Research Square

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Exposome-wide Association Patterns across Cardiovascular Disease Outcomes: Findings from the NHANES

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Abstract

Background

Environmental exposures may contribute to cardiovascular disease (CVD) through oxidative stress, inflammation, endothelial dysfunction and metabolic disruption, yet most studies still evaluate one exposure class or one cardiovascular endpoint at a time. Whether distinct cardiovascular outcomes share broader exposure signatures at the population level remains poorly resolved.

Methods

We conducted a cross-sectional exposome-wide association study in a CVD-augmented NHANES analytic dataset comprising 56,253 participants across ten survey cycles. Outcomes derived from the medical conditions questionnaire included composite CVD, coronary heart disease, heart attack, angina, heart failure and stroke. For each exposure-outcome pair, we fitted survey-weighted logistic regression models and estimated odds ratios per exposure doubling. Nominal *P*-values lower than 0.05 defined the main screening analysis. Furthermore, Benjamini-Hochberg correction, subgroup interaction models, never-smoker restriction, leave-one-cycle-out analyses and restricted cubic spline models were used as sensitivity analyses.

Results

The weighted prevalence estimates were 8.7% for composite CVD, 3.5% for coronary heart disease, 3.4% for heart attack, 2.4% for angina, 2.4% for heart failure and 2.8% for stroke. Across 138 fitted models per outcome, composite CVD yielded 27 nominal and 11 false discovery rate (FDR)-adjusted associations, and heart failure yielded 26 nominal and 16 FDR-adjusted associations. The leading composite CVD associations involved blood cadmium, serum cotinine, blood nitromethane, ethylene oxide hemoglobin adduct and triclosan. Recurrent signals across the principal cardiovascular outcomes included blood cadmium and linear perfluorooctanoate. Among the 92 nominal associations carried forward for robustness evaluation, 87 were directionally stable in leave-one-cycle-out analyses, 85 remained significant in the core covariate-adjusted model, and 30 remained nominally significant among never smokers.

Conclusions

In this cross-sectional NHANES analysis, cardiovascular outcomes showed heterogeneous but interpretable exposure signatures, with the clearest and most reproducible patterns observed for composite CVD and heart failure. These results help prioritize both endpoints and biomarkers for future

longitudinal and mechanistic research for CVD, while remaining bounded by the cross-sectional design and self-reported disease histories.

Research Insights

What is currently known about this topic?

- Environmental exposures contribute to cardiovascular disease beyond traditional risk factors.
- Comprehensive exposome-wide analyses across multiple cardiovascular disease phenotypes are scarce.

What is the key research question?

Which environmental exposure biomarkers are associated with different cardiovascular disease outcomes, and are these associations shared or outcome-specific?

What is new?

- We performed an exposome-wide association study of 138 environmental biomarkers across six cardiovascular disease outcomes.
- Blood cadmium, serum cotinine, and several tobacco-related biomarkers showed reproducible associations across multiple cardiovascular outcomes, whereas other exposures displayed outcome-specific patterns.
- Robustness analyses demonstrated that many associations remained consistent across demographic subgroups and sensitivity analyses.

How might this study influence clinical practice?

The findings help prioritize environmental exposures for cardiovascular risk assessment and provide a foundation for future studies evaluating environmental prevention strategies for cardiovascular disease.

Background

Cardiovascular disease (CVD) remains the leading cause of death worldwide and continues to impose a substantial burden on healthcare systems and population health [1]. Although considerable progress has been achieved in the prevention and management of traditional cardiovascular risk factors such as obesity, hypertension, dyslipidemia and diabetes, a substantial proportion of cardiovascular risk remains unexplained [2]. In recent years, increasing attention has been directed toward environmental determinants of cardiovascular health [3]. Accumulating epidemiological and mechanistic evidence suggests that exposure to environmental chemicals, including heavy metals [4], air pollutants [5], and persistent organic pollutants (POPs) [6], may contribute to the development and progression of cardiovascular disease through pathways involving oxidative stress, systemic inflammation, endothelial

dysfunction, and metabolic disturbances [7, 8]. Identifying environmental exposures that contribute to cardiovascular risk therefore represents an important step toward a more comprehensive understanding of CVD etiology and may reveal novel opportunities for disease prevention and population-level intervention.

To better characterize the environmental contribution to chronic diseases, the concept of the exposome was proposed, encompassing the totality of environmental exposures encountered throughout the life course [9]. Unlike traditional approaches that investigate individual risk factors in isolation, the exposome framework recognizes that humans are simultaneously exposed to a diverse array of chemical, physical, social, and behavioral factors that may interact to influence disease susceptibility [10, 11]. Importantly, because individuals are exposed to complex mixtures of environmental chemicals rather than isolated agents, evaluating exposures within an exposome framework may provide a more comprehensive understanding of CVD etiology and facilitate the identification of previously unrecognized environmental determinants of cardiovascular health [12].

Despite growing evidence linking environmental exposures to CVD, several important limitations remain in the current literature. Firstly, most epidemiological studies have focused on individual chemicals or specific exposure classes, such as heavy metals, air pollutants, POPs, or polyfluoroalkyl substances (PFAS), rather than evaluating the broader environmental mixture to which individuals are simultaneously exposed [9, 13, 14]. Such reductionist approaches may overlook cumulative or correlated exposure patterns and limit the identification of previously unrecognized cardiovascular risk factors. Environmental studies commonly investigate a single cardiovascular outcome, such as coronary heart disease (CHD) [15], myocardial infarction [16], or heart failure [17], making it difficult to distinguish exposure signatures that are shared across cardiovascular conditions from those that are outcome-specific. To address these challenges, exposome-wide association studies (ExWAS) have emerged as a systematic and agnostic screening approach that evaluates large numbers of environmental exposures simultaneously, analogous to genome-wide association studies in genetic epidemiology [18, 19]. Although ExWAS methodologies have been increasingly applied to metabolic disorders [18], cancer [20], and mortality [21], comprehensive exposome-wide investigations spanning multiple cardiovascular outcomes remain limited. Consequently, a systematic evaluation of environmental exposure patterns across diverse CVD phenotypes is needed to advance understanding of the environmental architecture of CVD.

Building on these knowledge gaps, we hypothesized that established environmental toxicants such as heavy metals, air pollutants, POPs, and PFAS, would show consistent associations across multiple cardiovascular outcomes. And an exposome-wide screening approach would identify both known and previously underrecognized environmental determinants of CVD that may not be captured through conventional hypothesis-driven analyses. In this study, we aimed to systematically evaluate the associations between a broad panel of environmental exposures and multiple CVD outcomes using a large, nationally representative sample from the National Health and Nutrition Examination Survey (NHANES). Specifically, we conducted an ExWAS of 138 curated exposure biomarkers across six

cardiovascular outcomes, including composite cardiovascular disease, CHD, heart attack, angina, heart failure, and stroke. Given the heterogeneity of cardiovascular phenotypes and the complex nature of environmental mixtures, we further sought to characterize both shared and outcome-specific exposure patterns, as well as to assess the consistency and robustness of observed associations across demographic subgroups and survey cycles. By leveraging repeated cross-sectional NHANES cycles and a harmonized exposome framework, this study provides a comprehensive evaluation of environmental exposure patterns in relation to CVD at the population level.

