obesity-related markers in offspring, as evidenced by a Danish cohort study on 665 pregnant women between 1988 and 1989, which found that PFOA exposure during pregnancy correlated with increased BMI and waist circumference in female offspring two decades later [57]. Conversely, recent studies present a more nuanced perspective, with some research indicating negative associations between prenatal PFAS exposure and BMI in young children, suggesting complex interactions between PFAS exposure and growth [41,55].

In adults, particularly females, PFOA exposure has been speculated to enhance steroid hormone synthesis in the ovaries, potentially predisposing them to greater adiposity [104]. However, a retrospective analysis within the C8 Health cohort project, which encompassed data from 8,764 individuals aged between 20 and 40 years, collected from 2008 to 2011, did not find a significant correlation between early-life PFOA exposure and increased risk of overweight or obesity in adulthood [105]. These contradictory conclusions highlight the need for further research to elucidate the intricate relationship between PFAS exposure and obesity outcomes, as well as underscore the complexity of PFAS's impact on human health and the imperative for continued investigation to inform regulatory policies and public health strategies effectively.

Despite studies suggesting PFAS is a contributing factor in the increased risk of childhood obesity, the data is mixed and insignificant at best. The associations between PFAS and the risk of obesity become more complex to make as children age. Jin and colleagues (2020) collected data from seventy-four children diagnosed with nonalcoholic fatty liver disease in the Atlanta area. Researchers showed an increased risk of progression to nonalcoholic steatohepatitis with higher plasma concentrations of PFOS and PFHxS; more specifically, PFHxS was associated with an increased risk of liver fibrosis [106]. However, in this study, most participants were boys despite many studies showing that PFAS exposure disproportionately affects girls. The progression of liver disease in adolescents may be due to confounding factors other than plasma PFAS concentration. In a continuation of the Health Outcomes and Measures of the Environment (HOME) study, researchers investigated prenatal and post-natal PFAS exposure to adolescent adiposity [18].. Importantly, in the original study, mothers had PFAS plasma concentrations nearly double that of the national average from ingesting contaminated drinking water caused by a nearby industrial plant [18,107]. The effects of PFAS from this study may be overstated due to the extraordinary exposure rates of the mothers and children. In this longitudinal study, 212 preteen children presented only a modest positive correlation between increased PFOA and PFHxS exposure with greater body fat and obesity risk in adolescent children. They corroborated the association between PFOA concentration and increased adiposity in female children. However, prenatal and post-natal PFAS concentrations were weakly correlated; therefore, instances of adolescent adiposity may rely more heavily on other factors like maternal gestational BMI, and environmental and socioeconomic factors [18].

3. Toxicokinetic of PFAS in the Human Body

PFASs are well absorbed by the human body; however, they are excreted slowly. PFOA and PFOS, particularly, are known for their persistence in the human body due to their chemical stability

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and resistance to metabolic breakdown. PFOA accumulates primarily within the liver and plasma [108,109]. Upon exposure, PFAS exhibits a strong affinity for binding to plasma proteins, particularly albumin, rendering the bloodstream a significant site for PFAS accumulation [110–113]. This results in relatively long half-lives in humans, with average serum half-lives estimated to be about 3 years for PFOA, indicating significant accumulation rather than rapid excretion [114]. Furthermore, other extensively bioaccumulated PFAS compounds and metabolites are predominantly excreted through urine; however, this process can be significantly impaired in individuals with kidney disease, leading to reduced excretion of all wastes, including PFAS from the body [115].The toxicokinetic profiles of PFAS in humans, as well as their modes of action, have been extensively discussed [116]; however, their modes of action in humans are very complex and not fully understood, with differences in accumulation and distribution observed across various tissues [108]. The use of *in vitro* methods, particularly human cell-based models, has been proposed as a way to better understand the toxicokinetics and potential health effects of PFAS, including the newer short-chain alternatives [117]. Nonetheless, these substances can accumulate in various human tissues, with different compounds showing varying prevalence and concentrations [108]. These compounds are then widely disseminated throughout the organism *via* the circulatory and enterohepatic systems, predominantly accumulating in the blood, liver, and kidneys. Unlike traditional organic pollutants such as polychlorinated biphenyls, certain pesticides, and dioxins, which tend to accumulate in adipose tissue, PFAS are transported into cellular structures through both passive diffusion and active transport mechanisms. This transport is mediated by specific proteins, including organic anion transporters and the apical sodium-dependent bile acid transporter, facilitating their cellular uptake [11,118–120].The uptake, accumulation, and metabolism of PFASs in plants have also been studied, with the potential risk of human exposure through plant-origin food being highlighted [121]. These findings underscore the need for further research on the distribution and metabolism of PFASs in the human body.

