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Full length article

Serum per- and polyfluoroalkyl substances and prevalent heart disease: cross-sectional analysis of the multi-site study

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ABSTRACT

Previous research investigating exposure to per- and polyfluoroalkyl substances (PFAS) and their association with heart disease has found conflicting results, with recent studies reporting null or inverse associations. We estimated associations between serum PFAS and prevalent heart disease in a large, cross-sectional, multi-site study of adults residing in communities in the United States with historic PFAS drinking water contamination. Participants were recruited from communities in eight states (2019–2023), where they completed questionnaires and provided blood samples. PFAS concentrations were measured in serum. Four PFAS detected in > 70% of participants were modeled as continuous variables, and three less-frequently detected PFAS were modeled as binary variables (above/below the limit of detection). Logistic regression models estimated associations between each PFAS and prevalent heart disease based on self-report, adjusting for demographics, smoking and alcohol consumption, waist-to-hip ratio, estimated glomerular filtration rate (eGFR), and study site. Quantile g-computation models were used to estimate the joint effects of PFAS as a mixture. A total of 5,761 adult participants were included in the analysis, with a mean age of 56 years and 60.2% female. The prevalence of self-reported heart disease was 9.2%. Adjusted analyses showed no statistically significant associations between all PFAS and self-reported heart disease. Our mixture analysis revealed no association between PFAS as a mixture and prevalent self-reported heart disease (OR: 0.99, 95% CI: 0.95–1.03). Our findings suggest largely null associations between serum PFAS and self-reported heart disease.

1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally (Hamza Khalifa Ibrahim, 2025). CVD are a group of conditions that affect the heart (e.g. coronary heart disease, heart failure, arrhythmias, and congenital heart defects) (Benjamin et al., 2019). In this study, we focus on heart disease, a subset of CVD that affects the heart and

accounts for the majority of CVD-related fatalities in the United States. During 2010–2022, 75% of all CVD deaths in the United States were attributable to heart disease (Woodruff et al., 2024). In 2024, heart disease alone accounted for 683,037 deaths in the United States as reported by the Centers for Disease Control and Prevention (CDC) (Provisional Report, 2024).

Per- and polyfluoroalkyl substances (PFAS) are characterized by

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their stable carbon–fluorine bonds, which make them resistant to heat, oil, grease, and water (Buck et al., 2011). Due to their chemical stability, PFAS are used extensively in industry and consumer products (Glüge et al., 2020). Despite the phasing out of production of “legacy” PFAS in the United States and Europe – such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonic acid (PFHxS) – their long half-lives (e.g., PFOA [range 1.48 to 5.1 years]; total PFOS [range 3.4 to 5.7 years]) have resulted in their accumulation and continued presence in the environment and accumulation in human tissue (Rosato et al., 2024). As a result, several legacy PFAS have been detected in the blood of over 98% of Americans (Calafat et al., 2007). PFAS contamination in drinking water occurs from various sources, including industrial facilities, military bases, and airports that used aqueous film-forming foam as a fire suppressant, and wastewater treatment (Hu et al., 2016). Widespread PFAS contamination of drinking water was reported in the United States in 2016, with more recent estimates suggesting over 200 million residents in the United States have drinking water containing more than 1 ng/L for PFOA or PFOS (Hu et al., 2016; Andrews and Naidenko, 2020). While this concentration was substantially below the EPA’s 2016 lifetime health advisory of 70 ng/L, the agency has since established enforceable Maximum Contaminant Levels (MCLs) of 4 ng/L for PFOA and PFOS in 2024, highlighting the increasing recognition of potential health risks at much lower exposure levels (Register, 2024).

Experimental evidence indicates that PFAS can induce cardiac toxicity *in vivo* and *in vitro*, characterized by cardiac dysfunction, morphologic abnormalities, mitochondrial damage, myocardial apoptosis, and impaired myocardial differentiation (Wen et al., 2023). Some epidemiological studies and meta-analysis have found evidence for associations between exposure to PFAS and increase in blood lipids (Lind et al., 2018; Liu et al., 2023), elevated blood pressure (Pitter et al., 2020; Pan et al., 2023), and markers of impaired glucose metabolism, including insulin resistance, altered pancreatic β -cell function, and, less consistently, type 2 diabetes (India Aldana et al., 2026), all of which are established risk factors for cardiovascular disease. Yet, epidemiologic studies that have directly examined associations between PFAS and CVD have found mixed results. Analyses of National Health and Nutrition Examination Survey (NHANES) data have reported positive associations of serum PFAS concentrations with prevalent cardiovascular disease (including coronary heart disease, congestive heart failure, angina pectoris, heart attack, and stroke) and peripheral artery disease using data collected from 1999 to 2014 (Shankar et al., 2012; Huang et al., 2018). The C8 Health Project – a large cohort study in a community with PFOA-contaminated drinking water in the United States – observed elevated hazards of incident stroke in retrospective analyses using pre-enrollment exposure histories, particularly in mid-range exposure quintiles; however, prospective analyses restricted to follow-up after cohort entry showed no association between cumulative PFOA exposure and stroke (Simpson et al., 2013). Other cohort studies, such as the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study and Young Taiwanese Cohort, found positive associations between PFAS concentrations and subclinical cardiovascular measures – including carotid intima-media thickness (e.g. PFOS, perfluoroundecanoic acid [PFUnDA]) – and left ventricular geometry (e.g., perfluorononanoic acid [PFNA]) (Liu et al., 2023; Lin et al., 2013; Mobacke et al., 2018). More recently, a 2023 study of two population-based longitudinal cohorts found no significant associations between PFAS concentrations (PFOA, PFOS, and PFHxS) and incident CVD (combined myocardial infarction, ischemic stroke, and heart failure) overall, and an inverse association between PFOS and incident CVD among men (Dunder et al., 2023). The same study conducted a meta-analysis of previously published epidemiologic studies and found an inverse association between PFOA and incident CVD (odds ratio [OR] = 0.80, 95% confidence interval [CI]: 0.66–0.94). One potential contributor to inconsistencies in associations between PFAS and CVD could be variations in the population’s PFAS exposure type and concentrations

from different sources of exposure, as well as heterogeneity in covariate adjustment across studies.