Materials and Methods

Study design and data source

We conducted a cross-sectional analysis using a CVD-augmented analytic dataset derived from the NHANES. The dataset was constructed by appending questionnaire-based cardiovascular history variables to a harmonized exposome analysis dataset developed in the parallel project. The analytic dataset retained the full survey design structure, including pooled sampling weights, strata, and primary sampling unit identifiers, as well as harmonized demographic and lifestyle covariates and the curated biomarker panel. After data harmonization, the final analytic sample included 56,253 participants across ten NHANES cycles from 1999–2000 through 2017–2018. The sample derivation process is summarized in the study flowchart (Fig. 1) and follows the same analytic framework used in the companion cardiometabolic study, with cardiovascular outcomes incorporated as an additional outcome domain.

Cardiovascular outcome definitions

Cardiovascular outcomes were defined from self-reported physician-diagnosed conditions recorded in the NHANES medical conditions questionnaire [22]. Five individual outcomes were considered: CHD, heart attack, angina, heart failure and stroke. A composite CVD outcome was additionally defined as the presence of at least one of these five conditions. Because several of these diagnoses overlap clinically and are not mutually exclusive, all six outcomes were analyzed within a common framework. For the main results narrative, we placed greater interpretive emphasis on composite CVD, heart failure, heart attack and stroke, because these outcomes yielded the clearest and most reproducible signal patterns in the present dataset. CHD and angina were retained to preserve the breadth of the screening analysis, but were discussed more cautiously.

Exposure biomarkers and covariates

The candidate exposure panel was carried forward from the curated exposome workflow used in the companion cardiometabolic analyses. In total, we included 138 exposure biomarkers distributed across blood, serum and urine matrices. The retained panel covered multiple chemical categories, including

heavy metals, PFAS, POPs, hemoglobin adducts, phenols, arsenic species, pesticide metabolites, tobacco-related biomarkers and volatile organic compound metabolites. Core covariates were age, sex, race or ethnicity, educational attainment, family income-to-poverty ratio (PIR) and NHANES cycle. Smoking status, body mass index (BMI), current alcohol use, and physical activity were additionally included when available and when not structurally incompatible with the outcome model. Covariates with a single observed level after complete-case restriction were removed automatically for the corresponding exposure-outcome model.

Statistical analysis

The data analysis workflow was summarized in Fig. 1. Each exposure-outcome association was estimated with a survey-weighted logistic regression model. Exposure concentrations were standardized to numeric values, non-positive measurements were treated as missing, and the remaining values were log2-transformed so that model coefficients could be interpreted as odds ratios (ORs) per exposure doubling. For model stability, we required at least 100 complete-case observations, at least 20 outcome events and at least four unique positive exposure values after transformation. Models that did not meet these criteria or produced invalid estimates were excluded from inference.

The primary screening analysis was based on a nominal significance threshold of $P < 0.05$, which was prespecified as the main discovery criterion in the exposome-wide screening framework. Within each outcome, false discovery rate (FDR) correction was applied as a sensitivity-based analysis rather than as the primary inferential threshold. Accordingly, results are reported based on nominal associations, with FDR-significant findings interpreted as more conservative evidence of robustness. To illustrate the distribution of outcome-specific associations across the full exposure panel, we generated radial association maps for all six cardiovascular outcomes. These maps preserve the direction, magnitude, and confidence intervals (CIs) of each exposure while enabling direct comparison of outcomes within a unified visual framework.

Several additional analyses were conducted to assess robustness and explore heterogeneity. First, sex-stratified analyses and exposure-by-sex interaction tests were performed for the principal cardiovascular outcomes. Second, age-stratified analyses (< 60 v.s. ≥ 60 years) were conducted, followed by formal exposure-by-age interaction testing. Third, given the prominence of smoking-related biomarkers among top associations, analyses were repeated among never smokers, and exposure-by-ever-smoking interaction tests were performed. Fourth, the robustness of the results wwas evaluated by comparing the fully adjusted models with models only adjusted for core covariates, and stability across NHANES cycles was assessed using leave-one-cycle-out analyses. Fifth, for nominally significant associations in the four main cardiovascular outcomes that met prespecified data-quality criteria, restricted cubic spline models were fitted to evaluate potential non-linear associations. Finally, enrichment analysis of chemical classes among nominal and FDR-significant associations across all six outcomes was performed.

Results

Participant characteristics

A total of 56,253 participants were included in the present study, comprising 48.3% of males and 51.7% were females. The average ages of the males and females were 45.14 ± 17.14 and 46.63 ± 17.78 years, respectively (**Table 1**). The weighted prevalence of cardiovascular outcomes was 8.7% for composite CVD, 3.5% for CHD, 3.4% for heart attack, 2.4% for angina, 2.4% for heart failure, and 2.8% for stroke. Across NHANES cycles, participant representation remained broadly balanced, with each 2-year cycle contributing approximately 8.9% to 11.0% of the weighted sample (**Table 1**).

Overview of exposome-cardiovascular disease associaitons

All six cardiovascular outcomes were successfully evaluated across the full panel of 138 exposure biomarkers, and no outcome-specific analyses were excluded because of insufficient data. The number of nominally significant associations varied across outcomes, ranging from 18 for heart attack to 27 for composite CVD. Specifically, composite CVD yielded 27 nominal associations, followed by heart failure (26), CHD (24), angina (21), stroke (21), and heart attack (18) (Fig. 2 **& Table S1**). After FDR adjustment, heart failure showed the largest number of robust associations, followed by composite cardiovascular disease. Four associations remained significant for each of heart attack, stroke, and angina, whereas none of the CHD associations survived FDR correction (Fig. 2 **& Table S1**). The consistency patterns of exposure associations across primary CVD outcomes were summarized using a heatmap in **Figure S1**. Overall, the strongest and most reproducible exposome-wide associations were observed for composite CVD and heart failure.

Composite cardiovascular disease

Composite cardiovascular disease yielded 27 nominal associations, 11 of which remained significant after FDR adjustment (Fig. 3). The strongest positive associations were observed for blood cadmium (OR 1.15, 95% CI: 1.08–1.21), serum cotinine (OR 1.03, 95% CI: 1.02–1.04), blood nitromethane (OR 1.49, 95% CI: 1.23–1.80) and ethylene oxide hemoglobin adduct (EO-Hb) (OR 1.29, 95% CI: 1.14–1.46) (**Table 2**). The negative associations were observed for triclosan (OR 0.94, 95% CI: 0.91–0.97) and linear perfluorooctanoate (n-PFOA) (OR 0.72, 95% CI: 0.60–0.86) (**Table 2 & Table S1**).