In silico toxicokinetic models gave insight into the mechanism of the uptake of PFOS and its alternatives into phospholipid bilayers using the MDS approach. Cellular membrane lipid models shed light on the adsorption, transport, and residence time of PFOS, along with two emerging PFAS alternatives within DPPC bilayers [122]. Studies reveal that PFOS, 6:2 Cl-PFESA, and OBS readily adhere to the DPPC bilayer surface through interactions with DPPC headgroups, showcasing a thermodynamically favorable and stable adsorption process [122]. Where the sulfonic groups of PFOS, 6:2 Cl-PFESA, and OBS interact mainly with the $-N^{+}(CH_3)_3$ groups of DPPC molecules, forming a stable complex [122]. These compounds navigate a minimal free energy barrier (2-3 kcal/mol $^{-1}$) to integrate into the bilayers, propelled predominantly by their thermal movement on the bilayer surface, with PFOS presenting the lowest and OBS the highest energy barrier among them [123]. The calculated energy barriers for the three compounds into the bilayer were low, suggesting that these compounds can seamlessly enter the bilayer of the cell membrane. Upon entering the bilayer, the interactions between the sulfonate head groups of PFAS and the cationic N-atoms within the bilayer lead to a constrained movement of the bilayer's head groups and an alteration in the bilayer's orientation. Moreover, the incorporation of PFAS into the bilayer results in a decreased area per lipid, akin to the effects of cholesterol, causing the bilayer to contract laterally and subsequently widen, which further modifies the bilayer's structural dynamics [122].. Following this initial interaction, PFAS compounds predominantly settle in the upper leaflet of the DPPC bilayers, demonstrating a limited inclination to either return to the surface or delve further into the bilayer's core. This sustained presence within the upper leaflet is shaped by the intricate molecular interactions between PFAS and DPPC, as well as the displacement of water molecules by the PFAS compounds, which further influences the bilayer's structural dynamics and orientation [124].

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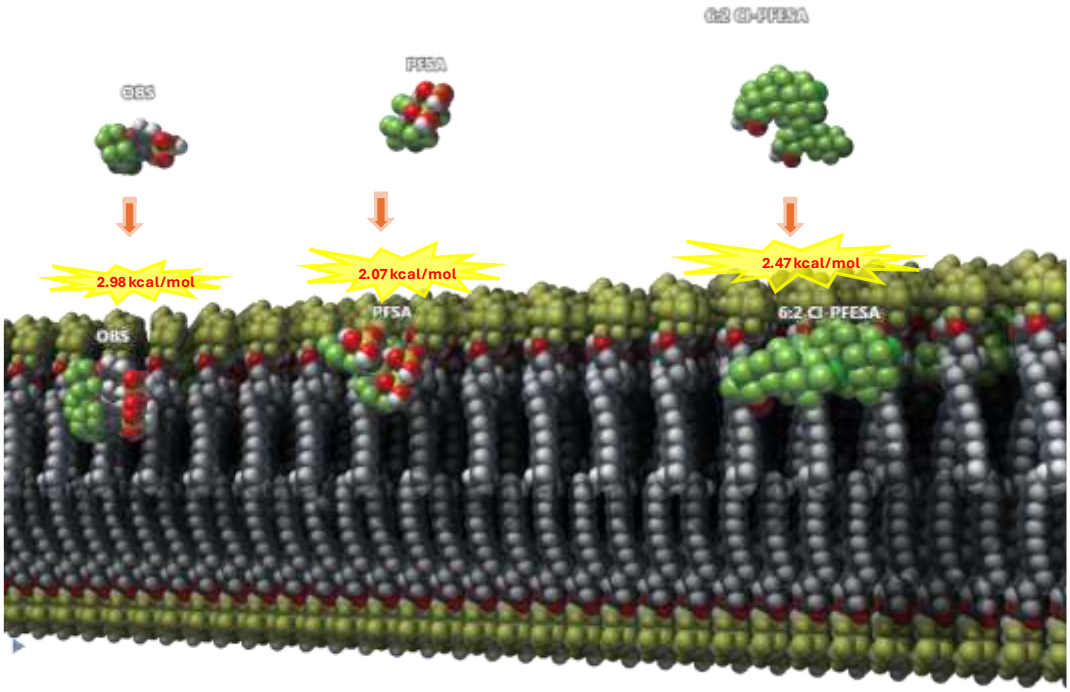

