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Urinary Nicotine Metabolites and Self-Reported Tobacco Use Among Adults in the Population Assessment of Tobacco and Health (PATH) Study, 2013–2014

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Abstract

Introduction: The Population Assessment of Tobacco and Health (PATH) Study is a longitudinal cohort study on tobacco use behavior, attitudes and beliefs, and tobacco-related health outcomes, including biomarkers of tobacco exposure in the U.S. population. In this report we provide

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Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

Declaration of Interests

Maciej Goniewicz has received a research grant from Pfizer and served as a member of scientific advisory board to Johnson & Johnson, pharmaceutical companies that manufacture smoking cessation medications. No other authors report potential conflicts of interest.

a summary of urinary nicotine metabolite measurements among adult users and non-users of tobacco from Wave 1 (2013–2014) of the PATH Study.

Methods: Total nicotine and its metabolites including cotinine, *trans*-3′-hydroxycotinine (HCTT), and other minor metabolites were measured in more than 11 500 adult participants by liquid chromatography tandem mass spectrometry methods. Weighted geometric means (GM) and least square means from statistical modeling were calculated for non-users and users of various tobacco products.

Results: Among daily users, the highest GM concentrations of nicotine, cotinine and HCTT were found in exclusive smokeless tobacco users, and the lowest in exclusive e-cigarette users. Exclusive combustible product users had intermediate concentrations, similar to those found in users of multiple products (polyusers). Concentrations increased with age within the categories of tobacco users, and differences associated with gender, race/ethnicity and educational attainment were also noted among user categories. Recent (past 12 months) former users had GM cotinine concentrations that were more than threefold greater than never users.

Conclusions: These urinary nicotine metabolite data provide quantification of nicotine exposure representative of the entire US adult population during 2013–2014 and may serve as a reference for similar analyses in future measurements within this study.

Implications: Nicotine and its metabolites in urine provide perhaps the most fundamental biomarkers of recent nicotine exposure. This report, based on Wave 1 of the Population Assessment of Tobacco and Health (PATH) Study, provides the first nationally representative data describing urinary nicotine biomarker concentrations in both non-users, and users of a variety of tobacco products including combustible, e-cigarette and smokeless products. These data provide a urinary biomarker concentration snapshot in time for the entire US population during 2013–2014, and will provide a basis for comparison with future results from continuing, periodic evaluations in the PATH Study.

Introduction

Tobacco use remains the leading preventable cause of morbidity and mortality in the United States,[1] with active smoking and exposure to secondhand smoke (SHS) responsible for more than 480 000 US deaths annually.[1,2] Current exposures to nicotine extend beyond traditional cigarettes and related combustible tobacco sources to include various forms of smokeless products, e-cigarettes, emerging heated products, and other alternative delivery products. In 2011, the Population Assessment of Tobacco and Health (PATH) Study was initiated by the US Food and Drug Administration Center for Tobacco Products (FDA CTP) and the National Institutes of Health, National Institute on Drug Abuse (NIH NIDA) as a longitudinal study of tobacco use, its determinants, and its impacts.[3,4] The PATH Study consists of a nationally representative cohort of tobacco product users, never users, and former users including youth aged 12–17 and adults aged 18 and older. The weighted response rate for the household screener was 54%. Among screened households the overall weighted response rate was 74% for the Adult Interview, and among those completing the Adult Interview, the weighted response rate for providing a urine sample was 63.6%.[3] During the PATH Study, information on tobacco type and use patterns, and urine samples from selected adults, are collected approximately every 12 months for the first four waves,

and biannually thereafter. Primary objectives of the PATH Study are to generate longitudinal epidemiologic data on the status and trends in the types, extent and nature of tobacco product use and biomarker assessed exposure by the US population, and to inform and monitor the impact of FDA's regulatory actions under the terms of the 2009 Family Smoking Prevention and Tobacco Control Act (TCA).[4]

NICT (NICT, COTT, and HCTT are defined as the total urinary content of nicotine, cotinine and *trans*-3′-hydroxycotinine, respectively) is perhaps the most fundamental biomarker of exposure to tobacco products. The presence of NICT and its metabolites in biospecimens indicates systemic exposure, either through active use of tobacco products, secondhand, and/or thirdhand tobacco smoke (SHS, THS), or in some cases, the use of NICT replacement therapy (NRT). Many biological matrices can be used for measurements of NICT and its metabolites, but urine provides the most complete, concentrated, and comprehensive assessment of NICT metabolites in a readily accessible, noninvasive matrix.[5,6] Following absorption, NICT undergoes extensive metabolism in the body with two major metabolites, cotinine (COTT) and *trans*-3′-hydroxycotinine (HCTT), excreted in urine, with lower concentrations of NICT itself and several other, minor metabolites.[7,8] All three main analytes (NICT, COTT and HCTT) form *N*-glucuronides, and HCTT also forms an *O*-glucuronide which is the predominant form for this analyte.