Considering the prominence of smoking-related exposures, the analysis was further performed in never smokers. In this subgroup, 10 of the 27 nominal associations remained nominally significant. These included serum cotinine, blood nitromethane, n-PFOA, tungsten, urinary nitrate, 2-aminothiazoline-4-carboxylic acid (ATCA), oxychlordane and trans-nonachlor. In contrast, blood cadmium, EO-Hb and triclosan were attenuated in the never-smoker subset (**Table 3 & Table S2**).

Heart failure

Heart failure yielded 26 nominal associations and 16 FDR-adjusted associations (Fig. 4). The leading negative associations included urinary nitrate (OR 0.81, 95% CI: 0.75–0.88), perfluorohexane sulfonic acid (PFHxS) (OR 0.83, 95% CI: 0.75–0.91) and glycidamide hemoglobin adduct (OR 0.77, 95% CI 0.67 to 0.88), whereas blood cadmium (OR 1.27, 95% CI: 1.14–1.40) and serum cotinine (OR 1.04, 95% CI: 1.02–1.06) were positively associated with heart failure (**Table 2 & Table S1**). Nine of the 26 nominal heart failure findings remained nominally significant among never smokers, six showed nominal evidence of exposure-by-sex interaction, and most remained directionally stable in leave-one-cycle-out analyses (**Table 3 & Table S2**).

Heart attack and stroke

Heart attack yielded 18 nominal associations, of which 4 remained significant after FDR correction (**Figure S2**). The top associations included blood cadmium (OR 1.22, 95% CI: 1.14–1.31), serum cotinine (OR 1.04, 95% CI: 1.03–1.06), o-toluidine (OR 1.42, 95% CI: 1.18–1.70) and n-PFOA (OR 0.73, 95% CI: 0.60–0.88) (**Table 2**). Stroke yielded 21 nominal associations and 4 FDR-adjusted associations (**Figure S3**), with leading associations that included blood cadmium (OR 1.16, 95% CI: 1.07–1.26), urinary inorganic mercury (OR 0.89, 95% CI: 0.83–0.95), n-PFOA (OR 0.71, 95% CI: 0.57–0.87) and blood total mercury (OR 0.90, 95% CI: 0.84–0.96) (**Table 2 & Table S1**). Several associations recurred across outcomes. Blood cadmium was nominally associated with all four principal outcomes, and n-PFOA showed negative associations across composite cardiovascular disease, heart attack, heart failure and stroke (**Table 2 & Figure S1**).

Angina and coronary heart disease

Angina yielded 21 nominal associations and 4 FDR-adjusted associations, including a urinary pesticide-related marker, polychlorinated biphenyls (PCB) 180, PCB170 and EO-Hb among the leading estimates (**Figure S4**). CHD yielded 24 nominal associations, but none survived false discovery rate correction (**Figure S5**). The nominal CHD profile still included trans-nonachlor, PCB-related biomarkers, serum cotinine and EO-Hb, but none of these associations remained significant after FDR correction (**Table 2 & Table S1**). Angina and CHD were therefore retained in the analytic scope, but were not used to anchor the main results narrative.

Sex-stratified and age-stratified heterogeneity

Across the 92 nominal associations carried forward for robustness evaluation, 24 showed nominal evidence of exposure-by-sex interaction (**Table S2**). In composite CVD, serum cotinine and blood total mercury retained FDR-adjusted interaction terms, whereas urinary nitrate, arsenous acid, N-acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA), and n-PFOA showed weaker nominal interaction evidence (**Table 3 & Table S2**). In heart attack, serum cotinine, EO-Hb and triclosan showed FDR-adjusted sex interaction. In heart failure, urinary barium and linear perfluorooctane sulfonate (n-PFOS) showed the clearest sex-

dependent association, and in stroke the strongest FDR-adjusted sex interaction was observed for dimethylarsonic acid (**Table 3 & Table S2**).

Age-related heterogeneity was less frequent overall. Eleven of the 92 nominal associations showed nominal exposure-by-age-group interaction, and the clearest association was blood cadmium in composite CVD (*P* for interaction = 8.26×10^{-5}) (**Table S2**). Stroke accounted for the largest share of age heterogeneity, including significant interaction signals for 1-naphthol and 1-aminonaphthalene after FDR correction, with additional near-threshold signals for blood cadmium and dimethylarsonic acid. Angina also showed strong age interaction for trans-nonachlor (**Table S2**).

Robustness and sensitivity analyses

Thirty of the 92 nominal associations remained nominally significant among never smokers, whereas several others lost support after restriction to never smokers or showed formal never-versus-ever interaction. Among the latter, blood cadmium, triclosan and glycidamide in composite CVD, and blood methylmercury in heart failure, showed FDR-adjusted effect modification by smoking history (**Table 3 & Table S2**).

Among the 92 nominal associations across composite CVD, heart failure, heart attack and stroke, 87 were directionally stable in all leave-one-cycle-out models, and 85 remained nominally significant in the core covariate-adjusted models (**Table S2**). Restricted cubic spline analyses identified 8 associations with evidence of non-linearity (**Table S2**). The representative restricted cubic spline plots for these outcomes were shown in **Figure S6-9**. Only five associations met the prespecified exploratory flag criteria, which combined small-sample or low-event structure, cycle restriction and leave-one-cycle-out instability. All five involved aromatic amine biomarkers with limited support across cycles (**Table S2**).

Chemical-category enrichment across cardiovascular outcomes

Chemical-category enrichment analysis revealed substantial heterogeneity in the distribution of nominally significant exposure-outcome associations across cardiovascular outcomes. Among the categories examined, blood heavy metals, urine metals, PCBs, and organochlorine pesticides exhibited significant enrichment for multiple cardiovascular outcomes (Fig. 5). Blood heavy metals were significantly enriched for heart failure and stroke, while urine metals showed significant enrichment for heart failure, indicating that metal-related exposures may play an important role in cardiovascular risk (Fig. 5). Similarly, PCBs and organochlorine pesticide categories demonstrated significant enrichment across several cardiovascular outcomes, including composite CVD, CHD, and angin, suggesting widespread associations of POPs with cardiovascular health (Fig. 5). Several other categories, including PFAS, hemoglobin adducts, smoking/nicotine biomarkers, and aromatic amines, displayed moderate hit rates across multiple outcomes but did not meet the threshold for category-level enrichment (**Table S3**).

Discussion

In this comprehensive ExWAS of 138 environmental exposure biomarkers across six cardiovascular outcomes, we identified a broad spectrum of environmental factors associated with CVD risk. Composite CVD and heart failure exhibited the largest number of robust associations, suggesting that these outcomes may be particularly sensitive to environmental influences. Several exposures, most notably blood cadmium and serum cotinine, showed consistent positive associations across multiple cardiovascular outcomes. Beyond individual chemicals, category-level enrichment analyses highlighted heavy metals, PCBs, and organochlorine pesticides as major exposure classes linked to cardiovascular health. Importantly, many associations remained evident among never smokers and were generally stable across demographic subgroups and NHANES survey cycles, supporting the robustness of the observed findings. The findings also highlight potentially modifiable environmental determinants of cardiovascular health, offering valuable evidence for future risk reduction policies and targeted public health interventions.