Figure 3. Graphical representation of the in-silico modeling of per- and polyfluoroalkyl substances (PFAS) uptake into a dipalmitoylphosphatidylcholine (DPPC) membrane bilayer. The simulation demonstrates the uptake process and the energy required for PFOS (perfluoro sulfonic acids): 2.07 kcal/mol $^{-1}$, 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA): 2.47 kcal/mol $^{-1}$, and sodium p-perfluorous nonenoxybenzene sulfate (OBS): 2.98 kcal/mol $^{-1}$ to cross into the membrane.

4. Regulatory Challenges and Risk Assessment of PFAS

[125–127]. However, due to the ongoing investigation into the full scope of exposure and associated risks of the diverse range of PFAS compounds, regulatory frameworks for PFAS management exhibit significant international variability. The lack of long-term health impact data and the current limitations of detection and remediation technologies further complicate the issue. Abunada (2020) emphasizes the global disparity in PFAS regulatory values, driven by scientific, technical, and societal factors [128]. Langenbach (2021) highlights the absence of federal regulations and standards in the United States, stressing the need for future epidemiological research [129].

The regulatory landscape for PFAS is currently evolving, with the EPA at the forefront, enacting measures under the Resource Conservation and Recovery Act. Various countries have adopted different strategies for regulating PFAS based on their risk assessments and public health priorities. The European Union, through the European Chemicals Agency (ECHA), has proposed restricting all PFAS, including firefighting foams, with several PFAS listed under the REACH Regulation [130]. Similarly, the German Ministry of Health has also recommended a threshold of 300 ng/L for these compounds [128,131]. Across the globe, Countries have developed their own guidelines for PFAS regulation. Australia, for instance, has in collaboration with USEPA, set a conservative drinking water guideline of 70 ng/L for the sum of PFOS and PFOA. Japan has established regulatory values for PFOS and PFOA in drinking water and industrial emissions and has adopted strict standards for PFAS in consumer products. China, on the other hand, is phasing out specific PFAS and implementing emission controls. This diversity in approaches underscores the complexity of the PFAS issue and the need for a unified global strategy [132]. While Health Canada has set drinking water guidelines of 200 ng/L for PFOS and 600 ng/L for PFOA [133].

New (EPA) initiatives take steps towards the regulation of specific PFAS entities, including perfluorohexane sulfonic acid (PFHxS) and hexafluoropropylene, in potable water, marking a

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transition towards more stringent regulatory paradigms [134,135]. However, some U.S. states have deemed these guidelines insufficient, adopting more stringent criteria. Such state-level actions are crucial, as demonstrated by the Department of Environment, Great Lakes, and Energy, which has implemented standards that exceed those of the EPA and cover a broader spectrum of PFAS compounds [136]. In 2022, the EPA introduced more rigorous health advisory limits (HALs) of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS [137] based on a thorough review of the latest scientific research and considerations of lifetime exposure risks. This strategy reflects an understanding of the long-term health effects of PFOA and PFOS, underscoring the need to evaluate the impact of these substances on human health. The EPA established these advisory levels by examining human health data to identify non-carcinogenic toxicity benchmarks, determining the lowest exposure levels associated with adverse effects on essential physiological functions such as immune response, thyroid function, liver health, and fetal development [131]. The advisory levels also incorporated a detailed calculation of the relative source contribution factor, set at 0.20, allocating 20% of the total allowable exposure to drinking water and thus recognizing the complex nature of environmental exposure to these chemicals [138].

The discrepancies in regulatory standards for PFAS arise from the absence of synchronous analytical methods and the intricate nature of their toxicological characteristics [139]. The Stockholm Convention on Persistent Organic Pollutants has designated PFOS and its derivatives for regulatory oversight, catalyzing a range of regulatory measures globally. The FDA is also involved in overseeing the presence of PFAS in food products, focusing on their uptake by food and the development of chemical standards for accurate identification [140]. This multifaceted strategy underscores the urgent and complex challenge of addressing PFAS contamination and subsequent human exposure across various products. Establishing permissible exposure limits (PELs) and mitigation strategies for PFAS compounds has been complex and challenging. As such, there is a pronounced need for additional research to bridge the gaps in our understanding of PFAS toxicology and to elucidate the relationship between exposure and health outcomes. Undeniably, long-chain PFASs have outstanding performances that are hard to match without fluorine, however they also pose serious environmental and health risks. Therefore, the consensus is that a more reasonable, more selective use of these compounds is indispensable in order to reduce exposure while preserving their societal benefits, all without penalizing developing countries [141]. As a result, remediation/"clean up" tasks are necessary to manage PFAS pollution [141].

