Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Blood metals, serum per- and polyfluoroalkyl substances, and serum butyrylcholinesterase activity and concentration in US adults: NHANES August 2021–August 2023

Dongda Han
Peking University
Meiyi Du
13125484570@163.com
Dalian Medical University

Research Article

Keywords: butyrylcholinesterase, cadmium, PFAS, biomonitoring, NHANES, liver

Posted Date: November 16th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11604282/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Blood metals, serum per- and polyfluoroalkyl substances, and serum butyrylcholinesterase activity and concentration in US adults: NHANES August 2021–August 2023

Dongda Han[a,b] and **Meiyi Du**[c,*]

[a] Department of Medical Genetics, School of Basic Medical Sciences, Peking University, Beijing 100191, China

[b] State Key Laboratory of Female Fertility Promotion, Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing 100191, China

[c] Dalian Medical University, Dalian 116000, Liaoning Province, People's Republic of China

*Corresponding author: Meiyi Du, Dalian Medical University, Dalian 116000, Liaoning Province, People's Republic of China. Email: 13125484570@163.com

Dongda Han: ORCID 0009-0006-3882-6213; amekokoro@shakuameji.com

Abstract

Butyrylcholinesterase (BChE) is a liver-derived plasma enzyme used to monitor anticholinesterase exposure, but its relationships with background environmental chemicals are poorly characterised. We analysed the National Health and Nutrition Examination Survey August 2021–August 2023, which measured serum BChE activity and protein concentration. We first described survey-weighted BChE reference distributions by age, sex and race in participants aged ≥6 years (n=7,061). Among adults aged ≥20 years, survey-weighted linear regression related five blood metals (n=4,217) and five serum PFAS (n=1,644) to BChE, adjusting for demographic, socioeconomic and lifestyle factors and body mass index. Weighted geometric mean activity was 3.85 U/mL and was highest at ages 6–11 years, higher in males, and lowest at ≥80 years. Blood cadmium was inversely associated with BChE activity (−0.122 U/mL per interquartile-range increase; 95% CI: −0.197, −0.048; false-discovery-rate q=0.008), persisting after adjustment for liver and metabolic markers, among never-smokers, with cotinine adjustment and after multiple imputation. All five PFAS and selenium were positively associated with activity but were attenuated after adjustment for liver and metabolic markers. Each association with activity was mirrored by a proportional difference in BChE concentration, no exposure was associated with the activity-to-concentration ratio, and hepatic fat (controlled attenuation parameter) did not mediate the cadmium or PFAS associations. Background cadmium exposure was therefore associated with less circulating enzyme rather than with lower activity per unit of protein, arguing against direct catalytic inhibition. These cross-sectional findings warrant longitudinal confirmation.

Keywords: butyrylcholinesterase; cadmium; PFAS; biomonitoring; NHANES; liver

1. Introduction

Butyrylcholinesterase (BChE, EC 3.1.1.8) is synthesised mainly in the liver and secreted into plasma, where it hydrolyses choline esters and ester-containing drugs and binds organophosphate and carbamate compounds [1]. Serum BChE activity is therefore measured to detect exposure to anticholinesterase pesticides and to assess hepatic synthetic function, although erythrocyte acetylcholinesterase and plasma BChE can classify pesticide-related depression differently [2]. Circulating BChE also varies with metabolic status: activity is higher in non-alcoholic fatty liver disease, obesity and metabolic imbalance [3–5]. A change in BChE in a population survey can thus reflect enzyme inhibition, altered hepatic synthesis or metabolic differences, and these possibilities need to be separated before BChE is interpreted as an exposure biomarker.

Metals are one plausible class of non-pesticide determinants. Cadmium, zinc and aluminium inhibit purified human serum BChE, and inorganic mercury inhibits cholinesterases through several mechanisms [6, 7]. These experiments used concentrations far above those found in the blood of the general population. Population evidence is limited and inconsistent. In 536 residents of southern Spain, serum BChE activity was inversely related to serum manganese and zinc [8]. In community studies in Bangladesh, environmental manganese and arsenic exposure were associated with lower plasma cholinesterase activity [9, 10], whereas a pilot study of 100 women in Saudi Arabia found no significant correlation between serum cadmium and cholinesterase activity [11]. These studies measured enzyme activity only. Without a measure of enzyme quantity, they could not distinguish lower activity per enzyme molecule, as expected from inhibition, from fewer circulating enzyme molecules.

Per- and polyfluoroalkyl substances (PFAS) raise a different question. PFAS perturb hepatic lipid metabolism and are associated with markers of liver injury in human studies [12, 13], and in vitro work reported increased BChE activity after exposure to perfluorooctanoic acid

and related compounds [14]. Because BChE production is linked to hepatic and metabolic state, PFAS could plausibly be associated with higher circulating BChE. Whether this occurs at population exposure levels has, to our knowledge, received little study.

The August 2021–August 2023 cycle of the US National Health and Nutrition Examination Survey (NHANES) measured both serum BChE activity and BChE protein concentration [15]. A concurrent preprint used these measurements to study hepatic steatosis [16], but associations with environmental chemicals have not, to our knowledge, been reported. Having both measurements allows a direct test of whether exposure-related differences in activity are accompanied by proportional differences in enzyme concentration.

We therefore described nationally weighted reference distributions of serum BChE activity and concentration by age, sex and race; estimated associations of five blood metals and five serum PFAS with BChE activity in US adults; compared associations with activity, concentration and their ratio in the same participants, as a way to distinguish a change in the amount of circulating enzyme from a change in activity per enzyme molecule; and tested whether hepatic fat mediated the associations.

2. Methods

2.1. Study population

NHANES is a continuous, multistage probability survey of the civilian non-institutionalised US population. Data collection resumed in August 2021 after the pandemic suspension, under an updated design [17]. Participants were interviewed at home and examined in mobile examination centres, where blood was collected. The National Center for Health Statistics (NCHS) Ethics Review Board approved the survey protocols, and participants provided written informed consent. This secondary analysis used only public, de-identified files.

Serum BChE was measured in examined participants aged ≥6 years [15]. We restricted analyses to examined adults aged ≥20 years because educational attainment is recorded only at these ages. We excluded participants with a positive pregnancy test or self-reported pregnancy; participants whose pregnancy status was unknown or not applicable were retained. Each analysis required measured BChE activity, all five exposures of the corresponding family, and a positive survey weight (Supplementary Table S1).

2.2. Outcomes and exposures

Serum BChE activity (U/mL) and BChE protein concentration (ng/mL) were measured by the CDC Division of Laboratory Sciences [15]. Activity was the primary outcome. Concentration and the activity-to-concentration ratio were secondary outcomes.

Lead, cadmium, total mercury, selenium and manganese were measured in whole blood by inductively coupled plasma mass spectrometry [18, 19]. Serum PFAS were measured in a random one-half subsample by high-performance liquid chromatography–tandem mass spectrometry [20]. We analysed the five PFAS detected in at least 94% of the subsample: perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), linear perfluorooctanoate (n-PFOA), linear perfluorooctane sulfonate (n-PFOS) and the sum of branched perfluorooctane sulfonate isomers (Sm-PFOS). Concentrations below the limit of detection were used as released by NHANES (limit divided by $\sqrt{2}$). All exposures were natural-log transformed.

2.3. Covariates and adjustment sets

Adjustment sets were specified from assumed causal relationships rather than statistical significance. Model M1 included age (natural cubic spline, knots at 40 and 60 years), sex, race and Hispanic origin, educational attainment and family income-to-poverty ratio. The primary model, M2, additionally included smoking status (never, former, current), past-year

alcohol frequency (none, less than monthly, monthly, weekly, daily or nearly daily) and body mass index. These variables can influence both exposure (for example, smoking and cadmium; diet and PFAS) and BChE. M0 was unadjusted.

The extended model, M3, added sedentary time, any prescription medication use, alanine and aspartate aminotransferase, γ-glutamyl transferase, albumin, triglycerides, estimated glomerular filtration rate (CKD-EPI 2021) [21], glycated haemoglobin and C-reactive protein; skewed markers (aminotransferases, γ-glutamyl transferase, triglycerides, C-reactive protein) were log-transformed. Several of these markers may lie on pathways from exposure to BChE; PFAS, for example, are associated with liver enzymes and lipids, and BChE tracks hepatic fat. Adjusting for them may remove confounding by liver and metabolic status, but it may also remove part of the effect of interest. We therefore report M3 alongside M2 rather than treating either as definitive.

This specification was revised after initial analyses of these data. An earlier version adjusted simultaneously for waist circumference, body mass index and the M3 markers as the primary model. We changed the primary adjustment set to M2 because of the possible mediator role of these markers and the redundancy of waist circumference with body mass index. The analysis was not preregistered, and the extended model is presented in full.

Refused and "don't know" responses were set to missing. Alcohol frequency was modelled as an unordered categorical variable.

2.4. Statistical analysis

All estimates accounted for the complex survey design through masked strata and primary sampling units, with Taylor-series linearisation. Survey designs were constructed for all participants with a positive weight before restriction to analytic domains, so that variance estimates reflected the full design. We used the phlebotomy weight (WTPH2YR) for analyses of BChE and metals, following NCHS guidance that this weight applies to blood laboratory

components in this cycle [22, 23]. PFAS analyses used the PFAS subsample weight (WTSPF2YR), as specified in the component documentation [20]. The design has 30 primary sampling units in 15 strata. Because regression models contained more parameters than design degrees of freedom, all confidence intervals and coefficient tests used the t distribution with 15 degrees of freedom, and joint tests used F distributions with 15 denominator degrees of freedom [24].

