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Isoprene Exposure in the United States based on Urinary IPM3: NHANES 2015-2016

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Abstract

Isoprene is the 2-methyl analog of 1,3-butadiene and is a possible human carcinogen (IARC Group 2B). We assessed isoprene exposure in the general US population by measuring its urinary metabolite, N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPM3) in participants (≥3 years old) from the 2015-2016 National Health and Nutrition Examination Survey. Spot urine samples were analyzed for IPM3 using ultra-high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Exclusive tobacco smokers were distinguished from non-users using a combination of self-reporting and serum cotinine data. IPM3 was detected in 80.2% of samples. The median IPM3 level was higher for exclusive cigarette smokers (39.8 μg/g creatinine) than for non-users (3.05 μg/g creatinine). Sample weighted regression analysis—controlling for creatinine, sex, age, race, body mass index, and diet—showed that IPM3 was positively and significantly associated with serum cotinine. Smoking 1-10 cigarettes per day (CPD, 0.5 pack) was significantly associated with an IPM3 increase of 596% ($p < .0001$), and smoking >20 CPD (>1 pack) was significantly associated with an IPM3 increase of 1,640% ($p < .0001$), controlling for confounding variables. Drinking beer/ale at median and 90th percentile levels (compared to zero consumption) was associated ($p < 0.05$) with 0% and 2.9%, respectively, increase in IPM3 in non-users. We conclude that tobacco smoke is a major source of isoprene exposure in the US population. This study provides important public health biomonitoring data on isoprene exposure in the general US population.

Keywords

Isoprene; IPM3; tobacco smoke exposure; NHANES; biomonitoring; VOC metabolites

Introduction

Isoprene is the 2-methyl analog of 1,3-butadiene and is a colorless, volatile liquid at room temperature.[1] Isoprene is classified by the International Agency for Research on Cancer as a

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Supporting Information

USDA Food Codes and Logic for Apportioning Dietary Intake (Table S1), Summary of Sample-weighted Medians, Geometric Means, and 95th Percentiles for Creatinine-adjusted Values (μg/g creatinine) and Non Creatinine-adjusted Values (ng/mL) for Urinary IPM3 (Table S2), Overview of IPM3 Full model vs. Reduced model (Table S3).

Group 2B possible human carcinogen.[2] Tobacco smoke contains isoprene, and it is the primary source of isoprene in indoor environments.[1, 3] Mainstream smoke from 24 US commercial cigarettes had an isoprene yield between 100-587 μg/cigarette.[4] Isoprene also occurs naturally in the environment from plant and tree emissions.[5, 6] Isoprene is manufactured to make polymers and copolymers, including cis-polyisoprene for vehicle tires, styrene-isoprene-styrene block polymers, and butyl rubber.[2] Additionally, isoprene is emitted from automobile exhaust, gasoline, biomass combustion and wood pulping, and it is the byproduct of ethylene production by naphtha cracking.[2] Isoprene comprises 50% of the total non-methane hydrocarbon emissions from the biosphere.[7] Global isoprene emissions range from 440-660 teragrams of carbon (Tg C), contributing one third to the total volatile organic compound (VOC) emissions.[8]

In humans, isoprene is produced endogenously at a rate of 0.15 μmol/kg of body weight per hour.[9] The mean endogenous blood concentration of isoprene was 2.52 μg/L, but can range from 1.0 to 4.8 μg/L.[10] According to one study, about 90% of endogenous isoprene is metabolized and 10% is exhaled unchanged.[11] Isoprene comprises between 30 - 70% of total exhaled hydrocarbons or 2-4 mg/24 hrs in human breath, making it the major endogenous hydrocarbon.[12] Endogenous isoprene production has been linked to cholesterol biosynthesis.[13, 14] Studies on mice and rats suggest that isoprene is metabolized by the liver cytochrome P450-dependent monooxygenase to form monoepoxide and diepoxide intermediates.[15, 16] A study reported that while isoprene without metabolic activation did not damage cells, isoprene's monoepoxide intermediate EPOX I caused a time- and dose-dependent increase in DNA damage to peripheral blood mononuclear cells and human leukemia cells in vitro.[17] Another study showed that increasing employment durations in isoprene rubber production, which would likely expose workers to isoprene, increased subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract.[2] Numerous studies on rats and mice have shown that inhaling isoprene caused tumors and cancer, including in the lung, liver, and Harderian gland adenomas.[1] However, available data are insufficient to prove carcinogenicity in humans, and thus isoprene is classified as a Group 2B potential human carcinogen.[2]

Despite the potential health impact of isoprene, no studies have been published characterizing isoprene exposure biomarkers in humans on a large scale. Our laboratory was the first to publish an assay to examine isoprene exposure by quantitating the urinary biomarkers N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPM3) and a mixture of N-acetyl-S-(1-hydroxymethyl-2-methyl-2-propen-1-yl)-L-cysteine and N-acetyl-S-(2-hydroxy-3methyl-3-buten-1-yl)-L-cysteine (IPM1).[18] In this report, we focus on IPM3 because of its selectivity and high detection rate. We assess isoprene exposure in participants of the 2015-2016 National Health and Nutrition Examination Survey (NHANES) to obtain population-based biomonitoring data of the United States civilian, non-institutionalized population. This paper characterizes U.S. population exposure to an important air toxicant, and thus adds to a growing literature on biomarkers of exposure to environmental toxicants.[19-21] We use multiple linear regression models to examine the impact of tobacco smoke, select demographic variables, and diet on isoprene exposure.

Study Design

NHANES is a population-based survey that assesses the health and nutritional status of the civilian, noninstitutionalized US population based on data collected from questionnaires, physical examinations and biological samples. This cross-sectional study is facilitated by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). The CDC/NCHS Research Ethics Review Board reviewed and approved the study, and all adult participants gave informed written consent to participate in the survey while parents or guardians provided written assent for participants younger than 18 years. Following interviews in the homes, physical exams were performed and biological specimens were collected in mobile examination centers (MEC). A total of 9,971 participants age 3 years and older provided spot urine samples for NHANES cycle 2015-2016, and we quantified IPM3 in a one-third subset.