Few studies have evaluated associations between PFAS and CVD across geographically diverse communities in the United States. To address this gap, we pooled data from adults residing in communities with documented PFAS-contaminated drinking water to investigate associations between seven serum PFAS and self-reported prevalent heart disease across different exposure sources, PFAS profiles, and serum concentration distributions.

2. Methods

2.1. Study population and exposure measurement

The Agency for Toxic Substances and Disease Registry’s (ATSDR) Multi-site study (MSS) is a nationwide cross-sectional study designed to investigate health outcomes linked to PFAS exposure in U.S. communities with known drinking water contamination. Detailed descriptions of the study design, recruitment strategies, and site selection criteria can be found in the previously published cohort profile (Pavuk et al., 2025). Briefly, participants were recruited from communities in California, Colorado, Massachusetts, Michigan, New Hampshire, New Jersey, New York, and Pennsylvania, during 2019–2023. New Hampshire served as the pilot site led by ATSDR from 2019 to 2021 to develop all protocol procedures and tools. The other seven participating MSS sites followed the established protocol for data collection, storage, shipping, and analysis starting in 2021. For the present analysis, we included participants aged ≥ 18 years with at least one serum PFAS measurement. Those with incomplete self-reported heart disease diagnosis were excluded from analysis ($n = 63$).

2.2. Exposure assessment

Blood samples were collected by phlebotomists during study clinic visits. Serum was aliquoted into polypropylene cryovials and stored at -80°C until shipment. Serum samples for PFAS analysis were shipped frozen with dry ice to the Centers for Disease Control and Prevention (CDC) laboratory. A second frozen serum sample was shipped to the CDC biorepository for storage for future use. Serum PFAS analysis was conducted at the CDC laboratory within the National Center for Environmental Health (NCEH). All materials for serum collection were provided by the CDC to ensure consistency among sites.

Quantification of serum PFAS, including 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA), PFHxS, linear PFOS (n-PFOS), sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS), linear PFOA (n-PFOA), sum of branched isomers of PFOA (Sb-PFOA), PFNA, perfluorodecanoic acid (PFDA), and PFUnDA, was performed using solid-phase extraction and isotope dilution, followed by high-performance liquid chromatography coupled with tandem mass spectrometry, based on established analytical methods (Kato et al., 2011; Kato et al., 2018). The limit of detection (LOD) for all PFAS was 0.1 ng/mL, and concentrations below LOD were replaced with $\text{LOD}/\sqrt{2}$ for statistical analyses (Hornung and Reed, 1990). After imputing concentrations below LOD with $0.1/\sqrt{2}$, the concentrations of PFOS and PFOA were calculated as the sum of n-PFOS and Sm-PFOS and the sum of n-PFOA and Sb-PFOA, respectively, and these summed measures were used in all analyses. PFAS detected in more than 70% of the study population were modeled as continuous variables ($\sum\text{PFOA}$, $\sum\text{PFOS}$, PFHxS, and PFNA), whereas PFAS detected in less than 70% (PFDA, PFUnDA, and MeFOSAA) were modeled as binary variables (detect versus non-detect). A sum of seven PFAS variable was calculated by summing the results of $\sum\text{PFOA}$, $\sum\text{PFOS}$, PFHxS, PFNA, PFDA, PFUnDA, and MeFOSAA (including imputed values replaced with $\text{LOD}/\sqrt{2}$) (Committee on the Guidance on PFAS Testing and Health Outcomes, 2022).

2.3. Health outcome

The primary health outcome – self-reported prevalent heart disease (self-reported heart disease) – was based on the question: “Have you ever been told by a medical professional or health care provider that you have or had the following – heart disease?” (Response options: yes, no, don’t know, refuse to answer) collected during the medical history portion of the questionnaire. All participants were asked for consent to request medical record information from their primary medical provider. For consenting participants, providers were asked to complete a medical record abstraction form that included the 25 prespecified clinical conditions assessed in the questionnaire, including heart disease, and recorded whether each condition was documented in the medical record and the year of diagnosis or treatment when available. For heart disease, the form included a single question – “Heart disease?” (Response options: yes, no) and “Year of Diagnosis or Treatment.” No prespecified cardiovascular diagnoses or ICD codes were designated for inclusion or exclusion; therefore, the medical record measure represents provider-recorded heart disease as a broad category and could not be further classified into specific cardiovascular conditions. For the vast majority of responses, the form was completed by health care providers or medical office staff and returned directly to the study team. If complete medical records were provided instead of the abstraction form (<0.1%), trained site research staff abstracted the requested disease information. Medical record abstraction was conducted between September 2023 and November 2024. Because completed abstraction forms were not available for all consenting participants, medical record information was used in sensitivity analyses rather than to define the primary outcome.

2.4. Covariates

Participants completed questionnaires in person during the clinical visit or over the phone with trained staff. During the questionnaire, participants provided information on demographic characteristics, health-related behaviors, and medical history. Age, sex, race and ethnicity, household income, education attained, alcohol history, smoking history, hormonal contraceptive usage, menopausal status, and parity were collected during questionnaires. Race and ethnicity were combined into the following categories: “Hispanic”, “Non-Hispanic, American Indian or Alaskan Native”, “Non-Hispanic, Asian or Pacific Islander”, “Non-Hispanic, Black”, “Non-Hispanic, Multiracial”, and “Non-Hispanic, White.” These categories were derived from two separate questions on the questionnaire: 1) “What is your race?” (Response options: American Indian or Alaskan Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or Multiracial and 2) “Are you Hispanic?” (Response options: No, Yes, Refuse to Answer). Missing values for race and ethnicity were imputed and then combined into a single variable for analysis.

During the clinic visit, body measurements and serum samples were taken by trained staff and phlebotomists. Serum samples for effect biomarkers were picked up daily or stored at -20°C , and shipped to LabCorp, who conducted the analyses of effect biomarkers and clinical tests. Waist-to-hip ratio was calculated by taking the waist circumference (measured in inches) divided by the hip circumference (measured in inches), both of which were collected during the clinic visit. Lastly, estimated glomerular filtration rate (eGFR) was derived from creatinine analyzed in serum samples collected during the clinic visit using the 2021 CKD-EPI creatinine equation (Inker et al., 2021).