Primary models. Survey-weighted linear regression related each log-transformed exposure, one at a time, to BChE activity on its original scale. Coefficients are differences in activity (U/mL) per natural-log unit of exposure; we also scaled them to the weighted interquartile range (IQR) of each log exposure. Benjamini–Hochberg false-discovery-rate q values were calculated separately within the metal and PFAS families of five tests.

Activity, concentration and ratio. Among participants with both measurements, we fitted M2 models for log activity, log concentration and log(activity/concentration). Because the same participants, design and covariates were used, the ratio coefficient equals the difference between the activity and concentration coefficients and provides a formal test of whether exposure is associated with activity per unit of measured enzyme protein. Coefficients were expressed as percentage differences.

Reference distributions. In participants aged ≥6 years with a measured outcome and a positive phlebotomy weight, we estimated survey-weighted geometric means (with t-based 95% confidence intervals on the log scale) and weighted percentiles (5th, 25th, 50th, 75th, 95th) of BChE activity and concentration, overall and by age group, sex, and race and Hispanic origin. These are weighted population distributions, not clinical reference intervals for a healthy population.

Mediation through hepatic fat. We tested whether liver fat mediated the cadmium, PFNA and selenium associations with log activity, using the controlled attenuation parameter (CAP)

from vibration-controlled transient elastography as the mediator (log-transformed); the median CAP for hepatic steatosis is well characterised [25]. Liver stiffness was used as a second mediator in sensitivity analyses. For each exposure we estimated the total effect, the exposure–mediator association, and the mediator and direct effects from survey-weighted models in the same participants, and obtained the indirect effect as their product with a delta-method (Sobel) confidence interval [26]. The bootstrap was not used because of the small number of primary sampling units. Mediation estimates are associational, not causal, given the cross-sectional design.

Model dependence. Models M0–M3 were compared in the common sample with complete M3 covariates.

Missing covariates. To assess the effect of complete-case selection, we imputed missing M2 covariates (education, income-to-poverty ratio, smoking, alcohol frequency, body mass index) by chained equations [27]. Imputation was carried out separately within each exposure family's analysis-eligible domain, restricted to participants with both BChE measurements. We used predictive mean matching for continuous variables and multinomial regression for categorical variables. Predictors included the age spline, sex, race and Hispanic origin, all five log exposures, log BChE activity and concentration, the 30 primary sampling units and the log survey weight. Outcomes, exposures and design variables were not imputed, and PFAS values were not imputed for participants outside the PFAS subsample. We created 20 imputations with 20 iterations, assessed convergence from trace plots and between-chain diagnostics, and combined survey-weighted estimates using Rubin's rules with Barnard–Rubin degrees of freedom based on 15 complete-data degrees of freedom [28, 29].

Dose–response. Restricted cubic splines with four knots at the survey-weighted 5th, 35th, 65th and 95th percentiles of each log exposure were fitted for all ten exposures under M2

[30]. We report overall and non-linearity F tests, with Benjamini–Hochberg adjustment across the ten exposures; these analyses were exploratory.

Joint quartile-score models. Within each family, the five exposures were entered simultaneously as weighted quartile scores (0–3) in one M2 model. The joint contrast estimated the difference associated with a one-quartile increase in all five exposures. It assumes linear and additive scores. This is a survey-weighted joint model, not weighted quantile sum regression or g-computation. We did not use unweighted mixture methods, which ignore the sampling design [31].

Sensitivity analyses. We repeated M2 models (i) among never-smokers (fewer than 100 lifetime cigarettes), (ii) excluding participants positive for hepatitis B surface antigen or hepatitis C RNA, (iii) adding log serum cotinine, and (iv) for metals, using the examination weight (WTMEC2YR) in the same participants. Among participants eligible for each analysis, we compared those included and excluded by covariate completeness using weighted standardised mean differences.

Analyses used R 4.6.2 with the survey (4.5), mice (3.19.0) and haven packages [32]. Reporting follows the STROBE statement [33].

3. Results

3.1. Participants

Of 6,023 examined adults aged ≥20 years who were not known to be pregnant, 5,569 had measured BChE activity and a positive phlebotomy weight (Fig. 1). Of these, 4,217 had complete M2 covariates and all five metals, and formed the metal sample. In the PFAS subsample, 2,179 eligible adults had all five PFAS measured and 1,644 had complete M2 covariates (Supplementary Table S1). In the metal sample, the weighted median age was 49 years (IQR 34–63) and 50.3% were women (Table 1). Weighted median BChE activity was

3.88 U/mL (IQR 3.33–4.48), and median concentration was 59.3 ng/mL (50.4–70.1). Median blood cadmium was 0.239 µg/L (0.155–0.408); in the PFAS sample, median n-PFOS was 1.9 ng/mL (1.1–3.2) and PFNA 0.3 ng/mL (0.2–0.5).

Participants excluded for missing covariates had similar BChE activity and blood cadmium to those included (standardised mean differences 0.05 and <0.01 in the metal sample). Among those with an observed value, excluded participants had a lower income-to-poverty ratio (standardised difference 0.42), and non-Hispanic Black and Mexican American adults were slightly over-represented among the excluded (Supplementary Table S2).

3.2. Reference distribution of serum BChE

Among 7,061 participants aged ≥6 years, the weighted geometric mean BChE activity was 3.85 U/mL (95% CI: 3.74, 3.95), with weighted 5th–95th percentiles of 2.61–5.53 U/mL; concentration had a geometric mean of 58.9 ng/mL (57.7, 60.2) and 5th–95th percentiles of 38.7–86.5 ng/mL (Table 4). Activity was highest at ages 6–11 years (4.23 U/mL), lower through adolescence and early adulthood (3.78 at 12–19 and 20–39 years), slightly higher at 40–59 years (3.92), and lowest at ≥80 years (3.40); concentration followed the same pattern (Fig. 2). Activity was higher in males (3.99 U/mL) than females (3.71) overall, but the sex difference narrowed or reversed after age 60 (Fig. 2). Geometric mean activity was highest in Mexican American (4.08 U/mL) and lowest in non-Hispanic Black participants (3.59). These are weighted population distributions and should not be read as clinical reference intervals for a healthy population.

3.3. Primary associations with BChE activity

In the primary model, blood cadmium was inversely associated with BChE activity: β=−0.126 U/mL per natural-log unit (95% CI: −0.204, −0.049; q=0.008), or −0.122 U/mL per IQR increase, about 3% of median activity (Table 2; Fig. 4). Selenium was positively

associated (+0.105 U/mL per IQR; 95% CI: 0.058, 0.152; q=0.001). Lead, total mercury and manganese were not associated (all q>0.30).

All five PFAS were positively associated with activity. Per IQR increase, differences ranged from +0.104 U/mL for PFHxS (95% CI: 0.012, 0.197; q=0.030) to +0.136 U/mL for Sm-PFOS (95% CI: 0.059, 0.214; q=0.007). PFNA, n-PFOA and n-PFOS each had q=0.007 (Table 2).

3.4. Activity, concentration and the activity-to-concentration ratio

The distinction between a change in the amount of circulating enzyme and a change in activity per enzyme molecule is shown schematically in Fig. 3. In participants with both measurements, the associations with log activity were mirrored by associations of similar size with log concentration (Table 3; Fig. 5). For cadmium, each natural-log unit was associated with 2.97% lower activity (95% CI: −4.80, −1.10) and 2.52% lower concentration (95% CI: −4.62, −0.38). The activity-to-concentration ratio differed by −0.46% (95% CI: −1.28, 0.37). The lower confidence limit for the ratio lay well above the activity estimate, so the data are inconsistent with the whole activity difference arising from lower activity per unit of measured protein. Selenium (+21.2% activity, +21.8% concentration) and each PFAS (activity +2.3% to +3.7%, concentration +3.0% to +3.4%) showed the same pattern. No exposure was associated with the ratio (all q≥0.53).

3.5. Mediation through hepatic fat

Hepatic fat did not explain the cadmium or PFAS associations (Fig. 7; Supplementary Table S11). For cadmium, the indirect effect of log activity through log CAP was −0.0020 per natural-log unit (95% CI: −0.0058, 0.0017; 7% of the total effect; p=0.26), and for PFNA it was essentially zero (0.4%; p=0.90). Only selenium showed a non-null indirect effect through CAP (0.016; 95% CI: 0.008, 0.024; 9%; p<0.001); because selenium was positively

associated with both hepatic fat and activity, this most likely reflects shared nutritional and hepatic-synthetic correlates rather than a hepatotoxic pathway. Liver stiffness mediated none of the associations (all indirect effects near zero).

3.6. Dependence on adjustment and missing data

In the common sample with complete extended covariates (n=4,061 metals; 1,613 PFAS), the cadmium estimate changed little from M0 to M2 (−0.158, −0.145 and −0.136 U/mL for M0, M1 and M2). It was attenuated but remained inverse after adding liver, lipid and metabolic markers (M3: −0.089; 95% CI: −0.144, −0.033; q=0.020) (Supplementary Table S3). By contrast, selenium (M2 +0.659, M3 +0.081) and the PFAS (for example, PFNA M2 +0.146, M3 +0.095) were attenuated in M3, and no PFAS association remained statistically significant after correction (M3 q=0.12–0.23). Lead was inversely associated in M0 and M1 (M1 −0.123 U/mL; p<0.001), but the estimate moved toward the null after adjustment for smoking, alcohol and body mass index (M2 −0.042; p=0.11).