In terms of corporate responsibility and industry actions, a significant milestone in the evolution of the PFAS regulatory framework was the decision by 3M Company, a major PFAS manufacturer, to voluntarily cease the production of PFOA and PFOS in the early 2000s [137]. Following this, DuPont also ended its production and use of PFOA in 2013, in accordance with an agreement with the EPA. This move was part of a broader trend of discontinuation among other global companies [137]. More recently, 3M has announced plans to completely halt PFAS production by the end of 2025. Despite these proactive measures by leading manufacturers towards phasing out long-chain PFAS and the implementation of regulatory frameworks in regions such as the United States, Japan, and Western Europe, new production entities, primarily in continental Asia, have continued to manufacture long-chain PFAS and their precursors [142,143]

Regulatory interventions to curtail PFAS exposure present substantial opportunities to attenuate their obesogenic effects and reduce obesity prevalence, particularly in pediatric populations [55]. Systematic reviews have emphasized the necessity for stringent regulatory measures to limit exposure to PFAS. Such measures could yield dual advantages: reducing obesity incidence and mitigating other health hazards associated with PFAS, including reproductive anomalies and dyslipidemia [55,135,144]. Such legislative action could incur stricter PFAS emission controls, enhanced surveillance of PFAS concentrations in consumer goods and food supplies, and spur the innovation of safer alternatives to PFAS-infused materials. By targeting the fundamental sources of PFAS exposure, these regulations bolster public health by reducing the prevalence of a potential obesogen, thus averting a range of additional adverse health outcomes linked to PFAS.

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However, federal implementation must navigate political, economic, and scientific challenges. In regions like the US Northeast, where local governments monitor PFAS contamination in drinking water aquifers, these regulatory hurdles are particularly pronounced [145]. For instance, California has introduced progressive regulations that classify PFAS as a chemical group in consumer products, a necessary and forward-thinking approach [146]. Despite these challenges, there are opportunities for collaborative efforts and new technologies to effectively address PFAS contamination [146]. Currently, the most studied PFAS compounds are PFOS and PFOA, even though they were phased out in the US decades ago [137]. The development of next-generation PFAS is outpacing researchers' ability to study them. The obesogenic mechanisms of older-generation PFAS remain unclear, complicating the assessment of newer PFAS's potential [17]. While many in vivo and in vitro models have examined individual PFAS molecules, few have studied aggregates found in technical mixtures and their associated health effects [35]. This complexity arises from the thousands of identified PFAS combinations. *In silico* modeling could link specific PFAS molecules and mixtures to physiological pathways, but without in vivo and in vitro investigations, these associations remain hypothetical [147]. Epidemiological studies often overlook associations between PFAS exposure, gestational weight gain, and childhood obesity. Key factors like diet, activity level, socioeconomic status, geographical location, and water and food sources are not considered but would help uncover PFAS exposure patterns and mechanisms. Therefore, risk assessment should focus on the most susceptible population sectors, exposure routes, and prevalent PFAS molecules and mixtures.

5. Strategies and Challenges in PFAS Remediation and Detoxification

PFAS presents formidable challenges in environmental remediation due to their chemical stability and persistence, attributed to the robust carbon-fluorine bonds (460 kJ/mol) [148]. Innovative approaches, such as photocatalytic degradation leveraging advanced oxidation processes, have been explored to counteract these resilient compounds [149]. Additionally, policy-driven strategies, including regulatory frameworks and the promotion of safer alternatives, are being considered to mitigate PFAS pollution [150]. The integration of technologies like constructed wetland-microbial fuel cell systems offers a novel pathway for PFAS removal from aqueous environments, highlighting the interdisciplinary efforts required to address PFAS contamination [151].