COTT and HCTT are generally preferred over NICT as the primary biomarkers of exposure because of their higher concentrations and longer elimination half-lives.[6–8] In all cases, the extent of NICT exposure based on the analysis of biomarkers can be variably affected by the user's choice and pattern of tobacco use, and by timing, demographic characteristics, and potential differences in metabolic activity.[6]

Here, we report the measurement of total NICT, its various metabolites, and three derived Total Nicotine Equivalents (TNE) values in urine samples from Wave 1 of the PATH Study, representative of never, current, and recent (past 12 months) former tobacco users in the civilian, non-institutionalized US population. Results from the analyses of serum COTT and HCTT have been described elsewhere.[9]

Methods

Interview Data

The PATH Study is a nationally representative, longitudinal cohort study of 45 971 adults and youth, ages 12 years and older. The urinary biomarker results described here are from adults aged 18 and older selected to have their Wave 1 biospecimens analyzed as described in the Wave 1 Biomarker Restricted Use File User Guide.[10] A stratified probability sample of 11 522 adults who completed the Wave 1 (W1) adult interview and who provided a urine specimen were selected for laboratory analyses. Several tobacco use groups were defined, and the weighting procedures used to assure that the biomarker results are nationally representative of members of their respective tobacco use category are described in detail in Section 3 of reference 10 (pp. 7–18). Thus, all weighted biomarker estimates are representative of never, current, and former (past 12 months) users of tobacco products in the US adult population at the time of W 1 (September 12, 2013

to December 14, 2014). Further details of the PATH Study design and methods have been provided previously.[3,4] Survey interview procedures, questionnaires, sampling, weighting and information on accessing the data are available at https://doi.org/10.3886/Series606. The Westat (Rockville MD) Institutional Review Board (IRB) approved the study design and protocol for the PATH Study.

Population Classification

Wave 1 adult participants with urinary biospecimen data were classified into three distinct sets of tobacco user groups for analysis based mainly on the tobacco use constructs outlined by Kasza et al.,[11] and an additional "combined polyuser category" that represents the combination of the three primary sets. These groups were chosen to examine the impact of differential patterns and timeframes of product use on the resulting estimates of exposure. The classification scheme, additional selection steps and definitions used for tobacco users and non-users are outlined in Table 1. Non-users are subdivided between those who reported working or living with smokers, and those who reported no known exposure sources to SHS (non-SHS).

Detection Rates

Summaries of the detection rates are given in Supplementary Table S1. Nearly all biomarkers had detection rates of >92% among tobacco users. The lowest detection rates were for nornicotine which was 92% among all users, and 87% for exclusive e-cigarette users. The COTT and HCTT detection rates were 98% or higher among all participants including all non-users.

Analytical and Statistical Procedures

Nicotine metabolites and analogs were analyzed by LC/MS/MS.[12–14] COTT and HCTT in low concentration samples were measured by a separate method, also by using LC/MS/MS.[15] The two methods were cross validated periodically to assure comparable results. Urinary creatinine was measured by using a Roche Cobas analyzer. These methods are described in detail in the Supplementary Material. All assays adhered to the rigorous requirements of the QC/QA program in place at the CDC NCEH Division of Laboratory Sciences.[16] Samples with creatinine concentrations less than 10 or greater than 370 mg/dL (hydration/dilution outliers) were excluded from any further evaluations to avoid including data from either overly dilute or hyper concentrated urine samples.[17,18]

Statistical analyses were sample weighted using Wave 1 urine weights as described in the Biomarker Restricted Use Files User Guide,[10] and performed using version 9.4 of SAS (SAS Institute Inc, Cary, NC) and SUDAAN version 11.0.0 (Research Triangle Institute, Research Triangle Park, Cary NC). Additional details of these analyses are provided in the Supplementary Material.

Results

Demographics for tobacco users and non-users in this study are summarized in Supplementary Table S2. In addition to user and non-user groups, the participants were

further classified by race/ethnicity, age, sex, and educational attainment. Tobacco users were divided into established daily users *vs.* intermittent users (defined in Table 1), and further included in major sub-categories based on self-report as shown in Table 2 including users of: (a) exclusively combustible products (62.7%), (b) exclusively smokeless tobacco (5.4%), (c) exclusively e-cigarettes (2.9%), and (d) mixed use or "polyusers" (29%) who regularly used more than one type of product.

Non-users of tobacco were further subdivided in some cases into never users of tobacco (73.5%), and recent (past 12 months) former tobacco users. No biomarker samples were analyzed from former users whose last use of tobacco was more than 12 months prior to evaluation. Biomarker concentrations among non-users are subdivided between those who reported working or living with smokers, and those who reported no known exposure sources to SHS.

Table 2 provides the GM and 95% CI, adjusted for creatinine, for NICT, COTT, and HCTT among (a) all tobacco users, (b) those who used combustible, smokeless or e-cigarettes exclusively, and (c) polyusers. Separate results for exclusive cigarette smokers are also provided in Table 2. GM of all analytes among tobacco users overall, and among each of the sub-categories, were substantially higher for daily compared to intermittent users. Among daily users, NICT, COTT and HCTT concentrations all varied by tobacco source. For example, the GM for urinary COTT among daily exclusive users of combustible tobacco was 2776 μg/g creatinine (95% CI 2629, 2932), whereas it was 4323 μg/g (95% CI 3781, 4943) in smokeless tobacco users, and 1691 μg/g (95% CI 1217, 2351) in e-cigarette users. In statistical models of daily users, exclusive users of smokeless tobacco products had significantly higher least-square mean (LSM) COTT concentrations in comparison to exclusive users of combustible tobacco (p value < .0001, Supplementary Table S7). Similar differences in LSM were found for NICT and HCTT (Supplementary Tables S5 and S9). Daily polyusers had adjusted LSM NICT concentrations that were significantly higher than those of exclusive users of combustible tobacco (p value = .0032, Supplementary Table S5). COTT and HCTT LSM concentrations were also significantly higher in polyusers (p value < .0001 in both cases, Supplementary Tables S7 and S9).