The composite CVD outcome likely benefited from its broader capture of cardiovascular history. By aggregating coronary heart disease, heart attack, angina, heart failure and stroke, this endpoint may better represent cumulative cardiovascular injury or healthcare-recognized disease burden than any single diagnosis alone. In the present dataset, that broader capture was accompanied by repeated associations across several exposure classes, including metals, smoking-related biomarkers, hemoglobin adducts and PFAS-related markers. Among the individual outcomes, Heart failure showed a related but distinct pattern, and retained the largest number of FDR-supported findings and also remained comparatively stable across never-smoker restriction, sex interaction analyses and leave-one-cycle-out models.

Several of the leading associations identified in the present ExWAS are consistent with biological pathways that have been extensively implicated in cardiovascular toxicology. In particular, blood cadmium, serum cotinine, and reactive hemoglobin adduct biomarkers showed reproducible positive associations across multiple cardiovascular outcomes. Cadmium is a well-established environmental toxicant and a major constituent of tobacco smoke that has been linked to atherosclerosis, endothelial dysfunction, vascular inflammation, and oxidative injury [22]. Meta analysis evidence indicates that higher cadmium exposure is associated with increased risks of CVD, CHD, and stroke, even among never-smokers, suggesting that its cardiovascular effects extend beyond smoking behavior alone [13]. Similarly, cotinine, the principal metabolite of nicotine and a sensitive biomarker of active and passive tobacco exposure, has been associated with increased risks of cardiovascular events and cardiovascular mortality [23, 24]. The observed associations of EO and other reactive hemoglobin adduct biomarkers further support a potential contribution of electrophilic and oxidative toxicants to cardiovascular injury. These compounds can induce oxidative stress, DNA damage, endothelial dysfunction, and chronic inflammatory responses, all of which are recognized mechanisms underlying CVD development [25]. Taken together, our findings reinforce existing evidence that tobacco-related toxicants and oxidative stress pathways represent important components of the environmental burden contributing to CVD risk.

The inverse associations observed for several PFAS-related biomarkers, particularly n-PFOA and PFHxS, as well as selected urinary biomarkers such as nitrate, warrant cautious interpretation. Most epidemiological and experimental studies have linked PFAS exposure to adverse cardiometabolic outcomes [14, 26]. Consequently, the protective directions observed in the present study are unlikely to indicate a direct cardioprotective effect of these compounds. Rather, they may reflect residual confounding, reverse causation, exposure-related behavioral differences, or the complex pharmacokinetic properties of PFAS in populations with CVD [27]. The inverse association observed for urinary nitrate is also biologically plausible, given that dietary nitrate can serve as a precursor of nitric oxide and may improve endothelial function, vascular homeostasis, and blood pressure regulation [28, 29]. Taken together, these findings highlight the complexity of environmental exposure–disease relationships and underscore the need for future longitudinal and mechanistic studies to clarify whether these recurrent inverse associations reflect causal biological effects, exposure mixtures, or methodological features inherent to cross-sectional analyses.

The subgroup analyses further indicated that exposome–CVD associations were not uniform across population strata. Sex-dependent interactions were observed for several of the principal associations, including serum cotinine and blood mercury in composite CVD, tobacco-related and hemoglobin adduct biomarkers in heart attack, and PFAS- or arsenic-related biomarkers in heart failure and stroke. Age-related heterogeneity was less common overall but was concentrated in composite cardiovascular disease and stroke, with particularly strong evidence of age-dependent associations for blood cadmium. Although the present cross-sectional analysis cannot establish the biological mechanisms underlying these interaction patterns, the findings are consistent with growing evidence that susceptibility to environmental exposures may differ according to gender [30]. Sex-specific differences in toxicant absorption, metabolism, hormone regulation, body composition, and inflammatory responses have been proposed as potential determinants of heterogeneous cardiovascular responses to environmental exposures [31]. Future studies incorporating longitudinal exposure assessment and formal investigation of susceptible populations may therefore provide a more nuanced understanding of the environmental determinants of CVD.

Several limitations constrain interpretation. First, the study is cross-sectional and therefore cannot establish temporality between exposure and cardiovascular history. This also limits the interpretability of mediation analysis, which is why mediation was not prioritized in the present paper. Second, cardiovascular outcomes were based on self-reported physician diagnoses rather than adjudicated incident events, and several diagnoses may overlap within the same participant. Third, residual confounding remains possible despite adjustment for demographic and lifestyle covariates. Finally, some exposure biomarkers were available only in restricted subsamples or a limited number of survey cycles, which is why we flagged unstable small-sample signals separately.

These limitations should be considered alongside several important strengths of the study. We leveraged a large, nationally representative NHANES dataset spanning ten survey cycles to systematically evaluate a broad range of environmental exposures across multiple cardiovascular outcomes. The use of a

consistent survey-weighted analytical approach enhanced comparability across outcomes, while extensive robustness assessments, including subgroup analyses, interaction testing, never-smoker restrictions, leave-one-cycle-out analyses, and nonlinearity evaluations, provided multiple layers of validation for the observed associations. By integrating outcome-specific, subgroup-specific, and chemical-category analyses within a unified ExWAS framework, the study was able to distinguish exposure patterns that were consistently reproduced across cardiovascular phenotypes. Collectively, these strengths provide a comprehensive characterization of the environmental landscape of CDV and generate a robust set of hypotheses for future longitudinal, mechanistic, and causal investigations.

Conclusions

In this ExWAS of 138 environmental exposure biomarkers across six cardiovascular outcomes, we identified multiple environmental exposures associated with CVD, with the strongest and most reproducible associations observed for composite CVD and heart failure. Blood cadmium, serum cotinine, and several tobacco-related biomarkers demonstrated consistent associations across multiple cardiovascular outcomes, while chemical-category enrichment highlighted heavy metals, PCB, and organochlorine pesticides as exposure classes enriched for cardiovascular associations. Subgroup analyses further suggested that some exposure–CVD relationships vary by sex and age, underscoring the importance of considering population heterogeneity in environmental health research. These findings provide a comprehensive overview of the environmental exposure landscape associated with CVD and establish a foundation for future prospective, mechanistic, and causal investigations aimed at identifying modifiable environmental determinants of cardiovascular risk.

Abbreviations

ATCA, 2-aminothiazoline-4-carboxylic acid; BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; CYMA, N-acetyl-S-(2-cyanoethyl)-L-cysteine; EO-Hb, ethylene oxide hemoglobin adduct; ExWAS, exposome-wide association study; FDR, false discovery rate; NHANES, National Health and Nutrition Examination Survey; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; OR, odds ratio; PCB, polychlorinated biphenyls; PFAS, polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonic acid; PIR, income-to-poverty ratio; POP, persistent organic pollutant.