The intrinsic resistance of PFAS to conventional degradation methods underscores the complexity of effectively dismantling these compounds. Detoxification of PFAS within the human body adds further complex layers, with current strategies being limited and largely ineffective in expediting the elimination process [152]. The variability in elimination kinetics, influenced by factors such as molecular structure and biological variables, necessitates a deeper understanding and development of targeted detoxification methods [109,153].

As of now, there are limited studies and no clinical trials specifically aimed at evaluating treatments to reduce the PFAS burden, even in cases of very high exposure [154]. Unfortunately, there is a significant gap in available treatment options for PFAS exposure in humans. Moving forward, ongoing research efforts are crucial to developing effective strategies for PFAS detoxification and removal from the human body.

Environmental strategies for PFAS degradation encompass a spectrum of techniques, from thermal and chemical treatments to advanced oxidation methods [155]. Despite their potential, these strategies face limitations such as specificity to certain PFAS structures and concerns over incomplete degradation leading to the formation of shorter-chain PFAS [148,155]. The optimal PFAS remediation strategy necessitates consideration of factors such as PFAS characteristics, water properties, and the cost-effectiveness of available technologies [156]. Commonly employed methods include activated carbon adsorption, which is particularly effective against long-chain PFAS but requires regular carbon renewal [157]. Ion exchange resins, capable of extracting both long- and short-chain PFAS, may face competition from other waterborne ions and also require periodic resin regeneration or replacement. Advanced treatment technologies, such as electrochemical oxidation and activated persulfate oxidation, have shown promise in degrading PFASs in water [158]. Using a UV/$S_2O_8^{2-}$ system, Lutze and Coworker showed that PFCAs are degraded by sulphate radicals [159].

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Furthermore, High-pressure membrane systems, encompassing nano-filtration and reverse osmosis, offer broad-spectrum PFAS removal but generate concentrated waste and demand significant energy and maintenance investments.

Emerging or less conventional approaches, such as photocatalytic degradation [160] and plasma treatment, hold promise for complete PFAS decomposition but may yield undesirable by-products and incur substantial energy and equipment costs [161,162]. Biological treatments, leveraging microorganisms or plants, offer a more natural remediation route but are constrained by PFAS's inherent resistance to biodegradation and warrant stringent biological process management. The exploration of enzymatic degradation, particularly through enzymes capable of cleaving the carbon-fluorine bond like fluoroacetate dehalogenase, presents a promising avenue for targeted PFAS breakdown [148,163].

Exploring *in silico* enzyme design emerges as a promising approach for the degradation of PFAS. A major limitation of enzymatic bioremediation, is the scarcity of naturally occurring enzymes capable of breaking down PFAS, underscoring the labyrinthian efforts to find viable remediation methods. However, the potential of computational strategies, including homology modeling and molecular dynamics, to facilitate the rational design of enzymes, optimizing their interaction with PFAS for effective degradation, offers a reason for confidence in the future of PFAS remediation. Chemical redox systems, despite their potential to generate bond-breaking radicals, face challenges such as pH sensitivity and inefficient defluorination leveraging enzymes like fluoroacetate dehalogenase, horseradish peroxidase, and laccase, which offers a targeted approach to catalyze PFAS degradation. Deploying radical-generating enzymes like laccase and horseradish peroxidase presents a viable strategy for degrading resilient carbon-fluorine bonds because these enzymes are capable of generating high-energy radical's adept at targeting and breaking down the robust C–F linkages in PFAS. Augmentation through metal ions or mediators can further enhance their efficacy, facilitating complex formation with PFAS or reducing the energy threshold for radical initiation. Advances in computational design and directed evolution techniques offer pathways to refine these enzymes, optimizing their selectivity, efficiency, and robustness. Furthermore, integration with nanozymes, or metal-organic frameworks to engineer bionanocatalysts, holds promise for the sequestration and decomposition of PFAS in environmental matrices, offering a multifaceted approach to mitigating PFAS pollution. Computational strategies, including homology modeling and molecular dynamics, facilitate the rational design of enzymes, optimizing their interaction with PFAS for effective degradation. This multidisciplinary approach combines the precision of enzymatic action with the power of computational design to address the persistent challenge of PFAS pollution.