Among non-users of tobacco, mean COTT and HCTT concentrations were less than 1 μg/g creatinine (Table 2). It is interesting that former users had GM concentrations of both metabolites that were more than 3 times greater than the concentrations measured in never users.

TNE2 values for both users and non-users of tobacco, and TNE3 values for tobacco users, are summarized in Table 2. The results for these derived values were in all cases similar to those observed for the individual analytes.

Log distributions and a contour plot matrix for all daily tobacco users are shown in Supplementary Figure S1. COTT and HCTT were strongly correlated (r = 0.81), and NICT was also correlated with both COTT and HCTT (r = 0.78 and r = 0.59, respectively). As would be expected, TNE2 and TNE3 were strongly correlated with each other, as was the

correlation between TNE2 and HCTT (r = 0.97). The plot of NICT versus HCTT had the greatest degree of scatter.

Comparison of the TNE2 distribution plots among various categories of daily tobacco users indicates that exclusive combustible product users, exclusive cigarette users, and polyusers, all had similar profiles (Supplementary Figure S2). Conversely, the distribution among exclusive smokeless users was skewed towards higher concentrations. Daily exclusive e-cigarette users had a distribution maximum similar to that of established cigarette smokers, but the e-cigarette users' distribution was notably broader, and displayed more tailing into the lower concentration range than other users.

Urinary total NICT, COTT, and HCTT concentrations among daily tobacco users in groups subdivided by gender, age, race/ethnicity, and educational attainment are given in Table 3. GM concentrations were similar for men and women among smokeless tobacco users, although the number of female smokeless tobacco users was relatively small. The creatinine-adjusted GM concentrations of all three analytes were higher (30%–40%) in women compared to men for both combustible tobacco and among polyusers, although male e-cigarette users had slightly higher COTT and HCTT means. Each age group had progressively higher GM NICT metabolite concentrations, and analyte concentrations were generally higher among those with lower educational attainment in both the smokeless and e-cigarette categories. This difference was attenuated in the exclusive combustible and polyuser categories.

When statistical models were evaluated on the basis of race/ethnicity after adjusting for covariates, total NICT, COTT, and HCTT concentrations in Hispanic people were all significantly lower than in Non-Hispanic White people (NHW) (p value < .0001 in each case, Supplementary Tables S5, S7, and S9). However, comparison of NHW and Non-Hispanic Black people (NHB) was more complex. NHB had significantly lower LSM NICT concentrations (p value < .0001, Supplementary Table S5), slightly higher but not significantly different COTT concentrations (p value = .7731, Supplementary Table S7), and significantly higher HCTT concentrations (p value = .0333, Supplementary Table S9) compared to NHW. By contrast, in a comparison of the Other Race/Multiracial category with NHW, the LSM of NICT was insignificantly higher (p value = .8353, Supplementary Table S5) whereas both COTT and HCTT were significantly lower than in NHW (p value = .0442 and p value < .0001, respectively, Supplementary Tables S7 and S9). However, the Other Race/Multiracial category is both relatively small and heterogeneous relative to the other two groups.

Summary results among daily tobacco users for the minor urinary metabolites nornicotine, nicotine 1′-oxide and cotinine N-oxide in both creatinine-adjusted and unadjusted form are provided in Table 4. Although the GM concentrations were much lower overall, the results were similar to those observed with the major metabolites. Thus, nornicotine, nicotine 1′-oxide, and cotinine N-oxide had similar GM values among daily combustible tobacco and polyusers, whereas the GM were lower in daily exclusive e-cigarette users and about 30% higher in exclusive smokeless tobacco users. Exclusive cigarette smokers had slightly higher concentrations than all combustible product users for all three analytes. Similar results were

seen in both the creatinine-adjusted and unadjusted groups (Table 4). Values for urinary TNE6, the NICT equivalents estimate that is based on NICT and five other major and minor metabolites, should provide the best available estimate of recent NICT intake. As shown in Table 4, TNE6 averaged 42.2 μmol/g creatinine for all users and was 140% higher (101 μmol/g creatinine) in smokeless users. TNE6 was similar for exclusive combustible tobacco product users and polyusers and averaged about 50%–60% higher than for exclusive e-cigarette users in both cases.

Discussion

This study summarizes urinary NICT metabolite measurements in Wave 1 (2013–2014) of the PATH Study. All urinary NICT metabolites reported here are the total concentrations, i.e., they are measured following a prior hydrolysis of the glucuronides, rather than measuring only the free forms. Since the relative amounts of the free and glucuronide forms can vary among individuals, measuring total concentrations should be more reflective of recent prior exposure to NICT, subject to the inherent limitations of spot urine sampling.

It is reasonable to assume that daily tobacco users on average would have higher NICT biomarker concentrations than intermittent users, and that is consistent with our results. Among all users, and among exclusive users of each tobacco type, daily users had substantially higher GM of urinary NICT metabolites than did intermittent users (Table 2) and the adjusted LSM differences were statistically significant in all cases (p value ≤ .001, Supplementary Tables S6, S8, and S10). Similarly, TNE2 GM among intermittent users was on average less than 10% of the value for daily tobacco users in all categories (Table 2).