Multiple imputation increased the analytic samples to 5,567 (metals) and 2,178 (PFAS). Chains mixed well without trend (potential scale reduction factors 0.98–1.06; no logged events), and the fraction of missing information was 0.12–0.13. Pooled estimates were close to the complete-case estimates: cadmium −0.119 U/mL per natural-log unit (95% CI: −0.171, −0.067), selenium +0.678, and PFAS +0.080 to +0.134 U/mL, with all PFAS confidence intervals excluding zero (Table 2).

3.7. Dose–response and joint models

Spline models showed overall associations for all exposures except mercury, and non-linearity for lead, cadmium, selenium, manganese and PFNA (non-linearity q=0.001–0.034; Fig. 6; Supplementary Table S4). The cadmium curve declined most steeply at low concentrations and more gradually above the median. Lead and manganese showed inverted-

U shapes, and selenium and PFNA rose and then plateaued. These analyses were exploratory, and the curves are imprecise at the extremes.

When the five PFAS were modelled jointly, a one-quartile increase in all five was associated with +0.095 U/mL activity (95% CI: 0.010, 0.181). Individual PFAS coefficients in this model were imprecise because the exposures were strongly correlated (Spearman ρ up to 0.87). In the joint metal model, the inverse cadmium coefficient (−0.065 U/mL per quartile; 95% CI: −0.114, −0.015) and the positive selenium coefficient (+0.100; 0.058, 0.141) offset each other, and the joint contrast was null (+0.022; −0.037, 0.081) (Supplementary Table S5).

3.8. Sensitivity analyses

The cadmium association was stronger among never-smokers (−0.172 U/mL per natural-log unit; 95% CI: −0.284, −0.060; n=2,460). It changed little with cotinine adjustment (−0.129; −0.205, −0.054), after excluding participants with active hepatitis B or C (−0.125; −0.200, −0.051), or with the examination weight (−0.128; −0.205, −0.052). PFAS estimates were similar among never-smokers (PFNA, n-PFOA, n-PFOS and Sm-PFOS q=0.047; PFHxS q=0.11; n=955) and in the other sensitivity analyses (Supplementary Table S6).

4. Discussion

In US adults, blood cadmium was associated with lower serum BChE, and PFAS and selenium with higher BChE. The cadmium association was the most consistent: it persisted after adjustment for liver and metabolic markers, among never-smokers, with cotinine adjustment and after multiple imputation. The PFAS and selenium associations depended on whether liver and metabolic markers were included in the model. For every exposure, differences in activity were accompanied by proportional differences in BChE protein concentration, no exposure was associated with activity per unit of measured protein, and

hepatic fat did not mediate the associations. We also provide the first nationally weighted reference distributions for this newly introduced assay.

4.1. Reference distribution

Serum BChE activity and concentration varied with age, sex and race. Activity was highest in children, fell through adolescence, and declined again in old age, broadly tracking the growth, metabolic and hepatic-synthetic changes across the life course. The distributions provide a population reference for a biomarker that was added to NHANES only in this cycle, and a context for interpreting the exposure associations; they are weighted population distributions and not clinical reference intervals for a healthy population.

4.2. Cadmium

The direction of the cadmium association agrees with experiments in which cadmium inhibited purified human serum BChE [6]. Those experiments, however, used sub-millimolar to millimolar cadmium, whereas the median blood cadmium here, 0.24 μg/L, corresponds to roughly 2 nmol/L. Our activity-to-concentration analysis also argues against direct inhibition as the main explanation. Inhibition would lower activity per enzyme molecule, but the ratio was unrelated to cadmium, and its confidence interval excluded a reduction as large as the reduction in activity. The association instead reflected lower circulating enzyme concentration. Possible explanations include reduced hepatic synthesis or secretion, since cadmium accumulates in the liver as well as the kidney [34], or confounding by unmeasured determinants of both cadmium burden and BChE. The data cannot distinguish these explanations, and the ratio interpretation assumes that the concentration assay measures circulating enzyme protein comparably across participants.

Smoking is the main non-dietary source of cadmium and could confound the association. The estimate was larger among never-smokers and unchanged by cotinine adjustment, which

makes confounding by active smoking an unlikely sole explanation, although diet-related confounding remains possible. Previous population studies of cadmium were small or examined other enzymes. In 100 Saudi women, serum cadmium was not significantly correlated with cholinesterase activity [11], and in a Spanish general-population study the inverse cadmium association was reported for paraoxonase 1 rather than BChE [8]. The present association is modest, about 3% of median activity per IQR, and is unlikely to matter for an individual BChE result. It may, however, contribute to between-person variation when BChE is used as a baseline in biomonitoring.

4.3. PFAS

Positive associations of PFAS with both activity and concentration are consistent with experimental effects of PFAS on hepatic lipid metabolism [12], with associations of PFAS with hepatic fibrosis and steatosis indices in NHANES [13], and with higher BChE in fatty liver disease [3, 16]. The attenuation after adjustment for liver enzymes, triglycerides, glycated haemoglobin and related markers fits either of two interpretations. These markers may be confounders, in which case M3 is preferable, or they may lie on a pathway from PFAS to BChE through hepatic metabolism, in which case M3 removes part of the association of interest. A cross-sectional design cannot separate these, so we regard the PFAS findings as suggestive. Formal mediation through hepatic fat was essentially zero for the PFAS, so if liver-related markers lie on the pathway, the relevant step is not the amount of liver fat measured by elastography. An in vitro study reported increased activity of purified horse-serum BChE with perfluorooctanoic acid [14], but at concentrations well above those in human serum. The null ratio in our data does not support altered activity per enzyme molecule. The joint PFAS model gave a positive association of similar size, so the result does not depend on a single congener, but the correlation among PFAS prevents attribution to any one compound.

4.4. Selenium and other metals

The strong selenium association was largely removed by adjustment for liver and metabolic markers. A large fraction of plasma selenium is carried in selenoprotein P, which is secreted mainly by the liver [35], so selenium and BChE may share hepatic and nutritional determinants. We therefore do not interpret the selenium association as an effect of selenium on BChE. Lead was associated with lower BChE only before adjustment for smoking, alcohol and body mass index, which suggests confounding. Blood manganese showed no linear association, in contrast to the inverse association with serum manganese in Spain [8] and with environmental manganese in Bangladesh [10]. The exposure matrices, exposure levels and populations differ, and the inverted-U spline for manganese was exploratory. In the joint metal model, inverse cadmium and positive selenium associations cancelled, so that a single index of all metals would have suggested no association. Grouping elements with opposite associations can obscure informative component associations.

4.5. Strengths and limitations

This study used a probability sample with design-based inference and objective biomarkers of exposure and outcome. It had two independent BChE measurements, which allowed a formal test of activity per unit of enzyme protein. We reported several adjustment sets in full and evaluated missing covariates by multiple imputation.

The study also has important limitations. First, it is cross-sectional, so temporality cannot be established, and reverse causation or confounding by liver and metabolic state cannot be excluded. Second, the analytic specification was revised after initial analyses of these data. Under the earlier specification, the cadmium association was at the margin of the false-discovery-rate threshold ($q=0.051$) and the PFAS associations did not reach it ($q\geq0.07$), consistent with the M3 results reported here. The PFAS findings in particular therefore depend on the choice of adjustment set. Third, the survey design provides only 15 degrees of

freedom, which limits precision. Spline, joint and sensitivity analyses added comparisons beyond the two primary families of five tests. Fourth, exposures and BChE were each measured once. Fifth, we could not account for BCHE genotype, which strongly influences enzyme activity [1], or for exposure to anticholinesterase pesticides. Sixth, the multiple imputation assumed that covariates were missing at random given the variables in the imputation model, and neither complete-case analysis nor imputation addresses participants without a BChE result. Finally, the results apply to adults aged ≥20 years; public-use age is top-coded at 80 years.

4.6. Conclusions

Blood cadmium was consistently associated with lower serum BChE in US adults. The association reflected lower circulating enzyme concentration rather than lower activity per unit of enzyme protein, which argues against direct inhibition at background exposure levels. PFAS were associated with higher BChE in a manner that depended on adjustment for liver and metabolic markers. Studies of BChE as an exposure biomarker should consider cadmium and hepatic metabolic status as sources of variation, and longitudinal studies with repeated measurements are needed to establish temporality.

Declarations

Data and code availability. NHANES data are publicly available from the National Center for Health Statistics (https://wwwn.cdc.gov/nchs/nhanes/). The analysis code, aggregate results and run logs are openly available at Zenodo (https://doi.org/10.5281/zenodo.23831479; version 0.3.0-IJHEH, CC BY 4.0).

Ethics. NHANES protocols were approved by the NCHS Ethics Review Board, and participants provided written informed consent. This secondary analysis used only publicly available, de-identified data, and additional ethical approval was not required.

Funding. This research was funded by the National College Students' Innovation and Entrepreneurship Training Program (Grant No. 202510161011). The funder had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRediT authorship contribution statement. Dongda Han: Conceptualization, Methodology, Data curation, Formal analysis, Software, Visualization, Writing – original draft. **Meiyi Du:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements. We thank the NHANES participants and the staff of the National Center for Health Statistics and the CDC Division of Laboratory Sciences.

