Review

Per- and Polyfluoroalkyl Substances Exposure as Emerging Cardiovascular Risk Factors: Implications for Ischemic Heart Disease

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Posted Date: 24 March 2026

doi: 10.20944/preprints202603.1796.v1

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 doi:10.20944/preprints202603.1796.v1

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Per- and Polyfluoroalkyl Substances Exposure as Emerging Cardiovascular Risk Factors: Implications for Ischemic Heart Disease

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Abstract

Ischemic heart disease (IHD), a chronic and progressive condition marked by restricted blood flow predominantly arising from atherosclerosis, is currently the leading cause of mortality within cardiovascular disease. In recent years, per- and polyfluoroalkyl substances (PFAS), ubiquitous, highly persistent environmental contaminants and well-established endocrine disruptors, have emerged as potential risk factors for IHD, given their documented associations with hypercholesterolemia, hypertriglyceridemia, and insulin resistance. Despite the still limited number of epidemiological studies and the inconsistent findings from investigations conducted in occupational settings, there is growing evidence that elevated exposure to certain PFAS compounds may increase the risk of IHD and vascular dysfunction, in some cases displaying dose-response relationships and sex-specific patterns. Mechanistic studies support these epidemiological signals. Dysregulation of peroxisome proliferator-activated receptors alpha promotes vascular inflammation and oxidative stress, thereby contributing to endothelial dysfunction and the establishment of a pro-thrombotic milieu. Epigenetic modifications, together with telomere shortening, and alterations in mitochondrial DNA copy number, provide additional pathways linking PFAS exposure to atherogenesis. Future opportunities offered by novel approaches and intelligent techniques might revolutionize the research in this field attempting to address the existing knowledge gaps and to clarify the mechanistic relationships linking PFAS exposures with clinical cardiovascular outcomes.

Keywords: PFAS; ischemic heart disease; coronary heart disease; atherosclerosis; epigenetics; telomere; mitochondrial DNA copy number; single nucleotide polymorphisms; oxidative stress; artificial intelligence

1. Introduction

Cardiovascular disease (CVD) has persisted as a major public-health burden over recent decades, consistently ranking among the leading causes of mortality on a global scale [1,2]. In 2022, CVD remained responsible for approximately 32% of all deaths, with the highest impact observed in low- and middle-income countries, as well as among males and older adults [3,4]. Among CVD, ischemic heart disease (IHD) is the leading cause of death, accounting for more than 315 million prevalent cases and approximately 9 million deaths globally in 2022 [5], and its overall disease burden is projected to continue rising through 2050 due to population aging and widening economic disparities [6]. IHD is a chronic, progressive condition characterized by restricted cardiac blood flow, most often resulting from coronary heart disease (CHD) driven by atherosclerosis, which leads to an imbalance between myocardial oxygen supply and demand [2,7]. It may present with a spectrum of manifestations generally referred to as chronic coronary syndromes, or it may evolve into acute clinical entities such as unstable angina and myocardial infarction (MI) [7,8].

 doi:10.20944/preprints202603.1796.v1

Recent analyses conducted across 204 countries and territories from 1990 to 2021 identified metabolic risk factors as accounting for approximately 52% of the IHD burden in high-socioeconomic index (SDI) regions [9,10]. High systolic blood pressure, elevated low-density lipoprotein (LDL) cholesterol, high fasting plasma glucose, and increased body mass index (BMI) together contributed to roughly half of the total disease load [10]. Conversely, in low-SDI regions, behavioral risks (e.g., smoking) and environmental exposures (e.g., air pollution) constitute a larger share of the IHD burden compared with metabolic risks [10].

Atherosclerosis, the primary cause of IHD, progresses from the lipid-streak phase—characterized by lipid accumulation within the intima, foam-cell formation, and macrophage infiltration—to the fibrous plaque phase, marked by the development of a fibrous cap composed of vascular smooth muscle cells [11]. Rupture of this cap can expose the underlying necrotic core of foam cells to the bloodstream, ultimately leading to thrombosis and, consequently, MI and stroke [11,12].

While dyslipidemia has long been recognized as a central driver of atherosclerosis—together with hypertension, obesity, diabetes mellitus, chronic kidney disease, inadequate diet, and sleep deprivation—accumulating evidence suggests that exposure to endocrine-disrupting chemicals (EDCs) may also contribute to disease development [11–14]. EDCs, which are widely distributed across environmental media, comprise a group of compounds capable of interfering with hormone homeostasis even at low doses, thereby contributing to a range of hormone-mediated biological alterations [15]. Per- and polyfluoroalkyl substances (PFAS), widely used over the past decades in industrial application—including firefighting foams, solvents, personal care products, textile-protective coatings (e.g., Gore-Tex), non-stick cookware (e.g., Teflon), and food-packaging materials—are persistent and bioaccumulative synthetic chemicals, resulting in measurable PFAS concentrations across multiple human biological matrices [15–17]. Toxicological and epidemiological studies have linked PFAS exposure to a range of adverse health effects, including reproductive and developmental toxicity, immunosuppression, thyroid dysfunction, liver and kidney disease, and cancer [18]. Furthermore, given the positive associations between serum PFAS levels and hypercholesterolemia, hypertriglyceridemia, and insulin resistance [19–22], it is reasonable to hypothesize that PFAS may contribute to atherosclerosis, thereby representing a potential novel risk factor for IHD. Conversely, findings from human studies examining the relationship between PFAS exposure and CVD remain somewhat inconsistent [21].

In this narrative critical review, we examine the current evidence on the association between PFAS exposure in both the general population and in occupational settings and the risk of IHD and related biomarkers, discuss the plausible biological mechanisms underlying this relationship, and outline the implications and future strategies for the primary prevention of one of the most impactful diseases worldwide.

2. Per- and Polyfluoroalkyl Substances: An Overview

PFAS, a diverse group of synthetic chemicals used across multiple industrial sectors (e.g., agriculture, chemical manufacturing, electronics, military applications, packaging, and textiles), are characterized by one of the strongest bonds in chemistry—namely the carbon–fluorine single bond covalently attached to the alkyl chain—and are therefore considered highly persistent environmental contaminants [23,24]. Their thermal and chemical stability, together with the extensive use of PFAS over more than 80 years—now spanning over 200 applications—has led to these compounds being referred to as “forever chemicals” and has resulted in their ubiquitous presence in the environment [23,25]. The accumulation of PFAS in environmental media—either directly or via sewage from electroplating operations, laundries, dry cleaners, and cosmetics from private households—is estimated to reach 4.4 million tons over the next thirty years unless their production is substantially reduced [26,27]. While more than 4,000 PFAS have been manufactured and hundreds detected in environmental samples, only a subset—classified as long-chain PFAS (those with more than 7 fully fluorinated carbon atoms), such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS),

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perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS)—has received the greatest attention due to their persistence, long-distance mobility, and ecotoxicological relevance [23,28–30]. These compounds can bioconcentrate in aquatic organisms through binding to proteins in the blood, liver, and kidney, and consequently biomagnify along the associated food webs, posing human health hazards [27,31]. As a result, human exposure primarily occurs through consumption of drinking water and seafood, particularly in populations living near contaminated sites, followed by inhalation of air and indoor dust, and, to a lesser extent, dermal contact [28,32]. Importantly, PFAS can also transfer from the placenta to the fetus during pregnancy and from mothers to infants through breastfeeding, thereby potentially influencing critical stages of human development [33,34] (Figure 1).

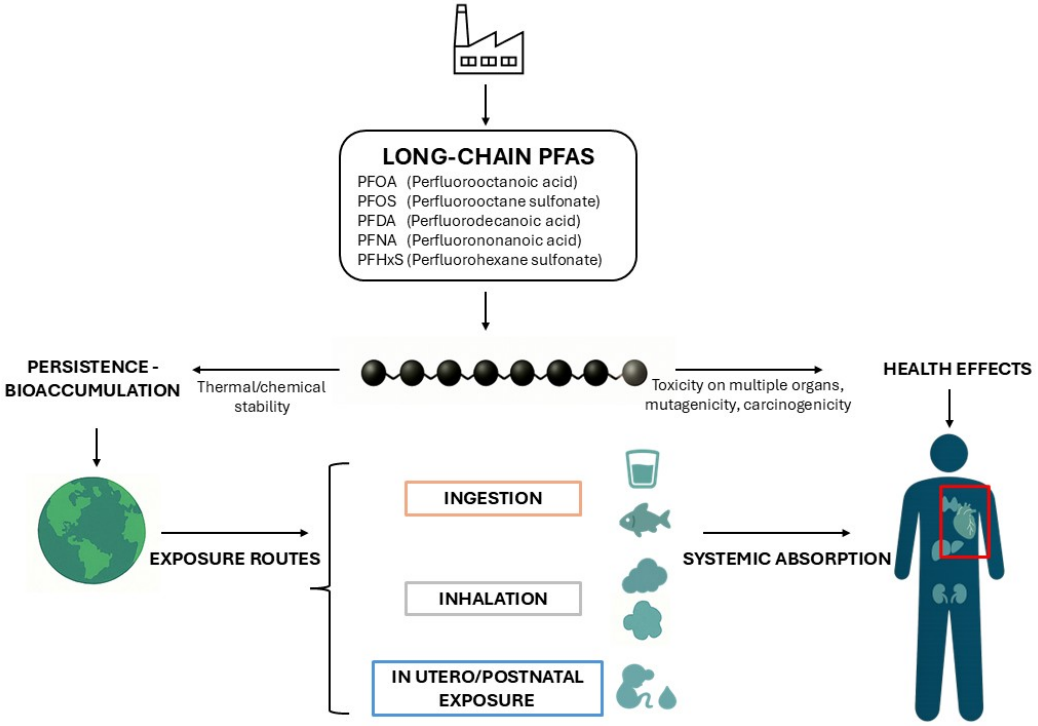

Figure 1. Lifecycle of per- and polyfluoroalkyl substances: from emission to human health effects. Image partially generated with AI Microsoft Copilot 365. Abbreviations: PFAS: per- and polyfluoroalkyl substances.