Smokeless tobacco users were consistently highest in the concentration of all urinary NICT biomarkers in comparison to exclusive users of other tobacco products. In the adjusted LSM models, the mean NICT, COTT, and HCTT concentrations were consistently and significantly higher among exclusive smokeless daily users than nearly all other categories (p value ≤ .0001 in all but two comparisons, Supplementary Tables S6, S8, and S10). Similar significant differences were seen for TNE2 and TNE3 with exclusive smokeless daily users compared to all other categories including polyusers (p values < .0079, Supplementary Tables S12 and S14).

Several previous studies have also found that smokeless users have relatively higher urinary NICT biomarker concentrations than do cigarette smokers.[19–22] The US smokeless user category is a broad one that essentially encompasses all noncombustible tobacco sources (excluding Electronic Nicotine Delivery Systems). Thus “smokeless” includes traditional chewing tobacco such as plug, twist and loose leaf “spit” tobacco, dry and moist snuff, Snus, and dissolvables, all with differing contents and delivery of NICT,[23,24] and with significant variations in pH. With respect to pH, it should be noted that some forms of smokeless tobacco such as Snus and dissolvables also have the highest percentage of unprotonated nicotine (the most absorbable form) of all smokeless products,[23] increasing nicotine bioavailability.

Currently, information on potential differences between urinary NICT metabolite levels between adult daily exclusive combustible product users and exclusive e-cigarette users remains limited. Many previous studies of e-cigarettes have focused on youth and occasional users, and dual use of both cigarettes and e-cigarettes is common. Furthermore, this is an actively evolving area which complicates evaluations. For example, the data reported here were collected about 6 years ago in 2013–2014, prior to subsequent changes such as the 2015 introduction of the currently popular nicotine salts devices and other variants with higher NICT content. However, the longitudinal aspect of the PATH Study is well suited to monitoring the changing landscape of the tobacco market.

Daily exclusive e-cigarette users in this evaluation had lower urinary NICT metabolite levels than exclusive daily users of combustible tobacco products. Some prior studies have reported similar concentrations of urinary NICT metabolites in smokers and e-cigarette users.[25] Conversely, Lorkiewicz et al.[26] reported lower urinary NICT metabolite concentrations in first generation e-cigarette users compared to conventional smokers. Hecht et al.[27] studied 28 mostly daily exclusive e-cigarette users in a study contemporaneous with Wave 1 of the PATH Study. They found urinary COTT GM in the e-cigarette users that were comparable to those reported in one prior study of cigarette smokers, but significantly lower than those reported in a separate, larger study of smokers.[27] The e-cigarette users' urinary COTT GM found by Hecht et al.[27] of 1880 ng/mL was similar to but somewhat higher than the COTT GM found in this study of 1472 ng/mL. It is possible that future Waves of the PATH Study may find increases in NICT metabolite concentrations among exclusive users of newer tobacco products that more efficiently deliver nicotine.[28]

GM of urinary NICT, COTT, and HCTT varied by gender, age, and race-ethnicity among PATH Study Wave 1 tobacco users in a manner that is generally consistent with prior reports.[6,29–31] GM total NICT, COTT and HCTT concentrations were similar between men and women among all tobacco use categories. Gender is known to influence nicotine metabolism.[6] Men typically have higher urinary creatinine concentrations than women which could have biased the ratio, but similar results were found in both the creatinine-adjusted and unadjusted data (Table 3; Supplementary Table S4). Furthermore, in the covariant model adjusting for age, race-ethnicity, creatinine and all forms of tobacco usage, LSM concentrations of all three urinary NICT metabolites were significantly higher among women than men: NICT (p value = .0110, Supplementary Table S5), COTT (p value = .0006, Supplementary Table S7) and HCTT (p value < .0001, Supplementary Table S9).

Age was also associated with higher GM of NICT metabolite concentrations. Among all tobacco use categories, the GM for COTT and HCTT were approximately twice as high in those aged >55 compared to the 18–24 age group, and mean NICT was approximately 4-fold higher in the older group. Overall, a consistent trend of increasing concentrations by age category was seen for all three analytes (Supplementary Tables S5, S7, and S9). When age was included as a continuous variable, the adjusted natural log models for NICT, COTT, and HCTT all had positive slopes ranging from 0.0268 to 0.0305 and were significant in all three cases (p value < .0001). The basis for this trend might include more intensive NICT intake among older users through changes in the number of cigarettes or other tobacco source used per day, more intensive smoking behavior, metabolic changes with age, or self-selection

over time through attrition of lighter smokers who have quit. Evaluation of data from future waves of the PATH Study comparing data from the same participants may help to clarify this matter.

The higher urinary metabolite GM concentrations among NHW vs. NHB users of combustible products and in polyusers (Table 3) differs from the results in prior reports from NHANES in which serum COTT was highest in NHB, albeit by relatively small differences.[32] This appears to reflect a difference between studies rather than biospecimen matrix since serum COTT concentrations in NHW were also higher in the PATH Study than in prior NHANES.[9] Differences in study design or in the relative timing of the studies may account for this. It might be noted that in the serum COTT analyses, the mean differences between the two studies were found to apply only to cigarette smokers and not to all combustible users in general.[9]

Although NHB typically smoke fewer cigarettes per day (CPD), they often have higher serum COTT concentrations per cigarette smoked, probably reflecting differences in smoking topography and NICT metabolic activity.[6,32–34] In Wave 1 of the PATH Study, the average number of cigarettes up to 100 CPD (a small number of reports >100 CPD were excluded as presumed erroneous entries) self-reported by NHB daily exclusive cigarette users was 9.4 (95% CI: 7.9, 10.9, $n = 273$), whereas among NHW daily exclusive cigarette users it was 15.2 (95% CI 14.4, 16.0; $n = 1414$) CPD. However, comparison of NHANES and the PATH Study is complicated by the more detailed product classification scheme used in the PATH Study. Again, the longitudinal nature of the PATH Study may help to confirm and/or clarify these differences.