6. Insights from In Silico Studies of PFAS

In silico studies play a crucial role in understanding the molecular interactions and mechanisms of PFAS [164] by leveraging computational algorithms and molecular modeling techniques. These methodologies provide a cost-effective and time-efficient approach to assess the binding potencies and mechanisms of PFAS with biological targets [165], such as receptors and enzymes involved in thyroid hormone transport and metabolism. By predicting binding probabilities and elucidating structural requirements for receptor binding, in-silico studies enable the identification of potential ligands or antagonists. This approach minimizes the need for invasive human sampling and complements *in vitro* and *in vivo* research, thereby contributing to a comprehensive understanding of PFAS toxicity and aiding in evidence-based policymaking [166].

In silico studies offer several significant advantages over other approaches in PFAS research. They provide a cost-effective and time-efficient approach to assessing the potential binding potencies with biological targets and mechanisms of PFAS toxicity, remediation, and degradation [164]. Moreover, *in silico* studies provide insights into the structural requirements of PFAS for binding to specific receptors, enabling the identification of potential ligands or antagonists [40,164]. Recent *in silico* research includes studies on PFAS toxicity, sequestration, degradation, and endocrine-disrupting effects. For example, a 2023 study by Dharpure and colleagues focused on the transthyretin (TTR) binding and thyroid-disrupting effects of PFAS. This analysis aimed to decode

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molecular complexity into how PFAS compounds interact with TTR, potentially leading to thyroid hormone disruption. Understanding these molecular mechanisms is crucial for assessing the endocrine-disrupting properties of PFAS and their implications for human health [167], such studies utilize computational methods to analyze the binding potencies and molecular interactions of PFAS with important biological targets, including the TTR and NHRs like PPARs and TRs [167,168]. However, current *in silico* studies have primarily focused on a limited number of receptors and PFAS compounds (such as PFOA, PFOS, and PFBS), and have not fully explored the diversity and complexity of the PFAS family and the thyroid hormone system highlighting a need for broader studies [40,164]. A diverse array of computational strategies, including molecular docking, molecular dynamics simulations, and Quantitative Structure-Activity Relationship (QSAR) modeling, are employed in *in silico* PFAS research [168–170]. For instance, Zhang and coworkers (2021) used a QSAR–ICE–SSD model to predict the no-effect concentrations (PNECs) of PFASs and assess their ecological risks near electroplating factories [171]. Molecular docking examines the binding interactions between PFAS compounds and target proteins, such as transthyretin (TTR) and nuclear hormone receptors (NHRs) [172]. It predicts the binding orientation and affinity of PFAS compounds towards specific targets. Subsequently, molecular dynamics simulations evaluate the stability and behavior of PFAS-target complexes within a solvated environment. These simulations provide insights into the temporal stability, persistence of interactions, and impact of mutations, structural modifications, and environmental factors on PFAS interactions [124]. Detailed analyses of parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bond dynamics during these simulations have provided in-depth insights into the interactions within PFAS-target complexes over time. Additionally, predictive tools like the mCSM server and the MM/GBSA method explore the potential effects of genetic mutations on the binding efficiency and stability of these complexes [170,173,174].

QSAR models predict the binding probabilities of a wide array of perfluoroalkyl compounds to specific receptors, such as TTR and peroxisome proliferator-activated receptor gamma (PPARγ), based on docking scores and structural features, including carbon chain length, molecular weight, and polarity [167,168]. These models have been validated by predicting binding energies of additional perfluoroalkyl compounds, closely aligning with experimental findings, highlighting their potential as predictive tools for identifying endocrine-disrupting compounds and aiding in the development of safer chemical alternatives with diminished affinity towards TTR and PPARγ [167,168]'

An interesting challenge within *in silico* PFAS research is identifying PFAS molecules with the highest binding affinities to receptors and enzymes implicated in thyroid hormone disruption [175,176]. Elucidating the structure-activity relationships and the structural determinants of PFAS binding potencies is crucial for assessing the potential health risks posed by specific PFAS molecules [144,177]. Combining *in silico* and experimental data is vital for enhancing the precision and reliability of predictions. This amalgamation offers a more holistic understanding of PFAS behavior and toxicity [167,178,179]. Rowan-Carroll and colleagues (2021) combined high-throughput transcriptomic data with the benchmark concentration modeling with the BMDExpress fit model to analyze concentration-response relationships of PFAS compounds [180] leveraging the strengths of both high-throughput data collection and sophisticated modeling to provide a more accurate and reliable assessment of chemical toxicity.