Covariates were selected based on a *a priori* review of literature and development of a directed acyclic graph (Fig. S1) (Textor et al., 2016).

2.5. Statistical analysis

Associations between PFAS (Σ PFOA, Σ PFOS, PFHxS, PFNA, PFDA, PFUnDA, MeFOSAA, and the sum of all measured seven PFAS) and self-reported heart disease were analyzed using logistic regression models.

Adjusted models were used to control for potential confounders: age (continuous), sex (male [reference], female, missing or refused), race and ethnicity (Hispanic, Non-Hispanic American Indian or Alaskan Native, Non-Hispanic Asian/Pacific Islander, Non-Hispanic Black, Non-Hispanic Multiracial, Non-Hispanic White [reference], missing or refused), education (high school graduate or less [reference], some university/college, technical or trade, university/college graduate, graduate school or higher, missing), income (<\$25,000 [reference], \$25,000 to \$69,999, \$70,000 to \$149,000, $\geq$ \$150,000, missing/refused/did not know), smoking history (never smoker [reference], former smoker, current smoker), alcohol history (never drinker [reference], former drinker, current drinker), waist-to-hip ratio (continuous), eGFR (continuous), and study site (California [reference], Colorado, Massachusetts, Michigan, New Jersey, New York, Pennsylvania, New Hampshire). Primary analysis included both all counts of self-reported heart disease and a case-restricted definition of recent case diagnosed (2019–2023), with participants reporting a diagnosis before 2019 excluded from analysis.

Multiple imputation was used to address missing data for covariates with incomplete observations, income (6.2%), race and ethnicity (1.3%), sex (0.1%), education (0.1%), alcohol use (0.2%), smoking status (0.2%), hormonal contraceptive usage (0.6%), and menopausal status (0.3%) with imputed 1,000 datasets and five iterations (Buuren and Groothuis-Oudshoorn, 2011). Additional variables such as high blood pressure diagnosis, kidney disease diagnosis, and high cholesterol were included in the multiple imputation by chained equations (MICE) model (Buuren and Groothuis-Oudshoorn, 2011) as predictors due to expected correlations with variables with some missing values but were not included in the primary analysis.

We also evaluated the joint association of the seven- PFAS mixture (Σ PFOA, Σ PFOS, PFHxS, PFNA, PFDA, PFUnDA, and MeFOSAA) with self-reported heart disease using logistic quantile g-computation (Keil et al., 2020). For this analysis, all PFAS were treated as continuous, with concentrations below the LOD assigned a value of $\text{LOD}/\sqrt{2}$ (Hornung and Reed, 1990). PFAS exposure measures were internally discretized into quartiles ($q = 4$) as part of the quantile g-computation procedure. For PFAS with a high proportion of values below the detection limit, many imputed values were identical, resulting in fewer distinct quantile cutpoints; these PFAS therefore contributed limited variability to the mixture analysis (PFUnDA and MeFOSAA). Primary mixture models were fitted using 500 bootstrap iterations to obtain confidence intervals and improve the stability of estimates (Efron and An, 1994). We estimated associations between the PFAS mixture and self-reported heart disease.

Sensitivity analyses assessed the robustness of findings by stratifying by sex, varying covariate adjustment, restricting cases to participants with self-reported heart disease documented in medical record abstraction data, and conducting leave-one-out site analyses. We conducted sex-stratified analyses to evaluate whether associations between PFAS exposure and prevalent heart disease differed between male and female participants. Models among male participants were adjusted for age, race and ethnicity, education, income, smoking history, alcohol history, waist-to-hip ratio, eGFR, and study site. Models among female participants were adjusted for the same covariates and additionally adjusted for hormonal contraceptive use, menopausal status, and parity to account for female-specific factors that may influence PFAS toxicokinetics and cardiovascular risk.

We evaluated the influence of covariate selection using two alternative adjustment models. First, Model A excluded waist-to-hip ratio from the primary covariate set because body composition may lie on the causal pathway between PFAS exposure and cardiovascular disease. Second, Model B additionally adjusted the primary model for serum albumin concentration, given the potential influence of albumin binding and physiological factors on measured serum PFAS concentrations.

An additional sensitivity analysis restricting cases to participants with heart disease documented in medical record abstraction data was

conducted to evaluate whether associations differed when the outcome was limited to provider-documented cases. Because medical record abstraction was limited and varied across sites (ranging from 10% to 88% of total participants with medical record abstraction performed (Pavuk et al., 2025), this analysis was interpreted as a sensitivity analysis rather than the primary analysis. Analyses included associations between individual PFAS, the sum of seven, and heart disease, as well as adjusted models controlling for the potential confounders listed above.

To evaluate whether a single site drove findings, we also conducted a leave-one-out sensitivity analysis. Using our MICE dataset, we sequentially removed each study site, refit the regression models, and compared the resulting effect estimates to evaluate whether findings

were driven by any single site or influenced by site-specific population characteristics or exposure distributions.

Lastly, to characterize the overlap between prevalent heart disease and other cardiometabolic conditions, we descriptively summarized the prevalence of self-reported high cholesterol, high blood pressure, diabetes, and kidney disease among participants with and without prevalent heart disease. We additionally summarized the proportions of participants with at least one and at least two of these comorbid conditions. Percentages for individual conditions were calculated among participants with non-missing information for that condition, whereas summaries of multiple comorbidities were restricted to participants with complete information for all four conditions.

Table 1

Baseline Characteristics of Adult Study Participants in the Multi-Site Study – Prevalent Heart Disease^a (n = 5,824) by case status.