The correlation of NICT with COTT is strong among daily tobacco users (Supplementary Figure S1; $r = 0.78$), and stronger than the relationship between NICT and HCTT ($r = 0.59$), which had the most scatter overall. Conversely, the correlation between COTT and HCTT ($r = 0.81$) was the strongest among the three major analytes. This is reasonable considering the pathway of metabolism of NICT → COTT → HCTT. The strong correlation between TNE2 and HCTT ($r = 0.97$) was the highest observed overall other than the relation between TNE2 and TNE3. TNE3 in urine would be expected to provide the best overall estimate of recent nicotine exposures based on the three major analytes, and TNE3 was strongly correlated with each, ranging from $r = 0.80$ for NICT to $r = 0.93$ for COTT. TNE6 which is based on the three major NICT analytes, plus additional minor metabolites should provide the best available estimate of recent NICT intake, accounting for 80%–90% of the daily NICT dose when measured in 24-h urine at steady-state.[6,35]

As expected, the lowest NICT metabolite concentrations observed in this study were from non-users of tobacco products. The low concentrations measured in this group represent exposure to environmental sources of NICT including SHS and THS, and those who lived or worked with smokers consistently had higher mean concentrations than those who did not. The highest concentrations overall among non-users were found in SHS-exposed former users, and the lowest among non-exposed never users. Former users had urine levels that were higher relative to never users such that the mean concentration of COTT among *non-exposed* former users was similar to the mean concentration observed among the SHS-

exposed group in the never users' category. However, since the time from the most recent use of tobacco is not specified for the group, former users who only quit relatively recently may have influenced this result. Furthermore, since both smoking status and exposure to SHS were based solely on self-report, the possibility of incorrect assignment from false reporting cannot be excluded. Although a firm basis for this difference is not yet clear, these results do suggest that evaluations of non-users should distinguish between never users and currently abstinent but former users when assessing current exposure to SHS, and also suggest that recording the time since cessation for former users would be helpful to distinguish those who have only recently quit from other non-users.

Urinary NICT metabolites are fundamental biomarkers of nicotine exposure in people and this article presents the mean concentrations among both non-users and users of various types of tobacco products in Wave 1 of the PATH Study. These results document important differences in biomarker levels according to both frequency of use and type of products used.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data are available in a public, open-access repository, the National Addiction and HIV Data Archive: https://www.icpsr.umich.edu/web/NAHDAP/studies/36498.

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Table 1.

Tobacco User Classification

Tobacco user group	Definition
All tobacco users (*n* = 8875)	All current established every day or intermittent cigarette, cigar, cigarillo, filtered cigar, pipe, hookah, smokeless, snus pouches, dissolvable, and e-cigarette users.
Exclusive combustible product user (*n* = 5565)	
Daily (*n* = 3180)	Current users who are both established and every day users of only cigarette, cigar, cigarillo, filtered cigar, pipe, and/or hookah; not users of any other tobacco products.
Intermittent (*n* = 2385)	Current experimental or someday users who use only cigarette, cigar, cigarillo, filtered cigar, pipe, and/or hookah; not users of any other tobacco products.
Exclusive smokeless product user (*n* = 476)	
Daily (*n* = 355)	Current users who are both established and every day users of only smokeless, snus, and/or dissolvable products; not using any other tobacco products.
Intermittent (*n* = 121)	Current experimental or someday users who use only smokeless, snus, and/or dissolvable products; not using any other tobacco products.
Exclusive e-cigarette user (258)	
Daily (*n* = 152)	Current users who are both established and every day users of only e-cigarettes; not using any other tobacco products.
Intermittent (*n* = 106)	Current experimental or someday users of only e-cigarettes; not using any other tobacco products.
Polyuser (*n* = 2576)	
Daily (*n* = 1987)	Current established every day user of combustibles, smokeless, and/or e-cigarettes and daily or intermittent user of at least one other category.
Intermittent (*n* = 589)	Current experimental or someday users of combustibles, smokeless, and/or e-cigarettes and intermittent user of at least one other category.
All non-users (*n* = 2098)	The combination of former users and never users.
Former (*n* = 557)	Former established or experimental cigarette, cigar, cigarillo, filtered cigar, pipe, hookah, smokeless tobacco, snus, dissolvable, and/or e-cigarette users, including former Nicotine replacement therapy (NRT) users, all with the last use within the previous 12 months.
Never (*n* = 1541)	Never used any tobacco product (cigarette, cigar, cigarillo, filtered cigar, pipe, hookah, smokeless tobacco, snus pouches, dissolvable, and/or e-cigarette) or NRT.

1. These sample sizes are for cotinine, *trans*-3′-hydroxycotinine, and TNE2. Sample sizes are smaller for nicotine and the minor metabolites due to participants missing measurements for nicotine or minor metabolites.

2. Tobacco use classifications are based on coding self-reported data PRIOR to weighting.

3. Established cigarette user: used more than 100 cigarettes in lifetime and currently smokes every day or some days; established user of product other than cigarettes: used product fairly regularly and currently uses every day or some days.