Reported maximum PFAS concentrations in drinking water reached 47 ng/L in the Netherlands [35], 60.4 ng/L in China [36], and up to 618 ng/L in the United States [37]. A recent study estimated that the median global concentration of C8-PFAS (the sum of PFOA and PFOS) in 212 marine fish species sampled between 2010 and 2021 was 0.34 ng/g wet weight, with Asia and Oceania showing the highest levels and cod-like and herring-like species contributing most to daily dietary intake due to their widespread consumption [38]. Furthermore, PFOS appears to exceed PFOA by approximately one order of magnitude in terms of exposure [38] Such exposure levels have been associated with developmental, hepatic, renal, and hormonal disorders, as well as increased risk of immunotoxicity, cancer, and cardiometabolic effects [18,23,26,39,40]. Human biomonitoring, which serves as a key approach for assessing environmental contaminants in tissues and biological matrices, is generally based on the measurement of organic fluorine in human blood for PFAS [28,32]. PFOS and PFOA have been detected in the blood of nearly all individuals assessed, demonstrating their ubiquitous exposure [41]. Once ingested, they are rapidly absorbed in the gastrointestinal tract, transported predominantly to the liver, and ultimately excreted in unmetabolized forms through urine and feces, with no significant differences in clearance rates between genders [37,41]. Notably,

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most PFAS exhibit slow elimination from the human body, with geometric mean serum half-lives estimated at 3.5 years for PFOA and 4.8 years for PFOS, respectively [42]. Nonetheless, median serum PFAS concentrations vary widely across geographic regions, likely reflecting differences in exposure levels and duration, as well as the industrialization characteristics of the study area [17].

2.1. Legislation of Per- and Polyfluoroalkyl Substances

While until 2000 PFAS were considered inert and harmless to both the environment and humans, emerging data on the risks associated with their exposure for ecosystems and human health have led to the progressive restriction of PFAS production (Figure 2) [43]. The discovery, at the beginning of the current century, of the extensive distribution of PFOS in the tissues of wild animals such as albatrosses, bald eagles, polar bears, and various seal species raised global concern about the bioaccumulation of this compound in higher trophic levels of the food chain [44]. Consequently, in 2000, 3M, the only US manufacturer of PFOS, announced the phase-out of PFOS production by the end of 2002 [43,45]. Six years later, the US Environmental Protection Agency (EPA) invited eight leading PFAS-producing companies, including 3M and DuPont, to join the PFOA Stewardship Program, which aimed to reduce PFOA emissions and PFOA-based products by 95% by 2010 and to achieve their complete elimination no later than 2015 [46]. Following these restrictions, blood PFOS and PFOA levels declined by more than 87% and 74%, respectively, in the US general population aged 12 years and older over two decades (from 1999–2000 to 2017–2020), although the vast majority of the population still shows detectable blood levels of these legacy PFAS, including individuals born after the initial phase-out of their production [47]. Notably, the geometric mean concentrations of PFHxS and PFNA, two additional legacy PFAS, also declined—by 52% and 16%, respectively—yet at slower rates, owing to their longer estimated half-lives and the later implementation of removal measures [48].

At the beginning of 2024, the Food and Drug Administration (FDA) announced that all PFAS-containing grease-proofing agents were no longer being sold in the US [48]. Subsequently, in January 2025, the FDA published a Notice in the Federal Register stating that 35 food contact notifications for PFAS-containing grease-proofers used in paper and paperboard food packaging were considered ineffective, as these uses had been fully abandoned [48]. In 2024, the EPA established legally enforceable limits, known as Maximum Contaminant Levels (MCLs), for five PFAS in drinking water—PFOA, PFOS, PFHxS, PFNA, and hexafluoropropylene oxide dimer acid (commonly known as GenX chemicals)—with allowable concentrations ranging from 4 to 10 ng/L [49]. MCLs were also set for mixtures containing at least two of these PFAS [49].

The European Union (EU) took its first steps toward restricting the production and commercialization of PFOS in 2004 under the EU Persistent Organic Pollutants (POPs) Regulation [50]. This regulatory framework was later replaced by Regulation (EU) 2019/1021, which established a ban on the manufacture and placing on the market of substances listed in Annex I, including PFOS and its derivatives [51]. In 2013, the European Chemicals Agency identified PFOA, along with five other substances, as substances of very high concern (SVHCs), based on their persistence, mobility, and toxicity, which contribute to their potential reproductive toxicity, mutagenicity, and carcinogenicity [52].

Based on the risk assessment of combined exposure to PFOA, PFNA, PFHxS, and PFOS—which together account for approximately 46% of total PFAS exposure—conducted by the European Food Safety Authority (EFSA), toddlers and other young children showed mean intake levels nearly twice those of older age groups due to their higher food intake per kilogram of body weight (bw) [53]. In 2020, EFSA established a daily intake of 0.63 ng/kg bw per day, corresponding to a tolerable weekly intake (TWI) of 4.4 ng/kg bw for the sum of PFOA, PFNA, PFHxS, and PFOS [53]. Given that mean lower-bound exposures for adolescents, adults, the elderly, and the very elderly ranged between 3 and 22 ng/kg bw per week, the highest value of exposure exceeds the TWI by a factor of five [53]. In early 2023, the Forever Pollution Project revealed the existence of nearly 23,000 sites contaminated by PFAS (≥10 ng/L), along with an additional 21,426 presumptive contamination sites across Europe [54].

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In addition, since January 2026, member states are required to systematically monitor PFAS levels in drinking water under the Directive (EU) 2020/2184, which established a maximum limit of 0.5 μg/L for total PFAS and 0.1 μg/L for PFAS at high concern for human exposure and report exceedances, incidents, and any granted derogations [55,56].

At the global level, since 2009, PFOS and its derivatives have been included in the international Stockholm Convention, which mandates their elimination [57]. This key decision was followed by the ban, under the POPs Regulation, of PFOA in 2019, PFHxS in 2022, and long-chain perfluorocarboxylic acids in 2025, together with their salts and derivatives [57]. Importantly, in November 2023, the International Agency for Research on Cancer classified PFOA and PFOS as Group 1 and Group 2B carcinogens, respectively [58].

Following the progressive restriction of long-chain PFAS, industry has shifted toward the use of short-chain PFAS compounds (4–7 fully fluorinated C-atoms)—such as perfluorobutane sulfonic acid (PFBS), perfluorobutanoic acid, perfluorohexanoic acid (PFHxA), perfluoropentanoic acid, and perfluoropentane sulfonic acid—which were initially considered safer due to their lower bioaccumulation potential [31]. However, emerging data indicate that, due to their higher water solubility, lower sorption affinity, and greater mobility, short-chain PFAS may disperse into all environmental media and have recently been associated with cytotoxicity and potential developmental toxicity [31,59]. Accordingly, under the Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH, the main EU framework aimed at protecting human health and the environment from risks posed by hazardous chemicals), PFBS, PFHxA, and 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid were classified as SVHCs between 2019 and 2023 [60,61] (Figure 2).

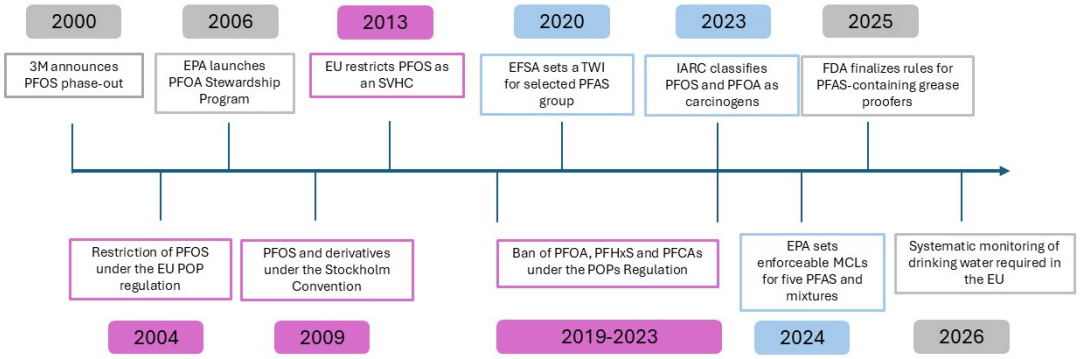

Figure 2. Regulation actions of per- and polyfluoroalkyl substances. Colors indicate the nature of each regulatory event: pink denotes bans and restrictions; light blue represents health-based limits, guidelines, or major toxicological evaluations; grey marks policy, regulatory programs, or industry actions. Abbreviations: EFSA: European Food Security Agency; EPA: Environmental Protection Agency; FDA: Food & Drug Administration; IARC: International Agency for Research on Cancer; MCL: maximum contaminant level; PFCA: perfluorocarboxylic acid; PFHxS: perfluorohexane sulfonate; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonic acid; POP: persistent organic pollutant; TWI: tolerable weight intake.

3. The Association Between Exposure to Per- and Polyfluoroalkyl Substances and Risk of Ischemic Heart Disease: The Epidemiological Evidence

To date, only a limited number of epidemiological studies have investigated the relationship between PFAS exposure and the risk of developing IHD in the general population, and these have predominantly focused on a restricted range of geographic regions. An even smaller number of studies have explored the potential contribution of PFAS to the development of atherosclerosis. Additional investigations conducted among PFAS-exposed worker populations, most of whom were employed prior to the progressive regulatory restrictions on PFAS production and use, have assessed

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IHD incidence and mortality; however, their findings have been inconsistent. Nonetheless, a substantial body of epidemiological and experimental evidence suggests that elevated serum PFAS concentrations may be associated with an increased risk of adverse cardiovascular outcomes. [62].

The following sections therefore summarize the main findings published to date, distinguishing between occupational PFAS exposure and exposure in the general population, the latter further stratified by geographic area and examined in relation to the risk of IHD and atherosclerosis-related biomarkers.