Study participants were identified as exclusive daily users of cigarette products (termed "exclusive smokers" in this report) if they responded "yes" to NHANES question SMDANY (tobacco use within 5 days prior to NHANES physical examination), "yes" to SMQ690a (cigarette use), "no" to SMQ690b – SMQ690J (use of pipes, cigars, chewing tobacco, snuff, patch/gum, hookah/water pipes, E-cigarettes, snus, and dissolvables), according to NHANES questionnaire data on recent tobacco use (NHANES dataset: SMQRTU_I) , and had serum cotinine > 10 ng/mL. Participants were identified as non-users if they answered "no" to SMDANY or had serum cotinine ≤ 10 ng/mL. The serum cotinine threshold of > 10 ng/mL has been identified as consistent with active use of combusted cigarette product,[22] and was used to stratify self-identified exclusive smokers and non-users in statistical analyses reported here. Laboratory data for 3,015 participants were reported for IPM3 (NHANES dataset: UVOC_I). Participants were excluded from analysis if they did not meet the criteria for either exclusive smoker or non-user (N=401), for missing serum cotinine data (N=109), for missing creatinine data (N=3) or for missing data for other variables used in the regression model (N=231). This attrition left 2,271 study participants eligible for statistical analysis. The work flow of the study design is shown in schematic 1.

Laboratory Method

Spot urine samples from NHANES 2015-2016 were analyzed for urinary IPM3 using ultra-high-performance liquid chromatography (UPLC; I-Classic Acquity, Waters Inc., Milford, MA) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS; Sciex 5500 Triple quad, Sciex, Framingham, MA).[23] Chromatographic separation was achieved using an Acquity UPLC® HSS T3, 100 Å, 1.8 μm, 2.1mm × 150mm column (Waters Inc., Milford, MA) with a Waters HSS T3 VanGuard pre-column (Waters Corporation, Milford, MA). The solvents consisted of a gradient of 15 mM ammonium acetate, pH 6.8 (mobile phase *A*) and acetonitrile (mobile phase *B*). Column and sample manager temperatures were set to 40 °C and 25 °C, respectively. The injection volume was 2 μL using full loop injection mode. The mass spectrometer was operated in negative ion ESI scheduled multiple reaction monitoring mode. The optimized ion source parameters were as follows: ESI voltage, −4000 V (negative mode); CAD gas, 7 psi; curtain gas flow, 45 psi; nebulizing gas (GS1) flow, 55 psi; heating gas (GS2) flow, 65 psi; and heater temperature, 650 °C. UPLC-MS/MS data was

acquired using Analyst software (Sciex, Framingham, MA), and the data was processed in MultiQuant 3.0.3 (Sciex, Framingham, MA). Urine specimens were prepared on a robotic liquid handler by diluting urine 1:10 in 15 mM ammonium acetate (pH 6.8) with a deuterated internal standard, IPM3-d_3. Sample concentrations were determined based on their relative response ratio (ratio of native analyte to stable isotope-labeled internal standard) against a calibration curve with known standard concentrations. IPM3 was monitored for transitions *m/z* 246→117 (quantitation ion), *m/z* 246→87 (confirmation ion), and *m/z* 249→117 (internal standard). The limit of detection (LOD) was 1.20 ng/mL.

Statistical Analysis

NHANES recruited participants through a multistage, probability sampling design. Accounting for the design (i.e., applying survey sample weights and using Taylor series linearization for variance estimation that respected strata and primary sampling units), we produced unbiased, nationally representative statistics with appropriate variance estimates. The SURVEYREG and SURVEYMEANS subroutines of SAS 9.4 were used to calculate estimates. Sample-weighted multiple linear regression models stratified by cigarette use status (exclusive smokers vs. non-users) were fit to data from NHANES cycle 2015-2016, where the dependent variable was urinary IPM3 concentration (ng/mL). Since the distribution of urinary IPM3 measurements was strongly right-skewed, which could have adversely affected hypothesis testing, we used natural log transformed IPM3 data for regression analysis. We report coefficients from these models along with their 95% confidence intervals (95% CI) and *p*-values. Stastical significance was set to $\alpha = 0.05$. Moreover, the exponentiated coefficient represent the proportional change of biomarker concentration and can be expressed mathematically as:

$$\%\ change\ of\ urinary\ IPM3\ concentration\ (\Delta IPM3) = \left(e^{coefficient} - 1\right) \times 100 \quad (1)$$

To interpret character predictors in the model, if the %(ΔIPM3) is > 1, it is indicative of the predictive percentage increase in IPM3. If %(ΔIPM3) is < 1, the predictive percentage is decreased in IPM3 concentration.

For numeric predictors, the %(ΔIPM3), which corresponds to increase in the predictor from zero to a percentile (median or 90th percentile), is calculated by exponentiating the product of the median percentile value and coefficient, treating the estimated percentile value as fixed, known values, not as estimates with quantified variability. Then, equation 1 can be expressed as:

$$\%\ (\Delta IPM3) = \left(e^{estimated\ percentile\ value \times coefficient} - 1\right) \times 100 \quad (2)$$

Sample-weighted regression models were stratified by cigarette use, and the following self-reported variables were included as predictors: urinary creatinine (g/L, laboratory data), diet, sex, age and race/ethnicity. Creatinine, a waste product of creatine and creatine phosphate (produced from muscle metabolism), is excreted in urine at a relatively constant rate.[24] Age was categorized into the following ranges and is consistent with the previous studies: 3 – 5,

6 –11, 12 – 19, 20 – 39, 40 – 59, and ≥60 years.[19, 25, 26] An additional predictor, body mass index (BMI), was calculated from measurements taken at the NHANES physical examination. Standard definitions for underweight (BMI < 18.5 kg/m^2), healthy weight (18.5 ≤ BMI < 25), and overweight/obese (BMI ≥ 25) apply to adults ≥ 20 years. Participants younger than 20 years were classified based on their BMI percentile for their sex and age: below the 5th percentile (underweight), between the 5th and 85th percentile (healthy weight), and above the 85th percentile (overweight/obese).

Food consumption information was collected by trained interviewers using structured questionnaires by NHANES. The intensive elicitation techniques were used to translate a participant's recollection of the type and amount of food consumed to a standardized numerical coding system and food mass. Dietary exposure was investigated by assessing the mass participants consumed within each US Department of Agriculture (USDA) food group for the 24-hour period (midnight to midnight) preceding the day of the in-person dietary recall interview and urine collection. The interviews were conducted in the MEC. Data for the 24-hour recall period are contained in the publicly available NHANES Individual Foods – First Day file (NHANES dataset: DR1IFF_I). This file lists each food, water, or beverage consumed by the participant, including the mass reported consumed and eight-digit USDA food code. Standardized hierarchical food groups can be identified from the USDA code, where the first digit represents one of nine major food groups, and each subsequent digit represents subgroups of increasing specificity.[27] The mass consumed in each food group was summed so that each participant was represented by a single record describing their dietary intake for the previous 24 hours. Each participant’s dietary intake was first apportioned over nine food groups: milk products; meat and poultry; eggs; legumes, nuts and seeds; grain products; fruits; vegetables; fats, oils and salad dressings; and sugars, sweets and beverages. In addition, we distinguished two food subgroups that possibly contain isoprene: (1) coffee and (2) beer and ales.[1] To avoid double counting, the mass consumed in each subgroup was subtracted from the mass consumed in its respective food group. Supplemental Table S1 describes the USDA food codes and logic for apportioning dietary intake.

Cotinine is a highly specific metabolite of nicotine—the primary addictive chemical in tobacco. Serum cotinine was a continuous predictor representing tobacco smoke exposure for both exclusive smokers and non-users. Among non-users, tobacco smoke exposure is attributable to inhalation of secondhand tobacco smoke (SHS), which can be quantified with serum cotinine. In addition, to provide an association between IPM3 and frequency of cigarette smoking, we ran an unstratified, sample-weighted regression model in which exposure among exclusive smokers was represented by the self-reported average number of cigarettes smoked per day (CPD) over the five days preceding the NHANES physical exam. This CPD regression model comprised the same predictors as the stratified models, except that tobacco smoke exposure was classified in the following mutually exclusive categories: ≤ 0.05 ng/mL serum cotinine and 0 CPD (unexposed to tobacco smoke), > 0.05 – ≤ 10 ng/mL serum cotinine and 0 CPD (presumptively exposed to second-hand tobacco smoke), > 10 ng/mL serum cotinine and 1 – 10 CPD (0.5 pack), > 10 ng/mL serum cotinine and 11 – 20 CPD (1 pack), and > 10 ng/mL serum cotinine and > 20 CPD (> 1 pack). The reference category was unexposed participants and was defined at ≤ 0.05 ng/mL serum cotinine. The analytic dataset for the CPD model comprised the same participants as the stratified models.

Results

Urinary IPM3 was detected in 80.2% of samples analyzed in NHANES 2015-2016. Sample-weighted demographic distributions of IPM3 are shown in Table 1. Sample-weighted summary statistics for IPM3 categorized by smoking status are presented in Table 2, categorized by sex, age, race/ethnicity and BMI. The IPM3 geometric means were higher for exclusive smokers (28.6 μg/g creatinine) than for non-users (3.23 μg/g creatinine) ($p < .0001$). This difference is depicted in a histogram of the percent distribution of natural logarithm creatinine-adjusted IPM3 concentrations of exclusive smokers (N=252) and non-users (N=2,019, Figure 1). The US population sample-weighted medians, geometric means, and 95th percentiles for IPM3 are shown in supplemental Table S2 using both μg/g creatinine and ng/mL units.

Sample-weighted multiple linear regression analyses for urinary IPM3 are shown for exclusive smokers in Table 3 and non-users in Table 4. The regression models include urinary creatinine, serum cotinine, sex, age, race/ethnicity, BMI and diet. Among exclusive smokers, serum cotinine positively predicted urinary IPM3. The median serum cotinine for exclusive smokers (192 ng/mL) predicted higher urinary IPM3 by 216% compared with participants with no smoke exposure ($p<0.0001$), controlling for other variables. In non-users, serum cotinine did not significantly affect IPM3. Sample-weighted medians and selected percentiles are shown in Table 5 to aid interpretation of the percentage of IPM3 changes for all numeric predictors.

Sample-weighted geometric means of urinary IPM3 for self-reported CPD are shown in Figure 2, adjusted for urinary creatinine, sex, age, race/ethnicity, BMI and diet. In the model, IPM3 concentrations increase with respect to increasing cigarettes smoked per day. Table 6 shows the sample-weighted multiple linear regression model with CPD instead of serum cotinine included in the model. Among the 2,271 participants, 7.92% smoked 1 – 10 CPD (0.5 pack), 2.86% smoked 11 – 20 CPD (1 pack), and 0.31% smoked > 20 CPD (> 1 pack). All participants who smoked cigarettes had significantly higher IPM3 levels ($p < 0.0001$) than unexposed participants (serum cotinine ≤ 0.05 ng/mL). Smoking 1-10 CPD (0.5 pack) increased IPM3 by 596% ($p <.0001$); smoking 11-20 CPD (1 pack) increased IPM3 by 1350% ($p <.0001$); and smoking > 20 CPD (> 1 packs) increased IPM3 by 1640% ($p <.0001$), compared with the reference group (serum cotinine ≤ 0.05 ng/mL) and controlling for confounders.

Demographic variables were evaluated in Tables 3 and 4. Females had higher urinary IPM3 levels than males, but this finding was statistically significant only for exclusive smokers. Using participants age 20-39 years as the reference, child non-users (age 3-5 and 6-11 years) and older adult non-users (age ≥ 60 years) had modestly, but statistically significantly higher IPM3 concentrations. Compared with non-Hispanic whites, non-Hispanic blacks had lower IPM3 among both exclusive smokers and non-users, but the p-value was not significant. BMI did not significantly affect IPM3.

Diet is also examined in Tables 3 and 4. Among both exclusive smokers and non-users, coffee consumption was a positive predictor of IPM3, but the effect was not statistically

significant. Beer/ale consumption was associated with increased IPM3 in non-users (*p*-value=0.048; the 90th percentile consumption predicted a 2.9% increase in urinary IPM3. In other words, after controlling for other variables, non-tobacco users who consumed 0.319 kg, equivalent to 12 oz, beer/ale daily (Table 5, 90th percentile) had a 2.9% increase in urinary IPM3 concentration compared to those who did not consume beer/ale. No other food group had a statistically significant association with urinary IPM3.

Additionally, we evaluated the correlation of IPM3 with its structural homologue, N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHB3, a urinary metabolite of 1,3-butadiene). Isoprene and 1,3-butadiene are formed during cigarette smoking.[4, 28] We evaluated the correlation of the urinary metabolites of these two smoke exposure biomarkers in 3,012 participants The number of participants for the correlation analysis was determined based on the available NHANES data for both IPM3 and MHB3. As expected, these metabolites show a strong correlation (coefficient = 0.80 that is shown in a weighted scatterplot diagram (Figure 3) of IPM3 (μg/g creatinine) versus MHB3 (μg/g creatinine) ($p < .0001$).

Discussion

This is the first large-scale, US population-representative study that evaluates isoprene exposure by assessing its urinary metabolite, IPM3. Our regression models show that cigarette smoke was a major source of isoprene exposure in the US population in NHANES 2015-2016 (Partial $R^2 = 0.33$, TableS3). The median IPM3 concentration for exclusive smokers (39.8 μg/g creatinine) was approximately thirteen times that of non-users (3.05 μg/g creatinine). The detection rate and median IPM3 concentrations were similar to a previously published small study: 82% detection rate and a median of 36.2 ng/mL for exclusive smokers and 2.31 ng/mL for non-users.[18] The high detection rate of IPM3 is likely the result of both endogenous isoprene formation and exogenous sources like tobacco smoke, plant emissions or gasoline fumes.[2, 12]

The sample-weighted, multiple linear regression models reveal that the median serum cotinine for exclusive smokers (192 ng/mL) was significantly associated with 216% higher urinary IPM3 compared with participants with no smoke exposure ($p < 0.0001$), controlling for confounding variables (Table 3). Similarly, smoking more cigarettes per day was associated with increased urinary IPM3 in a dose response pattern (Figure 2, Table 6). Compared with people who had no tobacco smoke exposure (serum cotinine ≤ 0.05 ng/mL) and controlling for confounders, smoking up to a half pack, 1 pack and > 1 pack was associated with 596%, 1350% and 1640% higher urinary IPM3 ($p < 0.0001$), respectively. People presumably exposed to secondhand smoke (serum cotinine >0.05 – ≤10 ng/mL) had nominally higher IPM3 compared with non-exposed non-smokers (serum cotinine ≤0.05 ng/mL), but the *p*-value was not significant. The observed increase in urinary IPM3 concentration with the increased tobacco smoke exposure is supported by previous studies identifying high microgram amounts of isoprene in cigarette smoke.[2, 3] Counts et al. found that mainstream smoke from 24 US commercial cigarettes had isoprene yields ranging from 100 to 587 μg/cigarette.[4] In addition, a pyrosynthetic mechanistic route that forms isoprene likely leads to the formation of 1,3-butadiene, albeit with lower yields (8.5-47.7 μg/cigarette), perhaps, from a different precursor.[4] Thus, we observe a strong correlation

between urinary levels of IPM3 and the 1,3-butadiene metabolite, MHB3 (Figure 3). Similar to the known metabolic pathway of MHB3, IPM3 could follow the glutathione metabolism and formed from acetylation of the S-(2-hydroxy-3-methyl-3-buten-l-y1)-glutathione conjugate.[18] Our finding of the importance of tobacco smoke as an isoprene exposure source is also consistent with a small study that found a 70% increase in exhaled breath isoprene after smoking one cigarette.[29]

Demographic variables were also evaluated for association with urinary IPM3 in the sample-weighted multiple linear regression models. Higher IPM3 in children (age 3-5 and 6-11 years) could result from their tendency to have higher secondhand smoke exposure than adults.[30] Modestly higher IPM3 in older adults (>60 years) could result from endogenous processes related to aging.[31] Alternatively, lower lean body mass in both children and elderly study participants may contribute to the appearance of higher IPM3 levels in creatinine-adjusted urinary data (Table S2). Positive predictors of IPM3 also include being female (compared with male) and non-Hispanic white (compared with non-Hispanic black). These findings may be the result of differing rates of endogenous isoprene production or isoprene absorption, depending on age, gender and/or ethnicity. Gender, for example, can affect blood cholesterol levels, which, in turn, would impact cholesterol synthesis and alter endogenous isoprene production.[32] Another explanation could be the effect of creatinine adjustments, shown in supplemental Table S2. While urinary creatinine excretion is relatively consistent in an individual, the amount excreted can vary significantly between individuals, based on lean body mass and a variety of genetic and physiological factors. Creatinine production is higher in people with more muscle mass, and tends to be higher in males compared with females and higher in non-Hispanic blacks compared with other races.[24]

We also examined diet, including nine food groups, plus coffee and beer/ale. We included coffee and beer/ale because isoprene is reportedly found in roasted coffee and the fruit of hops.[1, 9] Our regression models show that association between beer/ale consumption and IPM3 among non-tobacco users was statistically significant but the magnitude of association was limited (increased consumption from zero consumption to median and 90th percentile levels was associated only with 0% and 2.9% respective increase in IPM3). We found that coffee consumption was associated with nominally higher IPM3 in both exclusive smokers and non-users, but the *p*-value was not significant. While food of plant origin has been suspected to be a source of isoprene exposure,[1] vegetables and fruits were not significant positive predictors of IPM3. The percent changes in IPM3 predicted by dietary variables were of much lower magnitude compared with the percent changes associated with cigarette smoking.

In this analysis, we could not account for all sources of isoprene exposure. While we excluded tobacco users who were not exclusive tobacco smokers, marijuana use was not considered due to missing marijuana data from many participants. Occupational exposure is also a viable exposure route, but the NHANES population sampling design is unlikely to include a sizable proportion of US residents who have occupational isoprene exposure, and NHANES offers limited, publicly-available occupational information about its participants. Nevertheless, we found that the isoprene percent increase attributable to cigarette smoking is

greater than any dietary or demographic variable; hence, we conclude that tobacco smoke is a major source of isoprene exposure in the US population.

This study provides novel, US population-representative data about isoprene exposure based on the analysis of its urinary biomarker, IPM3. We found that isoprene exposure is widespread and, based on NHANES 2015-2016 data, tobacco smoke is a major source of isoprene exposure in the US population. Possible endogenous and dietary sources of isoprene exposure were insignificant compared with tobacco smoke. This paper provides important biomonitoring data to assess public health risk associated with isoprene exposure. These data add to our previous biomonitoring reports on exposure to other tobacco smoke-related VOCs. Future NHANES cycles can provide us with additional data on isoprene exposure trends in the US population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Highlights

- This paper is the first to evaluate urinary IPM3 as a biomarker of isoprene exposure in a large-scale, population-representative study.
- We found that tobacco smoke is a major source of isoprene exposure in the US population based on urinary IPM3 concentrations in NHANES 2015-2016.

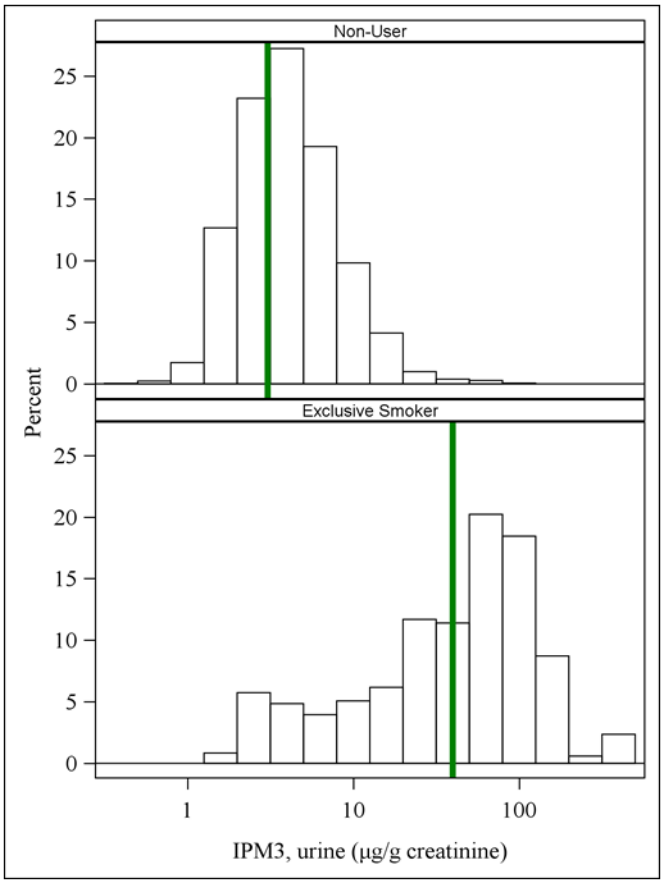


Figure 1.

Histogram (sample weighted) of IPM3 (µg/g creatinine) among non-users (N=2,019) and exclusive smokers (N=252) in NHANES 2015-2016. The vertical reference line represents the median concentration.

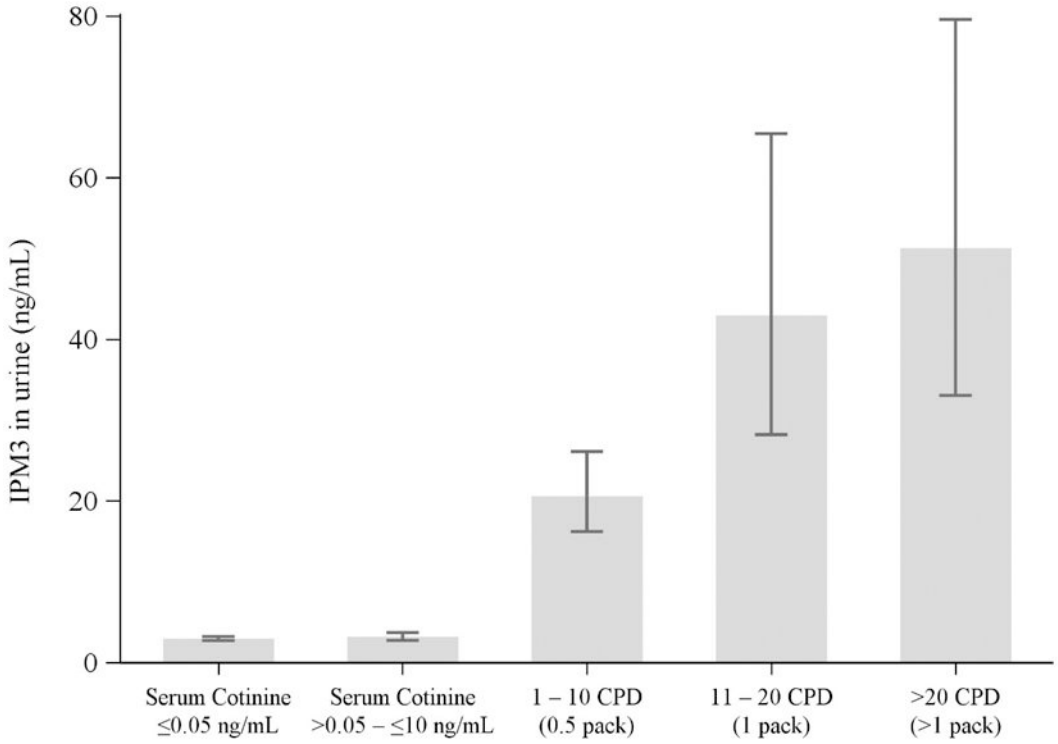


Figure 2.

Sample-weighted least squared geometric means (95% confidence intervals) for urinary IPM3 concentrations categorized by cigarette smoke exposure (N=2,271).

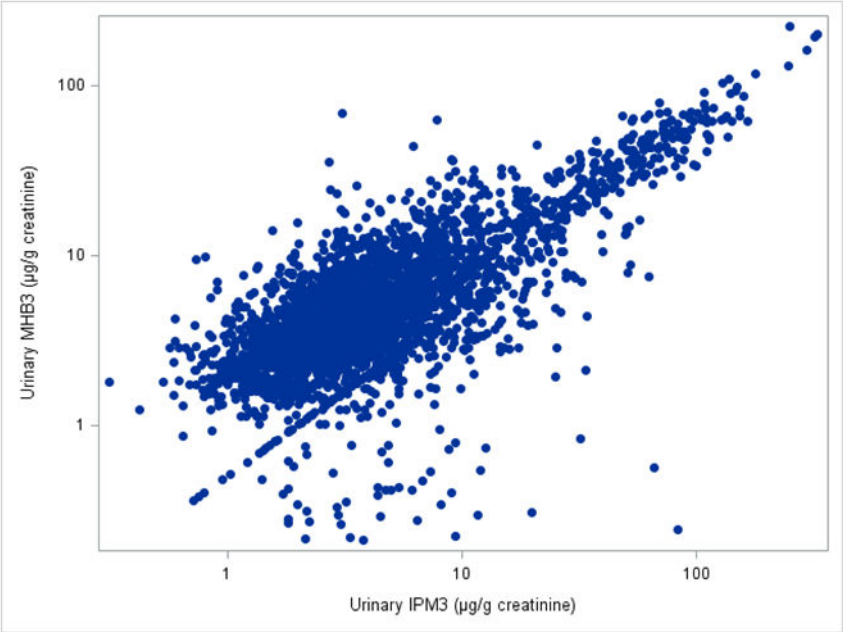
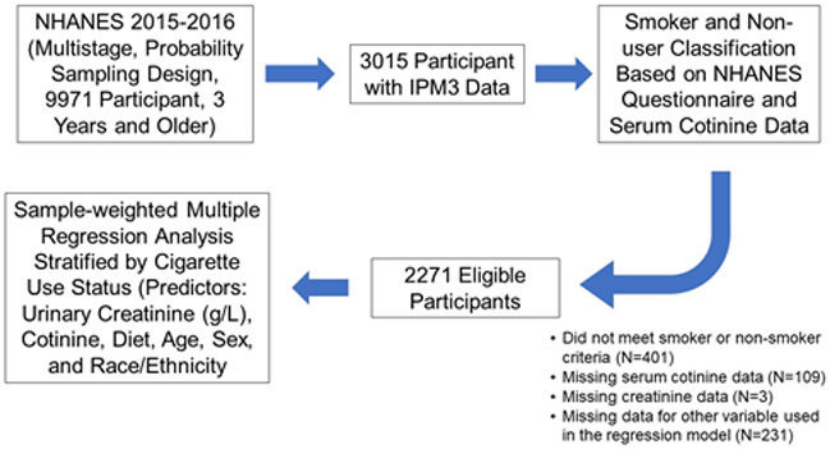


Figure 3.

Scatterplot diagram showing correlation of sample-weighted urinary IPM3 and urinary MHB3 (N=3,012). The Pearson correlation coefficient is 0.80.



Schematic 1.
Work flow of the study design.

Table 1.

Sample-weighted Demographic Distribution of NHANES 2015-2016 Participants (N = 2,271)[a]

Variable	Exclusive Smoker[b]		Non-user[c]	
	N[d]	% (SE[e])	N[d]	% (SE[e])
All	252	100 (0.00)	2,019	100 (0.00)
Sex				
Male	151	52.2 (3.47)	951	46.4 (1.69)
Female	101	47.9 (3.47)	1,068	53.6 (1.69)
Age (yr)				
3-5	0	N/A[f]	257	2.44 (0.23)
6-11	0	N/A[f]	261	7.78 (0.67)
12-19	8	1.88 (0.79)	315	12.7 (0.89)
20-39	87	40.2 (2.66)	385	25.6 (2.07)
40-59	92	39.0 (3.84)	376	27.2 (1.64)
≥60	65	18.9 (2.24)	425	24.3 (1.87)
Race/ethnicity				
Non-Hispanic White	98	63.2 (4.44)	588	61.2 (4.72)
Non-Hispanic Black	72	16.7 (3.08)	381	9.74 (2.01)
Mexican-American	37	8.39 (2.28)	431	11.2 (2.86)
Other Hispanic or Other/Multi Race	45	11.7 (1.89)	619	17.9 (2.09)
BMI				
Healthy weight	82	28.7 (3.22)	773	32.1 (1.74)
Underweight	4	1.53 (0.92)	25	1.25 (0.31)
Overweight/Obese	166	69.8 (3.03)	1,221	66.6 (1.82)

[a] Same data as in stratified serum cotinine regression models

[b] Participants reporting using cigarettes (and no other tobacco products) 5 days prior to physical examination and with serum cotinine measurement >10 ng/mL

[c] Participants reporting not using cigarettes during 5 days prior to physical examination or with serum cotinine measurement ≤10 ng/mL

[d] Sample size, not sample-weighted

[e] Standard error, sample-weighted

[f] N/A: not applicable

Table 2.

Sample-weighted Urinary IPM3 Concentrations (μg/g creatinine) Categorized by Smoking Status Among NHANES 2015-2016 Participants (N = 2,271)[a]

Variable	Exclusive Smoker[b] Median [25th, 75th percentile]	Non-user[c] Median [25th, 75th percentile]
All	39.8 [14.7, 72.3]	3.05 [2.03, 4.93]
Sex		
Male	31.1 [9.34, 58.8]	2.76 [1.84, 4.28]
Female	46.9 [18.1, 84.6]	3.40 [2.20, 5.23]
Age (yr)		
3-5	N/A[d]	6.06 [3.99, 8.86]
6-11	N/A[d]	4.11 [2.59, 6.49]
12-19	15.0 [3.60, 20.4]	2.40 [1.77, 3.60]
20-39	22.3 [7.60, 59.3]	2.58 [1.73, 4.25]
40-59	54.1 [18.0, 85.2]	3.15 [2.04, 4.54]
≥60	44.2 [26.4, 65.1]	3.41 [2.31, 5.21]
Race/ethnicity		
Non-Hispanic White	52.9 [20.6, 79.0]	3.19 [2.14, 4.97]
Non-Hispanic Black	19.4 [8.74, 38.0]	2.57 [1.68, 4.35]
Mexican-American	16.0 [5.94, 30.2]	2.90 [1.97, 4.69]
Other Hispanic or Other/Multi Race	23.1 [14.0, 66.4]	2.79 [1.86, 4.74]
BMI		
Healthy weight	38.5 [17.4, 71.2]	3.36 [2.29, 5.10]
Underweight	32.1 [22.0, 50.9]	3.47 [1.94, 6.25]
Overweight/Obese	41.0 [11.4, 72.7]	2.84 [1.93, 4.71]

[a] Same data as in stratified serum cotinine regression models

[b] Participants reporting using cigarettes (and no other tobacco products) 5 days prior to physical examination and with serum cotinine measurement >10 ng/mL

[c] Participants reporting not using cigarettes during 5 days prior to physical examination or with serum cotinine measurement ≤10 ng/mL

[d] N/A: not applicable

Table 3.

Sample-weighted Multiple Linear Regression Model Among Exclusive Smokers[a] (N = 252) for Urinary IPM3 (ng/mL) in NHANES 2015 – 2016