4. Experimental cigarette user: used less than 100 cigarettes in lifetime and currently smokes every day or some days; experimental user of product other than cigarettes: never used product fairly regularly and currently uses every day or some days.

5. All “user” categories (except for former user) exclude pharmaceutical nicotine sources such as nicotine replacement therapy (NRT).

6. All “user” categories (except for never user) can include former users of any tobacco products or pharmaceutical nicotine sources.

7. All retained subjects had urine creatinine levels (10 ≤ UCREAT ≤ 370) to eliminate abnormally dilute or concentrated samples.

8. All retained subjects had values for age, gender, race-ethnicity, educational level, and tobacco use status.

9. All retained subjects included COTT and HCTT measurements including those with non-detectable levels (such as nonsmokers).

10. Exclusions were made for: use of NRT = 114; creatinine <10 or >370 = 247; lack of information on product use group = 136; no measurement of COTT or HCTT available = 49; withdraw = 3. Wave 1 received subjects (11 522) – exclusions (549) = sample size of 10 973.

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Table 2.

Sample Weighted Geometric Mean Concentrations and 95% Confidence Intervals for Tobacco Users and Non-users Subdivided by Analyte and Category

	Nicotine (μg/g creat)		Cotinine (μg/g creat)		Hydroxycotinine (μg/g creat)		TNE2* (μmol/g creat)		TNE3* (μmol/g creat)	
	Daily	**Intermittent**	**Daily**	**Intermittent**	**Daily**	**Intermittent**	**Daily**	**Intermittent**	**Daily**	**Intermittent**
Tobacco users										
All users (*n*)	1281 [1218, 1347] 5610	177 [156, 201] 1900	2845 [2736, 2958] 5674	37.3 [31.1, 44.8] 3201	4877 [4655, 5110] 5674	65.0 [53.9, 78.4] 3201	43.7 [41.9, 45.5] 5674	0.58 [0.48,0.70] 3201	57.6 [55.6, 59.5] 5610	12.0 [10.9, 13.3] 1900
Combustible (*n*)	1281 [1192, 1377] 3150	172 [146, 202] 1374	2776 [2629, 2932] 3180	31.5 [25.9, 38.3] 2385	4800 [4492, 5129] 3180	54.1 [44.4, 66.0] 2385	42.9 [40.5, 45.4] 3180	0.49 [0.40, 0.59] 2385	56.3 [53.9, 58.7] 3150	11.6 [10.4, 13.0] 1374
Smokeless (*n*)	1931 [1690, 2206] 349	294 [167, 517] 98	4323 [3781, 4943] 355	292 [129, 660] 121	7923 [6949, 9034] 355	517 [229, 1167] 121	68.7 [60.4, 78.2] 355	4.57 [2.04, 10.2] 121	92.6 [83.8, 102] 349	21.6 [14.4, 32.4] 98
E-cigarette (*n*)	785 [620, 993] 148	77.5 [33.6, 179] 43	1691 [1217, 2351] 152	8.16 [4.17, 16.0] 106	2934 [2124, 4052] 152	16.1 [8.61, 30.0] 106	26.3 [19.2, 36.1] 152	0.14 [0.07, 0.26] 106	37.0 [30.2, 45.3] 148	4.69 [2.34, 9.41] 43
Polyuser (*n*)	1230 [1151, 1314] 1963	195 [147, 259] 385	2894 [2729, 3070] 1987	78.7 [54.7, 113] 589	4798 [4489, 5129] 1987	143 [99.3, 207] 589	43.4 [40.9, 46.1] 1987	1.25 [0.87, 1.80] 589	57.3 [54.4, 60.4] 1963	13.5 [11.0, 16.7] 385
Cigarette (*n*)	1451 [1325, 1590] 2021	303 [243, 378] 684	3063 [2876, 3263] 2037	272 [193, 383] 837	5242 [4826, 5694] 2037	485 [341, 688] 837	46.9 [43.8, 50.3] 2037	4.34 [3.07, 6.13] 837	61.5 [58.2, 65.0] 2021	18.7 [16.0, 21.8] 684

	Cotinine (μg/g creat)			Hydroxycotinine (μg/g creat)			TNE2* (μmol/g creat)		
	All	**Non-SHS**	**SHS**	**All**	**Non-SHS**	**SHS**	**All**	**Non-SHS**	**SHS**
Non-users									
All non-users (*n*)	0.49 [0.42, 0.57] 2098	0.34 [0.30, 0.40] 1373	1.13 [0.86, 1.48] 725	0.81 [0.70, 0.94] 2098	0.57 [0.49, 0.65] 1373	1.95 [1.45, 2.62] 725	0.007 [0.006, 0.008] 2098	0.005 [0.004, 0.006] 1373	0.017 [0.013, 0.023] 725
Never users (*n*)	0.42 [0.36, 0.49] 1541	0.31 [0.27, 0.35] 1082	0.95 [0.70, 1.27] 459	0.69 [0.59, 0.81] 1541	0.50 [0.43, 0.58] 1082	1.62 [1.16, 2.27] 459	0.006 [0.005, 0.007] 1541	0.005 [0.004, 0.005] 1082	0.015 [0.011, 0.020] 459
Former users (*n*)	1.36 [0.96, 1.92] 557	0.89 [0.57, 1.38] 291	2.64 [1.50, 4.65] 266	2.44 [1.70, 3.51] 557	1.58 [1.01, 2.48] 291	4.78 [2.69, 8.49] 266	0.021 [0.015, 0.030] 557	0.014 [0.009, 0.021] 291	0.042 [0.024, 0.074] 266

*TNE2 = (total cotinine/176.2151) + (total *trans*-3′-hydroxycotinine/192.2145) nmol/mL; TNE3 = (total nicotine/162.2316) + (total cotinine/176.2151) + (total *trans*-3′-hydroxycotinine/192.2145) nmol/mL.