References

1. Lockridge O (2015) Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. Pharmacol Ther 148:34–46. https://doi.org/10.1016/j.pharmthera.2014.11.011

2. Strelitz J, Engel LS, Keifer MC (2014) Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers. Occup Environ Med 71:842–847. https://doi.org/10.1136/oemed-2014-102315

3. Turecky L, Kupcova V, Durfinova M, Uhlikova E (2021) Serum butyrylcholinesterase activities in patients with non-alcoholic fatty liver disease. Comparison with liver proteosynthetic function and liver fibrosis. Bratisl Lek Listy 122:689–694. https://doi.org/10.4149/BLL_2021_110

4. Suriyaprom K, Songtrai S, Chowchaikong N, Sirikulchayanonta C (2025) Associations of Butyrylcholinesterase Activity and Lipid-Related Indices with Obesity in Thai Children. Int J Environ Res Public Health 22:1107. https://doi.org/10.3390/ijerph22071107

5. de Moura Bones DR, Ramos MM, Veigand NF, Araceli Joerin-Luque I, Sukow NM, Cupertino SE, dos Santos PI, de Oliveira Nunes HK, Gros AF, Barros VD, Tessaro JG, Garcia LL, Bucco IDO, Gonçalves LB, Alves ACG, de Moraes TR, Andrietta SÁ, de Cristo TF, Santos Ribeiro Zanette VD, de Paula AI, Hauser AB, Lopes CVG, Turek LV, Alle LF, Lehtonen Rodrigues Souza R, Beltrame MH (2026) Butyrylcholinesterase as a potential biomarker of metabolic imbalances in Afro-descendant populations. Int J Obes 50:1751–1760. https://doi.org/10.1038/s41366-026-02095-2

6. Sarkarati B, Çokuğraş AN, Tezcan EF (1999) Inhibition kinetics of human serum butyrylcholinesterase by Cd2+, Zn2+ and Al3+: comparison of the effects of metal ions on cholinesterases. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 122:181–190. https://doi.org/10.1016/S0742-8413(98)10102-0

7. Frasco MF, Colletier J-P, Weik M, Carvalho F, Guilhermino L, Stojan J, Fournier D (2007) Mechanisms of cholinesterase inhibition by inorganic mercury. FEBS J 274:1849–1861. https://doi.org/10.1111/j.1742-4658.2007.05732.x

8. Hernández AF, Gil F, Leno E, López O, Rodrigo L, Pla A (2009) Interaction between human serum esterases and environmental metal compounds. Neurotoxicology 30:628–635. https://doi.org/10.1016/j.neuro.2009.04.003

9. Ali N, Hoque MA, Haque A, Salam KA, Karim MR, Rahman A, Islam K, Saud ZA, Khalek MA, Akhand AA, Hossain M, Mandal A, Karim MR, Miyataka H, Himeno S, Hossain K (2010) Association between arsenic exposure and plasma cholinesterase activity: a population based study in Bangladesh. Environ Health 9:36. https://doi.org/10.1186/1476-069x-9-36

10. Anjum A, Biswas S, Rahman M, Rahman A, Siddique AE, Karim Y, Aktar S, Nikkon F, Haque A, Himeno S, Hossain K, Saud ZA (2019) Butyrylcholinesterase—a potential plasma biomarker in manganese-induced neurobehavioral changes. Environ Sci Pollut Res 26:6378–6387. https://doi.org/10.1007/s11356-018-04066-1

11. Hamouda AF, Felemban S (2023) A Bio-Indicator Pilot Study Screening Selected Heavy Metals in Female Hair, Nails, and Serum from Lifestyle Cosmetic, Canned Food, and Manufactured Drink Choices. Molecules 28:5582. https://doi.org/10.3390/molecules28145582

12. Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, Corton JC, Abbott BD (2017) Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378:37–52. https://doi.org/10.1016/j.tox.2016.12.007

13. Cheng W, Li M, Zhang L, Zhou C, Zhang X, Zhu C, Tan L, Lin H, Zhang W, Zhang W (2023) Close association of PFASs exposure with hepatic fibrosis than steatosis:

evidences from NHANES 2017–2018. Ann Med 55:2216943. https://doi.org/10.1080/07853890.2023.2216943

14. Obiako PC, Taylor BA, Clinger JA, Sayes CM (2025) Perfluorooctanoic acid, perfluorobutanoic acid, and undecafluoro-2-methyl-3-oxahexanoic acid disrupt neurotransmitter release and cholinesterase activity. Chemosphere 387:144678. https://doi.org/10.1016/j.chemosphere.2025.144678

15. National Center for Health Statistics (2026) National Health and Nutrition Examination Survey, August 2021-August 2023 Data Documentation, Codebook, and Frequencies: Butyrylcholinesterase Activity & Concentration (BCHE_L). Data file BCHE_L.xpt, first published August 2026. Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/BCHE_L.htm. Accessed 31 Oct 2026

16. Za'areer MHA, Jafari AA, Al-Za'areer CH (2026) Serum Butyrylcholinesterase and Hepatic Steatosis in U.S. Adults: A Nationally Representative NHANES Study. Research Square [preprint], version 1, posted 9 September 2026. https://doi.org/10.21203/rs.3.rs-10890673/v1

17. Paulose-Ram R, Graber JE, Woodwell D, Ahluwalia N (2021) The National Health and Nutrition Examination Survey (NHANES), 2021–2022: Adapting Data Collection in a COVID-19 Environment. Am J Public Health 111:2149–2156. https://doi.org/10.2105/AJPH.2021.306517

18. National Center for Health Statistics (2024) National Health and Nutrition Examination Survey, August 2021-August 2023 Data Documentation, Codebook, and Frequencies: Lead, Cadmium, Total Mercury, Selenium, & Manganese – Blood (PBCD_L). Data file PBCD_L.xpt, first published September 2024. Centers for Disease Control and Prevention.

https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/PBCD_L.htm. Accessed 31 Oct 2026

19. Centers for Disease Control and Prevention (2023) Laboratory Procedure Manual. Analytes: Cadmium, Lead, Manganese, Mercury, and Selenium; Matrix: Whole Blood; Method: Triple Quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-QQQ-MS). Method No. DLS 3040.1-05, revised 5 January 2023. Inorganic and Radiation Analytical Toxicology Branch, Division of Laboratory Sciences, National Center for Environmental Health. NHANES August 2021–August 2023 laboratory method file "Blood Lead, Cadmium, Total Mercury, Selenium and Manganese" (September 2024). https://wwwn.cdc.gov/nchs/data/nhanes/public/2021/labmethods/PBCD-L-MET-508.pdf. Accessed 31 Oct 2026

20. National Center for Health Statistics (2026) National Health and Nutrition Examination Survey, August 2021-August 2023 Data Documentation, Codebook, and Frequencies: Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS_L). Data file PFAS_L.xpt, first published August 2026. Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2021/DataFiles/PFAS_L.htm. Accessed 31 Oct 2026

21. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, Crews DC, Doria A, Estrella MM, Froissart M, Grams ME, Greene T, Grubb A, Gudnason V, Gutiérrez OM, Kalil R, Karger AB, Mauer M, Navis G, Nelson RG, Poggio ED, Rodby R, Rossing P, Rule AD, Selvin E, Seegmiller JC, Shlipak MG, Torres VE, Yang W, Ballew SH, Couture SJ, Powe NR, Levey AS, Chronic Kidney Disease Epidemiology Collaboration (2021) New Creatinine- and Cystatin C–Based Equations to Estimate GFR without Race. N Engl J Med 385:1737–1749. https://doi.org/10.1056/NEJMoa2102953

22. National Center for Health Statistics (2024) Brief Overview of Sample Design, Nonresponse Bias Assessment, and Analytic Guidelines for NHANES August 2021-August 2023. First published 20 September 2024. Centers for Disease Control and Prevention. https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/overviewbrief.aspx?Cycle=2021-2023. Accessed 31 Oct 2026

23. National Center for Health Statistics (n.d.) NHANES Tutorials – Weighting Module. Centers for Disease Control and Prevention. https://wwwn.cdc.gov/nchs/nhanes/tutorials/weighting.aspx. Accessed 31 Oct 2026

24. Lumley T (2004) Analysis of Complex Survey Samples. J Stat Softw 9:1–19. https://doi.org/10.18637/jss.v009.i08

25. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, Kumar M, Lupsor-Platon M, Han KH, Cardoso AC, Ferraioli G, Chan WK, Wong VWS, Myers RP, Chayama K, Friedrich-Rust M, Beaugrand M, Shen F, Hiriart JB, Sarin SK, Badea R, Jung KS, Marcellin P, Filice C, Mahadeva S, Wong GLH, Crotty P, Masaki K, Bojunga J, Bedossa P, Keim V, Wiegand J (2017) Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 66:1022–1030. https://doi.org/10.1016/j.jhep.2016.12.022

26. VanderWeele TJ (2016) Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health 37:17–32. https://doi.org/10.1146/annurev-publhealth-032315-021402

27. van Buuren S, Groothuis-Oudshoorn K (2011) mice: Multivariate Imputation by Chained Equations in R. J Stat Softw 45:1–67. https://doi.org/10.18637/jss.v045.i03

28. Rubin DB (1987) Multiple Imputation for Nonresponse in Surveys. Wiley, New York. https://doi.org/10.1002/9780470316696

29. Barnard J, Rubin DB (1999) Small-sample degrees of freedom with multiple imputation. Biometrika 86:948–955. https://doi.org/10.1093/biomet/86.4.948

30. Harrell FE Jr (2015) Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis, 2nd edn. Springer, Cham. https://doi.org/10.1007/978-3-319-19425-7

31. Heeringa SG, West BT, Berglund PA (2017) Applied Survey Data Analysis, 2nd edn. Chapman and Hall/CRC, New York. https://doi.org/10.1201/9781315153278

32. R Core Team (2026) R: A Language and Environment for Statistical Computing (Version 4.6.2). R Foundation for Statistical Computing, Vienna, Austria. https://doi.org/10.32614/R.manuals. https://www.R-project.org/. Accessed 31 Oct 2026

33. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative (2007) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 370:1453–1457. https://doi.org/10.1016/S0140-6736(07)61602-X

34. Agency for Toxic Substances and Disease Registry (2012) Toxicological Profile for Cadmium. Agency for Toxic Substances and Disease Registry, Atlanta, GA. https://www.ncbi.nlm.nih.gov/books/NBK158838/. Accessed 31 Oct 2026

35. Burk RF, Hill KE (2015) Regulation of selenium metabolism and transport. Annu Rev Nutr 35:109–134. https://doi.org/10.1146/annurev-nutr-071714-034250

Figure captions

Fig. 1. Participant flow. Derivation of the reference-distribution sample (aged ≥6 years) and of the adult metal and PFAS analysis samples, NHANES August 2021–August 2023. Counts are unweighted.