Characteristic	Category	No Heart Disease (n = 5,233)	Heart Disease (n = 528)	Missing/Don't Know (n = 63)	Overall (n = 5824)
Age	years	55 (18–100)	68 (26–92)	64 (26–89)	56 (18–100)
Sex	Male	2,024 (38.7%)	269 (50.9%)	23 (36.5%)	2,316 (39.8%)
	Female	3,206 (61.3%)	259 (49.1%)	40 (63.5%)	3,505 (60.2%)
	Missing or Refused	3 (0.1%)	0 (0.0%)	0 (0.0%)	3 (0.1%)
Race and ethnicity	Hispanic	341 (6.5%)	15 (2.8%)	7 (11.1%)	363 (6.2%)
	Non-Hispanic American Indian or Alaskan Native	13 (0.2%)	4 (0.8%)	1 (1.6%)	18 (0.3%)
	Non-Hispanic Asian/Pacific Islander	121 (2.3%)	6 (1.1%)	1 (1.6%)	128 (2.2%)
	Non-Hispanic Black	592 (11.3%)	53 (10.0%)	5 (7.9%)	650 (11.2%)
	Non-Hispanic Multiracial	85 (1.6%)	9 (1.7%)	3 (4.8%)	97 (1.7%)
	Non-Hispanic White	4,011 (76.6%)	434 (82.2%)	43 (68.3%)	4,488 (77.1%)
	Ethnicity Refused, White	4 (0.1%)	1 (0.2%)	1 (1.6%)	6 (0.1%)
Education	Missing or Refused	66 (1.3%)	6 (1.1%)	2 (3.2%)	74 (1.3%)
	High School graduate or less	1,027 (19.6%)	117 (22.2%)	9 (14.3%)	1,153 (19.8%)
	Some university/college	1,033 (19.7%)	130 (24.6%)	17 (27.0%)	1,180 (20.3%)
	Technical or trade	250 (4.8%)	34 (6.4%)	5 (7.9%)	289 (5.0%)
	University/college graduate	1,763 (33.7%)	137 (25.9%)	15 (23.8%)	1,915 (32.9%)
	Graduate school or higher	1,157 (22.1%)	110 (20.8%)	15 (23.8%)	1,282 (22.0%)
	Missing	3 (0.1%)	0 (0.0%)	2 (3.2%)	5 (0.1%)
Annual household income	<\$25,000	699 (13.4%)	81 (15.3%)	9 (14.3%)	789 (13.5%)
	\$25,000 to \$69,999	1,435 (27.4%)	182 (34.5%)	19 (30.2%)	1,636 (28.1%)
	\$70,000 to \$149,000	1,834 (35.0%)	182 (34.5%)	14 (22.2%)	2,030 (34.9%)
	> \$150,000	948 (18.1%)	51 (9.7%)	7 (11.1%)	1,006 (17.3%)
	Missing, Refused, or Did Not Know	317 (6.1%)	32 (6.1%)	14 (22.2%)	363 (6.2%)
Smoking status	Never Smoker	3,034 (58.0%)	253 (47.9%)	34 (54.0%)	3,321 (57.0%)
	Former Smoker	1,701 (32.5%)	236 (44.7%)	26 (41.3%)	1,963 (33.7%)
	Current Smoker	492 (9.4%)	37 (7.0%)	2 (3.2%)	531 (9.1%)
	Missing	6 (0.1%)	2 (0.4%)	1 (1.6%)	9 (0.2%)
	Never Drinker	417 (8.0%)	44 (8.3%)	5 (7.9%)	466 (8.0%)
Alcohol use	Former Drinker	1,083 (20.7%)	171 (32.4%)	20 (31.7%)	1,274 (21.9%)
	Current Drinker	3,726 (71.2%)	312 (59.1%)	37 (58.7%)	4,075 (70.0%)
	Missing	7 (0.1%)	1 (0.2%)	1 (1.6%)	9 (0.2%)
Waist-to-hip ratio		0.91 (0.57–1.53)	0.94 (0.64–1.25)	0.91 (0.70–1.10)	0.91 (0.57–1.53)
Estimated glomerular filtration rate	(mL/min/1.73 m ²)	92.76 (8.00–139.00)	80.28 (6.00–126.00)	88.41 (39.00–123.00)	91.58 (6.00–139.00)
Study site	California	472 (9.0%)	41 (7.8%)	5 (7.9%)	518 (8.9%)
	Colorado	819 (15.7%)	98 (18.6%)	8 (12.7%)	925 (15.9%)
	Massachusetts	608 (11.6%)	64 (12.1%)	17 (27.0%)	689 (11.8%)
	Michigan	376 (7.2%)	41 (7.8%)	4 (6.3%)	421 (7.2%)
	New Jersey	715 (13.7%)	59 (11.2%)	2 (3.2%)	776 (13.3%)
	New York	398 (7.6%)	63 (11.9%)	7 (11.1%)	468 (8.0%)
	Pennsylvania	1,108 (21.2%)	130 (24.6%)	13 (20.6%)	1,251 (21.5%)
	New Hampshire	737 (14.1%)	32 (6.1%)	7 (11.1%)	776 (13.3%)
	(g/dL)	4.52 (2.50–5.70)	4.43 (3.50–5.40)	4.45 (3.90–5.00)	4.51 (2.50–5.70)
Serum albumin					
Hormonal contraceptive use, females only (n = 3,467)	No	755 (23.5%)	74 (28.6%)	12 (30.0%)	841 (24.0%)
	Yes	2,436 (76.0%)	182 (70.3%)	26 (65.0%)	2,644 (75.4%)
	Missing, Don't Know, or Refused	15 (0.5%)	3 (1.2%)	2 (5.0%)	20 (0.6%)
Menopausal status, females only (n = 3,467)	Premenopausal	1,127 (35.2%)	11 (4.2%)	5 (12.5%)	1,143 (32.6%)
	Postmenopausal	2,070 (64.6%)	248 (95.8%)	33 (82.5%)	2,351 (67.1%)
	Missing	9 (0.3%)	0 (0.0%)	2 (5.0%)	11 (0.3%)
Parity ^b , females only (n = 3,467)	number of live births, median (Q1–Q3)	2 (1–3)	2.0 (1–3)	2 (1–3)	2 (1–3)

Values are n (%) for categorical variables and mean (minimum–maximum) for continuous variables unless otherwise noted.

^a Health outcome – self-reported prevalent heart disease – was based on the question: “Have you ever been told by a medical professional or health care provider that you have or had the following – heart disease?” (Response options: yes, no, don't know, refuse to answer).

^b Parity is presented as median (Q1–Q3). Parity, hormonal contraceptive use, and menopausal status were summarized among female participants only.