Declarations

Ethics approval and consent to participate

This study is a secondary analysis of publicly available de-identified NHANES data. The present manuscript did not involve new contact with human participants or new collection of identifiable data. The NHANES protocol was approved by the National Center for Health Statistics Research Ethics Review Board. All participants granted written informed consent before participating in NHANES.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

The study conceptualization and methodology were contributed by HW, YuZ, XL and ZG; The formal analysis were performed by HW and YuZ. Data curation were performed by JG, ZW, YaZ, and ZZ. The visualization were perfomed by HQ, LB, and AY. The original manuscript was written by HW and YZ. The draft was reviewed and edited by XL and ZG. All authors read and approved the final manuscript.

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Availability of data and materials

NHANES data used in this study are publicly available from the National Center for Health Statistics. Project-specific derived analysis tables, result summaries, and figure outputs are stored within the local project workspace used for this analysis.

References

1. Global Burden of Cardiovascular Diseases and Risks (2023) Collaborators Global, Regional, and National Burden of Cardiovascular Diseases and Risk Factors in 204 Countries and Territories, 1990–2023. J Am Coll Cardiol. 2025;86:2167–243

2. Artola Arita V, Beigrezaei S, Franco OH (2024) Risk factors for cardiovascular disease: the known unknown. Eur J Prev Cardiol 31:e106–e107
3. Zhang K, Brook RD, Li Y, Rajagopalan S, Kim JB (2023) Air Pollution, Built Environment, and Early Cardiovascular Disease. Circ Res 132:1707–1724
4. Pan Z, Gong T, Liang P (2024) Heavy Metal Exposure and Cardiovascular Disease. Circ Res 134:1160–1178
5. Khraishah H, Chen Z, Rajagopalan S (2024) Understanding the Cardiovascular and Metabolic Health Effects of Air Pollution in the Context of Cumulative Exposomic Impacts. Circ Res 134:1083–1097
6. Schillemans T, Yan Y, Ribbenstedt A, Donat-Vargas C, Lindh CH, Kiviranta H et al (2024) OMICs Signatures Linking Persistent Organic Pollutants to Cardiovascular Disease in the Swedish Mammography Cohort. Environ Sci Technol 58:1036–1047
7. Wen Z-J, Wei Y-J, Zhang Y-F, Zhang Y-F (2023) A review of cardiovascular effects and underlying mechanisms of legacy and emerging per- and polyfluoroalkyl substances (PFAS). Arch Toxicol 97:1195–1245
8. Wu H, Eckhardt CM, Baccarelli AA (2023) Molecular mechanisms of environmental exposures and human disease. Nat Rev Genet 24:332–344
9. Vermeulen R, Schymanski EL, Barabási A-L, Miller GW (2020) The exposome and health: Where chemistry meets biology. Science 367:392–396
10. Wild CP (2012) The exposome: from concept to utility. Int J Epidemiol 41:24–32
11. Niedzwiecki MM, Walker DI, Vermeulen R, Chadeau-Hyam M, Jones DP, Miller GW (2019) The Exposome: Molecules to Populations. Annu Rev Pharmacol Toxicol 59:107–127
12. Münzel T, Sørensen M, Hahad O, Nieuwenhuijsen M, Daiber A (2023) The contribution of the exposome to the burden of cardiovascular disease. Nat Rev Cardiol 20:651–669
13. Verzelloni P, Urbano T, Wise LA, Vinceti M, Filippini T (2024) Cadmium exposure and cardiovascular disease risk: A systematic review and dose-response meta-analysis. Environ Pollut 345:123462
14. Schlezinger JJ, Gokce N (2024) Perfluoroalkyl/Polyfluoroalkyl Substances: Links to Cardiovascular Disease Risk. Circ Res 134:1136–1159
15. Montone RA, Camilli M, Calvieri C, Magnani G, Bonanni A, Bhatt DL et al (2024) Exposome in ischaemic heart disease: beyond traditional risk factors. Eur Heart J 45:419–438
16. Ma Y, Li D, Cui F, Wang J, Tang L, Yang Y et al (2024) Exposure to Air Pollutants and Myocardial Infarction Incidence: A UK Biobank Study Exploring Gene-Environment Interaction. Environ Health Perspect 132:107002
17. Wang M, Zhou T, Song Y, Li X, Ma H, Hu Y et al (2021) Joint exposure to various ambient air pollutants and incident heart failure: a prospective analysis in UK Biobank. Eur Heart J 42:1582–1591
18. Ma J, Li P, Tao L, Yang X, Luo Y, Guo X et al (2025) Environment-wide association study of cardiovascular-kidney-metabolic syndrome based on NHANES 2013–2016. Ecotoxicol Environ Saf

303:118993
19. Uche UI, Suzuki S, Fulda KG, Zhou Z (2020) Environment-wide association study on childhood obesity in the U.S. Environ Res 191:110109
20. Soerensen SJC, Lim DS, Montez-Rath ME, Chertow GM, Chung BI, Rehkopf DH et al (2025) Pesticides and prostate cancer incidence and mortality: An environment-wide association study. Cancer 131:e35572
21. Fang Z, Zhou B, Zheng G, Chen X, Liu M, Zhang H et al (2025) Environment-wide association study of cardiovascular and all-cause mortality: Analysis of the National Health and Nutrition Examination Survey, 1999–2018. Environ Pollut 368:125770
22. Zhang Q, Xiao S, Jiao X, Shen Y (2023) The triglyceride-glucose index is a predictor for cardiovascular and all-cause mortality in CVD patients with diabetes or pre-diabetes: evidence from NHANES 2001–2018. Cardiovasc Diabetol 22:279
23. Pan D, Guo J, Wu S, Wang H, Wang J, Wang C et al (2024) Association of secondhand smoke exposure with all-cause mortality and cardiovascular death in patients with hypertension: Insights from NHANES. Nutr Metab Cardiovasc Dis 34:1779–1786
24. Wei R, Huang M, Zhang Y, He T, Zhu C, Piao H et al (2026) Tobacco exposure and peripheral artery disease: A multi-omics investigation integrating epidemiology, genetics, toxicology, and single-cell transcriptomics. Ecotoxicol Environ Saf 314:120083
25. Zhou C, Wang S, Ju L, Zhang R, Yang Y, Liu Y (2024) Positive association between blood ethylene oxide levels and metabolic syndrome: NHANES 2013–2020. Front Endocrinol (Lausanne) 15:1365658
26. Dunder L, Salihovic S, Varotsis G, Lind PM, Elmståhl S, Lind L (2023) Plasma levels of per- and polyfluoroalkyl substances (PFAS) and cardiovascular disease - Results from two independent population-based cohorts and a meta-analysis. Environ Int 181:108250
27. Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG (2019) A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 29:131–147
28. Volino-Souza M, de Oliveira GV, Pinheiro VDS, Conte-Junior CA, Alvares T (2024) da S. The effect of dietary nitrate on macro- and microvascular function: A systematic review. Crit Rev Food Sci Nutr. ;64:1225–36
29. Lundberg JO, Carlström M, Larsen FJ, Weitzberg E (2011) Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovasc Res 89:525–532
30. Vahter M, Akesson A, Lidén C, Ceccatelli S, Berglund M (2007) Gender differences in the disposition and toxicity of metals. Environ Res 104:85–95
31. Regitz-Zagrosek V, Kararigas G (2017) Mechanistic Pathways of Sex Differences in Cardiovascular Disease. Physiol Rev 97:1–37