Fig. 2. Weighted reference distribution of serum butyrylcholinesterase (BChE) by age group and sex, participants aged ≥6 years (n=7,061). Points are survey-weighted geometric means and vertical lines 95% confidence intervals (t distribution, 15 degrees of freedom). (A) Activity (U/mL); (B) protein concentration (ng/mL). These are weighted population distributions, not clinical reference intervals.

Fig. 3. Conceptual basis of the activity-to-concentration comparison. If an exposure reduces the amount of circulating enzyme (A), activity and enzyme concentration fall together and their ratio is unchanged; if an exposure inhibits the enzyme (B), activity falls while concentration is unchanged and the ratio decreases.

Fig. 4. Adjusted differences in serum BChE activity (U/mL) per weighted interquartile-range (IQR) increase in each blood metal (n=4,217) and serum PFAS (n=1,644), US adults aged ≥20 years. Points are estimates and horizontal lines 95% confidence intervals from survey-weighted linear regression (model M2: age, sex, race and Hispanic origin, education, income-to-poverty ratio, smoking, alcohol frequency and body mass index), using the t distribution with 15 degrees of freedom. Estimates per natural-log unit are given in Table 2. PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; Sm-PFOS, branched perfluorooctane sulfonate isomers.

Fig. 5. Percent differences in BChE activity (filled circles), BChE concentration (open circles) and the activity-to-concentration ratio (triangles) per weighted IQR increase in each exposure, in participants with both BChE measurements (model M2; metals n=4,216, PFAS

n=1,643). Horizontal lines are 95% confidence intervals. A ratio estimate near zero indicates that activity and concentration differ in proportion. Estimates per natural-log unit are given in Table 3.

Fig. 6. Restricted cubic spline curves for the difference in BChE activity (U/mL) relative to the weighted median of each exposure (model M2). Shaded bands are pointwise 95% confidence intervals; knots at the weighted 5th, 35th, 65th and 95th percentiles; curves are shown between the 5th and 95th percentiles on a logarithmic exposure axis. Panel titles give p values for the overall association and for non-linearity (F tests with 15 denominator degrees of freedom), unadjusted for multiple testing; adjusted values are in Supplementary Table S4. Exploratory analysis.

Fig. 7. Mediation of the association with BChE activity through hepatic fat (controlled attenuation parameter, CAP) and liver stiffness, for cadmium, PFNA and selenium (model M2). Points are indirect effects on ln(activity) per natural-log unit of exposure, with delta-method 95% confidence intervals; labels give the percent of the total effect mediated and the p value. Estimates are associational, not causal.

Table 1. Characteristics of the primary analysis samples, US adults aged ≥20 years, NHANES August 2021–August 2023

Characteristic	Metals sample (n=4,217)	PFAS sample (n=1,644)
Age, years, median (IQR)	49 (34–63)	50 (34–63)
Income-to-poverty ratio, median (IQR)	3.22 (1.71–5.00)	3.22 (1.76–5.00)
Body mass index, kg/m², median (IQR)	28.4 (24.7–33.4)	28.4 (24.5–33.3)
BChE activity, U/mL, median (IQR)	3.88 (3.33–4.48)	3.83 (3.29–4.43)
BChE concentration, ng/mL, median (IQR)	59.3 (50.4–70.1)	58.6 (49.9–68.8)
Lead, µg/dL, median (IQR)	0.68 (0.44–1.04)	—
Cadmium, µg/L, median (IQR)	0.239 (0.155–0.408)	—
Total mercury, µg/L, median (IQR)	0.54 (0.21–1.31)	—
Selenium, µg/L, median (IQR)	178 (166–194)	—
Manganese, µg/L, median (IQR)	8.84 (7.16–10.98)	—
PFHxS, ng/mL, median (IQR)	—	0.9 (0.5–1.6)
PFNA, ng/mL, median (IQR)	—	0.3 (0.2–0.5)
n-PFOA, ng/mL, median (IQR)	—	1.0 (0.6–1.5)
n-PFOS, ng/mL, median (IQR)	—	1.9 (1.1–3.2)
Sm-PFOS, ng/mL, median (IQR)	—	0.8 (0.4–1.4)
Women, n (%)	2,293 (50.3)	884 (48.8)
Race and Hispanic origin, n (%)		
Non-Hispanic White	2,679 (64.2)	1,068 (64.0)
Mexican American	260 (6.3)	89 (5.5)
Other Hispanic	385 (8.3)	146 (8.5)
Non-Hispanic Black	434 (9.9)	161 (9.9)
Non-Hispanic Asian	188 (5.2)	84 (6.5)
Other or multiracial	271 (6.0)	96 (5.6)
Education, n (%)		
Less than 9th grade	135 (2.5)	47 (2.5)
9th–11th grade	293 (5.8)	118 (5.9)
High school graduate	821 (24.3)	318 (23.5)
Some college	1,305 (30.5)	488 (29.8)
College graduate or above	1,663 (36.9)	673 (38.4)
Smoking, n (%)		
Never	2,460 (61.4)	955 (61.6)
Former	1,133 (23.8)	456 (24.8)
Current	624 (14.8)	233 (13.6)
Past-year alcohol frequency, n (%)		
None in past year	1,000 (20.9)	390 (20.6)
Less than monthly	1,006 (23.9)	377 (23.6)
1–3 times per month	698 (17.6)	274 (17.9)
1–4 times per week	1,120 (29.0)	446 (29.4)
Daily or nearly daily	393 (8.5)	157 (8.5)

Continuous variables are survey-weighted medians (interquartile range); categorical variables are unweighted counts with survey-weighted percentages. The metals sample was weighted with WTPH2YR and the PFAS sample with WTSPF2YR. Exposure rows apply only to the corresponding sample (—, not applicable).

Abbreviations: BChE, butyrylcholinesterase; IQR, interquartile range; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; Sm-PFOS, sum of branched perfluorooctane sulfonate isomers.

Table 2. Adjusted differences in BChE activity (U/mL) associated with blood metals and serum PFAS

Exposure	n	M2 β (95% CI)	p	q	M2 per IQR (95% CI)	MI β (95% CI)	MI n	M3 β (95% CI)	M3 q
Lead	4217	-0.034 (-0.087, 0.018)	0.185	0.309	-0.029 (-0.074, 0.016)	-0.043 (-0.098, 0.012)	5567	-0.027 (-0.081, 0.027)	0.766
Cadmium	4217	-0.126 (-0.204, -0.049)	0.003	0.008	-0.122 (-0.197, -0.048)	-0.119 (-0.171, -0.067)	5567	-0.089 (-0.144, -0.033)	0.020
Total mercury	4217	-0.004 (-0.043, 0.036)	0.848	0.848	-0.007 (-0.079, 0.066)	-0.009 (-0.045, 0.027)	5567	0.003 (-0.038, 0.043)	0.888
Selenium	4217	0.681 (0.377, 0.985)	<0.001	0.001	0.105 (0.058, 0.152)	0.678 (0.329, 1.028)	5567	0.081 (-0.247, 0.410)	0.796
Manganese	4217	-0.057 (-0.171, 0.057)	0.306	0.383	-0.024 (-0.073, 0.025)	-0.005 (-0.089, 0.078)	5567	-0.027 (-0.147, 0.093)	0.796
PFHxS	1644	0.090 (0.010, 0.170)	0.030	0.030	0.104 (0.012, 0.197)	0.080 (0.007, 0.153)	2178	0.044 (-0.032, 0.120)	0.232
PFNA	1644	0.143 (0.050, 0.236)	0.005	0.007	0.131 (0.046, 0.217)	0.134 (0.069, 0.200)	2178	0.095 (-0.000, 0.191)	0.122
n-PFOA	1644	0.115 (0.039, 0.191)	0.006	0.007	0.105 (0.036, 0.175)	0.100 (0.047, 0.153)	2178	0.066 (-0.010, 0.142)	0.122
n-PFOS	1644	0.122 (0.041, 0.204)	0.006	0.007	0.131 (0.044, 0.218)	0.097 (0.031, 0.162)	2178	0.075 (-0.001, 0.150)	0.122
Sm-PFOS	1644	0.109 (0.047, 0.171)	0.002	0.007	0.136 (0.059, 0.214)	0.090 (0.037, 0.144)	2178	0.051 (-0.011, 0.113)	0.122

β, difference in activity per natural-log unit increase in exposure; per IQR, β multiplied by the survey-weighted interquartile range of the log exposure. M2 (primary): age (natural spline), sex, race and Hispanic origin, education, income-to-poverty ratio, smoking, alcohol frequency and body mass index. MI: M2 after multiple imputation of missing covariates (20 imputations; Barnard–Rubin degrees of freedom). M3: M2 plus sedentary time, prescription medication use, liver enzymes, albumin, triglycerides, eGFR, HbA1c and C-reactive protein, in the M3 complete-case sample. Confidence intervals and p values from the t distribution with 15 degrees of freedom; q, Benjamini–Hochberg false-discovery-rate adjusted p value within the five metals or the five PFAS.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; IQR, interquartile range; MI, multiple imputation.