3.1. Occupational Exposure

The impact of occupational PFAS exposure on IHD was first evaluated by Leonard et al. [63] in a cohort of 6,027 men and women who had worked at the DuPont Washington Works (WW) polymer production facility in West Virginia between 1948 and 2002 (with a median serum PFOA concentration of 494 ng/mL measured among workers in 2004, [64]), where ammonium perfluorooctanoate (APFO) was used as a surfactant in industrial processes. The authors compared mortality among WW workers with three reference populations: the US population, the state population of West Virginia, and an eight-state regional employee population from the same company [63]. The latter comparison aimed to minimize the healthy worker effect (HWE), a potential bias that systematically leads to an underestimation of occupational health risks when compared with the general population [63]. IHD mortality was significantly lower than expected in relation to the US and West Virginia populations (Standardized Mortality Ratio, SMR = 81.2; 95% Confidence Interval, CI: 71.2–92.1; and SMR = 68.7; 95% CI: 60.2–77.9, respectively), but was not significantly elevated when compared with expected mortality in the DuPont regional worker population (SMR = 109.5; 95% CI: 96.1–124.4), thereby providing little evidence of an increased IHD mortality risk associated with occupational exposure to PFAS [63]. In a subsequent retrospective-cohort study performed on a cohort of 4,747 employees who had worked at WW plant between 1947 and 2002, IHD deaths accounted for 30.9% of all deaths in the cohort, with 63% of these due to MI [65]. In order to reduce HWE, the authors complemented the internal exposure-response analysis with the use of lagged exposure metrics, thereby limiting bias arising from the preferential retention of healthier workers in the workplace [65]. After dividing workers into four categories based on estimated cumulative exposure quartiles—defined either by the distribution of IHD mortality cases or by the distribution within the entire cohort—no significant increase in IHD mortality risk associated with increasing APFO exposure was observed [65]. The only exception was a borderline-significant positive trend (p=0.06) detected for the 10-year exposure lag in the two highest exposure categories (4,930-6,580 ng/mL and ≥6,580 ng/mL in the third and fourth categories, respectively) when quartiles cutpoints were based on the whole cohort [64]. An update of previous mortality analyses on a cohort of DuPont chemical plant workers [63], including 5,791 workers with follow-up through 2008 and an estimated average annual serum PFOA concentration of 350 ng/mL, reported no significant elevation in overall mortality nor significant positive trends in the exposure-response analysis by quartile of cumulative exposure for IHD with no lag when using DuPont referent rates [66]. In contrast with the findings of Sakr et al. [65], SMRs for IHD in 10-year- (0.95, 1.01, 0.93, 0.93) and 20- year-lag (1.00, 0.92, 1.05, 0.89) analyses, did not reveal any significantly positive trend with increasing quartiles of PFOA exposure [66]. A mortality study conducted retrospectively on a cohort of 3,993 workers (807 of whom died during the follow-up period ending in 2002) employed at a 3M Company manufacturing facility in Minnesota and exposed to APFO for six months or longer, found no increased risk of death from IHD compared with the mortality rate in the general population (SMR=0.8, 95%CI: 0.5-1.4) [67]. Furthermore, the Cox regression analysis showed no association between either intensity (workers highly exposed having median serum PFOA levels ranging from 2,600 to 5,200 ng/mL) or duration of exposure (from less of one year to 5 years or more) and the risk of IHD after adjusting for age, sex, smoking habits, and socioeconomic status [67]. Given the mean worker age of 60 years, the relatively small number of deaths during follow-up (due to the cohort's relatively young age) represents a limitation of the study [67].

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A longitudinal study including 32,254 subjects—3,713 DuPont workers (1948–2002) and 28,541 community residents exposed to PFOA for at least 12 years through contaminated drinking water, thus representing a mixed occupational and community cohort—assessed the association between modeled PFOA exposure and incident IHD [68]. At the time of the baseline C8 Health Project survey (2005–2006), the overall median serum concentration was 26.1 ng/mL [68]. Participants subsequently completed surveys between 2008 and 2011, providing demographic and medical history information [68]. In the primary retrospective analysis—based on yearly serum PFOA concentration estimates generated through environmental fate and transport modeling, residential and occupational exposure reconstruction, and a pharmacokinetic model—there was no clear evidence of an association between increasing quintiles of cumulative PFOA exposure and incident IHD, either in the combined cohorts or in the community cohort alone, suggesting that inclusion of workers did not affect results [68]. Primary retrospective analyses restricted to MI also showed patterns of association comparable to those observed for IHD [68]. Only males aged 20–39 years in the combined cohorts showed a higher risk of IHD in the second through fifth exposure (≥5,058 ng/mL per year) quintiles relative to the reference group (147 ng/mL per year); however, the test for trend was not statistically significant [68]. Similarly, the prospective analysis, which started at the participant's age one year after enrollment in the C8 Health Project, did not show any evidence of an increasing risk of IHD with increasing PFOA exposure.[68]. The absence of an association between PFOA exposure and IHD appears inconsistent with the reported evidence of an association between serum PFOA levels and hypercholesterolemia; however, treatment of participants for hypercholesterolemia may have attenuated any PFOA-related effects of elevated cholesterol on IHD risk [68]. Furthermore, because inclusion in the community cohort required participants to be alive in 2005–2006, some IHD cases may have been excluded from the study, potentially leading to an underestimation of IHD incidence [68]. Based on a subset of 3,713 workers—of whom 1,881 had measured serum PFOA concentrations in 2005 (median 112.7 ng/mL), used to validate the exposure model—a retrospective cohort study [69] evaluated whether PFOA exposure was associated with incident CHD. Exposure was estimated as lifetime cumulative dose, combining yearly occupational exposure (derived from more than 2,000 serum measurements collected from workers with at least one year in their job category) and non-occupational exposure from PFOA-contaminated drinking water through a multistage modelling procedure as reported in [69]. Between 1951 and 2011, 399 cases of CHD were validated, however the Cox regression models did not show any indication of a significantly positive trend for CHD across quartiles of cumulative exposure, with (800, 3,440 and 7,040 ng/mL for the second–fourth quartiles, respectively) or without a 10-year lag period (3,030, 6,160 and 11,420 ng/mL for the second–fourth quartiles, respectively), consistent with the lack of positive exposure-response trends for high cholesterol [69]. Notably, because workers had substantially higher PFOA exposure than the community cohort, the quartile cutpoints used in worker-only analyses were correspondingly higher [69]. These restrictions may have influenced the observed exposure-response trends [69].

In sum, the published studies do not support convincing evidence of an association between serum PFOA concentrations and increased IHD mortality, except for a suggestive trend emerging among the most highly exposed workers when a 10-year lag was applied. Likewise, investigations of IHD incidence in workers, community residents, and combined cohorts have not demonstrated a positive relationship with PFOA exposure, even though higher PFOA exposure was consistently associated with hypercholesterolemia in the pooled population. The limited and inconsistent findings may reflect methodological challenges common to retrospective studies, including imprecise exposure reconstruction, particularly for deceased workers with incomplete occupational and residential histories, and underscore the need for future research using more refined exposure assessment and validated clinical cardiovascular endpoints (Table 1).

 doi:10.20944/preprints202603.1796.v1

Table 1. Key findings and evidence gaps on exposure to per- and polyfluoroalkyl substances and ischemic heart disease in occupational settings.

References	Pitfalls	References	Clues
[63,65–67,69]	Retrospective studies	[63]	Non-significant increase in IHD mortality among WW workers exposed to APFO compared to the regional employee population from the same company
[63]	Significant decrease in SMR mortality for IHD among WW workers in comparison to US and West Virginia population	[64]	A significant trend of increasing IHD mortality risk across higher APFO exposure categories observed only at the 10-year lag
[63]	Potential underestimation of exposure for workers died before 1957		
[65]	No additional significant risk estimates found in any higher exposure category compared with the reference group, nor at any other exposure lags.		
[65]	No information available on individual risk factors for CVD and on medication use		
[65,69]	Possibility of selection bias		
[66]	No exposure-response trend observed for IHD mortality in both 10-year- and 20-year-lag analyses		
[67]	No increase in IHD mortality among employees at 3M Company' Minnesota manufacturing plant exposed to APFO		
[67–69]	Potential for misclassification of exposure, disease and covariates		
[67]	Limited smoking data		
[67]	Relatively small number of deaths in the follow-up period		
[68]	No association between PFOA exposure and incident IHD in either the combined community and worker cohorts or the community cohort		
[69]	No association between PFAS exposure (occupational and non-occupational) and incident CHD		
[68,69]	Underestimation of CHD incidence due to exclusion of deceased workers and requirement to be alive in 2005–2006.		
[68]	Possibility decreasing susceptibility to PFOA effects with increasing follow-up time		

Abbreviations: APFO: ammonium perfluorooctanoate; CHD: coronary heart disease; CVD: cardiovascular disease; PFAS: per- and polyfluoroalkyl substances; PFOA: perfluorooctanoic acid; SMR: standardized mortality ratio; WW: Washington Works plant facility in Parkersburg,.

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3.2. Exposure in the General Population: Risk of Ischemic Heart Disease

3.2.1. United States

An early investigation evaluated the health status of 599 adults recruited through information in the media from a source population of approximately 70,010 residents living in the vicinity of the DuPont plant in West Virginia and exposed to PFOA-contaminated drinking water at concentrations exceeding 0.05 ng/mL [70]. [70]. The exposed subjects showed statistically significant greater prevalence of angina and MI (Standardized Prevalence Ratio—SPR =8.07, 95% CI: = 6.54-9.95 and SPR =1.91, 95% CI:=1.40–2.62) comparing with data of NHANES 2001-2002 [70]. However, the lack of a true unexposed comparison group and the potential self-selection of participants may have introduced bias, possibly leading to an overestimation of the associations due to exaggerated reporting of symptoms [70]. Importantly, the use of contaminated water as a proxy for individual exposure may also have introduced exposure misclassification [70]. Using data from the NHANES 1999–2000, 2003–2004 and 2005–2006, including 3,974 adults representative of the US population, Melzer et al. [71] estimated the associations between serum PFOA and PFOS concentrations and thyroid disease, along with other health outcomes. Both mean serum PFOA and PFOS levels were significantly higher in men than in women (4.91 vs. 3.77 ng/mL and 25.08 vs. 19.14 ng/mL, respectively) [71]. In logistic regression models adjusted for age, ethnicity, study year, BMI, educational status, smoking status and alcohol consumption, neither higher PFOA nor higher PFOS concentrations were significantly associated with increased prevalence CHD, MI, or angina [71]. It should be noted, however, that despite the long half-life of PFOA and PFOS, these results are based on a single serum measurement used as a proxy for medium-term internal dose, which may lead to an underestimation of exposure and a weakening of the observed associations [71]. A subsequent study [72] based on merged data from NHANES 1999-2000 and 2003-2004 consisting of 1327 participants 40 years and older examined the independent relationship between serum PFOA levels and CVD outcomes, including CHD, stroke, and peripheral arterial disease (PAD), the latter characterized by chronic progressive accumulation of atherosclerotic lesions leading to varying degrees of arterial obstruction and ischemic symptoms in the affected extremities [73]. In multivariable logistic regression models, increasing serum PFOA concentrations across sex-specific quartiles (from <2.9 to >5.6 ng/mL in women and from <3.1 to >6.1 ng/mL in men) were significantly associated with the combined prevalence of CHD or PAD, with an Odds Ratio (OR) of 2.28 (95% CI: 1.40–3.71) in the highest quartile compared with the reference) [72]. In particular, the authors reported that participants in the highest PFOA quartile had a significantly higher prevalence of CHD (OR = 2.24, 95% CI: 1.02–4.94) and PAD (OR =1.78, 95% CI: 1.03–3.08) compared with those in the lowest quartile, independently of major confounders such as age, sex, BMI, diabetes, total cholesterol, hypertension, smoking and alcohol consumption [72]. In subgroup analyses, higher PFOA levels were significantly and positively associated with the presence of CHD or PAD in both sexes, in obese and non-obese individuals, and in never or former smokers [72]. The absence of an association among current smokers was likely attributable to the small sample size in this subgroup [72]. While these results are based on a representative, multiethnic sample of adequate size and include information on multiple confounders, the cross-sectional design does not allow for causal inference [72]. A US biomonitoring study conducted in 2012–2013 on 154 male anglers aged ≥50 years, a group with potentially elevated PFAS exposure due to high fish consumption, found no significant associations between serum concentrations of any of the seven PFAS detected in at least 30% of participants (perfluoroheptane sulfonate, PFDA, PFHxS, PFNA, PFOA, PFOS, PFuDA) and self-reported diagnoses of CHD (10.4% prevalence) or the grouped CVD outcome after adjustment for age, BMI, work status, and alcohol consumption, despite a modest but significant positive association of ΣPFAS and Σsulfonates with high cholesterol [74]. Consistently, among the few and weak associations observed between fish consumption and individual PFAS, Great Lakes and other locally caught fish appeared to be the major sources of PFAS exposure [74]. Furthermore, PFAS concentrations in this cohort were generally comparable to those of the US general population, except for PFOS, which was