Figures

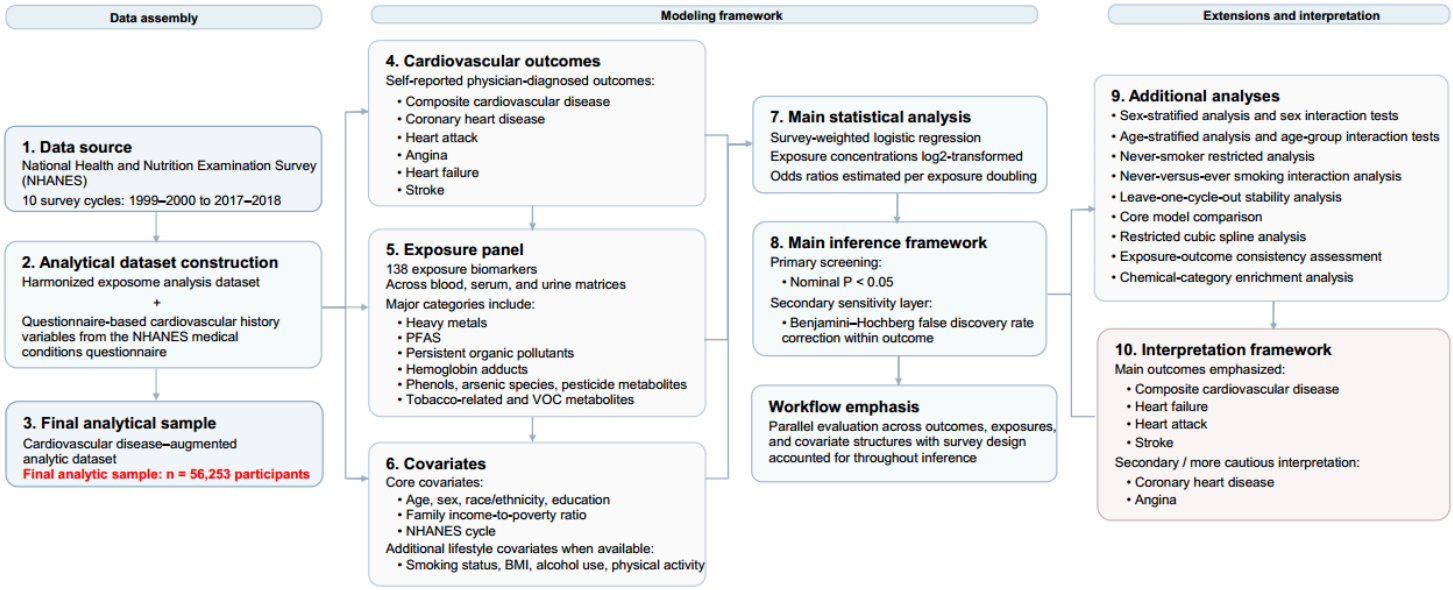


Figure 1

Study design and data analysis workflow

Note: The diagram summarizes construction of the pooled cross-sectional dataset used for the exposome-wide association study, beginning with eligible NHANES participants across 1999-2000 to 2017-2018 and ending with the harmonized analytic sample containing survey design variables, covariates, curated exposure biomarkers and questionnaire-derived cardiovascular outcomes.

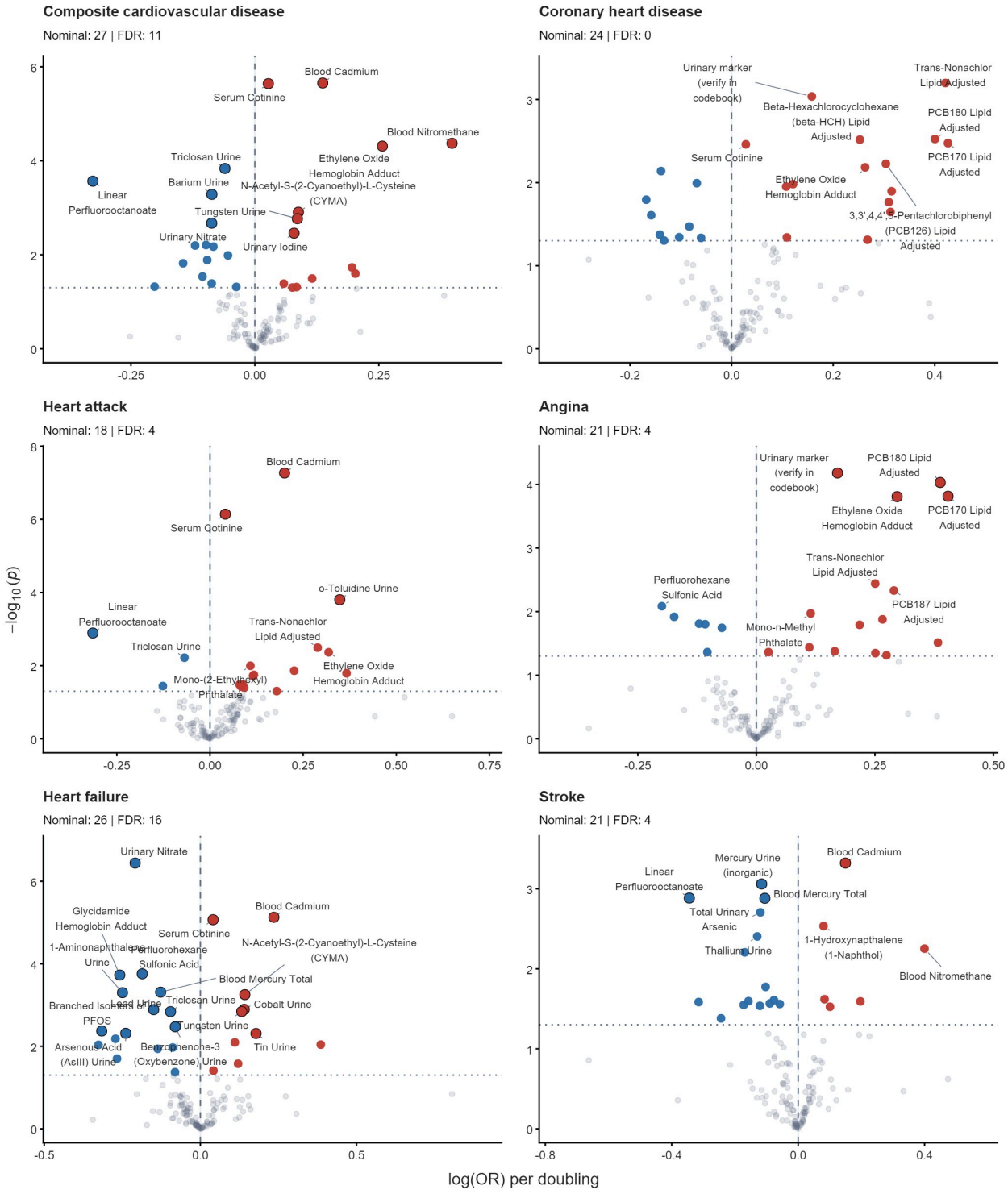


Figure 2

Exposome-wide volcano plots across the six cardiovascular outcomes

Note: Each point represents one exposure-outcome association from a survey-weighted logistic regression model parameterized per exposure doubling. The x-axis shows the log odds ratios and the y-axis shows -log10 *P*-values. Colored points indicate nominal associations (*P*< 0.05), and filled black-

outlined points indicate associations that remained significant after Benjamini-Hochberg false discovery rate correction within outcome.