Table 3. Percent differences in BChE activity, BChE concentration and the activity-to-concentration ratio per natural-log unit increase in exposure

Exposure	n	Activity % (95% CI)	q	Concentration % (95% CI)	q	Activity/concentration % (95% CI)	q
Lead	4216	-0.64 (-2.04, 0.78)	0.439	-1.27 (-2.50, -0.01)	0.080	0.64 (-0.39, 1.67)	0.637
Cadmium	4216	-2.97 (-4.80, -1.10)	0.011	-2.52 (-4.62, -0.38)	0.061	-0.46 (-1.28, 0.37)	0.637
Total mercury	4216	0.00 (-0.95, 0.97)	0.992	-0.16 (-1.02, 0.70)	0.692	0.17 (-0.35, 0.69)	0.833
Selenium	4216	21.24 (11.19, 32.20)	0.001	21.76 (8.84, 36.20)	0.010	-0.42 (-5.72, 5.17)	0.871
Manganese	4216	-1.65 (-4.86, 1.67)	0.439	-1.79 (-5.61, 2.20)	0.436	0.14 (-1.69, 2.01)	0.871
PFHxS	1643	2.31 (0.15, 4.51)	0.037	3.12 (1.08, 5.21)	0.008	-0.79 (-1.85, 0.28)	0.529
PFNA	1643	3.68 (1.34, 6.07)	0.010	2.99 (0.38, 5.67)	0.027	0.66 (-0.70, 2.05)	0.529
n-PFOA	1643	2.91 (0.92, 4.94)	0.010	3.44 (1.28, 5.65)	0.008	-0.51 (-2.51, 1.53)	0.735
n-PFOS	1643	3.15 (0.95, 5.39)	0.010	3.30 (1.07, 5.57)	0.008	-0.15 (-1.05, 0.76)	0.735
Sm-PFOS	1643	2.77 (1.10, 4.46)	0.010	3.36 (1.57, 5.19)	0.005	-0.58 (-1.51, 0.37)	0.529

Model M2 in participants with both BChE measurements. The ratio coefficient equals the activity coefficient minus the concentration coefficient on the log scale; a ratio estimate near zero indicates that activity and concentration differ in proportion. q, Benjamini–Hochberg adjusted within each outcome and exposure family.

Abbreviations: BChE, butyrylcholinesterase; CI, confidence interval.

Table 4. Weighted reference distribution of serum BChE activity and concentration, participants aged ≥6 years, NHANES August 2021–August 2023

Stratum	Outcome (unit)	n	GM (95% CI)	P5	P25	P50	P75	P95
Overall (≥6 y)	Activity (U/mL)	7061	3.85 (3.74, 3.95)	2.61	3.34	3.88	4.47	5.53
Overall (≥6 y)	Concentration (ng/mL)	7060	58.94 (57.69, 60.21)	38.7	50.5	59.6	69.7	86.5
Age 6-11 y	Activity (U/mL)	537	4.23 (4.08, 4.38)	3.15	3.73	4.20	4.73	5.81
Age 6-11 y	Concentration (ng/mL)	537	65.58 (63.80, 67.41)	46.8	58.3	65.3	73.5	91.6
Age 12-19 y	Activity (U/mL)	919	3.78 (3.64, 3.92)	2.67	3.31	3.75	4.32	5.22
Age 12-19 y	Concentration (ng/mL)	919	57.47 (55.67, 59.32)	39.5	49.3	57.9	66.6	81.0
Age 20-39 y	Activity (U/mL)	1417	3.78 (3.66, 3.91)	2.53	3.24	3.82	4.44	5.45
Age 20-39 y	Concentration (ng/mL)	1417	57.79 (56.12, 59.51)	37.4	49.2	58.0	69.4	87.2
Age 40-59 y	Activity (U/mL)	1626	3.92 (3.83, 4.02)	2.65	3.40	3.96	4.60	5.58
Age 40-59 y	Concentration (ng/mL)	1626	60.01 (58.77, 61.29)	40.0	51.5	60.6	71.2	88.2
Age 60-79 y	Activity (U/mL)	2253	3.82 (3.73, 3.91)	2.57	3.35	3.88	4.42	5.41
Age 60-79 y	Concentration (ng/mL)	2252	58.68 (57.58, 59.79)	37.9	50.9	59.6	69.0	84.4
Age >=80 y	Activity (U/mL)	309	3.40 (3.29, 3.51)	2.43	2.98	3.40	3.89	4.84
Age >=80 y	Concentration (ng/mL)	309	53.05 (50.59, 55.63)	36.4	44.7	52.9	62.4	78.6
Male	Activity (U/mL)	3252	3.99 (3.89, 4.10)	2.75	3.51	4.03	4.62	5.63
Male	Concentration (ng/mL)	3251	61.43 (60.15, 62.73)	41.2	52.8	62.1	72.0	89.3
Female	Activity (U/mL)	3809	3.71 (3.60, 3.82)	2.53	3.21	3.74	4.33	5.38
Female	Concentration (ng/mL)	3809	56.63 (55.32, 57.96)	37.6	48.7	57.2	67.1	83.2
Non-Hispanic White	Activity (U/mL)	3918	3.85 (3.74, 3.97)	2.62	3.35	3.88	4.47	5.52
Non-Hispanic White	Concentration (ng/mL)	3917	58.99 (57.49, 60.54)	38.9	50.8	59.5	69.5	87.4
Mexican American	Activity (U/mL)	599	4.08 (3.98,	2.86	3.56	4.16	4.69	5.60

			4.18)					
Mexican American	Concentration (ng/mL)	599	62.39 (60.86, 63.96)	43.5	53.8	63.8	73.1	87.1
Other Hispanic	Activity (U/mL)	810	3.89 (3.69, 4.09)	2.64	3.35	3.95	4.55	5.64
Other Hispanic	Concentration (ng/mL)	810	59.88 (57.14, 62.75)	39.0	51.8	60.5	70.6	88.0
Non-Hispanic Black	Activity (U/mL)	854	3.59 (3.44, 3.75)	2.38	3.09	3.62	4.20	5.34
Non-Hispanic Black	Concentration (ng/mL)	854	54.88 (53.24, 56.57)	34.8	46.0	55.1	65.9	81.9
Non-Hispanic Asian	Activity (U/mL)	396	3.98 (3.83, 4.12)	2.88	3.56	3.94	4.59	5.52
Non-Hispanic Asian	Concentration (ng/mL)	396	60.63 (58.78, 62.54)	41.7	52.9	62.0	69.8	84.8
Other/multiracial	Activity (U/mL)	484	3.77 (3.69, 3.84)	2.58	3.20	3.79	4.36	5.47
Other/multiracial	Concentration (ng/mL)	484	58.23 (56.63, 59.87)	38.4	49.1	59.5	70.5	82.6

Survey-weighted geometric means (GM) with 95% confidence intervals (t distribution, 15 degrees of freedom) and weighted percentiles (P5–P95), using the phlebotomy weight WTPH2YR. These are weighted population distributions, not clinical reference intervals for a healthy population. Age is top-coded at 80 years.

Abbreviations: BChE, butyrylcholinesterase; CI, confidence interval; GM, geometric mean.

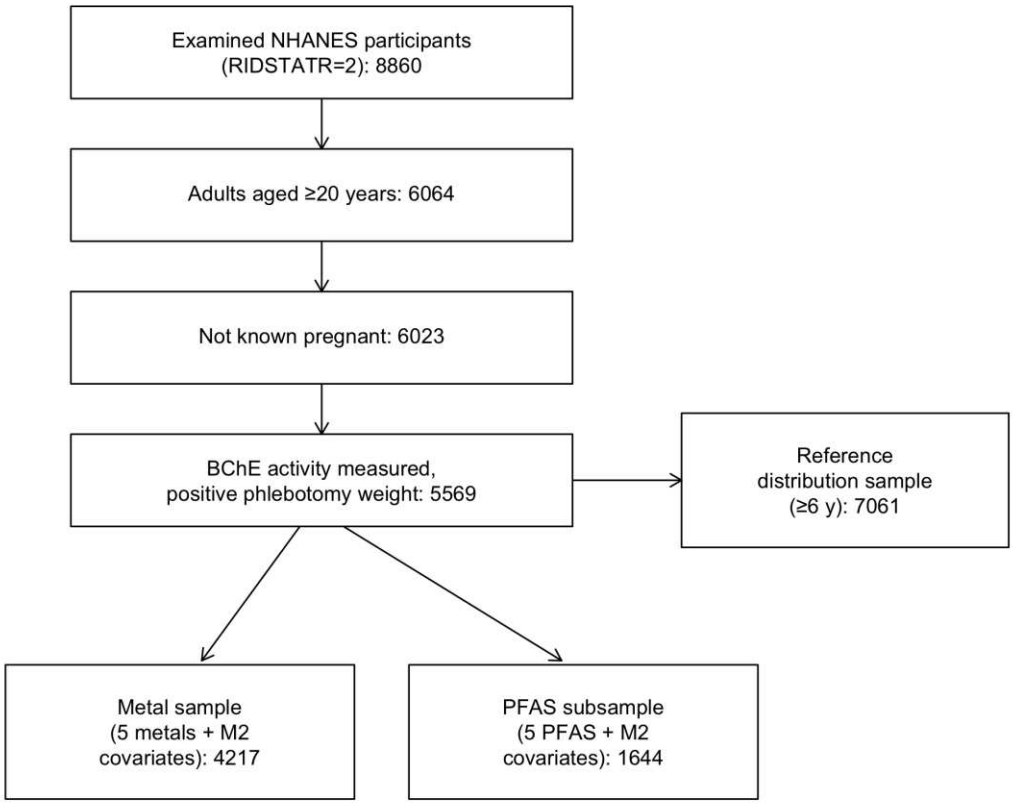


Fig. 1. Participant flow. Derivation of the reference-distribution sample (aged ≥6 years) and of the adult metal and PFAS analysis samples, NHANES August 2021–August 2023. Counts are unweighted.