Table 3.

Sample Weighted Geometric Mean Concentrations and 95% Confidence Intervals for Daily Tobacco Users Subdivided by Sex, Age, Race-Ethnicity and Education Attainment*

	Combustible, μg/g creat			Smokeless, μg/g creat			E-cigarette, μg/g creat			Polyuser, μg/g creat		
	NICT	COTT	HCTT	NICT	COTT	HCTT	NICT	COTT	HCTT	NICT	COTT	HCTT
All users (*n*)	1281 [1192,1377] 3,150	2776 [2629,2932] 3,180	4800 [4492,5129] 3,180	1931 [1690,2206] 349	4323 [3781,4943] 355	7923 [6949,9034] 355	785 [620,993] 148	1691 [1217,2351] 152	2934 [2124,4052] 152	1230 [1151,1314] 1,963	2894 [2729,3070] 1,987	4798 [4489,5129] 1,987
Female (*n*)	1477 [1332,1638] 1,558	3268 [3023,3533] 1,571	5790 [5295,6331] 1,571	1780 [1113,2848]* 19	4729 [2938,7610]* 19	9774 [6714,14229]* 19	824 [567,1198] 89	1541 [900,2641] 93	2751 [1595,4745] 93	1445 [1278,1633] 748	3329 [3029,3659] 759	5934 [5332,6603] 759
Male (*n*)	1117 [1015,1230] 1,592	2373 [2216,2542] 1,609	4009 [3698,4347] 1,609	1939 [1687,2228] 330	4305 [3741,4953] 336	7844 [6833,9005] 336	735 [554,976] 59	1930 [1498,2486] 59	3215 [2526,4091] 59	1116 [1018,1224] 1,215	2661 [2431,2913] 1,228	4224 [3875,4604] 1,228
Age 18–24 (*n*)	423 [343,521] 647	1638 [1428,1879] 652	2592 [2289,2936] 652	424 [284,632] 51	1115 [518,2396]* 55	1902 [918,3942]* 55	492 [239,1011]* 15	1325 [650,2703]* 15	2684 [1243,5795]* 15	491 [425,568] 592	1699 [1468,1966] 602	2729 [2302,3235] 602
Age 25–34 (*n*)	931 [792,1094] 672	2542 [2297,2814] 677	4394 [3894,4957] 677	1422 [981,2061] 60	3064 [2298,4085] 61	5342 [4010,7115] 61	603 [405,897]* 42	1532 [923,2543]* 43	2503 [1604,3908]* 43	1013 [868,1183]482	2739 [2418,3102] 485	4289 [3878,4744] 485
Age 35–54 (*n*)	1616 [1454,1796] 1,190	3004 [2764,3265] 1,201	5170 [4691,5698] 1,201	2452 [2123,2833] 161	5512 [4919,6176] 161	10227 [8938,11702] 161	920 [656,1290] 60	2385 [1728,3294] 60	4074 [2801,5926] 60	1818 [1627,2031] 658	3461 [3101,3863] 666	5781 [5127,6518] 666
Age 55+ (*n*)	1852 [1683,2038] 641	3293 [2925,3707] 650	5955 [5122,6923] 650	2317 [1864,2880] 77	5245 [3779,7279] 78	9903 [7147,13722] 78	955 [544,1678]* 31	1178 [543,2556]* 34	2177 [916,5174]* 34	2126 [1888,2393] 231	4044 [3586,4561] 234	7590 [6578,8758] 234
Non-Hispanic White (*n*)	1612 [1487,1746] 2,039	3515 [3337,3703] 2,053	5750 [5377,6150] 2,053	1959 [1694,2267] 297	4585 [3984,5277] 301	8199 [7115,9447] 301	899 [704,1149] 123	1911 [1408,2593] 126	3245 [2384,4417] 126	1393 [1300,1492] 1,481	3415 [3218,3623] 1,493	5602 [5239,5989] 1,493
Non-Hispanic Black (*n*)	695 [578,835] 544	1506 [1299,1745] 550	3413 [2818,4135] 550	1149 [378,3494]* 15	1148 [288,4587]* 16	3449 [753,15794]* 16	254 [96.9,664]* 9	455 [146,1423]* 10	1081 [345,3391]* 10	760 [574,1006] 136	1264 [971,1645] 140	2716 [2109,3498] 140
Hispanic (*n*)	770 [661,897] 348	1678 [1325,2126] 355	3150 [2400,4134] 355	2638 [1500,4638]* 10	6511 [4305,9847]* 10	11433 [7081,18459]* 10	222 [53.2,925]* 8	809 [150,4371]* 8	1651 [342,7975]* 8	581 [427,790] 174	1237 [918,1668] 180	2203 [1622,2993] 180
Other Race/ Multiracial (*n*)	1112 [839,1475] 219	2212 [1758,2782] 222	2677 [1884,3802] 222	1816 [1369,2410]* 27	3774 [1835,7764]* 28	7137 [3385,15052]* 28	1532 [648,3622]* 8	3693 [1981,6884]* 8	4347 [2028,9319]* 8	994 [744,1329] 172	2383 [1909,2973] 174	3130 [2355,4160] 174