 doi:10.20944/preprints202603.1796.v1

nearly twice as high among anglers compared with a similar NHANES subgroup (19 vs. 10 ng/mL) [74]. In agreement with [74], in a cross-sectional study of 5,270 adults aged ≥20 years with diabetes from the C8 Health Project, Honda-Kohmo et al. reported that serum concentrations of the four PFAS examined -PFOA, PFOS, PFHxS, and PFNA—were significantly (except for PFNA) and inversely associated with the prevalence of CHD with multivariable ORs ranging from 0.72 to 0.90 after adjustment for relevant confounders, including BMI, CRP, LDL and high-density lipoprotein (HDL) cholesterol, diabetes duration, acid uric, and estimated glomerular filtration rate [75]. A progressively stronger inverse association between PFAS concentrations and CHD was also observed across exposure quintiles, with higher quintiles showing increasingly lower odds of CHD [75]. In a post-hoc analysis evaluating the relationship between PFAS exposure and CHD in 49,161 individuals without diabetes, the inverse association between serum levels of each PFAS and CHD prevalence was similarly confirmed (ORs 0.92-0.95) [75]. The authors also excluded the possibility that the inverse association between PFAS and CHD in diabetic patients was attributable to reduced kidney function: although 22.2% of participants had chronic kidney disease (CKD), results remained consistent when analyses were stratified by CKD status, and no significant interaction between PFAS levels and CKD was observed [75]. Based on data of NHANES 1999-2014 for a total 10,859 participants aged ≥20 years who provided a serum sample for the measurement of 12 PFAS and a positive self-reported physician diagnosis of selected CVD outcomes, Huang et al. [76] reported borderline significant associations between increasing levels of serum PFAS levels and prevalence of CHD and MI (p for trend=0.0623 and 0.0636, respectively) after full adjustment for well-established risk factors for CVD. Additional significant associations emerged at the highest exposure levels for specific compounds: CHD was positively associated with PFNA, PFDA, and perfluoroundecanoic acid (PFUnDA) (p for trend = 0.0101, 0.0107, and 0.0008, respectively); MI with PFNA (p for trend = 0.0240); and angina pectoris with PFUnDA and perfluorododecanoic acid (PFDoA) (p for trend = 0.0408 and 0.0138, respectively) [76]. However, it should be noted that among the 12 PFAS analyzed, only four of these (PFOS, PFOA, PFNA and PFHxS) were detected in more than 98% of participants, whereas five compounds, including PFDoA, showed detectable levels in only 30-65% of samples [76]. On the other hand, the lack of significant associations for PFOS and PFOA is likely attributable to their progressive phase-out and the consequent decline in population exposure over time, although participants with CVD exhibited higher levels of total PFAS and PFOS compared to those without CVD [76]. In the analysis of the relationship between PFAS exposure (serum concentrations of PFOS, PFOA, PFNA, PFHxS, detected in >75% of participants) and CVD risk in 7,904 adults participating in NHANES 2003–2012, Feng et al. [77] reported that a log-unit increase in PFOS levels was weakly but significantly associated with a higher risk of MI among males (OR =1.01, 95% CI: 1.00–1.01, p = 0.040), while a log-unit increase in PFNA levels was associated with a 10% higher risk of CHD and MI (p = 0.022 and p = 0.028, respectively) among males. These findings support a stronger influence of PFAS exposure in males, likely reflecting sex-specific elimination pathways in females—primarily menstruation, followed by pregnancy and lactation—which facilitate faster clearance of PFAS [77]. Finally, using data from the Health Professionals Follow-up Study (HPFS, 51,529 male professionals aged 40-75 years at baseline) and Nurses' Health Study (NHS, 121,700 female nurses aged 30-55 years at baseline), Zhu et al. [78] conducted two nested case-control study by including participants who were initially free of CVD at blood collection (1990 in NHS and 1994 in HPFS) and subsequently developed CHD (n = 101). Each case was matched to a control on age, smoking status, and date of blood sampling [78]. After full adjustment for confounders including family history of MI and histories of hypertension, diabetes, and hypercholesterolemia, higher plasma levels of total PFOS (OR=3.66, 95%CI: 1.36-9.89), branched PFOS (OR=3.68, 95%CI: 1.55-8.76), and linear PFOS (OR=3.01, 95%CI: 1.16-7.86) were significantly associated with an increased risk of CHD, with all three PFOS measures exhibiting a clear linear dose–response relationship [78]. In contrast, no significant associations with CHD were observed for PFOA, PFDA, PFNA, or PFHxS [78]. The associations between PFOS and CHD risk do not appear to be mediated by blood lipids or lipid subfractions, given the absence of significant relationships between PFAS and apolipoprotein (apo)C-III levels, a key predictor of cardiovascular outcomes [79].

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Conversely, the significant positive associations of PFDA, PFNA, and PFHxS with apoE in HDL particles, with or without apoC-III, may help explain the non-significant inverse associations of these compounds with CHD risk [78].

3.2.2. Europe

Within a longitudinal setting, Mattsson et al. [80] recruited 253 men with a CHD diagnosis between 1992 and 2009, identified through national registers, along with an equal number of age-matched controls. All participants provided a baseline blood sample in 1990–1991, and 104 case–control pairs contributed a second sample at the 2002–2003 follow-up for the measurement of eight PFAS [80]. Serum levels of PFOS and PFOA significantly decreased over time, whereas those of PFDA, PFNA, and PFHxS increased; no significant changes were observed for perfluoroheptanoic acid (PFHpA), PFUnDA and PFDoA. Unlike the findings reported in [76], no differences were observed between cases and controls for any PFAS, suggesting that CHD does not substantially influence PFAS levels [80]. Considering the exposure levels from the first sampling point, the authors observed no significant associations between serum levels of any PFAS and risk of CHD, with the exception of a significantly higher risk associated with the third quartile of PFHpA compared to the first quartile (OR=2.58, 95%CI: 1.39-4.78) [80. They hypothesized that this effect was likely due to chance, given the much lower concentrations of PFHpA relative to the chemically similar compound PFOA [80]. On the other hand, because the study population consisted exclusively of men, and given the potential sex-specific differences in PFAS toxicokinetics and effects, this may have introduced sex-related bias in the observed associations [80]. More recently, in a nested case–control study, Schillemans et al. [81] investigated the association between PFAS exposure and the risk of incident MI and stroke using data from two Swedish cohorts: the Swedish Mammography Cohort-Clinical (SMC-C), including women residing in the Uppsala area, and the Cohort of 60-year-olds (60YO), consisting of men and women residing in Stockholm County, which provided baseline blood samples collected between 2003–2009 and 1997–1998, respectively. During follow-up (through 2017 for SMC-C and 2014 for 60YO), 345 incident MI cases were identified and matched with healthy controls in a 2:1 ratio in the SMC-C and a 1:1 ratio in the 60YO, according to sex, age, and sample date (yielding a total of 475 controls) [81]. The third tertile of both the PFAS sum (comprising eight compounds) and most individual compounds (including PFOS, PFNA, PFDA, and PFUnDA) was significantly and positively associated with higher total and LDL cholesterol levels [81]. At the same time, the vast majority of PFAS showed favorable metabolic effects, being associated with increased concentrations of HDL cholesterol and apoA1, the major protein component of HDL and a marker linked to reduced atherosclerotic risk, [82]) as well as with lower triglyceride levels [81]. After full adjustment for traditional cardiovascular risk factors, the ∑PFAS demonstrated a significantly inverse association with CVD risk in the pooled cohorts (OR=0.73; 95% CI: 0.55–0.97, comparing the third tertile with the reference group) [81]. A similar pattern emerged for MI, where the multivariable-adjusted risk was lower in the highest tertile of PFAS sum compared with the lowest (pooled OR=0.60; 95% CI: 0.39–0.92) [81]. Therefore, despite the strong and well-established association between LDL cholesterol and MI [83,84], and despite the adverse impact of PFAS on total and LDL cholesterol levels, this does not translate into increased risk of MI, possibly due to the counterbalancing favorable effects of PFAS on HDL cholesterol, apoA1, and triglyceride levels, which may attenuate or outweigh the potentially harmful influence of LDL elevations [81]. Alternatively, the PFAS-mediated increase in LDL cholesterol may not be sufficient to enhance cardiovascular risk, as supported by the observed lack of association between higher PFAS concentrations (both as a combined exposure metric and as individual compounds) and apoB, the principal structural protein of atherogenic lipoproteins and a stronger determinant of atherosclerotic risk than LDL cholesterol alone [81,85]. Furthermore, the inability to include PFOA and PFHpA in the analyses of the SMC-C cohort due to sample contamination may have resulted in an underestimation of the overall PFAS effect [81].