All analyses were conducted in R (version 4.5.0). Logistic regression models were fit using the `glm()` function in the base *stats* package, multiple imputation of missing covariate data was performed using the *mice* package, Spearman's rank correlation using *corrplot*, and *ggcomp* for mixture analysis (Buuren and Groothuis-Oudshoorn, 2011). All attenuation models were estimated using multiply imputed datasets and pooled using Rubin's rules (Rubin, 1987).

3. Results

Table 1 displays the baseline characteristics of the MSS heart disease study population. A total of 5,761 participants were included in the analysis. 528 participants reported having heart disease (9.2% prevalence rate). Participants were on average 56.1 years old (range: 18–100), with a mean waist-to-hip ratio of 0.91 (range: 0.57–1.53) and mean eGFR of 91.6 mL/min/1.73 m² (range: 6.0–139.0). Participants were predominantly female (60.2%), 284 (54.9%), had a household income of \$70,000 – \$149,000 (34.9%), current alcohol drinkers (70%), never smokers (57%), Non-Hispanic White (77.1%), and had an average waist-to-hip ratio of 0.91. However, participants with self-reported heart disease were more likely to be male (50.9% vs 38.7%), Non-Hispanic White (82.2% vs 76.6%), older (68.3 years vs 54.7 years), and have lower eGFR (80.3 vs 92.8) than participants with no heart disease.

Serum PFAS concentrations in this population are reported in Table 2, and results are reported stratified by heart disease status in Table S1. n-PFOA, n-PFOS, sb-PFOS, PFHxS, and PFNA all had high detection rates (96.4%–99.4%), whereas sb-PFOA, PFDA, PFUnDA, and MeFOSAA were detected at 1.3%, 43.8%, 56.1%, and 66.9% respectively. The highest serum concentration (ng/mL) was Σ PFOS (median: 4.3; interquartile range [IQR] = 2.3–7.7), followed by PFHxS (2.6; 0.1–6.3), Σ PFOA (1.77; 1.07–3.34), and PFNA (0.4; 0.3–0.62). Fig. 1 displays a Spearman correlation heat map for serum PFAS concentrations in this cohort. Σ PFOA– Σ PFOS, Σ PFOS–PFHxS, and PFNA–PFDA were all highly correlated, with their Spearman correlation coefficients greater than 0.6.

Our main findings are presented in Table 3. After adjustment for age, sex, race and ethnicity, education, income, smoking history, alcohol history, waist-to-hip ratio, eGFR, and study site, none of the individual PFAS or sum of seven were statistically associated with prevalent self-reported heart disease. Case restriction to recent diagnosis also produced results consistent with the primary analyses and did not support an association between PFAS exposure and recently diagnosed heart disease (2019–2023).

Quantile g-computation was used to estimate the joint effect of seven serum PFAS concentrations with self-reported heart disease. After adjustment for age, sex, race and ethnicity, education, income, smoking, alcohol, waist-to-hip ratio, eGFR, and study site the overall mixture

effect was null (OR: 0.99, 95% CI: 0.95–1.03). The mixture included positive contributions (relative weights adding to 1 in both positive and negative direction) for PFHxS (0.45), PFNA (0.39), MeFOSAA (0.12), and PFUnDA (0.05) and negative contributions for Σ PFOS (0.52), PFDA (0.39), and Σ PFOA (0.10) (Fig. 2A). These weights indicated that several PFAS contributed in positive and negative directions to the overall mixture effect; however, the opposing contributions canceled out, resulting in an overall null mixture effect after adjustment (Fig. 2B).

In sex-stratified analyses there was no evidence of associations between most PFAS and prevalent heart disease amongst males or females. PFNA was inversely associated with prevalent heart disease among males (OR = 0.68, 95% CI: 0.52, 0.91), whereas no association was observed among females (OR = 1.00, 95% CI: 0.85, 1.16). No statistically significant associations were observed for Σ PFOA, Σ PFOS, PFHxS, PFDA, PFUnDA, MeFOSAA, or the sum of seven in either sex.

Sensitivity analyses using alternative covariate adjustment models produced results consistent with the primary analyses (Table S3). Excluding waist-to-hip ratio from the adjustment set (Model A) or additionally adjusting for albumin (Model B) did not materially alter the estimated associations between PFAS and prevalent heart disease. Across these models, no statistically significant associations were observed for all PFAS and the summed PFAS variable.

In the medical record-restricted analysis, participants who had heart disease documented in medical record abstraction data were classified as cases (297 cases, 56.3% of self-reported cases; baseline characteristics are displayed in Table S4). Controls were defined as participants who self-reported no heart disease and either had medical record abstraction data indicating no heart disease or lacked medical record abstraction data. Participants with missing self-reported heart disease status and no heart disease documented in medical record abstraction data were excluded from the analysis. After adjusting for confounders and other covariates, no statistically significant associations were observed for all PFAS and the sum of seven.

Our leave-one-out sensitivity analysis showed that the estimates were consistent with our primary findings (Table S7, Fig. S2). For Σ PFOA, Σ PFOS, PFHxS, MeFOSAA, and the sum of seven, exclusion of individual sites produced odds ratios that were nearly identical to the full-sample estimates and remained close to the null, indicating that the overall findings were not driven by any single study site. Results for PFNA and PFDA were also generally consistent across analyses, although the inverse association for PFNA was found to be statistically significant when California was excluded (OR = 0.87, 95% CI: 0.77, 0.99). PFUnDA showed somewhat greater variation across site exclusions, with estimates ranging from OR = 0.73 (95% CI: 0.59, 0.91) when Colorado was excluded to OR = 0.89 (95% CI: 0.71, 1.13) when Pennsylvania was excluded, compared with OR = 0.82 (95% CI: 0.67, 1.02) in the full sample. However, the direction of the PFUnDA estimate remained

Table 2
Summary of Serum PFAS Concentrations (n = 5,824).