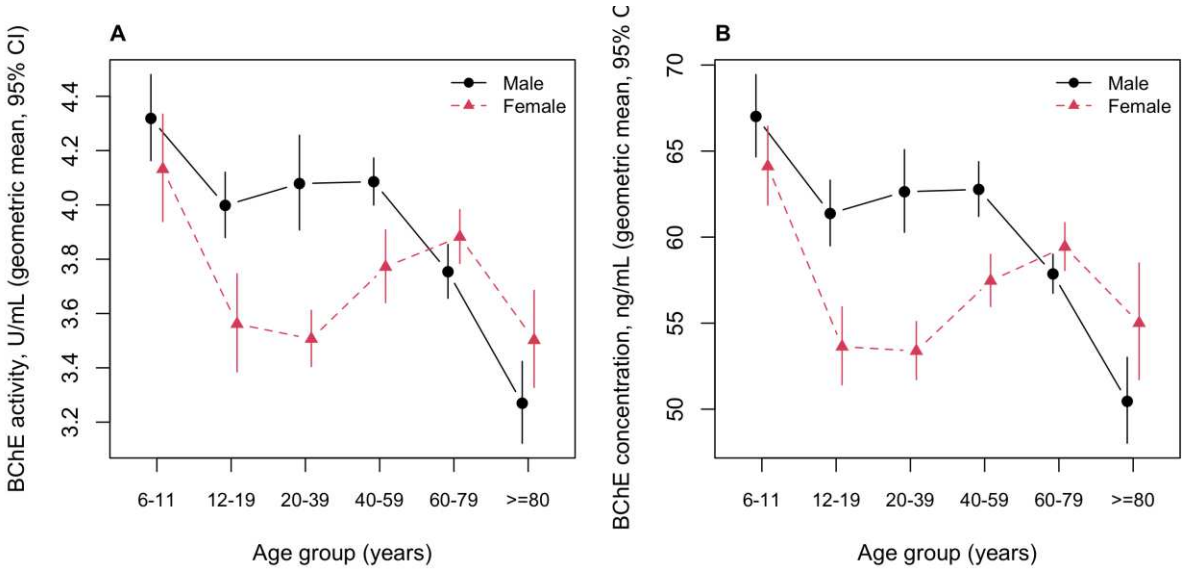


Fig. 2. Weighted reference distribution of serum butyrylcholinesterase (BChE) by age group and sex, participants aged ≥6 years (n=7,061). Points are survey-weighted geometric means and vertical lines 95% confidence intervals (t distribution, 15 degrees of freedom). (A) Activity (U/mL); (B) protein concentration (ng/mL). These are weighted population distributions, not clinical reference intervals.

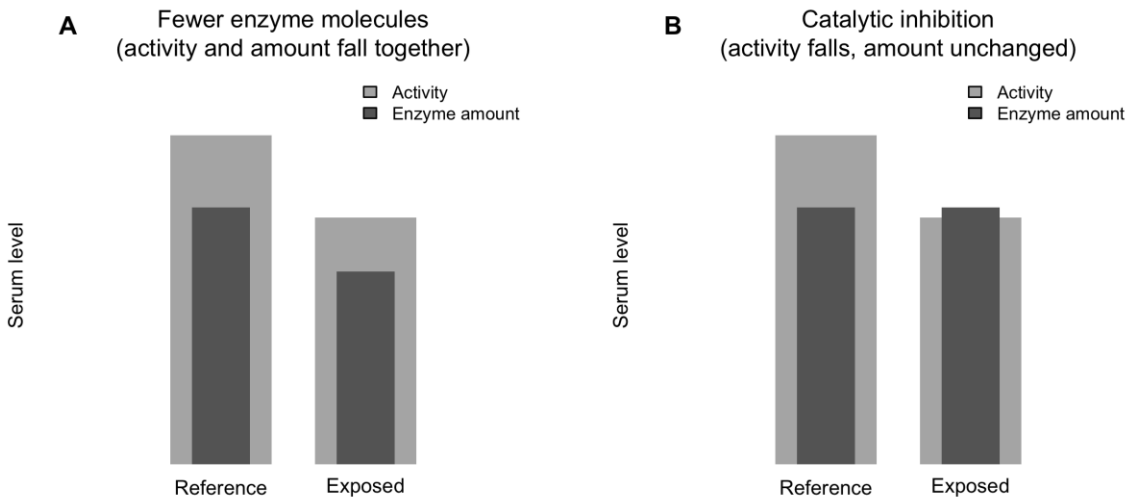


Fig. 3. Conceptual basis of the activity-to-concentration comparison. If an exposure reduces the amount of circulating enzyme (A), activity and enzyme concentration fall together and their ratio is unchanged; if an exposure inhibits the enzyme (B), activity falls while concentration is unchanged and the ratio decreases.

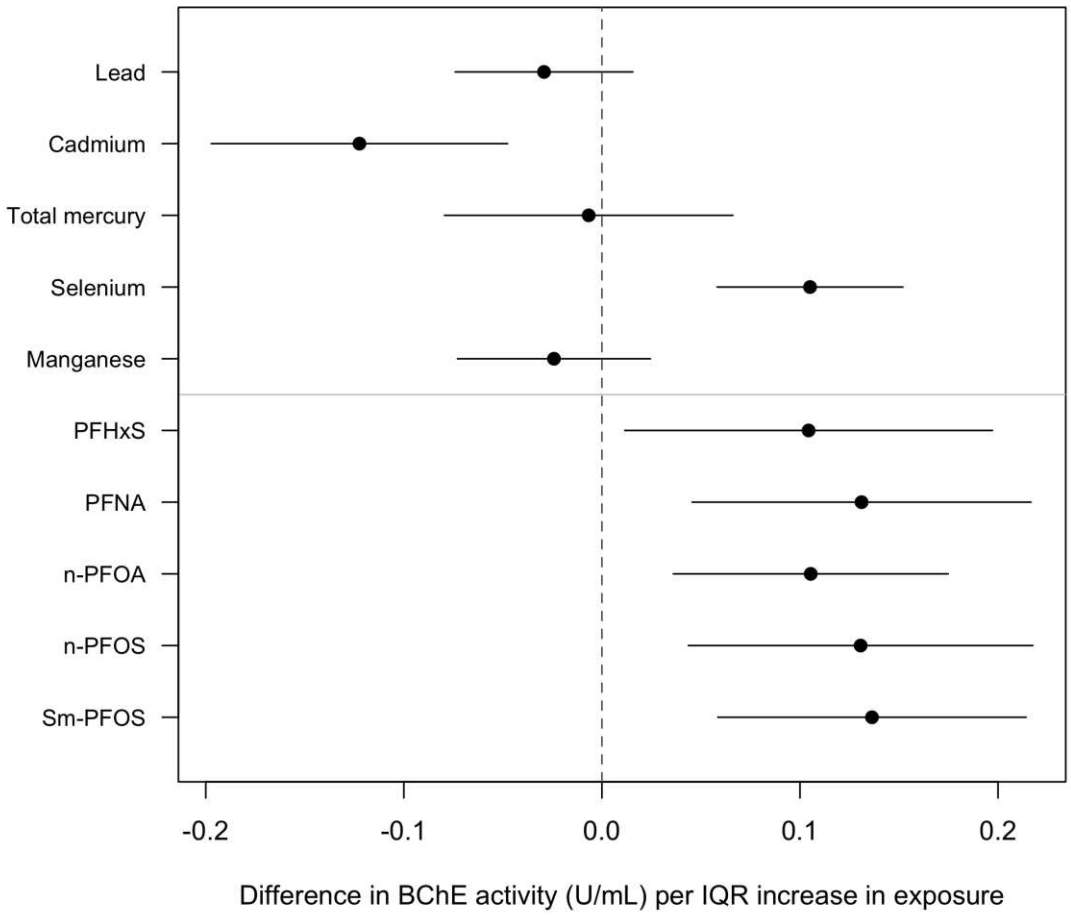


Fig. 4. Adjusted differences in serum BChE activity (U/mL) per weighted interquartile-range (IQR) increase in each blood metal (n=4,217) and serum PFAS (n=1,644), US adults aged ≥20 years. Points are estimates and horizontal lines 95% confidence intervals from survey-weighted linear regression (model M2: age, sex, race and Hispanic origin, education, income-to-poverty ratio, smoking, alcohol frequency and body mass index), using the t distribution with 15 degrees of freedom. Estimates per natural-log unit are given in Table 2. PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; Sm-PFOS, branched perfluorooctane sulfonate isomers.