 doi:10.20944/preprints202603.1796.v1

3.2.3. China

A hospital-based case-control study enrolling 355 newly diagnosed patients aged 18-75 years with acute coronary syndrome (ACS, including MI and instable angina) between January and May 2022, together with 466 controls matched for age (within 5 years) and sex, evaluated the association between exposure to six plasma PFAS concentrations and ACS risk [86]. In the fully adjusted logistic regression model, only plasma levels of PFOA (OR=2.43, 95%CI: 1.34-4.39, p=0.006), PFOS (OR=1.65, 95%CI: 1.14-2.38, p=0.013), and PFUnDA (OR=1.50, 95%CI: 1.07-2.09, p=0.024) were significantly positively associated with risk for ACS [86]. However, the multiple-PFAS model showed significantly positive associations exclusively related to PFOA and PFOS (OR=1.51, 95%CI: 1.07-2.15, p=0.049; OR=1.77, 95%CI: 1.15-2.72, p=0.034), with a clear dose-response pattern, possibly reflecting their markedly higher plasma concentrations—up to seven-fold greater than those of the other PFAS [86]. Nonetheless, the overall mixture effect on ACS risk was null, as only PFOA, PFOS, and PFUnDA contributed positively to the mixture estimate [86]. In the sex-stratified analysis, the authors reported a sex-dependent effect, with no associations detected in females and significant positive associations for PFOA, PFOS, PFHxS, and PFDA among males, likely attributable to both the lower PFAS concentrations and the lower prevalence of ACS in women [86]. Of interest, despite the significantly inverse associations of platelet count, mean platelet volume, and plateletcrit (the product of MPV and platelet count) and the significantly positive association of platelet distribution width with ACS risk, PFOS showed a significant inverse association only with platelet count, and platelet count appeared to attenuate its effect on ACS risk by more than 15%, suggesting a potential mechanistic pathway through which PFAS may contribute to CVD development [86]. A further study enrolling 571 consecutive patients aged 18–80 years with a new diagnosis of ACS and no history of occupational PFAS exposure assessed whether PFAS exposure may influence the degree of coronary stenosis at baseline, thereby affecting ACS prognosis during follow-up [87]. The degree of coronary stenosis was quantified through coronary angiography using the Gensini score (GS), categorized into four groups (0–3) reflecting increasing stenosis severity, and by the number of lesioned vessels (LVN), defined as the number of major coronary arteries exhibiting ≥50% angiographic stenosis [87]. Prognosis was evaluated through the incidence of major adverse cardiovascular events (MACE), including cardiovascular death, nonfatal MI, stroke, and coronary revascularization, over a median follow-up of 14.3 months. [87]. The results showed a significant positive association between plasma PFOS concentrations, modeled as a continuous variable, and both GS (Hazard Ratio, HR=1.33, 95% CI: 1.06–1.67, p=0.034) and LVN (HR = 1.36, 95% CI: 1.08–1.71, p = 0.023) after full adjustment for covariates (age, sex, BMI, smoking, alcohol consumption, and educational status). Furthermore, PFOS and total PFAS were significantly and positively associated with the occurrence of MACE (HR=1.96, 95% CI: 1.34–2.89, p=0.002; and HR=2.46, 95% CI: 1.51–4.01, p=0.002, respectively), and nonfatal MI (HR=3.86, 95% CI: 2.00–7.46, p=0.000; and HR=4.56, 95% CI: 1.99–2.45, p=0.001, respectively), with similar positive associations observed when comparing the highest tertile of exposure with the reference group [87]. Additionally, the authors identified threshold concentrations for PFAS and individual congeners for each endpoint examined, with PFOS thresholds of 4.65 ng/mL for GS, 4.54 ng/mL for LVN, 5.14 ng/mL for MACE, and 5.03 ng/mL for nonfatal MI, and with variability across compounds depending on differences in chemical structure, biological behavior, and bioaccumulation potential [87]. Inconsistent effects of PFOA compared with the authors' previous study [86] may be attributable to the lower PFOA concentrations in this population (4.39 ng/mL vs 4.99 ng/mL) [87]. Furthermore, the absence of associations between PFAS mixtures and the degree of coronary stenosis (GS and LVN scores) or coronary revascularization may depend on the differential susceptibility of these indicators to PFAS exposure, as well as on the heterogeneous effects of the various components within the mixtures [87].

Overall, current evidence regarding the contribution of PFAS exposure to the occurrence of IHD in the general population remains inconsistent. However, suggestive findings indicate associations with certain PFAS compounds, particularly PFOS and PFOA, which, despite regulatory restrictions, have historically reached the highest environmental levels and contribute most to human exposure.

 doi:10.20944/preprints202603.1796.v1

The predominantly cross-sectional design of most published studies, together with the lack of repeated PFAS measurements, prevents firm conclusions about causality, even when significant positive associations are observed. Moreover, differences in study populations (in some cases including only men and largely conducted in the United States), limited sample sizes, heterogeneous adjustment for confounders, and the variety of congeners examined complicate comparisons across studies. Future longitudinal investigations conducted across diverse geographic areas, including populations exposed to levels exceeding background concentrations and incorporating comprehensive adjustment for established cardiovascular risk factors, are needed to strengthen the current evidence base (Table 2).

Table 2. Key findings and evidence gaps on exposure to per- and polyfluoroalkyl substances and ischemic heart disease in the general population.

References	Pitfalls	References	Clues
[70,72,74–77]	Self-reported diseases/symptoms	[70]	PFOA exposure via drinking water significantly associated with increased self-reported prevalence of angina and MI
[70]	Potential selection bias	[72]	Increasing PFOA concentrations significantly associated with a higher prevalence of CHD and PAD, independent of traditional cardiovascular risk factors
[70]	No true unexposed control group for comparison	[76]	Signals of significant positive association between the highest exposure to PFAS sum and prevalence of CHD and MI
[70]	Potential exposure misclassification	[76]	Increasing quartiles of serum PFNA, PFDA, and PFUnDA significantly and positively associated with prevalence of CHD
[70]	No adjustment for confounders	[76]	Increasing quartiles of serum PFNA significantly and positively associated with prevalence of MI
[71]	No significant association between serum PFOA and PFOS concentrations and prevalence of IHD	[76]	Increasing quartiles of serum PFUnDA, and PFDoA significantly and positively associated with prevalence of angina pectoris
[71,72,74–78,81,86,87]	Single PFAS measurement	[77]	Log-unit change in PFOA levels modestly but significantly associated with increased MI risk among males
[13,71,72,74–78,86,87]	Cross-sectional design	[77]	Log-unit change in PFNA levels significantly associated with increased MI and CHD risk among males
[74,80]	No significant association between serum levels of any of PFAS examined and risk of CHD	[78]	Higher plasma levels of total PFOS, branched PFOS, and linear PFOS significantly associated with increased risk of CHD
[74,80]	Cohort composed solely of men	[80]	Significant association only between serum levels of PFHpA and higher risk of CHD for the third quartile compared to the lowest quartile
[75]	Serum PFAS levels significantly inversely associated with CHD in subjects with diabetes independently of the presence of CKD	[81]	Plasma levels of PFAS sum, PFOS, PFDA, PFNA, and PFUnDA significantly and positively associated with higher concentrations of total and LDL-cholesterol
[78]	No significant positive associations between PFAS with lipoprotein subspecies relevant for CHD risk	[86]	Plasma PFOA and PFOS significantly and positively associated with ACS risk

 doi:10.20944/preprints202603.1796.v1

[78]	Serum levels of PFNA, PFDA and PFHxS significantly and positively associated with total apoE among HDL particles with or without apoC-III	[86]	Dose-response relationship with increasing trend between PFOA and PFOS and ACS risk
[81]	Plasma levels of PFAS sum, PFOS, PFOA, PFDA, PFNA, and PFUnDA significantly and positively associated with higher concentrations of HDL-cholesterol and apoA1	[87]	Significant positive association between plasma PFOS concentration and coronary stenosis severity in patients with ACS
[81]	Plasma levels of PFAS sum, PFOS, PFOA, PFDA, PFNA, and PFUnDA significantly and positively associated with higher concentrations of HDL-cholesterol and apoA1	[87]	Significant positive association between plasma levels of total PFAS and PFOS with occurrence of MACE in patients with ACS
[81]	Plasma levels of PFAS sum, PFHxS, PFOS, PFDA, PFNA, and PFUnDA significantly and positively associated with lower concentrations of triglycerides		
[81]	No significant association between plasma levels of PFAS and apoB		
[81]	Plasma PFAS levels significantly and inversely associated with incident MI		
[78,81]	Cross-sectional design of lipid analyses		
[71,75,77,78,81,86]	Potential residual or unmeasured confounding		
[81]	Possibility of underestimation of MI risk due to the use of lipid-lowering medications during follow-up		
[86]	Plasma levels of PFNA, PFDA, PFHxS and PFUnDA not significantly associated or inversely correlated with ACS risk		
[86]	No significant association between plasma PFAS mixture levels and ACS risk		
[70,74,78,86]	Limited sample size		
[78]	Homogenous socioeconomic status among participants		

Abbreviations: ACS: acute coronary syndrome; Apo: apolipoprotein; CHD: coronary heart disease; CKD: chronic kidney disease; HDL: high-density lipoprotein; MI: myocardial infarction; MACE: major adverse cardiovascular effects; PAD: peripheral arterial disease; PFDA: perfluorodecanoic acid; PFDoA: perfluorododecanoic acid; PFHpA: perfluoroheptanoic acid; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluoro-octane sulfonic acid; PFUnDA: perfluoroundecanoic acid; PFHxS: perfluorohexane sulfonate; 3.3. Exposure in the general population: association with atherosclerosis development.

A limited number of studies have explored the potential link between PFAS exposure and atherosclerosis, the main contributor to IHD. In a cross-sectional study based on a nationwide mass