	Combustible, μg/g creat			Smokeless, μg/g creat			E-cigarette, μg/g creat			Polyuser, μg/g creat		
	NICT	**COTT**	**HCTT**	**NICT**	**COTT**	**HCTT**	**NICT**	**COTT**	**HCTT**	**NICT**	**COTT**	**HCTT**
Less than High School Diploma or GED (*n*)	1379 [1210,1570] 995	2770 [2485,3088] 1,009	4790 [4217,5441] 1,009	2274 [1843,2806] 88	4496 [3175,6366] 91	8723 [6172,12327] 91	1303 [942,1802]* 24	1850 [862,3974]* 25	2878 [1315,6301]* 25	1487 [1295,1707] 536	3126 [2733,3576] 541	5168 [4483,5958] 541
High School Diploma (*n*)	1363 [1185,1567] 826	2914 [2684,3163] 832	5133 [4638,5681] 832	2004 [1548,2595] 102	5061 [4117,6222] 103	8363 [6687,10459] 103	525 [347,794]* 36	1310 [816,2103]* 37	2524 [1597,3988]* 37	978 [842,1137] 490	2469 [2098,2906] 499	4228 [3600,4964] 499
Some College or Associates Degree (*n*)	1127 [975,1303] 1,119	2684 [2427,2967] 1,127	4683 [4181,5245] 1,127	1789 [1473,2173] 121	3734 [2812,4957] 123	7055 [5407,9206] 123	866 [561,1338] 65	1845 [1183,2878] 67	2958 [1760,4973] 67	1164 [1032,1313] 767	2900 [2648,3176] 777	4668 [4208,5179] 777
Bachelors or Advanced Degree (*n*)	1316 [1116,1551] 210	2648 [2263,3098] 212	4015 [3245,4968] 212	1506 [985,2304]* 38	3790 [2887,4976]* 38	7460 [5819,9563]* 38	743 [405,1365]* 23	1937 [1107,3388]* 23	3847 [2325,6367]* 23	1564 [1335,1831] 170	3408 [3007,3862] 170	5827 [4891,6942] 170

*Estimate should be interpreted with caution because it has low precision. It is based on a sample size of less than 50, or the relative standard error is larger than 30%.

Table 4.

Minor Metabolites and Total Nicotine Equivalents Among All Users and in Daily Users of Specific Tobacco Types

	Creatinine-adjusted weighted geometric mean concentrations and 95% confidence intervals for daily users			
	Nicotine 1′-oxide (μg/g creat)	**Cotinine *N*-oxide (μg/g creat)**	**Nornicotine (μg/g creat)**	**TNE6* (μmol/g creat)**
All users (*n*)	244 [232, 256] (7510)	236 [227, 247] (7510)	46.3 [44.3, 48.3] (7501)	42.2 [40.4, 44.0] (7501)
Combustible (*n*)	389 [368, 410] (3150)	344 [329, 360] (3150)	69.9 [66.6, 73.3] (3143)	61.6 [59.0, 64.2] (3143)
Smokeless (*n*)	659 [585, 741] (349)	516 [466, 573] (349)	99.5 [88.4, 112] (348)	101 [91.3, 111] (348)
E-cigarette (*n*)	284 [231, 350] (148)	209 [171, 255] (148)	29.9 [24.6, 36.4] (148)	40.4 [33.0, 49.4] (148)
Polyuser (*n*)	382 [363, 401] (1963)	347 [330, 364] (1963)	65.5 [62.2, 68.9] (1963)	62.4 [59.3, 65.6] (1963)
Cigarette (*n*)	432 [405, 461] (2021)	368 [348, 390](2021)	76.1 [71.6, 80.9](2014)	67.3 [63.8, 71.1](2014)
	Unadjusted weighted geometric mean concentrations and 95% confidence intervals for daily users			
	Nicotine 1′-oxide ng/mL	**Cotinine *N*-oxide ng/mL**	**Nornicotine ng/mL**	**TNE6* nmol/mL**
All users (*n*)	247 [234, 260] (7510)	239 [228, 251] (7510)	46.7 [44.7, 48.8] (7501)	42.6 [40.8, 44.4] (7501)
Combustible (*n*)	380 [357, 405] (3150)	336 [318, 356] (3150)	68.1 [64.7, 71.6] (3143)	59.9 [57.3, 62.7] (3143)
Smokeless (*n*)	662 [591, 742] (349)	519 [467, 577] (349)	99.8 [90.0, 111] (348)	101 [93.0, 110] (348)
E-cigarette (*n*)	249 [199, 313] (148)	183 [149, 226] (148)	26.3 [21.4, 32.2] (148)	35.4 [28.9, 43.5] (148)
Polyuser (*n*)	371 [349, 394] (1963)	337 [317, 358] (1963)	63.6 [59.4, 68.1] (1963)	60.6 [57.3, 64,1] (1963)
Cigarette (*n*)	406 [379, 435] (2021)	346 [324, 369](2021)	71.0 [67.0, 75.4](2014)	62.9 [59.6, 66.3](2014)

*TNE6 = (total nicotine/162.2316) + (total cotinine/176.2151) + (total *trans*-3′-hydroxycotinine/192.2145) + (total cotinine N-oxide/192.2145) + (nicotine 1′-oxide/178.231) + (nornicotine/148.2050) nmol/mL.