Insight into the molecular interaction and dynamics between PFAS compounds and nuclear hormone receptors, such as PPARs, TRs, and liver X receptors (LXRs), provides better understandings into the disruptions caused by PFAS in lipid metabolism and their role in promoting adipocyte differentiation, thereby contributing to obesity. *In silico* studies demonstrated that PFAS carbon chain length and the nature of the functional group play a crucial role in determining their affinity towards those receptors [164]. Molecules with longer carbon chains and higher degrees of fluorination and branching exhibit increased receptor binding efficacy, which is a crucial aspect considered in QSAR models. PFAS show receptor-specific binding affinities, with low affinity towards PPARα and moderate probabilities towards PPARβ and PPARγ, delineating their

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toxicological profile [181]. However, transcriptomics studies and *in silico* analysis, suggested that PPARα is the principal transcription factor regulated by PFOA, influencing not only lipid metabolism-related genes but also all differentially expressed genes (DEGs) in the liver [181].

Advances in integrative systems biology have facilitated the construction of molecular networks that illustrate the intricate interplay between PFAS exposure, signaling pathways, and gene expression alterations. These computational frameworks have been instrumental in identifying specific gene sets and regulatory modules implicated in PFAS-induced obesity, such as the HNF4α Pathway; *in silico* docking simulations have indicated that PFOA and PFOS can directly interact with HNF4α, similar to endogenous fatty acids [182]. This interaction suggests that PFAS can mimic natural ligands of HNF4α, potentially altering its activity PFOS and PFOA may suppress the HNF4α signaling pathway, which is crucial for liver function and lipid homeostasis. Similarly, comparative *in silico* transcriptome analyses have shown that both legacy and alternative PFAS can modulate molecular pathways associated with the sterol regulatory element binding protein (SREBP) signaling [183]. These changes can affect lipid metabolism and contribute to hepatic dysfunction. Additionally, high-throughput transcriptomics is used to derive toxicity points of departure (tPODs), cross-species responses, PFAS body burdens, and internal concentrations at multiple time points. Studies such as Addicks et al. (2023), Beccacece et al. (2023), Rericha et al. (n.d.), and Rudzanová et al. (2024) serve as valuable experimental input for model training datasets [184–187]. These datasets are curated in databases of pathways and reactions in human biology, such as REACTOME, and are used for Gene Set Enrichment Analysis (GSEA), gene networks analysis, and other *in silico* applications using resources like the STRING database and the National Library of Medicine's post-Toxicology Data Network (TOXNET) resources.

Machine learning algorithms have paved the way for the development of predictive models for assessing PFAS toxicity and obesogenic potential. Studies by Feinstein (2021) and Lai (2022) have employed deep transfer learning and molecular screening, respectively, to predict the toxicological profiles of PFAS compounds. Feinstein's comparative analysis of various machine learning methodologies highlighted the superior performance of the Deep Neural Network (DNN) model, which outperformed other algorithms such as Random Forest, Support Vector Machine, and Graph Convolutional Network in terms of prediction accuracy and generalizability [188]. The integration of transfer learning techniques into the DNN model significantly enhanced its predictive capabilities by leveraging a vast array of toxicity data from the broader organic chemical spectrum [188]. An uncertainty-informed approach employing the SelectiveNet architecture further refined the model's output by filtering uncertain predictions and providing confidence levels for each prediction. Concurrently, the Random Forest algorithm demonstrated notable efficacy in estimating the toxicokinetic half-lives of PFAS compounds across various species, drawing on a combination of physiological and structural characteristics [188]. Similarly, Lai's study, utilizing molecular descriptors and machine learning, screened and estimated the toxicity of over 260,000 PFAS molecules [189]. Similarly, an ML study showcasing the impressive Random Forest algorithm's ability to predict the toxicokinetic half-lives (t½) of PFAS across multiple species also reported an accuracy of 86.1% [190]. The model, built on a dataset comprising 119 chemical and physiological descriptors, effectively categorized the t½ of 11 PFAS compounds in humans, monkeys, rats, mice, and dogs into distinct temporal categories, demonstrating the model's broad applicability and high predictive accuracy [190]. Using Machine learning models, Singam and colleagues (2020) also investigated the interactions between over 5,000 PFAS compounds and human androgen receptors (HAR), identifying 23 PFAS that exhibited strong interactions with HAR [127,191]. The study pinpointed three PFASalternatives-9-(nonafluorobutyl)-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-11-one (NON), 2-(heptafluoropropyl)-3-phenylquinoxaline (HEP), and 2,2,3,3,4,4,5,5,5-nonafluoro-N-(4-nitrophenyl)pentanamide (NNN) as having notable impacts on HAR at environmentally relevant concentrations [127,191]. These PFAS were observed to inhibit HAR transactivation through competitive binding, leading to the upregulation of HAR and a consequent decrease in the expression of androgen-regulated genes such as PSA and FKBP5, indicative of antiandrogenic effects. The outcomes of these PFAS exposure experiments aligned with