 doi:10.20944/preprints202603.1796.v1

urine screening program, Lin et al. [13] investigated the relationship between PFAS exposure and the risk of IHD in 664 Taiwanese individuals aged 12–30 years, using carotid intima–media thickness (IMT) as a well-established surrogate marker of atherosclerosis and a strong predictor of CHD and MI [88]. Among the four PFAS examined—PFOA, PFOS, PFNA, and PFUnDA—only increasing serum PFOS concentrations were significantly and positively associated with thicker carotid in the full-adjusted multiple linear regression analysis (p for trend < 0.001) [13]. In contrast, a significant inverse association was reported between increasing quartiles of PFNA and carotid IMT [13]. Subgroup analyses further showed a significant negative association between PFNA and carotid IMT in females, individuals aged 12–19 years, those with BMI <24, non-smokers, and subjects carrying the *APOE* ε2 or ε2/ε3 genotype. [13]. *APOE* is a gene widely recognized as involved in the pathophysiology of CVD, diabetes, neurodegenerative disorders, and nephropathies [89]. The common apoE variants are associated with heterogeneous effects on health and cardiovascular risk [64]. Individuals who are homozygous for the *APOE2* allele or heterozygous *APOE3/2* typically exhibit a reduced risk of CVD, whereas carriers of at least one ε4 allele show increased carotid IMT and higher LDL cholesterol levels. [89,90]. These unexpected findings may be explained by hypothesizing that the effects of age, sex, obesity, smoking, and *APOE* genotype on carotid IMT are stronger than those of PFOS itself [13]. Notably, similar to other EDCs, the maximal effects of PFOS on carotid IMT appear to occur at the 50th–75th concentration percentiles, following a non-monotonic or biphasic dose–response pattern characterized by U-shaped or inverted U-shaped relationships [91]. Furthermore, although plasma PFNA levels were inversely associated with carotid IMT, the concurrent presence of higher PFOS concentrations (>50th percentile) and lower PFNA levels (≤60th percentile) was associated with a three-fold increase in the risk of having thicker carotid IMT (OR 3.01; 95%CI: 1.68–5.39) [13]. The same authors subsequently explored the relationship of PFAS exposure and carotid IMT with oxidative stress, circulating endothelial microparticles and platelet microparticles in a cohort of 848 subjects 12-30 years old [92]. Endothelial microparticles, small, anucleate vesicles (100–1000 nm) released from activated or apoptotic cells, may contribute to endothelial dysfunction by interfering with the nitric oxide production, thereby serving as sensitive biomarkers of endothelial injury and potential key players in the development and progression of CVD [92,93]. Increasing quartiles of serum PFOS concentrations—showing maximal effects at the 50th–75th percentiles—were significantly associated with higher levels of constitutive endothelial microparticles (CD31+/CD42a−; p for trend < 0.001), elevated CD31+/CD42a+ (a marker of platelet apoptosis; p=0.010), and greater IMT (p < 0.001), suggesting that PFOS may contribute to the progression of vascular dysfunction by inducing endothelial and platelet apoptosis [92]. In contrast, CD31+/CD42a− levels were inversely associated across increasing categories of PFOA, PFNA, and PFUnDA [92]. Additionally, a significant negative relationship was found between quartiles of PFUnDA and both CD62E, a marker of endothelial activation, and carotid IMT [92]. None of these PFAS were significantly associated with urinary oxidized nucleoside 8-hydroxydeoxyguanosine, a biomarker of oxidative DNA damage [92,94]. Notably, the highest risk of thicker carotid IMT (greater than the 50th percentile) was observed when both CD31+/CD42a− and CD31+/CD42a+ levels exceeded the 50th percentile (OR=2.86, 95% CI: 1.69–4.84, p < 0.001). However, as in previous research, the cross-sectional design does not allow inference on causal direction. Lind et al. [95] assessed three different indices of atherosclerosis in a cohort of 1,016 seventy-year-old subjects living in Uppsala, Sweden: carotid IMT and the echogenicity of the intima–media complex (also referred to as the intima–media grey scale median), both indicators of early carotid arterial changes, as well as the presence of plaques (defined as a local IMT increase of more than 50% compared with the surrounding IMT). All three measures are established predictors of cardiovascular events [49,96,97]. In the total sample, 26.9% of participants had bilateral carotid plaque, while 33.8% had unilateral plaque [95]. Eight PFAS—PFOA, PFOS, PFDA, PFNA, PFHxS, PFUnDA, PFHpA, and perfluorooctane sulfonamide (PFOSA)—showed measurable concentrations above the lower limit of detection in more than 80% of participants [95]. In contrast to the findings reported by Lin et al. [13], in linear regression models none of the examined PFAS were significantly associated with carotid

 doi:10.20944/preprints202603.1796.v1

IMT, either in sex-adjusted analyses (with men and women combined) or in sex-stratified analyses, with the exception of PFOSA, which was significantly associated with thicker IMT in the fully adjusted model for the entire cohort (p=0.01) and among females (p=0.004) [95]. These conflicting findings may be partly explained by differences in the age of the study populations, although Lin et al. [13] also reported a stronger relationship between PFAS exposure and IMT among females. Some of the selected PFAS showed a significant interaction with sex in their relationship with the intima–media grey scale median: PFNA was positively associated among females after full adjustment, whereas PFUnDA was inversely associated in men, with the latter effect appearing to be only minimally mediated by serum cholesterol [95]. No association was detected with the occurrence of carotid plaques in the overall cohort, except for an increased risk of atherosclerotic plaques observed in females in relation to elevated serum levels of PFUnDA (OR=1.59, 95% CI: 1.03–2.43), although this association did not appear to be mediated by cholesterol levels [95]. Based on data from 666 prediabetic adults enrolled in the US Diabetes Prevention Program trial who had provided two blood samples for PFAS measurement, one at baseline and one at the second annual visit as well as measurements of coronary artery calcium (CAC) and ascending (AsAC) and descending aorta-calcification (DAC) at the tenth annual visit, Osorio-Yáñez et al. [98] evaluated the relationship between PFAS exposure (for a total six compounds) and risk of CAC and thoracic aorta calcification (TAC). Quantification of CAC by computed tomography is considered the non-invasive gold standard for cardiovascular risk stratification in asymptomatic individuals, with elevated CAC indicating subclinical atherosclerosis and an independent predictor of CHD morbidity and mortality [99–101]. The Agatston score provides a standardized measure of coronary calcification based on both the extent and density of calcified plaques [100]. Similarly, TAC has been associated with atherosclerosis-related risk factors and with CHD risk [101]. The authors reported that each doubling in mean plasma total PFOS (defined as the sum of linear PFOS and PFOS isomers) and linear PFOS concentrations was associated with a higher risk of severe CAC (CAC Agatston score >400; OR= 1.49, 95% CI: 1.01–2.21 and OR=1.54, 95% CI: 1.05–2.50, respectively) after adjustment for confounders (including treatment assignment and statin use) [98]. In the fully adjusted regression model, N-ethyl-perfluorooctane sulfonamido acetic acid (EtFOSAA) demonstrated a clear dose–response relationship with CAC risk. Specifically, each doubling of EtFOSAA concentration was significantly associated with higher odds of moderate CAC (CAC Agatston score 100-400; OR=1.26, 95%CI: 1.08-1.47) and severe CAC (OR=1.37, 95%CI: 1.07-1.74) [98]. Furthermore, after adjustment for covariates, each doubling of plasma total PFOS was associated with a 67% higher probability of AsAC (95%CI: 1.10-2.54), an effect primarily driven by the linear PFOS isomer (OR=1.70, 95%CI: 1.13-2,56) [98]. In contrast, no significant associations were observed between EtFOSAA and either AsAC or DAC [98]. Importantly, although each quantile increase in plasma PFAS mixture concentration was associated with a 31% higher risk of moderate-to-high CAC compared with low CAC in the crude model, this effect became non-significant after full adjustment [98].

Collectively, exposure to certain PFAS congeners may contribute to the development of atherosclerosis, as suggested by significant positive associations reported with increased IMT, markers of endothelial and platelet dysfunction, CAC, and thoracic aortic calcification, independent of traditional cardiovascular risk factors, and in some cases displaying a sex specific pattern. In contrast, associations with other markers of vascular disease, such as the echogenicity of the intima–media complex and the number of carotid plaques, remain inconsistent. The number of studies published to date remains limited, and most available evidence is derived from cross-sectional designs relying on a single PFAS measurement. Future multicenter prospective studies including repeated exposure assessments and multiple vascular biomarkers are needed and should be conducted in larger, multiethnic populations spanning a wide age range.

 doi:10.20944/preprints202603.1796.v1

Table 3. Key findings and evidence gaps on exposure to per- and polyfluoroalkyl substances and atherosclerosis in the general population.

References	Pitfalls	References	Clues
[13,92,95]	Single PFAS measurement	[13]	Serum increasing quartiles of PFOS and PFNA significantly and positively associated with carotid IMT
[13,92,95]	Cross-sectional design	[92]	Significant positive associations between increasing serum PFOS concentrations, CD31+/CD42a− and CD31+/CD42a+ and extent of carotid IMT
[13]	Increasing levels of serum PFNA significantly and inversely associated with carotid IMT	[92]	Most relevant association between PFOS and carotid IMT observed when both levels of CD31+/CD42a− and CD31+/ CD42a+ are elevated
[13]	Most relevant associations between PFOS exposure and carotid IMT observed in younger, non-obese individuals and in those carrying *APOE* ε2 or ε2/ε3 genotype	[95]	Serum PFOSA levels significantly associated with thicker IMT in the whole cohort and among women
[13,92,95,98]	No adjustment for other environmental contaminants and medications	[95]	PFNA exposure significantly and positively associated with IM-GSM among women
[13,92]	Population study consisting of exclusively adolescents and young adults	[95]	PFUnDA significantly associated with increased risk with the number of carotid arteries plaques among females
[13]	Other unknown processes able to increase both serum PFOS levels and carotid IMT	[98]	Each doubling of mean sum of linear and branched isomers of PFOS significantly associated with severe CAC
[92]	Significant inverse associations between increasing serum PFNA levels and CD31+/CD42a− and CD62E	[98]	Each doubling of mean plasma concentration of linear PFOS significantly associated with severe CAC
[92]	Significant inverse relationship between increasing categories of PFOA and PFNA and CD31+/CD42a− levels	[98]	Each doubling of mean plasma concentration of EtFOSAA significantly associated with CAC in a dose-dependent manner
[92]	No significant association between serum PFAS and urinary 8-OHdG levels	[98]	Each doubling of both mean plasma concentration of PFOS and linear PFOS significantly associated with AsAC
[95]	No significant association between serum PFAS levels and IMT (except for PFOSA) and IM-GSM in the sex-combined analyses		
[95]	Serum PFUnDA concentration inversely associated with IM-GSM among men		
[95]	Sample limited on only Caucasian aged 70		
[98]	No significant association between mean concentration of plasma PFAS mixture and CAC		
[98]	No significant association between any PFAS compounds and DAC		
[98]	Lack of repeated CAC measurements		

Abbreviations: Apo: apolipoprotein; AsAC: ascendent aortic calcification; CAC: coronary artery calcium; DAC: descendent aortic calcification; EtFOSAA: N-ethyl-perfluorooctane sulfonamido acetic acid; IM-GSM: intima–media grey scale median; IMT: intima-media thickness; PFAS: per- and polyfluoroalkyl substances; PFNA: perfluorononanoic acid; PFOS: perfluoro-octane sulfonic acid; PFOSA: perfluorooctane sulfonamide.

 doi:10.20944/preprints202603.1796.v1

4. Epigenetic and Genetic Mechanisms Underlying PFAS-Induced Ischemic Heart Disease

Although epidemiological evidence increasingly links PFAS exposure to cardiovascular outcomes, including IHD, the molecular dynamics underlying these associations remain to be fully characterized. Described mechanisms include increased levels of total and LDL cholesterol [102], induction of platelet activation [103], inflammation and endothelial dysfunction [77], remodeling in cardiomyocytes [104], elevated blood pressure and increased arterial stiffness [105]. Beyond these alterations, deeper modifications may occur in cells that can change their assets, and that can be ultimately responsible for IHD risk (Figure 3). In this context, growing attention has been directed toward epigenetic regulation as potential mediator of PFAS-induced cardiovascular effects [106]. Moreover, genetic alterations in terms of telomere shortening and variations in mitochondrial DNA copy number (mtDNAcn) have been reported as markers of PFAS effects [107] and separately correlated to IHD risk [108–110]. However, direct and robust evidence linking PFAS-induced epigenetic and genetic variations to IHD is limited in medical literature, therefore this review aims to shed light on the mechanisms underlying the possible cause-effect correlation between PFAS exposure and cardiovascular risk, based on the current knowledge that need to be further investigated.