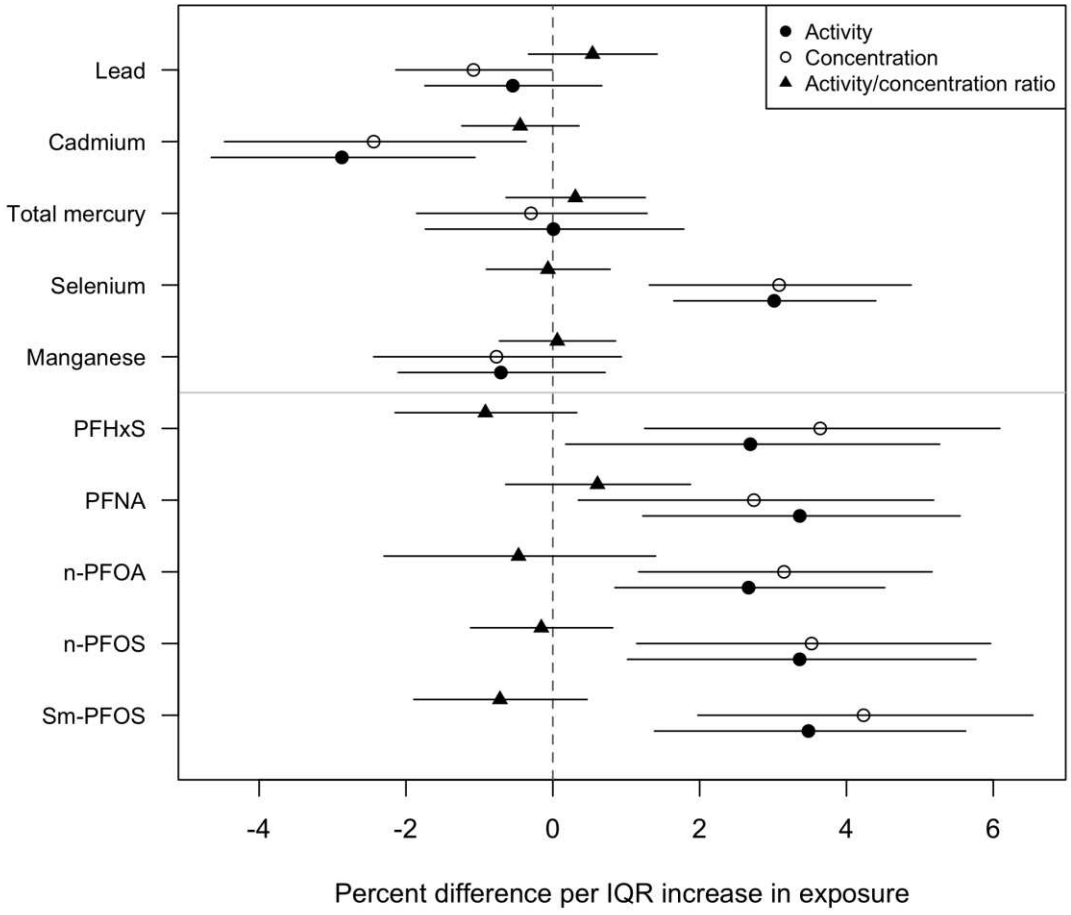


Fig. 5. Percent differences in BChE activity (filled circles), BChE concentration (open circles) and the activity-to-concentration ratio (triangles) per weighted IQR increase in each exposure, in participants with both BChE measurements (model M2; metals n=4,216, PFAS n=1,643). Horizontal lines are 95% confidence intervals. A ratio estimate near zero indicates that activity and concentration differ in proportion. Estimates per natural-log unit are given in Table 3.

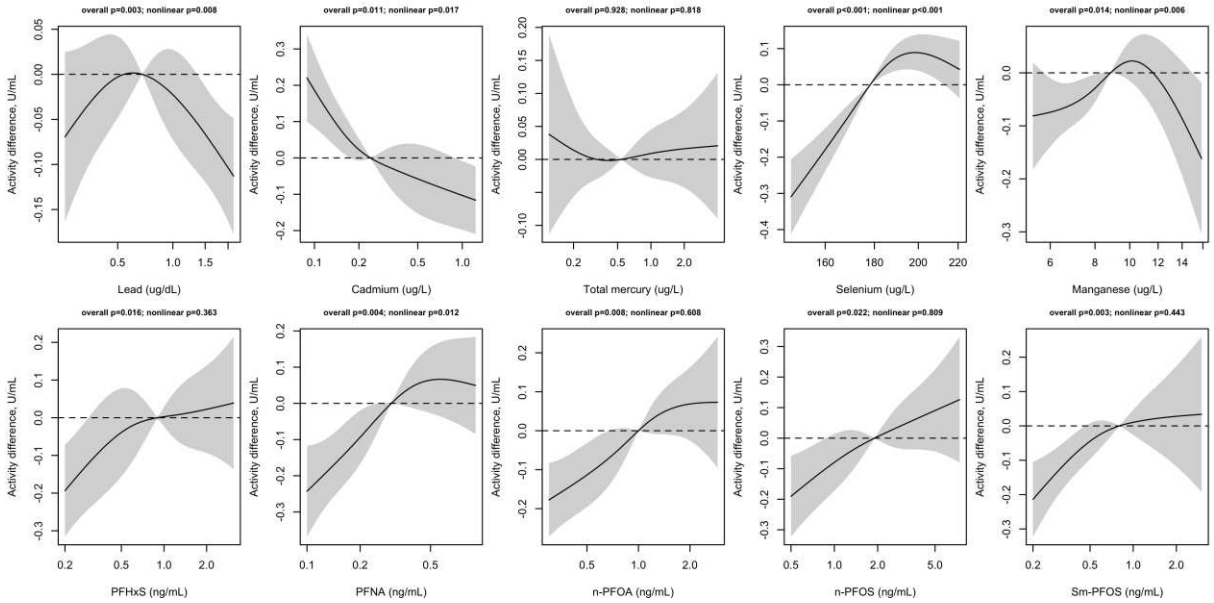


Fig. 6. Restricted cubic spline curves for the difference in BChE activity (U/mL) relative to the weighted median of each exposure (model M2). Shaded bands are pointwise 95% confidence intervals; knots at the weighted 5th, 35th, 65th and 95th percentiles; curves are shown between the 5th and 95th percentiles on a logarithmic exposure axis. Panel titles give p values for the overall association and for non-linearity (F tests with 15 denominator degrees of freedom), unadjusted for multiple testing; adjusted values are in Supplementary Table S4. Exploratory analysis.

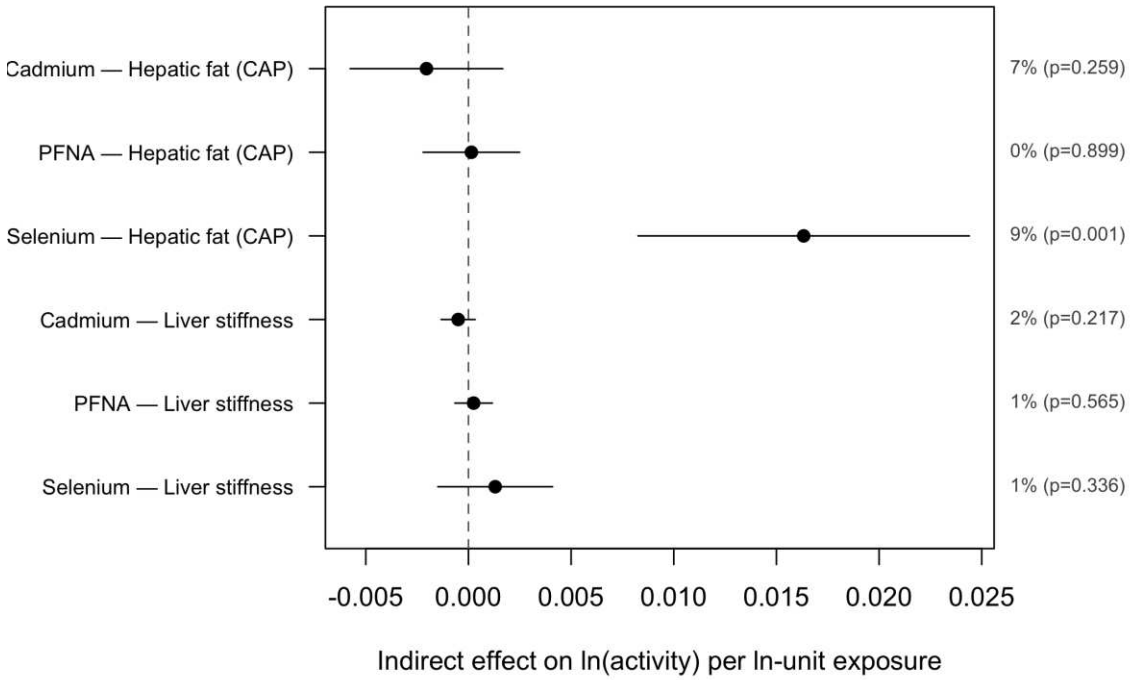


Fig. 7. Mediation of the association with BChE activity through hepatic fat (controlled attenuation parameter, CAP) and liver stiffness, for cadmium, PFNA and selenium (model M2). Points are indirect effects on ln(activity) per natural-log unit of exposure, with delta-method 95% confidence intervals; labels give the percent of the total effect mediated and the p value. Estimates are associational, not causal.

Supplementary Files

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

- BTERsupplementarytables.docx