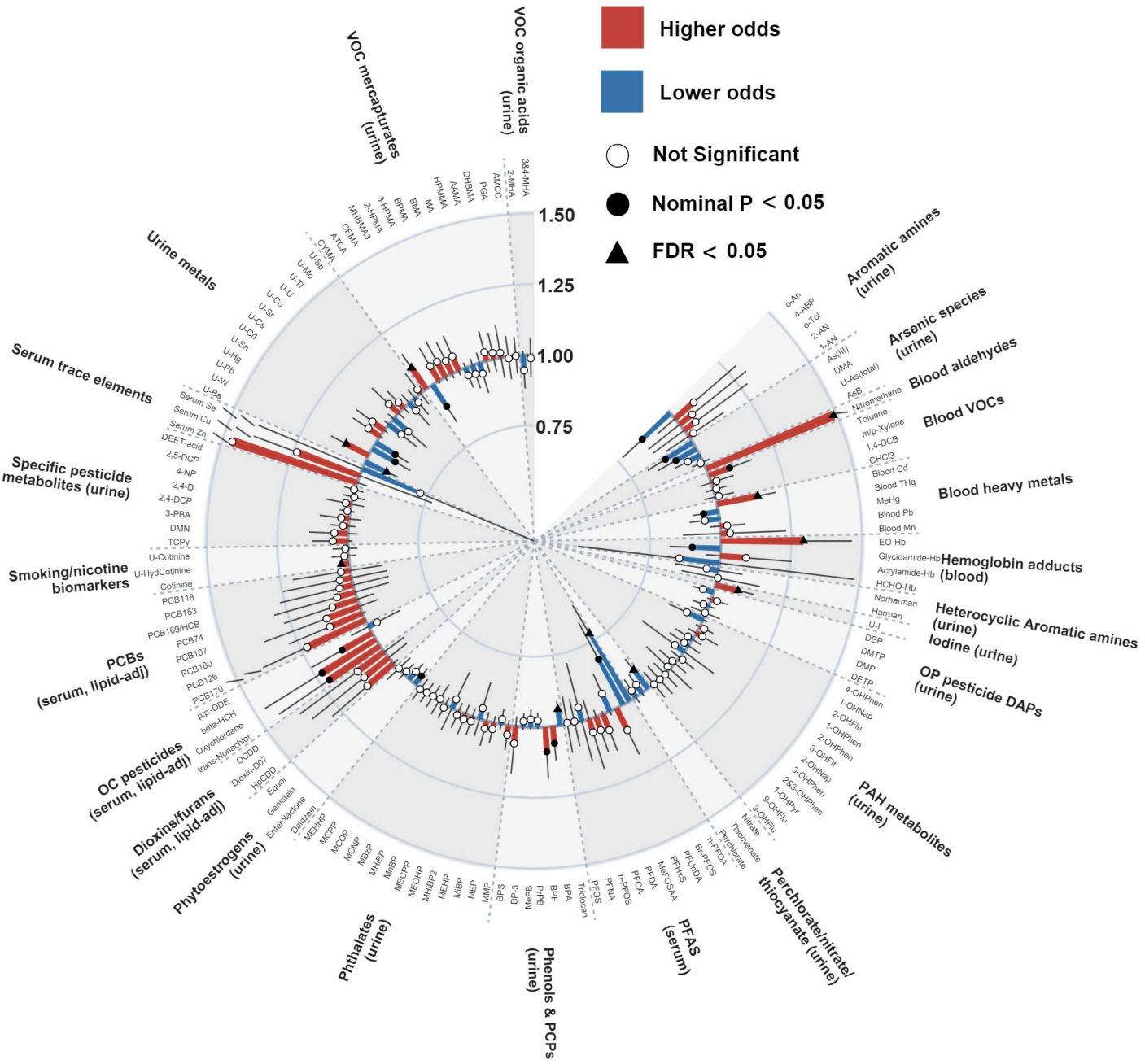


Figure 3

Radial map for the association between exposures and composite cardiovascular diseases

Note: Each radial bar represents one exposure biomarker, grouped by exposure category around the circumference. Bar direction and length encode the odds ratio per exposure doubling relative to OR = 1, black central markers denote statistical status (not significant, nominal *P*< 0.05, or FDR-adjusted *P* <

0.05), and radial line segments show 95% confidence intervals. Broken line ends indicate confidence intervals extending beyond the plotted range.