PFAS	% >LOD ^a	Serum concentrations [ng/mL]					
		25p	Median	Geo. Mean	Mean	75p	Max
n-PFOA	99.3	1.00	1.70	1.73	3.26	2.8	203
sb-PFOA	1.3	<LOD	<LOD	<LOD ^b	<LOD ^b	<LOD	3.40
Σ PFOA		1.07	1.77	1.84	3.34	2.87	203.07
n-PFOS	99.4	1.60	2.90	2.80	4.73	5.12	912
sm-PFOS	98.1	0.70	1.40	1.30	2.32	2.60	601
Σ PFOS		2.30	4.30	4.17	7.05	7.70	1513
PFHxS	98.2	1.10	2.60	2.62	6.21	6.30	596
PFNA	96.4	0.30	0.40	0.44	0.62	0.70	12.20
PFDA	43.8	<LOD	0.10	0.12	0.16	0.20	6.60
PFUnDA	56.1	<LOD	<LOD	0.11	0.15	0.20	4.10
MeFOSAA	66.9	<LOD	<LOD	0.10	0.16	0.10	5.60
Sum PFAS		6.18	11.04	10.98	17.67	19.61	2313.14

^a The limits of detection for all PFAS were 0.1 ng/mL. Levels below the limit of detection (LOD) were imputed with the LOD divided by the square root of 2.

^b Not calculated: proportion of results below limit of detection was too high to provide a valid result.

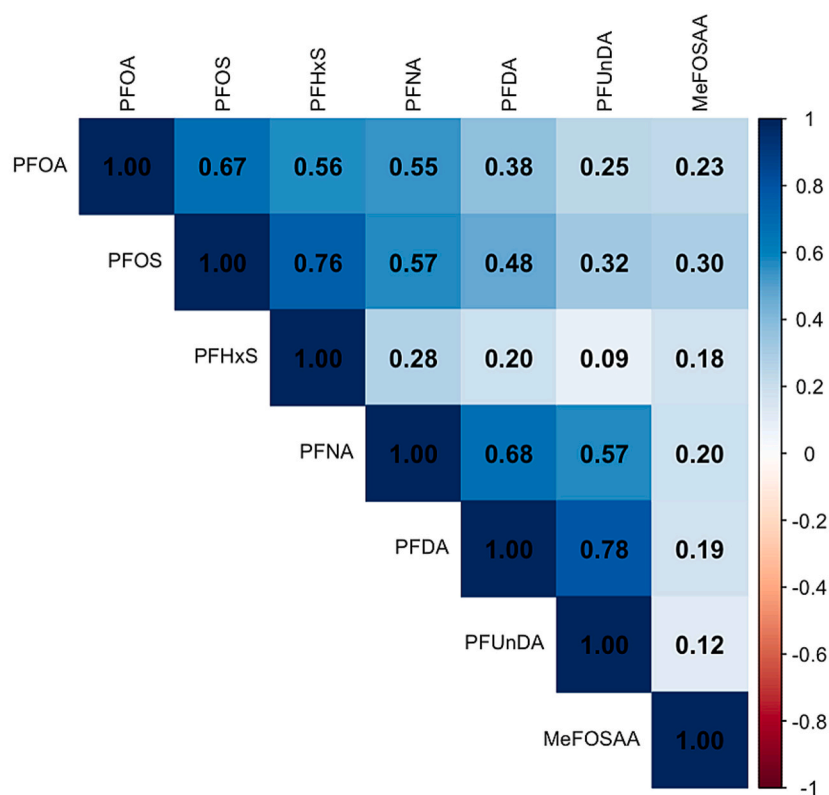


Fig. 1. Spearman Correlation Heat Map for PFAS Exposure in the Multi-Site Study – Prevalent Heart Disease.

Table 3

Association Between PFAS Exposure and Prevalent Heart Disease in the Multi-site study (n = 5,761): Odds Ratios (95% CI) from logistic regression models using all self-reported heart disease cases and recent cases diagnosed during 2019–2023.

PFAS	All heart disease cases ^a (n = 5,761) OR (95% CI)	p-value	Diagnosed 2019–2023 ^b (n = 5,355) OR (95% CI)	p-value
PFOA	0.99 (0.98, 1.01)	0.345	0.98 (0.95, 1.02)	0.334
PFOS	0.99 (0.98, 1.00)	0.187	1.00 (0.98, 1.02)	0.847
PFHxS	1.00 (1.00, 1.01)	0.516	1.00 (1.00, 1.01)	0.301
PFNA	0.89 (0.78, 1.02)	0.106	0.85 (0.66, 1.09)	0.195
PFDA	0.93 (0.76, 1.14)	0.504	0.90 (0.61, 1.34)	0.611
PFUnDA	0.82 (0.67, 1.02)	0.069	0.90 (0.60, 1.35)	0.608
MeFOSAA	1.03 (0.84, 1.26)	0.762	0.91 (0.61, 1.34)	0.623
Sum of PFAS	1.00 (1.00, 1.00)	0.580	1.00 (1.00, 1.01)	0.946

Note: Adjusted models controlled for potential confounders: age, sex, race and ethnicity, education, income, smoking history, alcohol history, waist-to-hip ratio, estimated glomerular filtration rate (eGFR), and study site. Missing covariate data were imputed using multiple imputation by chained equations (MICE).

^aCases defined as participants who self-reported ever having been diagnosed with heart disease (528 cases; analytic n = 5,761).

^bCases were restricted to participants reporting a heart disease diagnosis during 2019–2023 (122 cases; analytic n = 5,355); participants reporting a diagnosis before 2019 were excluded.

inverse across all leave-one-site-out analyses.

Cardiometabolic comorbidities were common among participants with prevalent heart disease (Table S7). Among participants with heart disease and non-missing information for each condition, 70.1% reported high cholesterol, 68.3% reported high blood pressure, 24.6% reported diabetes, and 8.8% reported kidney disease. Among participants with complete information for all four conditions, 84.9% of those with

prevalent heart disease had at least one of these comorbidities and 61.9% had at least two.

4. Discussion

In this multi-site study of 5,761 adults from communities with PFAS-contaminated drinking water, we found little evidence of an association between exposure to PFAS and prevalence of self-reported heart disease. After adjustment for potential confounders and other covariates – age, sex, race and ethnicity, education, income, smoking history, alcohol history, waist to hip ratio, eGFR, and study site – no statistically significant associations were observed for any individual PFAS or the sum of seven.

Our results contribute to a mixed and evolving literature on PFAS and CVD health outcomes. While several recent studies have found null or negative associations (Wen et al., 2023; Dunder et al., 2023; Schlesinger and Gokce, 2024; Al-Kindi et al., 2020), other investigations in highly exposed communities such as Ronneby, Sweden and the Veneto region in Italy have found raised mortality and morbidity from exposure to PFAS and cardiovascular disease (Biggeri et al., 2024; The, 2025; Zhou et al., 2025). Despite null findings for prevalent heart disease found in our study, consistent evidence has linked PFAS exposure to established cardiovascular risk factors such as increases in blood lipids (Liu et al., 2023; Wu et al., 2023), alterations in glycemic regulation and elevated risk of diabetes (India Aldana et al., 2026), and, to a lesser extent, blood pressure (Pitter et al., 2020). These cardiometabolic conditions frequently co-occurred with heart disease in our study; among participants with prevalent heart disease, approximately 70% reported high cholesterol, 68% reported high blood pressure, and 25% reported diabetes (Table S7). This substantial overlap highlights the clustering of cardiovascular risk factors and disease in this population, while also indicating that the heart disease outcome represents a distinct, though overlapping, group of participants. While we did find a nonsignificant inverse association between PFUnDA and prevalent heart disease, our

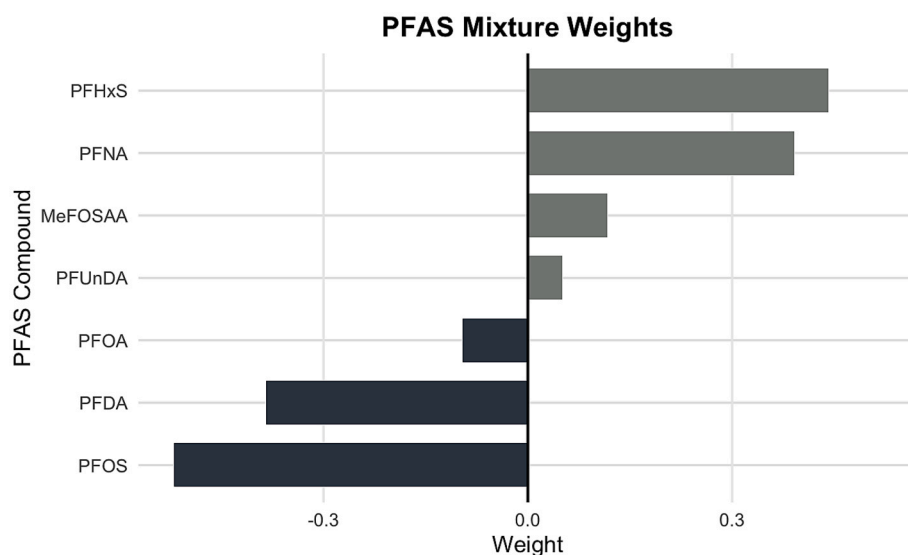


Fig. 2A. Relative weights of individual PFAS in the mixture model predicting prevalent self-reported heart disease. Positive relative weights (blue) indicate PFAS contributing to higher odds of heart disease, while relative negative weights (red) indicate PFAS associated with lower odds (both relative weights add to 1). Weights were derived from a quantile g-computation model adjusted for age at blood draw, sex, race/ethnicity, education, income, smoking, alcohol use, estimated glomerular filtration rate, and waist-to-hip ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

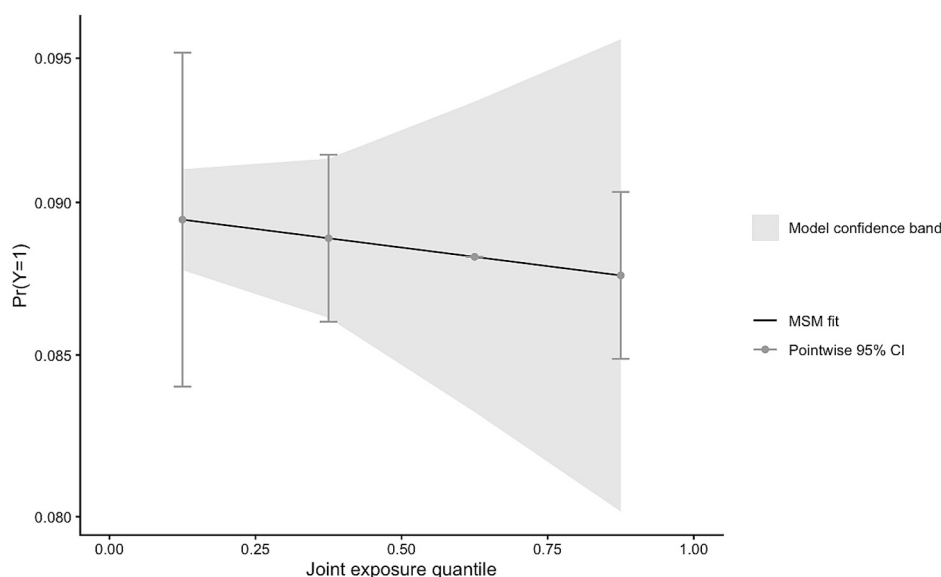


Fig. 2B. Predicted probability of self-reported heart disease ($Y = 1$) across quantiles of joint PFAS exposure distribution. The solid black line represents the marginal structural model fit, with the shaded gray area showing the 95% model-based confidence band. Gray points with error bars represent pointwise predicted probabilities and their 95% confidence intervals, adjusted for age, sex, race/ethnicity, education, income, smoking, alcohol use, estimated glomerular filtration rate (eGFR), and waist-to-hip ratio (WHR).

mixture modeling found no association when examining the overall joint effect of PFAS on self-reported heart disease.

Across multiple sensitivity analyses, our conclusions were largely unchanged. Alternative covariate adjustment models, leave-one-site-out analyses, and sex-stratified analyses did not meaningfully change the interpretation of the null findings. In sex-stratified analyses, most associations remained null; however, PFNA was inversely associated with prevalent heart disease among males but not females. The medical-record-restricted analysis found null associations across all PFAS. Of note, medical record abstraction was incomplete, varied substantially across sites, and assessed heart disease as a broad outcome without prespecified ICD codes or distinction among specific cardiovascular

diagnoses. Consequently, etiologically distinct cardiovascular conditions could not be differentiated, limiting the specificity and interpretation of this sensitivity analysis.

While our study contributes important evidence to the literature—particularly by leveraging a large sample size, capturing a wide exposure range, and incorporating both single-chemical and mixture approaches—several limitations should be considered when interpreting these findings. First, our cross-sectional design precludes causal inference, as the temporal relationship between PFAS serum concentrations at the time of the exposure assessment and heart disease cannot be established. However, given the long biological half-lives of several PFAS, measured concentrations may reflect exposures during the

etiologically relevant period for many participants (Nielsen et al., 2024; Pérez et al., 2013). Second, because PFAS concentrations in the United States population have generally declined over time following voluntary and more recently regulatory phaseouts of legacy PFAS (Sonnenberg et al., 2023), and because all sites in the present study received interventions to limit exposure to contaminated water, measured serum concentrations in our cohort may not fully reflect historic peak exposures. Third, reliance on self-reported heart disease may have introduced outcome misclassification, as undiagnosed individuals could have been misclassified as controls. Further, the questionnaire did not distinguish among specific clinical entities (e.g., myocardial infarction, arrhythmia, or congestive heart failure) and explicitly excluded stroke, leaving certain cardiovascular outcomes unexamined. Although self-reported heart disease has been found to be reliable when compared to direct measurements (Lee et al., 2023), and has been widely used in epidemiologic studies evaluating associations between PFAS concentrations and CVD (Meneguzzi et al., 2021), many contemporary epidemiologic studies incorporate additional sources of outcome ascertainment, such as administrative health records, medication data, or clinically adjudicated events. Thus, reliance on a single cross-sectional self-report measure represents an important limitation. Fourth, unmeasured confounding remains possible. Diet is a major source of PFAS in the general population (Pacyga et al., 2025) and a risk factor for heart disease (Chareonrungrueangchai et al., 2020). This may be particularly relevant for PFAS such as PFUnDA, which was not reported in the drinking water at most of our sites and thus may also be more susceptible to confounding by unmeasured dietary differences (such as consumption of seafood) than the PFAS for which exposure was predominantly driven by contaminated water. The relationship between PFAS, diet, and heart disease warrants further exploration.

Finally, survivor bias may have attenuated associations if individuals experiencing severe cardiovascular disease died before study enrollment (Anderson et al., 2011). Although some mortality studies have reported associations between PFAS-contaminated drinking water and elevated cardiovascular deaths (Biggeri et al., 2024; Zhou et al., 2025), the broader epidemiologic evidence for a large effect of PFAS on cardiovascular mortality remains limited and inconsistent, with many cohort studies showing null or weak associations with incident cardiovascular disease and few prospective mortality analyses outside highly exposed populations (Dunder et al., 2023).

Despite our limitations, our study has several strengths, including a large study population, high detection rates for the four major PFAS, inclusion of biomarker endpoints, and the application of both single-chemical and mixture modeling approaches. In addition to PFOA, PFOS, PFHxS and PFNA we also measured less frequently quantified PFAS such as PFDA, PFUnDA, and MeFOSAA. We were also able to capture a wide range of exposure in our cohort across participating MSS sites, focusing on communities with documented PFAS contamination in drinking water in the vicinity of former military sites, airports, industrial sites, or urban/suburban areas without clearly defined sources. This range of exposure reflects variation observed in the broader United States population. In addition, our banked serum samples provide an opportunity for future analyses.

Taken together, our findings contribute to a growing body of evidence suggesting that serum PFAS concentrations have null associations with the prevalence of heart disease, while also underscoring complexities that warrant continued investigation. Continued surveillance in affected communities—especially those with documented PFAS contamination in drinking water—may improve understanding of long-term cardiovascular risks and inform public health responses.

Disclaimer.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

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CRediT authorship contribution statement

Celina N. Phillipson: . **Marian Pavuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Yerin Jung:** Writing – review & editing, Validation, Investigation, Formal analysis. **Carolina Villanueva:** Writing – review & editing, Validation, Investigation, Formal analysis. **Robert J. Laumbach:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Linda M. Brown:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Anne P. Starling:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Veronica M. Vieira:** Writing – review & editing, Project administration, Investigation. **Michael Lewin:** Writing – review & editing, Investigation. **Teresa Wang:** Writing – review & editing, Investigation, Conceptualization. **Tamarra James-Todd:** Writing – review & editing, Investigation, Conceptualization. **Yuting Wang:** Writing – review & editing, Investigation. **Meghan M. Weems:** Writing – review & editing, Project administration, Investigation. **Karen S. Valladares:** Writing – review & editing, Investigation. **Adam M. Schaefer:** Writing – review & editing, Investigation, Data curation. **Scott M. Bartell:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SMB has served as a compensated expert witness in PFAS litigations.

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Author statement.

MP, SMB, LMB, RJL, and TJV carried out the conceptualization of the study; CNP, MP, and SMB designed the analytical methods and statistical models; CNP and CV implemented the formal analysis; MP and AMS were responsible for aggregated data curation; MP, SMB, LMB, RJL, and TJV were responsible for acquiring and managing the financial support for the project leading to this publication; MP, RJL, APS, MMW, AMS, VV, ML, TW, SMB were involved in project administration. All authors were involved in the investigation throughout the study; CNP wrote the manuscript and all authors reviewed and revised the manuscript.

Ethics declaration

Informed consent and patient details.

Written informed consent to take part in the study and to publish the

article has been obtained from all participants or their legal representatives. The privacy rights of participants have been observed.

Studies in Human.

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place.

This study was approved by the Centers for Disease Control and Prevention Institutional Review Board (CDC IRB). (Approval No. protocol #7207)

Appendix A. Supplementary data

Supplementary material includes additional descriptive data, sensitivity analyses, and figures supporting the main analyses, including sex-stratified analyses and alternative model analyses, medical record-restricted and leave-one-out analyses, cardiometabolic comorbidities, and a directed acyclic graph. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2026.110522>.

Data availability

The data that has been used is confidential.

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