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those observed for hydroxyflutamide, a recognized AR inhibitor, underscoring the antiandrogenic potential of these compounds. Remarkably, the alternative PFAS demonstrated more pronounced androgenic effects compared to their legacy counterparts, affirming the efficacy of the *in silico* model in forecasting the endocrine-disrupting impacts of these chemicals [127]. These collective efforts underscore the potential of machine learning in facilitating rapid and cost-effective hazard assessments of PFAS compounds, enabling the identification of structure-activity relationships and the design of new PFAS molecules with reduced obesogenic potential.

While *in silico* studies offer valuable insights, they also face challenges. One challenge is the need for accurate and validated computational models that can reliably predict the binding affinities and interactions of PFAS with biological targets [192]. The quality of QSAR models and the availability of experimentally verified data are critical in ensuring the accuracy of predictions [192].. Additionally, the vast structural diversity of PFAS compounds requires comprehensive libraries and databases for effective screening and analysis [192]. Interpreting the dynamic behavior of PFAS-protein complexes from molecular dynamics simulations requires substantial computational resources and expertise [192]. Addressing these challenges will improve the reliability and applicability of *in silico* studies in PFAS research. As such, *in silico* studies of PFAS are emerging as valuable tools to understand the molecular interactions, binding potencies, and mechanisms of PFAS with important biological targets. They play a significant role in assessing the potential health risks associated with PFAS exposure, including their disruption of the thyroid hormone system. By combining computational and experimental data, *in silico* studies contribute to evidence-based policymaking and aid in identifying potential ligands or antagonists to mitigate adverse effects. Despite this, *in silico* studies face major limitations, such as the availability and quality of data, the validity and accuracy of models, and the extrapolation of human health outcomes. Therefore, *in silico* studies should be complemented by *in vivo* and *in vitro* experiments to better understand the impacts of PFAS exposure. Currently, ongoing research and advancements in computational methods are expected to enhance the accuracy and applicability of *in silico* studies in PFAS research.

7. Conclusions

This review underscores the pervasive nature and multifaceted health implications of per- and poly-fluoroalkyl substances (PFAS), emphasizing their potential role as obesogens. Due to their persistent and bio-accumulative properties, PFAS present significant challenges for both environmental and public health. This comprehensive evaluation highlights the molecular mechanisms through which PFAS may contribute to obesity, focusing on their interactions with lipid metabolism, endocrine disruption, and regulatory pathways such as peroxisome proliferator-activated receptors (PPARs) and fatty acid binding proteins (FABPs).

Epidemiological studies suggest a correlation between PFAS exposure and an increased risk of obesity, particularly among vulnerable populations such as children and expectant mothers. These studies also illustrate the complexities in establishing causal relationships, given the heterogeneity of PFAS compounds and numerous confounding factors in human health research. *In vitro* and *in vivo* studies provide further insights into the biochemical pathways influenced by PFAS, reinforcing their potential to disrupt metabolic homeostasis and contribute to conditions like dyslipidemia and nonalcoholic fatty liver disease.

In silico models offer valuable insights into the binding affinities and interaction mechanisms of PFAS with biological targets, complementing traditional experimental methods. Although these computational tools enhance our understanding of PFAS toxicity and support the development of safer chemical alternatives, the limitations of *in silico* studies, including the need for validated models and comprehensive data sets, highlight the necessity of integrating these findings with empirical research.

The evolving regulatory frameworks for PFAS reflect a growing recognition of their health risks. The global variability in PFAS regulation underscores the need for a unified strategy to manage these contaminants effectively. Regulatory measures, combined with innovative remediation technologies

and policy-driven approaches, are crucial for mitigating PFAS pollution and reducing its obesogenic impact.

Future research should prioritize longitudinal studies to better understand the long-term health effects of PFAS exposure. Developing advanced methodologies for detecting and remediating PFAS in the environment is also essential. Addressing the multifaceted challenges posed by PFAS will help safeguard public health and foster more effective regulatory and remediation strategies to mitigate their impact.

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