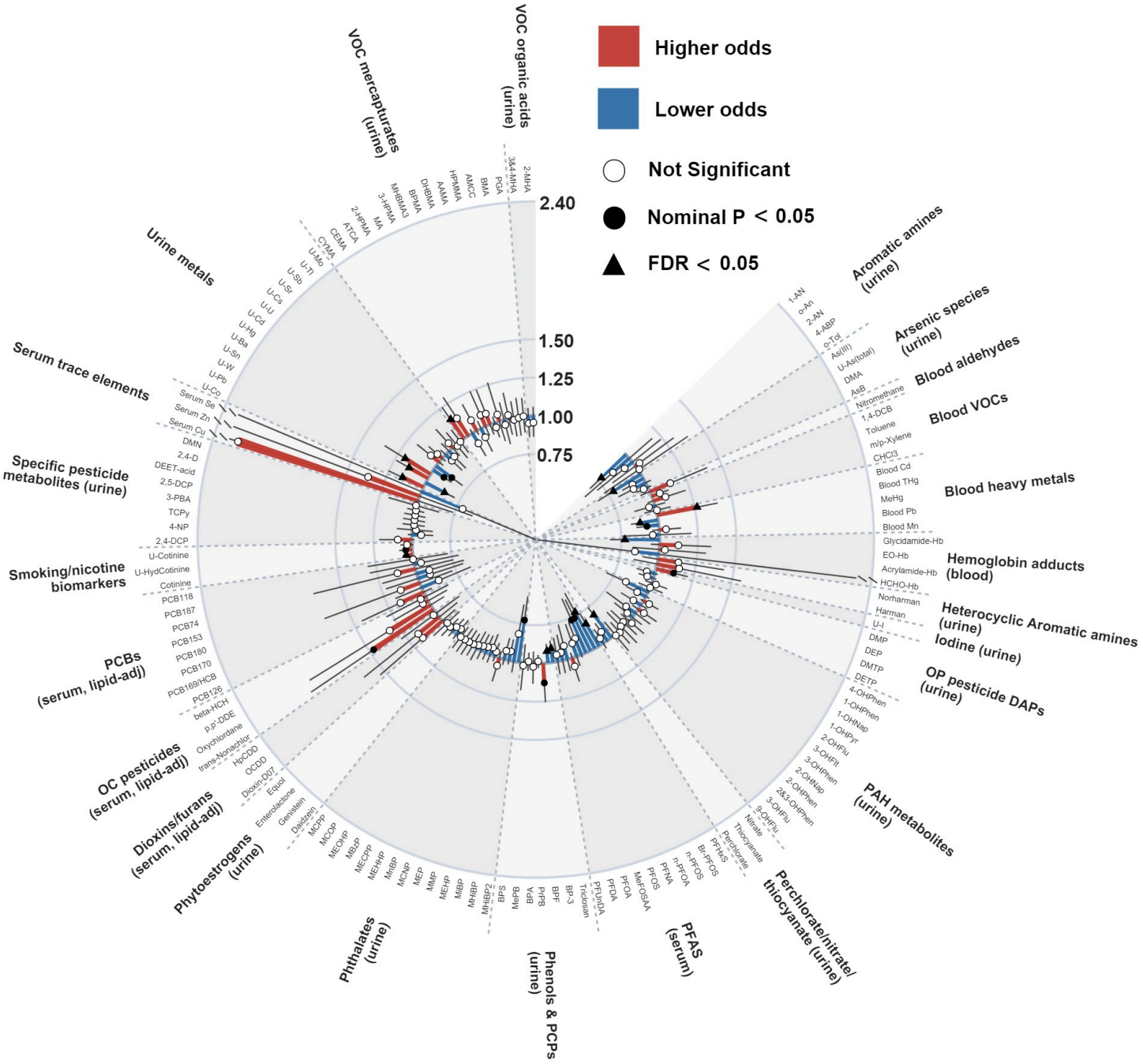


Figure 4

Radial map for the association between exposures and heart failure

Note: Each radial bar represents one exposure biomarker, grouped by exposure category around the circumference. Bar direction and length encode the odds ratio per exposure doubling relative to OR = 1, black central markers denote statistical status (not significant, nominal *P*< 0.05, or FDR-adjusted *P* <

0.05), and radial line segments show 95% confidence intervals. Broken line ends indicate confidence intervals extending beyond the plotted range.

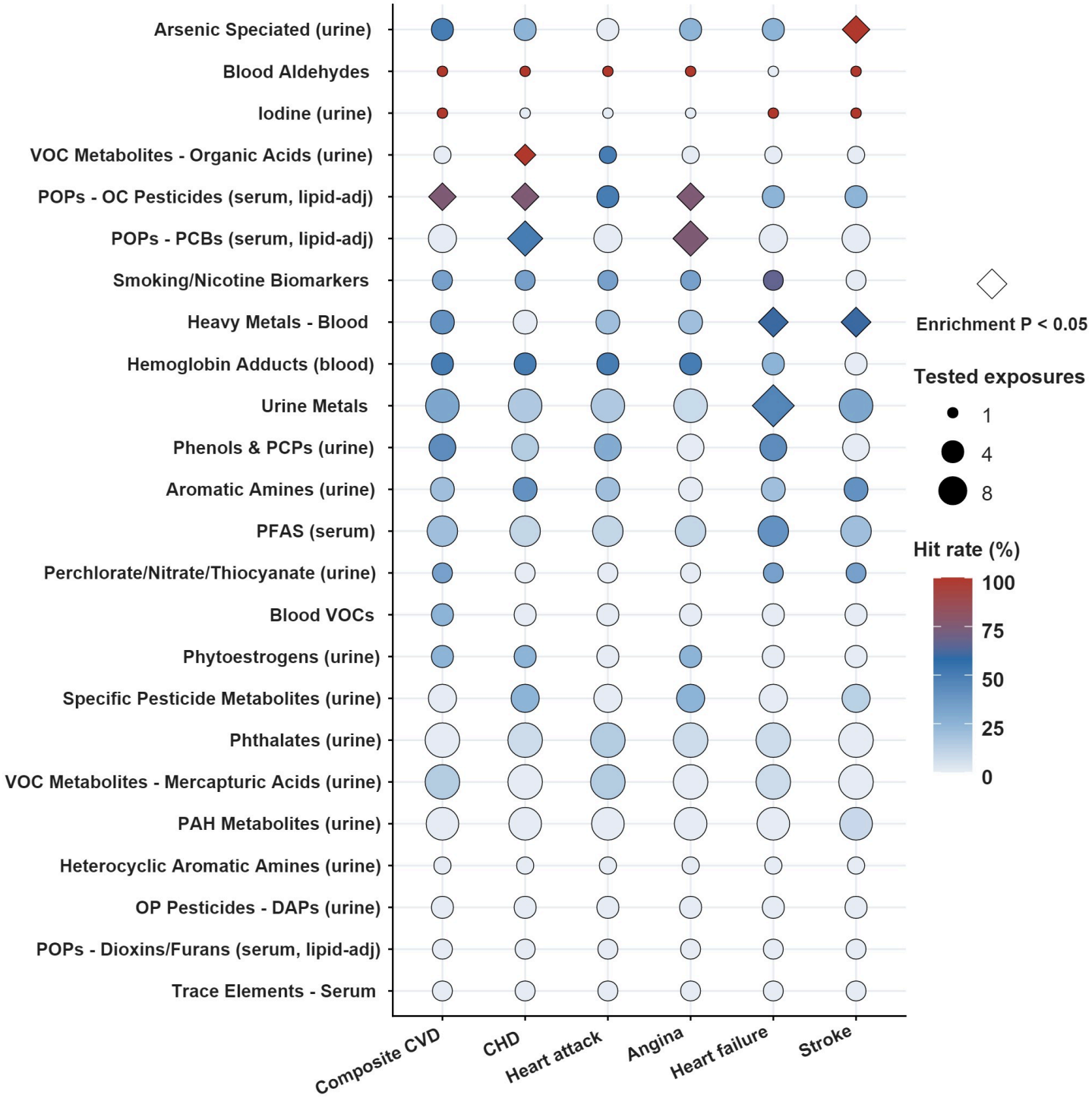


Figure 5

Chemical-category enrichment matrix across cardiovascular outcomes

Note: This figure summarizes Chemical-category enrichment matrix of associations. Bubble color indicates the nominal hit rate within each exposure category and outcome, bubble size indicates the number of tested exposures in that category, and diamond markers denote nominal category enrichment $P < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1CVDConsistencyHeatmap.pdf
- FigureS2RadialExWASHeartAttackR1.pdf
- FigureS3RadialExWASStrokeR1.pdf
- FigureS4RadialExWASCHDR1.pdf
- FigureS5RadialExWASAnginaR1.pdf
- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx