# Characterization of Acrylonitrile Exposure in the United States based on Urinary N-Acetyl-S-(2-cyanoethyl)-L-cysteine (2CYEMA): NHANES 2011–2016

**Víctor R. De Jesús**[*,1], **Luyu Zhang**[1], **Deepak Bhandari**[1], **Wanzhe Zhu**[1], **Joanne T. Chang**[2], **Benjamin C. Blount**[1]

[1]Centers for Disease Control and Prevention, Division of Laboratory Sciences, Tobacco and Volatiles Branch, Atlanta, GA 30341 USA

[2]Office of Science, Center for Tobacco Products, US Food and Drug Administration, Silver Spring, MD 20993 USA

## Abstract

**Background:** Acrylonitrile is a possible human carcinogen that is used in polymers and formed in tobacco smoke. We assessed acrylonitrile exposure in the US population by measuring its urinary metabolites N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (2CYEMA) and N-acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine (1CYHEMA) in participants from the 2011–2016 National Health and Nutrition Examination Survey.

**Objective:** To assessed acrylonitrile exposure using population-based biomonitoring data of the US civilian, non-institutionalized population.

**Methods:** Laboratory data for 8,057 participants were reported for 2CYEMA and 1CYHEMA using ultrahigh-performance liquid chromatography / tandem mass spectrometry. Exclusive tobacco smokers were distinguished from non-users using a combination of self-reporting and serum cotinine data. We used multiple linear regression models to fit 2CYEMA concentrations with sex, age, race/Hispanic origin, and tobacco user group as predictor variables.

**Results:** The median 2CYEMA level was higher for exclusive cigarette smokers (145 μg/g creatinine) than for non-users (1.38 μg/g creatinine). Compared to unexposed individuals (serum cotinine 0.015 ng/ml) and controlling for confounders, presumptive second-hand tobacco smoke exposure (serum cotinine > 0.015 – 10 ng/ml and 0 cigarettes per day, CPD) was significantly associated with 36% higher 2CYEMA levels ($p < 0.0001$). Smoking 1–10 CPD was significantly associated with 6,720% higher 2CYEMA levels ($p < 0.0001$).

[*]Corresponding Author: Víctor R. De Jesús, PhD, Tobacco and Volatiles Branch, Division of Laboratory Sciences, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mail Stop S103-3, Atlanta, GA 30341, USA, vdejesus@cdc.gov, Phone: 770-488-7963, Fax: 770-488-0181.

**Significance:** We show that tobacco smoke is an important source of acrylonitrile exposure in the US population and provide important biomonitoring data on acrylonitrile exposure.

## Keywords

Acrylonitrile; 2CYEMA; tobacco smoke exposure; NHANES; biomonitoring; VOC metabolites

## 1. Introduction

Acrylonitrile is a chemical with a sharp, onion- or garlic-like odor [1]. It is used mostly to make plastics, acrylic fibers, nitrile rubbers, and barrier resins [2]. Exposure to acrylonitrile in the general population is limited to tobacco smoke, accidental fires, and residual acrylonitrile in commercial polymeric material [3]. Human exposure to acrylonitrile at concentrations 16 parts per million (ppm) can cause headaches and nausea [1]. Moreover, the Occupational Safety and Health Administration has established a 2-ppm eight-hour time weighted average limit [4]. Tobacco smoke is the major non-occupational source for acrylonitrile exposure [5]. The International Agency for Research on Cancer has listed acrylonitrile as possibly carcinogenic to humans (Group 2B) [6]. It is also included in the US Food and Drug Administration's Established List of Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke [7]. Mainstream smoke from 50 US commercial cigarettes contained acrylonitrile levels between 0.90–15.34 μg/cigarette (ISO protocol) and 19.7–37.7 μg/cigarette (Canadian Intense protocol) [8].

Acrylonitrile is metabolized through epoxidation to glycidonitrile, resulting in several metabolites including cyanide, and glutathione conjugation [9]. The major metabolite formed by glutathione conjugation is urinary N-acetyl-S-(2-cyanoethyl)-L-cysteine (2CYEMA) [10]; a minor metabolite is urinary N-acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine (1CYHEMA) [3, 5]. Another minor acrylonitrile metabolite is urinary N-acetyl-S-(2-hydroxyethyl)-L-cysteine (2HEMA) [11], which suffers from low specificity as it can also result from exposure to ethylene oxide, ethylene dibromide, and vinyl chloride [12]. The mercapturic acid 2CYEMA has been recognized as a specific, suitable biomarker of exposure to acrylonitrile [13]. Chen et al. showed that creatinine-adjusted intra-class correlation coefficients for 2CYEMA and total nicotine equivalents (TNE) were 0.67, and 0.68, respectively, indicating good longitudinal consistency for 2CYEMA [14]. A strong correlation between 2CYEMA and TNE values was observed. The authors concluded that 2CYEMA is a reliable biomarker of tobacco smoke exposure, since the data indicated that 2CYEMA levels are consistent over time in cigarette smokers. In addition, data from the Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013–2014) showed that 2CYEMA levels in daily cigar-only smokers were comparable to those observed in daily cigarette-only smokers [15]. Another study examined the effect of concurrent use of combusted tobacco products and cannabis [16]. Across all tobacco user groups, those who also smoked cannabis exhibited significantly higher 2CYEMA levels compared to non-cannabis users (39% – 464%), suggesting potential additive toxicant exposures among smokers of tobacco and cannabis.

Urinary 2CYEMA levels have also been used to monitor short-term product switch from conventional cigarettes to either electronic cigarettes or nicotine gum (i.e., non-combustible tobacco products) [17]. This study found that the magnitude of biomarker reductions among subjects that switched to electronic cigarettes was similar to subjects switched to nicotine gum for non-combustible products. Specifically, observed decreases ranged from 30% to greater than 85% for constituents such as benzene and acrylonitrile. St. Helen et al. found that concentrations of volatile organic compound metabolites were higher during smoking compared with vaping [18]. The geometric mean ratio (95% confidence interval) of 2CYEMA concentrations when smoking relative to vaping was 7.09 (5.88–8.54), supporting their potential harm reduction potential among smokers who may want to switch to non-combustible tobacco product use. Foods likely to contain measurable acrylonitrile are high-fat or highly acidic items, such as luncheon meat, margarine, vegetable oil, or fruit juice, primarily due to contact with food packaging. Acrylonitrile polymer-containing materials are used to package food, and are a potentially relevant route of acrylonitrile exposure [19]. However, the US Food and Drug Administration's Total Diet Study found no acrylonitrile residue in any of the foods tested from 1991 to 2004.

To date, no studies have been published characterizing human exposure to acrylonitrile on a population-wide scale, despite its harmful properties. The present study examined acrylonitrile exposure in participants of the 2011–2016 National Health and Nutrition Examination Survey (NHANES) to obtain population-based biomonitoring data of the US civilian, non-institutionalized population. In addition, we used multiple linear regression models to examine the impact of tobacco smoke, select demographic variables, and diet on acrylonitrile exposure.

## 2. Materials and Methods

### 2.1. Study Design

NHANES is a household-based survey that assesses the health and nutritional status of the civilian, non-institutionalized US population based on data collected from questionnaires, physical examinations and biological samples [20–22]. This cross-sectional study with data released every 2 years, is conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC). We evaluated data from three cycles, NHANES 2011–2012, 2013–2014, and 2015–2016. Participants aged 3 years and older provided spot urine samples for NHANES cycles 2011–2016, and we quantified 2CYEMA (CAS 74514-75-3) and 1CYHEMA (CAS 116477-44-2) 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, [23] and

was used to stratify self-identified exclusive smokers and non-users in statistical analyses reported here. Laboratory data for 8,057 participants were reported for 2CYEMA and 1CYHEMA (NHANES datasets: UVOC_G, UVOC_H, UVOC_I). Participants were excluded from analysis if they did not meet the criteria for either exclusive smoker or non-user (N=1,027, either poly-users or non-combusted tobacco users), for missing serum cotinine data (N=226), for missing creatinine data (N=5) or for missing data for other variables used in the regression model (N=618). This attrition left 6,181 study participants eligible for statistical analysis.

### 2.2.   Laboratory Method

Spot urine samples from NHANES 2011–2016 were analyzed for urinary 2CYEMA and 1CYHEMA 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) [24] since urinary acrylonitrile metabolite concentrations are proportional to acrylonitrile exposure. Briefly, chromatographic separation was achieved using an Acquity UPLC® HSS T3, 100 Å, 1.8 μm, 2.1mm × 150 mm column (Waters Inc., Milford, MA) with a Waters HSS T3 VanGuard pre-column (Waters Corporation, Milford, MA). The mass spectrometer was operated in negative ion ESI scheduled multiple reaction monitoring mode [21, 25]. 2CYEMA was monitored using ion transitions $m/z$ 215→86 (quantifier), $m/z$ 215→162 (qualifier), and $m/z$ 218→165 (2CYEMA-$[^2H_3]$, internal standard). 1CYHEMA was monitored using ion transitions $m/z$ 231→84 (quantifier), $m/z$ 231→102 (qualifier), and $m/z$ 234→84 (1CYHEMA-$[^2H_3]$, internal standard). 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. The limit of detection (LOD) was 0.500 ng/ml for 2CYEMA and 2.6 ng/ml for 1CYHEMA.

Reported analytical results met the accuracy and precision specifications of the quality control/quality assurance program of the Division of Laboratory Sciences in the CDC National Center for Environmental Health. Measurements below the LOD were substituted with the quotient of the LOD divided by the square root of two $(LOD/\sqrt{2})$. Thus, 2CYEMA concentrations less than the LOD were imputed using 0.354 ng/ml $(LOD/\sqrt{2})$ [26].

### 2.3.   Statistical Analysis

NHANES recruited participants through a multistage, probability sampling design [20]. 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 procedures of SAS 9.4 were used to calculate estimates. Weighted multiple linear regression models stratified by cigarette use status (exclusive smokers vs. non-users) were fit to data from NHANES cycle 2011–2016, where the dependent variable was urinary 2CYEMA concentration (ng/ml). Since the distribution of urinary 2CYEMA measurements was strongly right-skewed, which could have adversely affected hypothesis testing, we used natural log transformed 2CYEMA data for regression analysis. We report coefficients from these models along with their 95% confidence intervals

(95% CI) and *p*-values. The exponentiated coefficients represent the proportional change of biomarker concentration [27]. An evaluation of statistical reliability was performed to ensure all proportions follow NCHS Data Presentation Standards [28]. Statistical significance was set to $\alpha = 0.05$. Regression modeling did not include 1CYHEMA because of low detection rates.

Weighted regression models were stratified by cigarette use, and the following self-reported variables were included as predictors: urinary creatinine (g/l, laboratory data), dietary information, fasting time, sex, age and race/Hispanic origin. Creatinine, a waste product of creatine and creatine phosphate (produced from muscle metabolism), is excreted in urine at a relatively constant rate [29]. 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 [21, 25, 30]. An additional predictor, weight status was classified by body mass index (BMI, weight in kilograms divided by height in meters squared) which was calculated from measurements taken at the NHANES physical examination. Standard definitions for underweight (BMI < 18.5), normal weight (18.5 BMI < 25), and overweight/obesity (BMI 25) apply to adults 20 years. Participants younger than 20 years were classified based on their BMI percentile from the CDC growth charts for their sex and age: below the 5th percentile (underweight), between the 5th and 85th percentile (normal weight), and above the 85th percentile (overweight/obesity). In addition, dietary exposure was investigated by assessing the amount 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 [25, 27].

To estimate an association between 2CYEMA and frequency of cigarette smoking, we performed an unstratified, 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: 0.015 ng/ml serum cotinine and 0 CPD (unexposed to tobacco smoke), > 0.015 – 10 ng/ml serum cotinine and 0 CPD (presumptively exposed to second-hand tobacco smoke), > 10 ng/ml serum cotinine and 1 – 10 CPD, > 10 ng/ml serum cotinine and 11 – 20 CPD, and > 10 ng/ml serum cotinine and > 20 CPD. The reference category was unexposed participants and was defined at 0.015 ng/ml serum cotinine. The analytic dataset for the CPD model comprised the same participants as the stratified models.

## 3. Results

Weighted detection rates for 2CYEMA and 1CYHEMA were 86.5% and 14.9%, respectively. Weighted demographic distributions of 2CYEMA are shown in Table 1. The 1CYHEMA detection rate in NHANES 2015–2016 was 14.9% (only one NHANES cycle had data for 1CYHEMA), which was insufficient for robust statistical analysis. Thus, we focus our analysis on 2CYEMA results. Weighted summary statistics for 2CYEMA categorized by smoking status are presented in Table 2, categorized by sex, age, race/Hispanic origin and weight status. The median concentration of 2CYEMA is 145 μg/g

creatinine for exclusive smokers, and 1.38 μg/g creatinine for non-users. Moreover, the US population weighted medians, 25th and 75th percentiles for 2CYEMA are shown in Table 2.

Weighted multiple linear regression analyses for urinary 2CYEMA are shown for exclusive smokers in Table 3 and non-users in Table 4. The regression models include urinary creatinine, serum cotinine, fasting time, sex, age, race/Hispanic origin, weight status and dietary groups. Among exclusive smokers, serum cotinine positively predicted urinary 2CYEMA. The higher serum cotinine levels were associated with higher urinary 2CYEMA among for exclusive smokers ($p < 0.0001$, Table 3) as well as among non-users ($p < 0.0001$, Table 4), controlling for other variables. Among non-users, serum cotinine (0.018 ng/ml) predicted higher urinary 2CYEMA ($p < 0.0001$), controlling for other variables. Among exclusive smokers, females had significantly (42%, $p=0.0139$) higher urinary 2CYEMA levels compared to males, controlling for other variables. Using participants age 20–39 years as the reference group, older adults 40–59 years and 60 years had significantly (25%, $p=0.0287$; 47%, $p=0.0012$, respectively) higher 2CYEMA levels, controlling for other variables. Dietary intake did not have a statistically significant effect on 2CYEMA levels among smokers. Among non-users, every additional hour of fasting time was associated with 0.8% lower 2CYEMA levels ($p=0.0142$).

Weighted geometric means of urinary 2CYEMA for self-reported CPD are shown in Figure 1, adjusted for urinary creatinine, fasting time, sex, age, race/Hispanic origin, weight status and diet. In the model, 2CYEMA concentrations increase with respect to increasing CPD. Table 5 shows the weighted multiple linear regression model with CPD. Compared to unexposed participants (serum cotinine 0.05 ng/ml) and controlling for confounders, being presumptively exposed to second-hand tobacco smoke (serum cotinine > 0.05 – 10 ng/ml and 0 CPD) was significantly associated with 36% higher 2CYEMA levels ($p < 0.0001$); smoking 1–10 CPD was significantly associated with 6,720% higher 2CYEMA levels ($p < 0.0001$); smoking 11–20 CPD was significantly associated with 11,300% higher 2CYEMA levels ($p < 0.0001$), and smoking > 20 CPD was significantly associated with 18,500% higher 2CYEMA levels ($p < 0.0001$). In addition, every additional hour of fasting time was associated with 0.8% lower 2CYEMA levels ($p = 0.0294$) in the CPD model.

## 4. Discussion

This is the first large-scale, US population-representative study that evaluates acrylonitrile exposure by assessing its urinary metabolite, 2CYEMA. Our regression models show that cigarette smoke was an important source of acrylonitrile exposure in the US population during 2011–2016. The median 2CYEMA concentration for exclusive smokers (145 μg/g creatinine) was approximately 100 times that of non-users (1.38 μg/g creatinine). Similarly, smoking more CPD was associated with increased urinary 2CYEMA in a dose response pattern (Figure 1).

The weighted, multiple linear regression models reveal that, compared with people who had no tobacco smoke exposure (serum cotinine 0.05 ng/ml) and controlling for confounders, people presumptively exposed to second-hand tobacco smoke (serum cotinine > 0.05 – 10 ng/ml and 0 CPD), smoking up 10 CPD, 11–20 CPD and > 20 CPD was associated with

36%, 6,720%, 11,300%, and 18,500% higher urinary 2CYEMA ($p$ <0.0001), respectively. In addition, serum cotinine was significantly and positively associated with urinary 2CYEMA in both non-users and smokers. The observed increase in urinary 2CYEMA concentration with the increased tobacco smoke exposure is supported by previous studies identifying high microgram amounts of acrylonitrile in cigarette smoke [8]. Our finding of the importance of tobacco smoke as an acrylonitrile exposure source is also consistent with other studies that found increased 2CYEMA levels resulting from tobacco smoke exposure [5, 31, 32].

Demographic variables were also evaluated for association with urinary 2CYEMA in the weighted multiple linear regression models. Higher 2CYEMA in children (age 3–5 and 6–11 years) than in non-users age   12 years could result from their propensity to have higher secondhand smoke exposure than adults [33]. Modestly higher 2CYEMA in older adults (>60 years) could result from endogenous processes related to aging, or smoking intensity. Being a female was a positive predictor of 2CYEMA levels, possibly related to differences in lean body mass complicating creatinine adjustment of hydration status [29].

We also examined dietary intake, including nine food groups. Our regression models found no significant association between consumption of foods from the nine dietary groups and 2CYEMA, regardless of tobacco product use. Increasing fasting time was modestly but statistically significant associated with higher urinary 2CYEMA among non-users (Table 4) and in the CPD model (Table 5). Some foods may contain acrylonitrile, albeit at concentrations much lower than tobacco smoke. Furthermore, while we excluded tobacco users who were not exclusive tobacco smokers, marijuana use was not considered due to extensive missing data on marijuana use for many participants. Nevertheless, we found that, in the models we used, tobacco smoke exposure variables were shown to be the only variables associated with urinary 2CYEMA in a statistically-significant manner in sizable magnitudes (with the exceptions of fasting time and some age indicators), indicating that tobacco smoke is far more important as a source of acrylonitrile exposures than diet in the US population.

There are important limitations to our study. The NHANES survey is cross-sectional, where measurements are sometimes repeated at different times to assess trends over time. Moreover, causality cannot be determined from cross-sectional data. Temporal bias is a concern; thus, causality cannot be inferred from the present study. In addition, we controlled for numerous confounding variables, including diet and fasting time. Dietary information was assessed using a 24-hour recall, which has limitations for estimating long-term dietary patterns [34]. Self-reported information could lead to misclassifications.

This study provides novel, US population-representative data about acrylonitrile exposure based on the analysis of its urinary biomarker, 2CYEMA. We found that acrylonitrile exposure, based on NHANES 2011–2016 data, is mainly due to tobacco smoke in the US population. Possible dietary sources of acrylonitrile exposure were insignificant compared with tobacco smoke. This paper provides important biomonitoring data to assess public health risk associated with acrylonitrile exposure and add to our previous biomonitoring reports on exposure to other tobacco smoke-related volatile organic compounds.

## Acknowledgements

## References

1. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrylonitrile. 1990.

2. U.S. Environmental Protection Agency. Acrylonitrile Hazard Summary. 2000.

3. Leonard A, Gerber GB, Stecca C, Rueff J, Borba H, Farmer PB et al. Mutagenicity, carcinogenicity, and teratogenicity of acrylonitrile. Mutat Res 1999; 436: 263–283. [PubMed: 10354525]

4. Occupational Safety and Health Administration. Acrylonitrile. In: Toxic and Hazardous Substances: U.S. Department of Labor, 2012.

5. Minet E, Cheung F, Errington G, Sterz K, Scherer G Urinary excretion of the acrylonitrile metabolite 2-cyanoethylmercapturic acid is correlated with a variety of biomarkers of tobacco smoke exposure and consumption. Biomarkers 2011; 16: 89–96. [PubMed: 21108560]

6. International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Monomers, Plastics and Synthetic Elastomers, and Acrolein,, 1979.

7. U.S. Food and Drug Administration. Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List. 2012.

8. Pazo DY, Moliere F, Sampson MM, Reese CM, Agnew-Heard KA, Walters MJ et al. Mainstream Smoke Levels of Volatile Organic Compounds in 50 U.S. Domestic Cigarette Brands Smoked With the ISO and Canadian Intense Protocols. Nicotine Tob Res 2016; 18: 1886–1894. [PubMed: 27113015]

9. Lambotte-Vandepaer M, Duverger-van Bogaert M, Rollmann B Metabolism and mutagenicity of acrylonitrile: an in vivo study. Environ Mutagen 1985; 7: 655–662. [PubMed: 3899631]

10. Langvardt PW, Putzig CL, Braun WH, Young JD Identification of the major urinary metabolites of acrylonitrile in the rat. J Toxicol Environ Health 1980; 6: 273–282. [PubMed: 7392095]

11. Linhart I, Smejkal J, Novak J N-acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine, a new urinary metabolite of acrylonitrile and oxiranecarbonitrile. Arch Toxicol 1988; 61: 484–488. [PubMed: 3190446]

12. Calafat AM, Barr DB, Pirkle JL, Ashley DL Reference range concentrations of N-acetyl-S-(2-hydroxyethyl)-L-cysteine, a common metabolite of several volatile organic compounds, in the urine of adults in the United States. J Expo Anal Environ Epidemiol 1999; 9: 336–342. [PubMed: 10489158]

13. Fennell TR, Kedderis GL, Sumner SC Urinary metabolites of [1,2,3–13C] acrylonitrile in rats and mice detected by 13C nuclear magnetic resonance spectroscopy. Chem Res Toxicol 1991; 4: 678–687. [PubMed: 1807451]

14. Chen M, Carmella SG, Sipe C, Jensen J, Luo X, Le CT et al. Longitudinal stability in cigarette smokers of urinary biomarkers of exposure to the toxicants acrylonitrile and acrolein. PLoS One 2019; 14: e0210104. [PubMed: 30608961]

15. Chang CM, Rostron BL, Chang JT, Corey CG, Kimmel HL, Sosnoff CS *et al* Biomarkers of Exposure among U.S. Adult Cigar Smokers: Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013–2014). Cancer Epidemiol Biomarkers Prev 2019; 28: 943–953. [PubMed: 30733305]

16. Smith DM, O'Connor RJ, Wei B, Travers M, Hyland A, Goniewicz MLNicotine and Toxicant Exposure among Concurrent Users ("Co-users") of Tobacco and Cannabis. Nicotine Tob Res 2019; e-pub ahead of print 2019/08/08; doi 10.1093/ntr/ntz122.

17. Round EK, Chen P, Taylor AK, Schmidt E Biomarkers of Tobacco Exposure Decrease After Smokers Switch to an E-Cigarette or Nicotine Gum. Nicotine Tob Res 2019; 21: 1239–1247. [PubMed: 30202883]

18. St Helen G, Liakoni E, Nardone N, Addo N, Jacob P 3rd, Benowitz NLComparison of Systemic Exposure to Toxic and/or Carcinogenic Volatile Organic Compounds (VOC) during Vaping, Smoking, and Abstention. Cancer Prev Res (Phila) 2020; 13: 153–162. [PubMed: 31554628]

19. Muncke J Endocrine disrupting chemicals and other substances of concern in food contact materials: an updated review of exposure, effect and risk assessment. J Steroid Biochem Mol Biol 2011; 127: 118–127. [PubMed: 21073950]

20. U.S. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey.

21. Bagchi P, Geldner N, deCastro BR, De Jesus VR, Park SK, Blount BC Crotonaldehyde exposure in U.S. tobacco smokers and nonsmokers: NHANES 2005–2006 and 2011–2012. Environ Res 2018; 163: 1–9. [PubMed: 29407484]

22. U.S. Centers for Disease Control and Prevention. NHANES Survey Methods and Analytic Guidelines. 2020.

23. Pirkle JL, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA 1996; 275: 1233–1240. [PubMed: 8601954]

24. Alwis KU, Blount BC, Britt AS, Patel D, Ashley DL Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). Anal Chim Acta 2012; 750: 152–160. [PubMed: 23062436]

25. Capella KM, Roland K, Geldner N, Rey deCastro B, De Jesus VR, van Bemmel D et al. Ethylbenzene and styrene exposure in the United States based on urinary mandelic acid and phenylglyoxylic acid: NHANES 2005–2006 and 2011–2012. Environ Res 2019; 171: 101–110. [PubMed: 30660916]

26. Hornung RW, Reed LD, Estimation of Average Concentration in the Presence of Nondetectable Values. Applied Occupational and Environmental Hygiene 1990; 5: 46–51.

27. Biren C, Zhang L, Bhandari D, Blount BC, De Jesus VR Isoprene Exposure in the United States Based on Urinary IPM3: NHANES 2015–2016. Environ Sci Technol 2020; e-pub ahead of print 2020/01/22; doi 10.1021/acs.est.9b06587.

28. U.S. Centers for Disease Control and Prevention. National Center for Health Statistics Data Presentation Standards for Proportions: Data Evaluation and Methods Research,. In: National Center for Health Statistics, 2017.

29. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005; 113: 192–200. [PubMed: 15687057]

30. Espenship MF, Silva LK, Smith MM, Capella KM, Reese CM, Rasio JP et al. Nitromethane Exposure from Tobacco Smoke and Diet in the U.S. Population: NHANES, 2007–2012. Environ Sci Technol 2019; 53: 2134–2140. [PubMed: 30672285]

31. Etemadi A, Poustchi H, Chang CM, Blount BC, Calafat AM, Wang L et al. Urinary Biomarkers of Carcinogenic Exposure among Cigarette, Waterpipe, and Smokeless Tobacco Users and Never Users of Tobacco in the Golestan Cohort Study. Cancer Epidemiol Biomarkers Prev 2019; 28: 337–347. [PubMed: 30622099]

32. Goniewicz ML, Smith DM, Edwards KC, Blount BC, Caldwell KL, Feng J et al. Comparison of Nicotine and Toxicant Exposure in Users of Electronic Cigarettes and Combustible Cigarettes. JAMA Netw Open 2018; 1: e185937. [PubMed: 30646298]

33. Centers for Disease Control and Prevention C Vital signs: nonsmokers' exposure to secondhand smoke - United States, 1999–2008. Morbidity and Mortality Weekly Report 2010; 59: 1141–1146. [PubMed: 20829748]

34. Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ Health 2008; 7: 27. [PubMed: 18522739]
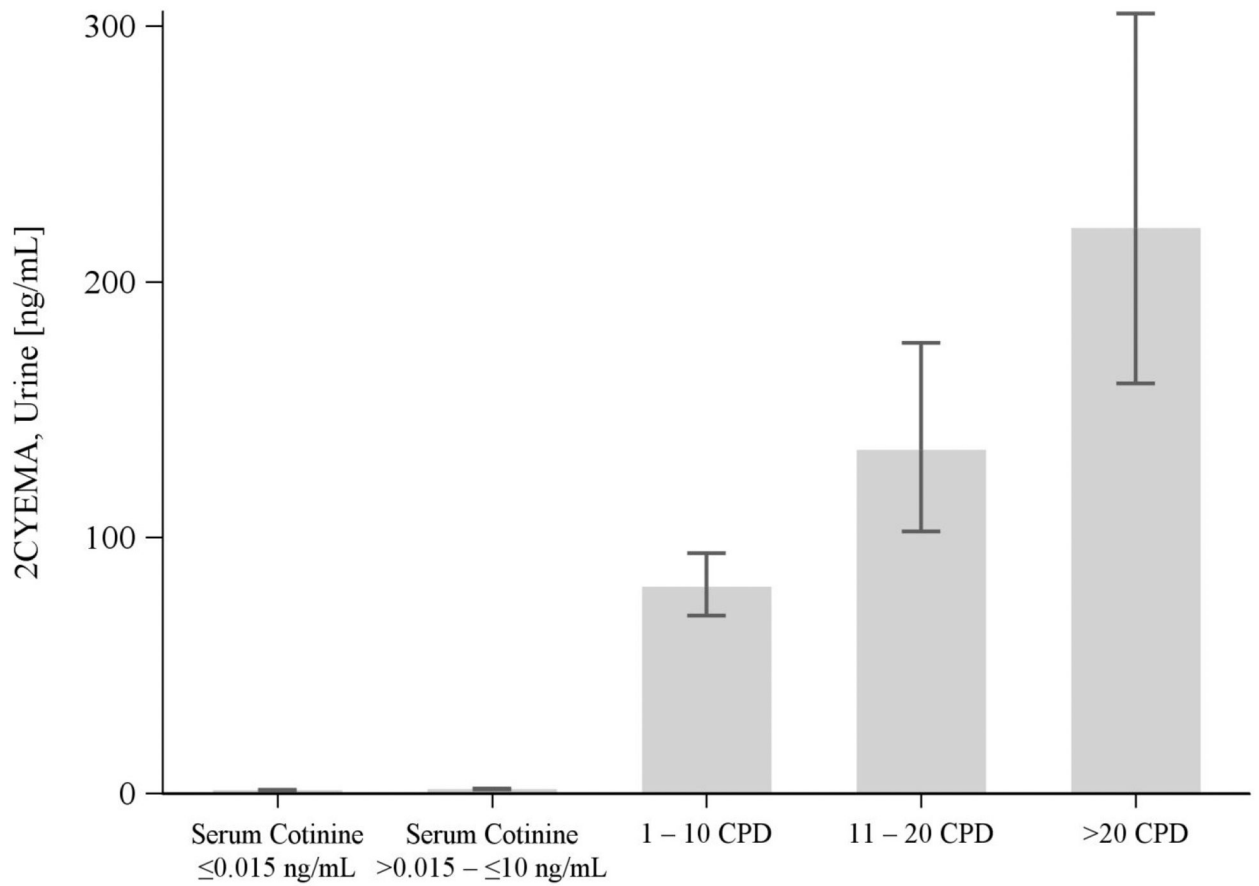
**Figure 1.**
Weighted least squared geometric means (95% confidence intervals) for urinary 2CYEMA concentrations categorized by cigarette smoke exposure (N=6,681).

**Table 1.**

Weighted demographic distribution of NHANES 2011–2016 participants (n = 6,181)[1].

| Characteristic | Level | N[2], Exclusive Smokers | Percent (SE)[3], Exclusive Smokers | N[2] Non-Users | Percent (SE)[3] Non-Users |
|---|---|---|---|---|---|
| Sex | Male | 428 | 50.8 (2.82) | 2,595 | 47.08 (0.78) |
| | Female | 313 | 49.2 (2.82) | 2,845 | 52.92 (0.78) |
| Age | 3 – 5 | 0 | N/A | 257 | 0.85 (0.07) |
| | 6 – 11 | 0 | N/A | 817 | 8.50 (0.41) |
| | 12 – 19 | 41 | 3.92 (0.62) | 939 | 12.84 (0.65) |
| | 20 – 39 | 273 | 38.49 (2.09) | 1,171 | 26.85 (1.06) |
| | 40 – 59 | 276 | 43.07 (2.50) | 1,088 | 27.88 (1.01) |
| | 60 | 151 | 14.52 (1.69) | 1,168 | 23.08 (1.03) |
| Race/Hispanic Origin | Non-Hispanic White | 337 | 67.25 (3.15) | 1,756 | 62.77 (2.40) |
| | Non-Hispanic Black | 199 | 14.39 (1.88) | 1,145 | 10.39 (1.17) |
| | Hispanic | 137 | 12.22 (1.88) | 1,676 | 18.37 (1.86) |
| | Other Race/Multi-Racial | 68 | 6.14 (0.89) | 863 | 8.47 (0.66) |
| Weight Status | Underweight | 21 | 2.91* (0.82) | 96 | 1.41 (0.20) |
| | Normal Weight | 247 | 31.58 (2.03) | 2,107 | 34.82 (1.26) |
| | Overweight/Obesity | 473 | 65.50 (1.89) | 3,237 | 63.77 (1.33) |
| NHANES Cycle | 2011 – 2012 | 259 | 37.19 (2.24) | 1,698 | 33.21 (1.90) |
| | 2013 – 2014 | 230 | 29.18 (1.79) | 1,723 | 31.92 (1.75) |
| | 2015 – 2016 | 252 | 33.62 (2.17) | 2,019 | 34.87 (1.99) |

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

[2]Not weighted

[3]Weighted

N/A: Not applicable

SE: Standard error

**Table 2.**

Sample-weighted urinary 2CYEMA median [25th, 75th percentile] concentrations (μg/g creatinine) categorized by smoking status among NHANES 2011–2016 participants (N = 6,181)[1].

| Characteristic | Level | Exclusive Smokers Median [25th, 75th Percentiles] | Non-Users Median [25th, 75th Percentiles] |
|---|---|---|---|
| | All | 145 [74.9, 240] | 1.38 [0.895, 2.27] |
| Sex | Male | 122 [67.0, 221] | 1.30 [0.850, 2.18] |
| | Female | 174 [92.0, 280] | 1.46 [0.940, 2.36] |
| Age | 3 – 5 | N/A | 2.17 [1.47, 3.60] |
| | 6 – 11 | N/A | 1.77 [1.18, 2.79] |
| | 12 – 19 | 79.3 [17.8, 200] | 1.26 [0.831, 2.10] |
| | 20 – 39 | 115 [58.2, 189] | 1.34 [0.826, 2.37] |
| | 40 – 59 | 188 [96.9, 270] | 1.37 [0.913, 2.21] |
| | 60 | 175 [97.8, 265] | 1.36 [0.885, 2.07] |
| Race/Hispanic Origin | Non-Hispanic White | 171 [92.7, 251] | 1.41 [0.903, 2.32] |
| | Non-Hispanic Black | 119 [66.8, 201] | 1.34 [0.848, 2.36] |
| | Hispanic | 93.4 [32.6, 170] | 1.35 [0.863, 2.11] |
| | Other Race/Multi-Racial | 121 [68.5, 297] | 1.38 [0.905, 2.33] |
| Weight Status | Underweight | 198 [124, 271] | 1.33 [1.01, 1.82] |
| | Normal Weight | 178 [87.2, 268] | 1.46 [0.962, 2.49] |
| | Overweight/Obesity | 130 [71.9, 223] | 1.34 [0.863, 2.21] |
| NHANES Cycle | 2011 – 2012 | 184 [92.1, 264] | 1.55 [0.989, 2.42] |
| | 2013 – 2014 | 126 [66.7, 239] | 1.47 [0.958, 2.58] |
| | 2015 – 2016 | 136 [70.2, 212] | 1.14 [0.783, 1.86] |

[1]Same data as in stratified serum cotinine regression models.

N/A: Not applicable

**Table 3.**

Weighted multiple linear regression model among exclusive smokers (n = 741) for urinary 2CYEMA (ng/ml) in NHANES 2011 – 2016 participants.

| Variable | Level | Exponentiated coefficient [95% CI][1] | p-Value |
|---|---|---|---|
| Intercept | Intercept | 18.6 [12.1, 28.7] | |
| Creatinine, Urine [g/l] | Slope | 1.95 [1.76, 2.17] | <0.0001 |
| Cotinine, Serum [ng/ml] | Slope | 1.00 [1.00, 1.00] | <0.0001 |
| Fasting Time [HH.00] | Slope | 1.00 [0.986, 1.02] | 0.7587 |
| Sex | Male | Ref | |
| | Female | 1.42 [1.08, 1.87] | 0.0142 |
| Age | 3 – 5 | N/A | |
| | 6 – 11 | N/A | |
| | 12 – 19 | 0.843 [0.602, 1.18] | 0.3151 |
| | 20 – 39 | Ref. | |
| | 40 – 59 | 1.25 [1.02, 1.53] | 0.0289 |
| | 60 | 1.47 [1.17, 1.84] | 0.0015 |
| Race/Hispanic Origin | Non-Hispanic White | Ref. | |
| | Non-Hispanic Black | 0.901 [0.724, 1.12] | 0.3441 |
| | Hispanic | 0.880 [0.686, 1.13] | 0.3054 |
| | Other Race/Multi-Racial | 1.01 [0.654, 1.56] | 0.9636 |
| Weight Status | Underweight | 1.21 [0.900, 1.62] | 0.2037 |
| | Normal Weight | Ref. | |
| | Overweight/Obesity | 0.933 [0.763, 1.14] | 0.4913 |
| Food Consumed [kg/d] | Milk Products | 1.03 [0.831, 1.28] | 0.7758 |
| | Meat, Poultry, Fish | 1.07 [0.677, 1.71] | 0.7563 |
| | Eggs | 0.742 [0.187, 2.94] | 0.6653 |
| | Legumes, Nuts, Seeds | 0.801 [0.258, 2.48] | 0.6948 |
| | Grain Products | 0.789 [0.593, 1.05] | 0.1021 |
| | Fruits | 0.994 [0.710, 1.39] | 0.9695 |
| | Vegetables | 1.10 [0.619, 1.95] | 0.7456 |
| | Fats, Oils, Salad Dressings | 0.239 [1.16E-04, 495] | 0.7080 |
| | Sugars, Sweets, Beverages | 1.02 [0.957, 1.08] | 0.6053 |

[1] 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).

N/A: Not applicable

**Table 4.**

Weighted multiple linear regression model among non-users (n = 5,440) for urinary 2CYEMA (ng/ml) in NHANES 2011 – 2016 participants.

| Variable | Level | Exponentiated coefficient [95% CI][1] | p-Value |
|---|---|:---:|:---:|
| Intercept | Intercept | 0.641 [0.505, 0.813] | |
| Creatinine, Urine [g/l] | Slope | 2.05 [1.90, 2.21] | <0.0001 |
| Cotinine, Serum [ng/ml] | Slope | 1.45 [1.37, 1.53] | <0.0001 |
| Fasting Time [HH.00] | Slope | 0.990 [0.983, 0.998] | 0.0135 |
| Sex | Male | Ref. | |
| | Female | 0.973 [0.889, 1.07] | 0.5509 |
| Age | 3 – 5 | 0.983 [0.837, 1.15] | 0.8327 |
| | 6 – 11 | 1.06 [0.914, 1.23] | 0.4248 |
| | 12 – 19 | 0.928 [0.786, 1.09] | 0.3664 |
| | 20 – 39 | Ref. | |
| | 40 – 59 | 1.04 [0.913, 1.19] | 0.5430 |
| | 60 | 1.00 [0.878, 1.14] | 0.9762 |
| Race/Hispanic Origin | Non-Hispanic White | Ref. | |
| | Non-Hispanic Black | 0.960 [0.855, 1.08] | 0.4860 |
| | Hispanic | 0.973 [0.882, 1.07] | 0.5833 |
| | Other Race/Multi-Racial | 0.913 [0.834, 0.999] | 0.0481 |
| Weight Status | Underweight | 0.971 [0.696, 1.36] | 0.8615 |
| | Normal Weight | Ref. | |
| | Overweight/Obesity | 0.963 [0.881, 1.05] | 0.3905 |
| Food Consumed [kg/d] | Milk Products | 1.00 [0.855, 1.17] | 0.9878 |
| | Meat, Poultry, Fish | 0.958 [0.768, 1.19] | 0.6958 |
| | Eggs | 0.653 [0.397, 1.08] | 0.0924 |
| | Legumes, Nuts, Seeds | 1.01 [0.715, 1.42] | 0.9627 |
| | Grain Products | 0.957 [0.827, 1.11] | 0.5516 |
| | Fruits | 1.04 [0.842, 1.27] | 0.7360 |
| | Vegetables | 0.819 [0.617, 1.09] | 0.1611 |
| | Fats, Oils, Salad Dressings | 10.6 [0.378, 299] | 0.1606 |
| | Sugars, Sweets, Beverages | 1.02 [0.977, 1.07] | 0.3498 |

[1]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).

**Table 5.**

Multiple linear regression modeling of urinary N-acetyl-S-(2-cyanoethyl)-L-cysteine (2CYEMA) on predictor variables in NHANES 2011 – 2016 participants.

| Variable | Level | Exponentiated slope [95% CI][1] | p-Value |
|---|---|---|---|
| Intercept | Intercept | 0.620 [0.494, 0.778] | |
| Creatinine, Urine [g/l][2] | Slope | 2.04 [1.92, 2.17] | <0.0001 |
| Fasting Time [HH.00] | Slope | 0.992 [0.985, 0.999] | 0.0269 |
| Tobacco Smoke Exposure | 0.015 ng/ml Serum Cotinine | Ref. | |
| | >0.015 – 10 ng/ml Serum Cotinine | 1.37 [1.27, 1.48] | <0.0001 |
| | 1 – 10 CPD | 68.3 [59.1, 78.9] | <0.0001 |
| | 11 – 20 CPD | 114 [86.8, 150] | <0.0001 |
| | >20 CPD | 186 [135, 255] | <0.0001 |
| Sex | Male | Ref. | |
| | Female | 0.987 [0.906, 1.07] | 0.7504 |
| Age | 3 – 5 | 0.969 [0.825, 1.14] | 0.6978 |
| | 6 – 11 | 1.03 [0.894, 1.20] | 0.6389 |
| | 12 – 19 | 0.912 [0.780, 1.07] | 0.2407 |
| | 20 – 39 | Ref. | |
| | 40 – 59 | 1.03 [0.906, 1.17] | 0.6555 |
| | 60 | 0.991 [0.869, 1.13] | 0.8966 |
| Race/Hispanic Origin | Non-Hispanic White | Ref. | |
| | Non-Hispanic Black | 0.986 [0.887, 1.10] | 0.7909 |
| | Hispanic | 0.947 [0.860, 1.04] | 0.2585 |
| | Other Race/Multi-Racial | 0.866 [0.781, 0.961] | 0.0076 |
| Weight Status | Underweight | 1.03 [0.772, 1.37] | 0.8408 |
| | Healthy Weight | Ref. | |
| | Overweight/Obesity | 0.942 [0.865, 1.03] | 0.1610 |
| Food Consumed [kg/d] | Milk Products | 0.987 [0.850, 1.15] | 0.8586 |
| | Meat, Poultry | 0.996 [0.795, 1.25] | 0.9714 |
| | Eggs | 0.726 [0.407, 1.29] | 0.2702 |
| | Legumes, Nuts, Seeds | 0.923 [0.639, 1.33] | 0.6632 |
| | Grain Products | 0.933 [0.815, 1.07] | 0.3083 |
| | Fruits | 0.999 [0.844, 1.18] | 0.9876 |
| | Vegetables | 0.847 [0.630, 1.14] | 0.2650 |
| | Fats, Oils, Salad Dressings | 4.90 [0.215, 112] | 0.3119 |
| | Sugars, Sweets, Beverages | 1.02 [0.982, 1.05] | 0.3270 |

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

[2]For each unit-increase in the predictor, the expected biomarker concentration in μg/ml is multiplied by the exponentiated coefficient (controlling for other predictors in the model).