In addition, emerging studies report that genetic background may modulate individual susceptibility to PFAS-related effects within a gene-environment interaction framework, thereby contributing to inter-individual variability in disease risk [111].

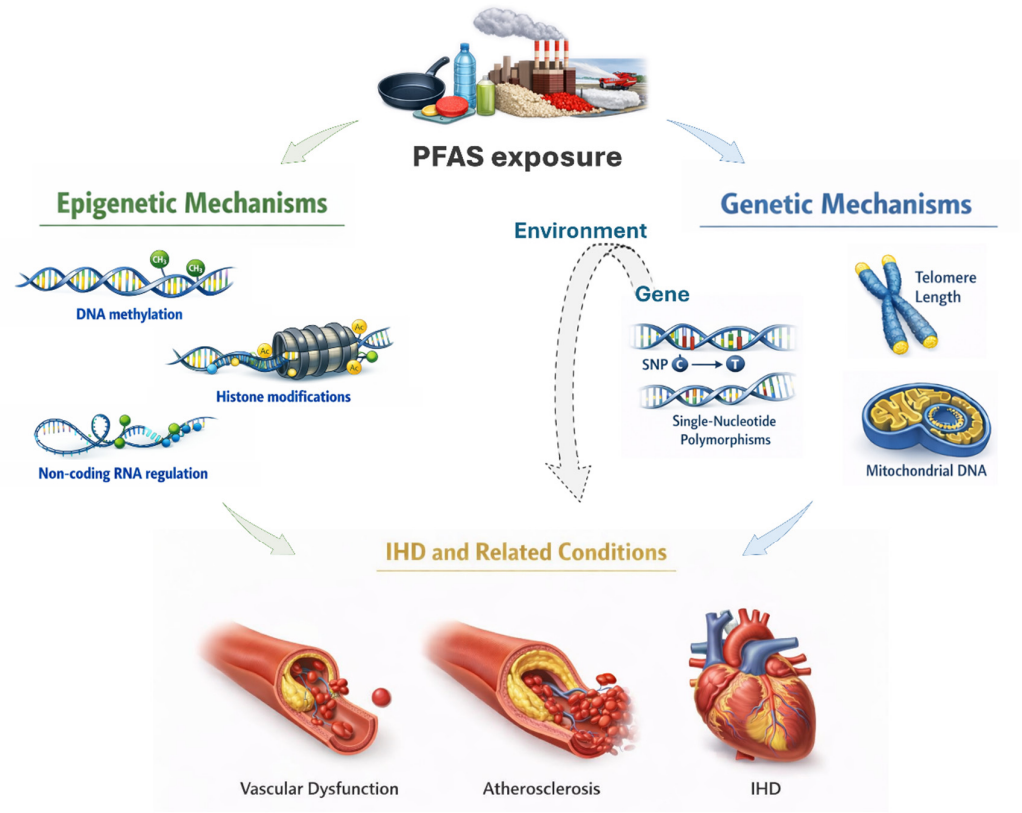

Figure 3. PFAS-induced epigenetic and genetic mechanisms related to IHD risk. Image partly generated with AI Microsoft Copilot 365. Abbreviations: DNA: deoxyribonucleic acid; IHD: Ischemic heart disease; PFAS: per- and polyfluoroalkyl substances; RNA: ribonucleic acid; SNP: single-nucleotide polymorphisms.

4.1. Epigenetic Alterations: DNA Methylation, Histone Modifications, and Non-Coding RNAs Regulation

 doi:10.20944/preprints202603.1796.v1

Epigenetic alterations are defined as mechanisms such as DNA methylation, histone modifications, and non-coding RNA regulation, which modulate gene expression without modifying DNA sequence and lead to changes in cellular functions, contributing to disease development. Epigenetic changes can be triggered by several endogenous and exogenous factors, including exposure to environmental pollutants like PFAS [106].

DNA methylation represents one of the major epigenetic mechanisms and consists in the covalent addition of a methyl group in 5′ position of cytosines in CpG regions by DNA methyltransferases (DNMT). This modification plays an essential role in gene regulation, typically leading to transcriptional repression. PFAS have been reported to alter global and gene-specific DNA methylation, in both a direct manner and indirectly by inducing oxidative stress and hormonal imbalances [112].

A large cross-sectional study including 1425 young and middle-aged individuals demonstrated a positive association between serum PFOS levels, increased global DNA methylation, and greater carotid IMT [113].

Alterations in DNA methylation patterns are incorporated into mathematical models known as epigenetic clocks that estimate biological aging and, consequently, age-related diseases. Higher serum concentrations of PFNA and PFSA were significantly associated with accelerated epigenetic aging, as assessed by DNA methylation-based epigenetic clocks, with a more pronounced effect in older and male adults [114].

Importantly, PFAS exposure during pregnancy, particularly to PFHxS, has been linked to extensive alterations in placental DNA methylation in genes involved in pathways related to fetal development and cardiometabolic risk, including lipid metabolism, inflammation, and vascular function, all processes that are mechanistically implicated in the development of IHD [115]. These findings are consistent with a previous longitudinal epigenome-wide association study by Liu et al., showing that gestational exposure to PFOA, PFOS, PFHxS, and, especially PFNA, promoted differential DNA methylation at 435 CpG sites in children, several of which mapped to genes implicated associated with CVD and other major health outcomes [116].

Histone modifications encompass a variety of chemical changes—such as acetylation, methylation, phosphorylation, ubiquitination—occurring primarily on histone tails and regulating gene expression by altering histone-DNA interactions and, consequently, chromatin structure. Emerging evidence suggests that PFAS may disturb this regulatory network by directly inhibiting enzymes responsible for histone modifications or by indirectly affecting their activity through mechanisms including endocrine disruption and oxidative stress [112]. PFAS exposure has been shown to influence histone-modification dynamics during pregnancy as well. In an in vivo model, maternal exposure to PFOA induced liver toxicity in offspring, accompanied by reduced histone acetylation and decreased expression of peroxisome proliferator-activated receptors alpha (PPAR-α) gene [117], events mechanistically linked to IHD via transcriptional repression of cardioprotective genes, enhanced vascular inflammation, oxidative stress, maladaptive cardiac remodeling, impaired lipid metabolism, and reduced anti-atherogenic activity [118,119].

Non-coding RNAs (ncRNAs) are epigenetic regulators of gene expression, acting through diverse mechanisms depending on their class: microRNAs (miRNAs) bind to complementary messenger RNA to block translation or promote degradation; long non-coding RNAs are larger molecules (>200 nucleotides) can function as “scaffolds,” bringing chromatin-remodeling complexes to specific DNA sites, or as “sponges” that sequester miRNAs; circular RNAs regulate intracellular availability of miRNAs. ncRNAs families play a central role in orchestrating cellular responses to environmental stressors, including PFAS, ultimately shaping disease susceptibility and progression [120].

PFOS and PFHxS exposure has been associated with downregulation of three serum microRNAs, miR-101-3p, miR-144-3p, and miR-19a-3p, which target genes, including *DNMT3a*, *PPAR-α*, *EGFR* (Epidermal Growth Factor Receptor), *HMGCR* (3-hydroxy-3-methylglutaryl-CoA reductase), NR1H3 (Nuclear Receptor Subfamily 1 Group H Member 3), *PTGS2* (Prostaglandin-

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Endoperoxide Synthase 2), and *TGF-α* (Transforming Growth Factor Alpha) [121]. Through effects on lipid metabolism, vascular inflammation, and endothelial function, these alterations may mediate PFAS-induced cardiovascular risk [121]. Moreover, in two independent cohorts of children, PFAS exposure correlated with decreased levels of miR-148b-3p and miR-29a-3p, both implicated in cardiovascular disease pathways and related chronic disease mechanisms [122].

4.2. Genetic Alterations and Susceptibility: Focus on Telomere Length, Mitochondrial DNA Copy Number, and Single-Nucleotide Polymorphisms

Telomere length and mtDNAcn have emerged as informative biomarkers of genomic stability and cellular aging, largely owing to their accessibility in peripheral blood. Accumulating evidence indicates that these markers are sensitive to environmental exposures and may reflect the biological impact of external stressors on human health, thereby providing insights into individual susceptibility to disease [123]. Telomeres, composed of repetitive TTAGGG nucleotide sequences located at the ends of linear chromosomes, preserve genomic integrity but in eukaryotic cells, shorten progressively with each cycle of DNA replication due to the so-called “end-replication problem”, ultimately limiting cellular replicative capacity. Telomere attrition is widely regarded as a hallmark of biological aging and cumulative cellular stress, and is associated with several conditions, including IHD [124].

A study recruiting 175 adults aged 50-65 years reported an inverse association between leukocyte telomere length (LTL) and PFOS exposure [125]. In a larger population including 453 individuals, telomere shortening was linked to PFOA, perfluoro-2,5-dimethyl-3,6-dioxanonanoic acid, and perfluoro-2-methoxyacetic acid concentrations [107]. Notably, a cohort study of 1,489 middle-aged and older Chinese adults demonstrated that high PFAS exposure (especially to PFOA, PFNA, PFDA, and PFUnDA) was associated with reduced LTL and genetic susceptibility and poor diet amplify this effect: telomere shortening becomes particularly significant when combined with low healthy diet score (determined on the basis of the consumption of eight major food components) and high polygenic risk score for telomere shortening (derived from 16 single-nucleotide polymorphisms identified in a genome-wide assay) [126].

PFAS cross the maternal-fetal barrier, and prenatal appears to influence telomere length, shaping susceptibility to age-related diseases later in life. This effect seems to be sex-specific and mediated by oxidative-stress–related mechanisms. Indeed, in, in a cohort of 581 newborns, higher PFOS and PFDA levels were associated with shorter LTL in female but not male infants, in parallel with increased reactive oxygen species [127]. A similarly sized Chinese study confirmed inverse associations between maternal PFAS exposure and newborn telomere length and identified spring birth season as a potential amplifier of PFAS effects [128]

Mitochondrial DNA (mtDNA) is an essential component of the cellular genome. It is organized as a double-stranded circular molecule and encodes genes that are critical for mitochondrial function, particularly oxidative phosphorylation and cellular energy metabolism. Unlike nuclear DNA, mtDNA lacks protective histone proteins and has limited repair capacity, which makes it especially susceptible to oxidative damage. Consequently, mtDNAcn is considered a sensitive indicator of mitochondrial dysfunction and oxidative stress, processes increasingly implicated in the development of cardiovascular diseases, including IHD)[129].