Variable	Coefficient [95% CI][b]	*p*-value[c]	Exponentiated Coefficient[d] [95% CI]
Intercept	0.712 [−0.0482, 1.47]		2.04 [0.953, 4.36]
Creatinine, urine [g/L][e]	0.653 [0.453, 0.853]	<.0001	1.92 [1.57, 2.35]
Cotinine, serum [ng/mL]	0.0060 [0.0045, 0.0074]	<.0001	1.01 [1.00, 1.01]
Sex			
Male	Ref.	.	Ref.
Female	0.567 [0.166, 0.967]	0.0087	1.76 [1.18, 2.63]
Age (yr)			
3-5	N/A	N/A	N/A
6-11	N/A	N/A	N/A
12-19	−0.348 [−0.980, 0.285]	0.2593	0.706 [0.375, 1.33]
20-39	Ref.	.	Ref.
40-59	0.181 [−0.0874, 0.449]	0.1714	1.20 [0.916, 1.57]
≥60	0.471 [−0.0869, 1.03]	0.0921	1.60 [0.917, 2.80]
Race/ethnicity			
Non-Hispanic White	Ref.	.	Ref.
Non-Hispanic Black	−0.422 [−0.921, 0.0760]	0.0910	0.655 [0.398, 1.08]
Mexican-American	−0.227 [−0.683, 0.229]	0.3060	0.797 [0.505, 1.26]
Other Hispanic or Other/Multi Race	−0.157 [−0.636, 0.322]	0.4957	0.855 [0.529, 1.38]
BMI			
Healthy weight	Ref.	.	Ref.
Underweight	−0.282 [−1.06, 0.495]	0.4513	0.754 [0.347, 1.64]
Overweight/Obese	0.0724 [−0.184, 0.328]	0.5553	1.08 [0.832, 1.39]
Food Group			
Milk Products [kg/d]	0.300 [−0.520, 1.12]	0.4475	1.35 [0.594, 3.07]
Meat, Poultry [kg/d]	−0.148 [−1.071, 0.775]	0.7366	0.862 [0.343, 2.17]
Eggs [kg/d]	0.666 [−1.30, 2.63]	0.4817	1.95 [0.272, 13.9]
Legumes, Nuts, Seeds [kg/d]	−0.598 [−2.49, 1.30]	0.5110	0.550 [0.0828, 3.65]
Grain Products [kg/d]	0.418 [−0.0048, 0.841]	0.0523	1.52 [0.995, 2.32]
Fruits [kg/d]	0.0996 [−0.549, 0.749]	0.7481	1.10 [0.577, 2.11]
Vegetables [Kg/d]	−0.130 [−1.46, 1.205]	0.8389	0.879 [0.231, 3.34]
Fats, Oils, Salad Dressings [kg/d]	0.891 [−6.17, 7.95]	0.7917	2.44 [2.09E-03, 2.84E+03]
Sugars, Sweets, Beverages [kg/d]	−0.007 [−0.123, 0.109]	0.8990	0.993 [0.885, 1.11]
Coffee [kg/d]	0.165 [−0.135, 0.465]	0.2590	1.18 [0.874, 1.59]
Beers and Ales [kg/d]	0.0165 [−0.156, 0.189]	0.8412	1.02 [0.856, 1.21]

[a]Participants reporting using cigarettes (and no other tobacco products) 5 days prior to physical examination or with serum cotinine measurement >10 ng/mL

[b]95% confidence interval

[c]The dependent variable, biomarker concentration, was natural log-transformed for the regression model

[d]For each unit-increase in the variable, the expected biomarker concentration in ng/mL is multiplied by the exponentiated coefficient (controlling for other predictors in the model)

[e]Although urinary creatinine is reported in mg/dL, using g/L simplifies the slope to a more readily interpretable scale of μg/g creatinine

Table 4.

Sample-weighted Multiple Linear Regression Model Among Non-users[a] (N = 2,019) for Urinary IPM3 (ng/mL) in NHANES 2015 – 2016

Variable	Coefficient [95% CI][b]	*p*-value[c]	Exponentiated Coefficient[d] [95% CI]
Intercept	0.0128 [−0.160, 0.186]		1.01 [0.852, 1.20]
Creatinine, urine [g/L][e]	0.847 [0.747, 0.947]	<.0001	2.33 [2.11, 2.58]
Cotinine, serum [ng/mL]	−0.0156 [−0.0706, 0.0393]	0.5539	0.985 [0.932, 1.04]
Sex			
Male	Ref.	.	Ref.
Female	0.0723 [−0.0076, 0.152]	0.0728	1.08 [0.992, 1.16]
Age (yr)			
3-5	0.591 [0.412, 0.770]	<.0001	1.81 [1.51, 2.16]
6-11	0.463 [0.353, 0.573]	<.0001	1.59 [1.42, 1.77]
12-19	−0.0208 [−0.159, 0.118]	0.7530	0.979 [0.853, 1.12]
20-39	Ref.	.	Ref.
40-59	0.116 [−0.0540, 0.287]	0.1661	1.12 [0.947, 1.33]
≥60	0.267 [0.117, 0.416]	0.0018	1.31 [1.12, 1.52]
Race/ethnicity			
Non-Hispanic White	Ref.	.	Ref.
Non-Hispanic Black	−0.127 [−0.290, 0.0356]	0.1164	0.880 [0.748, 1.04]
Mexican-American	0.0007 [−0.0981, 0.0996]	0.9878	1.00 [0.907, 1.10]
Other Hispanic or Other/Multi Race	−0.0376 [−0.167, 0.0913]	0.5437	0.963 [0.847, 1.10]
BMI			
Healthy weight	Ref.	.	Ref.
Underweight	0.113 [−0.170, 0.396]	0.4093	1.12 [0.843, 1.49]
Overweight/Obese	−0.0711 [−0.169, 0.0269]	0.1427	0.931 [0.844, 1.03]
Food Group			
Milk Products [kg/d]	−0.191 [−0.392, 0.0100]	0.0610	0.826 [0.675, 1.01]
Meat, Poultry [kg/d]	0.0047 [−0.197, 0.207]	0.9607	1.00 [0.821, 1.23]
Eggs [kg/d]	−0.0584 [−0.693, 0.576]	0.8469	0.943 [0.500, 1.78]
Legumes, Nuts, Seeds [kg/d]	0.0720 [−0.545, 0.689]	0.8068	1.07 [0.580, 1.99]
Grain Products [kg/d]	−0.0844 [−0.214, 0.0454]	0.1861	0.919 [0.807, 1.05]
Fruits [kg/d]	−0.0959 [−0.336, 0.1442]	0.4080	0.909 [0.715, 1.16]
Vegetables [kg/d]	−0.208 [−0.488, 0.0726]	0.1350	0.812 [0.614, 1.08]
Fats, Oils, Salad Dressings [kg/d]	1.77 [−0.854, 4.40]	0.1707	5.90 [0.426, 81.7]
Sugars, Sweets, Beverages [kg/d]	0.0208 [−0.0186, 0.0603]	0.2781	1.02 [0.982, 1.06]
Coffee [kg/d]	0.0926 [−0.118, 0.303]	0.3622	1.10 [0.889, 1.35]
Beers and Ales [kg/d]	0.0896 [0.0014, 0.178]	0.0468	1.09 [1.00, 1.19]

[a]Participants reporting not using cigarettes (and no other tobacco products) 5 days prior to physical examination and with serum cotinine measurement ≤10 ng/mL

[b]95% confidence interval

[c]The dependent variable, biomarker concentration, was natural log-transformed for the regression model

[d]For each unit-increase in the variable, the expected biomarker concentration in ng/mL is multiplied by the exponentiated coefficient (controlling for other predictors in the model)

[e]Although urinary creatinine is reported in mg/dL, using g/L simplifies the slope to a more readily interpretable scale of μg/g creatinine

Table 5.

Sample-weighted Median and Selected Percentiles of All Numerical Predictors in the Regression Model

Variable Label	50 th	75 th	90 th	95 th
Creatinine, Urine [g/L]	0.934	1.53	2.06	2.47
Cotinine, Serum [ng/mL]: Exclusive Smokers	192	268	357	393
Cotinine, Serum [ng/mL]: Non-user	0.0166	0.0485	0.297	1.20
Milk Products [kg/d]	0.124	0.309	0.544	0.729
Meat, Poultry [kg/d]	0.142	0.270	0.430	0.550
Eggs [kg/d]	0	0	0.107	0.150
Legumes, Nuts, Seeds [kg/d]	0	0.0282	0.112	0.180
Grain Products [kg/d]	0.257	0.474	0.714	0.886
Fruits [kg/d]	0.0728	0.229	0.410	0.542
Vegetables [kg/d]	0.0967	0.231	0.399	0.506
Fats, Oils, Salad Dressings [kg/d]	0	0.00944	0.0294	0.0580
Sugars, Sweets, Beverages [kg/d]	1.46	2.37	3.41	4.26
Coffee [kg/d]	0	0.379	0.720	1.04
Beers and ales [kg/d]	0	0	0.319	0.718

Table 6.

Sample-weighted Multiple Linear Regression Model with Tobacco Smoke Exposure for Urinary IPM3 Concentrations (ng/mL) among NHANES 2015 – 2016 (N = 2,271)

Variable	Coefficient [95% CI][a]	*p*-value[b]	Exponentiated Coefficient [c] [95% CI]
Intercept	−0.0291 [−0.190, 0.132]		0.971 [0.827, 1.14]
Creatinine, urine [g/L][d]	0.798 [0.696, 0.900]	<.0001	2.22 [2.01, 2.46]
CPD Group[e]			
≤0.05 ng/mL cotinine, serum	Ref.	.	Ref.
>0.05 - ≤10 ng/mL cotinine,	0.0733 [−0.0445, 0.191]	0.2047	1.08 [0.956, 1.21]
1-10 CPD (0.5 pack)	1.94 [1.69, 2.20]	<.0001	6.96 [5.39, 9.00]
11-20 CPD (1 pack)	2.68 [2.28, 3.07]	<.0001	14.5 [9.78, 21.6]
>20 CPD (>1 pack)	2.85 [2.40, 3.31]	<.0001	17.4 [11.0, 27.3]
Sex			
Male	Ref.	.	Ref.
Female	0.127 [0.0534, 0.200]	0.0022	1.14 [1.05, 1.22]
Age (yr)			
3-5	0.565 [0.364, 0.766]	<.0001	1.76 [1.44, 2.15]
6-11	0.454 [0.301, 0.606]	<.0001	1.57 [1.35, 1.83]
12-19	−0.0212 [−0.185, 0.143]	0.7867	0.979 [0.831, 1.15]
20-39	Ref.	.	Ref.
40-59	0.175 [0.0153, 0.335]	0.0338	1.19 [1.02, 1.40]
≥60	0.305 [0.166, 0.443]	0.0003	1.36 [1.18, 1.56]
Race/ethnicity			
Non-Hispanic White	Ref.	.	Ref.
Non-Hispanic Black	−0.149 [−0.325, 0.0268]	0.0909	0.861 [0.722, 1.03]
Mexican-American	−0.0494 [−0.155, 0.0563]	0.3352	0.952 [0.856, 1.06]
Other Hispanic or Multi Race	−0.0572 [−0.162, 0.0481]	0.2650	0.944 [0.850, 1.05]
BMI			
Healthy weight	Ref.	.	Ref.
Overweight/Obese	0.121 [−0.158, 0.401]	0.3704	1.13 [0.853, 1.49]
Underweight	−0.0798 [−0.184, 0.0247]	0.1243	0.923 [0.832, 1.02]
Food Group			
Milk Products [kg/d]	−0.0733 [−0.270, 0.123]	0.4392	0.929 [0.764, 1.13]
Meat, Poultry [kg/d]	0.104 [−0.0638, 0.272]	0.2063	1.11 [0.938, 1.31]
Eggs [kg/d]	−0.0299 [−0.738, 0.678]	0.9294	0.970 [0.478, 1.97]
Legumes, Nuts, Seeds [kg/d]	0.0448 [−0.503, 0.593]	0.8641	1.05 [0.605, 1.81]
Grain Products [kg/d]	−0.0493 [−0.213, 0.114]	0.5296	0.952 [0.808, 1.12]
Fruits [kg/d]	−0.118 [−0.362, 0.127]	0.3207	0.889 [0.696, 1.13]

Variable	Coefficient [95% CI][a]	*p*-value[b]	Exponentiated Coefficient [c] [95% CI]
Vegetables [kg/d]	−0.194 [−0.443, 0.0561]	0.1192	0.824 [0.642, 1.06]
Fats, Oils, Salad Dressings [kg/d]	1.38 [−0.704, 3.46]	0.1786	3.97 [0.495, 31.9]
Sugars, Sweets, Beverages [kg/d]	0.0137 [−0.0309, 0.0584]	0.5222	1.01 [0.970, 1.06]
Coffee [kg/d]	0.108 [−0.0834, 0.299]	0.2478	1.11 [0.920, 1.35]
Beer and ales [kg/d]	0.0214 [−0.0640, 0.107]	0.6008	1.02 [0.938, 1.11]

[a]95% confidence interval

[b]The dependent variable, biomarker concentration, was natural log-transformed for the regression model

[c]For each unit-increase in the variable, the expected biomarker concentration in ng/mL is multiplied by the exponentiated coefficient (controlling for other predictors in the model)

[d]Although urinary creatinine is reported in mg/dL, using g/L simplifies the slope to a more readily interpretable scale of μg/g creatinine

[e]CPD: cigarettes smoked per day (exclusive smokers). Among all participants, 7.92% of participants smoked 1 – 10 CPD, 2.86% smoked 11 – 20 CPD, and 0.31% smoked >20 CPD.