The overall influence of PFAS exposure on mtDNAcn appears to be inconsistent and dependent on the specific PFAS congeners considered. In the study by Vriens et al., mtDNA content was higher in individuals with elevated PFOS serum concentrations, whereas exposure to PFHxS was associated with lower mtDNAcn [125]. Similarly, a cross-sectional study conducted in China involving 453 participants reported positive correlations between perfluoro-3,5,7,9-butaoxadecanoic acid and perfluoro-3,5,7,9,11-pentaoxadodecanoic acid levels and mtDNAcn. An increase in mtDNAcn may reflect enhanced mitochondrial biogenesis or a compensatory response to mitochondrial stress, whereas reduced mtDNAcn is generally interpreted as a sign of impaired mitochondrial replication or increased mitochondrial damage [107]

 doi:10.20944/preprints202603.1796.v1

Analogous to telomeres, mtDNAcn variations have also been detected in cord blood in association with maternal PFAS exposure. In a cohort of 572 mother-newborn pairs, prenatal exposure to PFOS resulted in an inverse association with mtDNAcn, while nonlinear associations were found for 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid, PFDA, and PFNA. A clear sex-specific pattern emerged since reductions in mtDNAcn were more pronounced in female newborns of mothers exposed to PFOS, whereas lower mtDNAcn was observed in male offspring when mothers were exposed to PFHxS. The authors also highlighted potential mitigating role of folate status in modulating PFAS-related mitochondrial effects [130]. Consistently, a decrease in mtDNAcn was reported in a panel study including 284 children, where exposure to PFAS mixture significantly reduced mtDNAcn, with PFOA identified as the major contributor [131].

As previously reported, PFAS exposure has been associated with dyslipidemia, increased oxidative stress, and vascular dysfunction. However, not all individuals exposed to PFAS develop CVD, suggesting a role for genetic predisposition in modifying risk. Gene–environment interactions may help explain inter-individual variability in disease onset and progression. Genetic variants, particularly single-nucleotide polymorphisms, are common alterations in the DNA sequence that can influence gene function or expression and contribute to inter-individual variability in susceptibility to environmentally induced diseases, including the cardiovascular impacts of PFAS [111]. In a study of Kobayashi et al. on 504 pregnant women, variants in *PPARD*, a nuclear receptor regulating fatty acid oxidation and lipid homeostasis, were shown to modulate the effects of PFOS exposure on lipid levels [132]. Notably, the rs1053049 TT genotype, which is associated with elevated LDL cholesterol as well as altered triglyceride and fatty acid profiles, may heighten susceptibility to PFAS-related cardiometabolic distirbamces that contribute to IHD [132]. Similarly, in a cohort of 665 Faroese adults followed from birth, Valvi et al. identified several genetic variants, including *ABCA1* rs3890182, *FTO* rs9939609, *FTO* rs3751812, *PPARG* rs170036314, and *SLC12A3* rs2289116, that modify susceptibility to PFOS- and, to a lesser extent, PFOA-induced reductions in insulin sensitivity. These genes are involved in lipid transport (*ABCA1*), energy balance and obesity (*FTO, PPARG*), and blood pressure regulation (*SLC12A3*), and individuals carrying these risk alleles may be more prone to PFAS-related metabolic disruptions linked to IHD [133].

5. Promising New Approaches to PFAS Research and Related Cardiovascular Risk Assessment

Digital Health represents the actual frontier of technology research in the field of healthcare and well-being [134], and in such framework Artificial Intelligence (AI) presents a novel manner to look after common questions of the healthcare universe. More specifically, in the sector of environmental pollutants, it can be leveraged to improve understanding of PFAS-related cardiovascular toxicity and to enhance exposure assessment, risk prediction, and disease monitoring, particularly where early detection of exposure effects and related subclinical dysfunction gets critical [135].

5.1. Potentialities of Artificial Intelligence for PFAS Exposure Assessment and Environmental Modeling

In the framework of PFAS-related research, it becomes of utmost importance to ensure proper classification of exposure due to the lack of completeness of historical data and, consequently, to heterogeneity in exposure pathways [136]. To cope with that, the possibility to integrate multiple heterogeneous data sources by means of AI, can improve the whole exposure framework reconstruction [137], ultimately leading to the development of models for accurately estimating the individual exposure trajectories to PFAS, overtaking typical methodological limitations related to the single biomarker measurement.

In addition, the increasing availability of high dimensional biological data, such as epigenomic, transcriptomic, proteomic, metabolomic, and microbiome, requires all the facilities to identify complex associations between the exposure to PFAS and molecular pathways significantly concerned with cardiovascular disease [138,139].

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AI, and Machine Learning in particular, as happening with other conditions in cardiovascular field [140], as well as in cancer research [141] and beyond, offers the possibility to detect signatures of PFAS-induced endothelial dysfunction, mitochondrial impairment, lipid perturbation, and pro-inflammatory phenotypes, and enable the integration of genomic susceptibility factors, with PFAS exposure data potentially useful for the identification of subgroups at higher risk. All in all, AI modeling is foreseen to be capable of playing a role in clarifying causal pathways and mediating factors related to the cardiovascular conditions eventually associated with such exposures. For example, Neural Networks and ensemble learning techniques might be used to embed PFAS-related biomarkers along with traditional risk factors (lipids, blood pressure, glucose metabolism), lifestyle variables, medication history, and sociodemographic characteristics, to support early identification of individuals with subclinical endothelial dysfunction, increased arterial stiffness or, in general, enhanced vascular aging [142,143]. Longitudinal modeling, fostered by AI, could help clarify the dose-response patterns and highlight the windows of susceptibility [144], particularly during critical life phases, such as prenatal or perinatal periods, adolescence or older age.

5.2. Future Perspectives for Consumer Technologies and Digital Health in PFAS-Related Effects Monitoring

Wearable tools are, like AI, solutions whose relevance in the market and in the research is continuously growing, opening a completely new, unprecedented opportunity in the effort of collecting continuous physiological data related to health and well-being of an individual, particularly, in this case, for cardiovascular health. Currently, modern biosensors and smartwatches have the capability of monitoring physiological parameters such as Heart Rate Variability blood pressure, oxygen saturation, sleep quality, physical activity and, indirectly, checking arrhythmia burden, sensitive features to early signs of autonomic imbalance, endothelial dysfunction, and systemic inflammation, in turn potentially influenced by PFAS exposure [145,146]. All this considered, the convergence of AI, multi-omics and consumer technologies leads to the implementation of precision medicine—precision cardiology in this case—approaches, which also turn out to be environmentally informed. Although some issues and challenges remain, such as data privacy, standardization, explainability and equitable access, those approaches, when merged, have the potential to enable early detection of PFAS-related cardiovascular impairments, ultimately leading to targeted prevention strategies.

6. Conclusions

Despite global regulatory efforts to restrict the production of legacy PFAS and limit their dispersion and accumulation across environmental media, PFAS exposure remains a worldwide concern due to their environmental persistence, their long biological half-life in humans, and the partly unknown spectrum of their toxicological effects. Their role in the occurrence of IHD has yet to be fully established, with studies conducted in the general population reporting positive, null, or even inverse associations. The variety of congeners assessed and the variability in exposure profiles across study populations have likely contributed to these conflicting results. Notably, the significant positive associations described in the literature largely involve the most widespread congeners—PFOS, PFDA, and PFNA—although these same compounds have also shown an apparent protective effect against IHD in some analyses, further contributing to the inconsistency of current evidence. Moreover, studies conducted in occupational settings have not reported any association between PFAS exposure and IHD mortality, while evidence on disease incidence remains sparse and characterized by substantial uncertainty. By contrast, more consistent findings have emerged from research evaluating the relationship between PFAS and markers of atherosclerosis and vascular dysfunction, particularly PFOS and PFNA. Nonetheless, these indications require confirmation through large, multicenter prospective studies that include repeated exposure measurements and standardized protocols for PFAS congener selection, as well as careful adjustment for key confounders. Particular attention should be given to potential effect modification by age, sex, and comorbidities to improve the interpretability and generalizability of findings.

 doi:10.20944/preprints202603.1796.v1

Current evidence suggests that PFAS may contribute to cardiovascular risk through a combination of metabolic, inflammatory, and structural alterations, along with more subtle molecular alterations involving epigenetic regulation and genetic integrity. Changes in epigenetic signature, telomere length and mtDNAcn point to possible underlying biological effects; however, their direct causal role in the development of IHD remains insufficiently established. In addition, inter-individual variability linked to genetic background highlights the importance of gene–environment interactions in shaping susceptibility to PFAS-related cardiovascular damage.

Taken together, these observations underscore the need for integrated, multidisciplinary research to clarify causal mechanisms, validate reliable biomarkers of effect, and identify population groups at increased risk—ultimately informing targeted prevention and public health strategies. Future studies should also address the combined effects of PFAS mixtures on both intermediate and clinical cardiovascular outcomes. This effort should be supported by mechanistic investigations in experimental models and by leveraging emerging opportunities offered by artificial intelligence and advanced analytical tools to better elucidate biological pathways and strengthen causal inference.

Author Contributions: Conceptualization, F.G., A.T. and A.B.; methodology, F.G. and A.B; writing—original draft preparation, F.G., A.T., M.P. and A.B.; writing—review and editing, F.G., A.T., M.P., E.B., F.M. and A.B.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACS	Acute coronary syndrome
APFO	Ammonium perfluorooctanoate
Apo	Apolipoprotein
BMI	Body mass index
EDC	Endocrine disrupting chemical
CHD	Coronary heart disease
CIMP	Carotid intima-media thickness
CRP	C-reactive protein
CVD	Cardiovascular disease
HDL	High-density lipoprotein
HWE	Healthy worker effect
IHD	Ischemic heart disease
IMT	Intima-media thickness
LDL	Low-density lipoprotein
LTL	Leukocyte telomere length
MI	Myocardial infarction
MtDNAcn	Mitochondrial DNA copy number
OR	Odds Ratio
PFAS	Per- and polyfluoroalkyl substances
PFBS	Perfluorobutane sulfonic acid
PFDA	Perfluorodecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFDoA	Perfluorododecanoic acid

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PFOS	Perfluoro-octane sulfonic acid
PFOSA	Perfluorooctane sulfonamide
PFUnDA	Perfluoroundecanoic acid
POP	Persistent organic pollutant
SMR	Standardized Mortality Ratio
SVHC	Substance of very high concern
TWI	Tolerable weekly intake

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