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Original Investigation

Tobacco-Specific Nitrosamines (NNAL, NNN, NAT, and NAB) Exposures in the US Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013–2014)

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Abstract

Introduction: The tobacco-specific nitrosamines (TSNAs) are an important group of carcinogens found in tobacco and tobacco smoke. To describe and characterize the levels of TSNAs in the Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013–2014), we present four biomarkers of TSNA exposure: *N*′-nitrosonornicotine, *N*′-nitrosoanabasine, *N*′-nitrosoanatabine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) which is the primary urinary metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

Methods: We measured total TSNAs in 11 522 adults who provided urine using automated solid-phase extraction coupled to isotope dilution liquid chromatography–tandem mass spectrometry. After exclusions in this current analysis, we selected 11 004 NNAL results, 10 753 *N*′-nitrosonornicotine results, 10 919 *N*′-nitrosoanatabine results, and 10 996 *N*′-nitrosoanabasine results for data analysis. Geometric means and correlations were calculated using SAS and SUDAAN.

Results: TSNA concentrations were associated with choice of tobacco product and frequency of use. Among established, every day, exclusive tobacco product users, the geometric mean urinary NNAL concentration was highest for smokeless tobacco users (993.3; 95% confidence

interval [CI: 839.2, 1147.3] ng/g creatinine), followed by all types of combustible tobacco product users (285.4; 95% CI: [267.9, 303.0] ng/g creatinine), poly tobacco users (278.6; 95% CI: [254.9, 302.2] ng/g creatinine), and e-cigarette product users (6.3; 95% CI: [4.7, 7.9] ng/g creatinine). TSNA concentrations were higher in every day users than in intermittent users for all the tobacco product groups. Among single product users, exposure to TSNAs differed by sex, age, race/ethnicity, and education. Urinary TSNAs and nicotine metabolite biomarkers were also highly correlated.

Conclusions: We have provided PATH Study estimates of TSNA exposure among US adult users of a variety of tobacco products. These data can inform future tobacco product and human exposure evaluations and related regulatory activities.

Introduction

More than 70 carcinogens, including tobacco-specific nitrosamines (TSNAs), have been identified in tobacco and cigarette smoke.[1–3] Prevalent TSNAs include 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*′-nitrosonornicotine (NNN), *N*′-nitrosoanabasine (NAB), and *N*′-nitrosoanatabine (NAT). A predominant metabolite of NNK-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is the main TSNA measurable in urine. TSNAs play an important role in carcinogenesis in tobacco product users and nonusers who are exposed to tobacco. The World Health Organization (WHO) Study Group on Tobacco Product Regulation has identified NNK and NNN as major contributors to tobacco smoke carcinogenicity.[3,4] The International Agency for Research on Cancer (IARC) categorizes NNK and NNN as IARC Group I carcinogens in humans, a designation used when there is sufficient evidence of carcinogenicity in humans.[3,5] NNK is regarded as an important cause of lung cancer in humans, and NNN has been shown to induce cancers of the oral cavity, esophagus, nasal cavities, and respiratory tract in laboratory animals.[6,7]

Exposure to TSNAs can be assessed by measuring the sum of the free and glucuronide conjugated forms of TSNAs and their metabolites in human urine.[4,8,9] Specifically, urinary NNAL (the primary urinary metabolite of NNK) has been used widely as a biomarker of human exposure.[6,10,11] In the United States, population exposure to NNK has been assessed since 2007 by measuring total NNAL in urine collected as part of the National Health and Nutrition Examination Survey (NHANES).[8,9] These data indicate widespread NNK exposure among the general population.[8,9] General population exposure data for NNN, NAT, and NAB have not been previously reported. In addition, changing tobacco usage patterns may modify TSNA exposures, especially as electronic nicotine delivery devices become more prevalent.[12–14]

The Population Assessment of Tobacco and Health (PATH) Study began data collection in 2013 to generate longitudinal epidemiologic data on tobacco use behaviors, including patterns of use, attitudes, beliefs, exposures, and health outcomes among the US population to inform and to monitor the impact of U.S. Food and Drug Administration (FDA) regulatory actions to reduce tobacco-related population harm.[15,16] The health impact of tobacco use depends on many factors, including the specific products which are used. The PATH Study assesses a broad distribution of tobacco products (eg, cigarettes, e-cigarettes, traditional cigars, cigarillos, filtered cigars, pipe tobacco, hookah tobacco, smokeless tobacco [SLT], snus pouches, and dissolvable tobacco).[15–17] Tobacco products can be grouped into combustible (cigarettes, traditional cigars, cigarillos, filtered cigars, pipes, and hookah tobacco) and noncombustible products (noncombustible) (e-cigarettes, SLT, snus pouches, and dissolvable tobacco).

Total urinary TSNA concentrations were measured in Wave 1 (W1) of the PATH Study among adults, which was conducted from September 12, 2013 to December 15, 2014. Extensive information on tobacco-use patterns and other demographic data were collected from all study participants.[6] In this report, we describe the PATH Study W1 TSNA exposure data which provides population exposure levels during the time frame of Wave 1, and which also will provide a useful reference point for further evaluations of TSNA exposures in future waves of the PATH Study data and potentially other studies as well.

Materials and Methods

Study Design

The PATH Study is a nationally representative, longitudinal cohort study of approximately 46 000 US adults and youth, ages 12 years and older. The National Institutes of Health, through the National Institute on Drug Abuse, is partnering with the U.S. Food and Drug Administration's Center for Tobacco Products to conduct the PATH Study under a contract with Westat (Rockville, MD). The PATH Study used audio-computer assisted self-interviews (ACASI) available in English and Spanish to collect information on tobacco-use patterns and associated health behaviors. This current analysis draws from the 32 320 W1 adult interviews (all participants aged 18 years or older), including both users and nonusers of tobacco. Recruitment employed address-based, area-probability sampling, using an in-person household screener to select youth and adults. In the PATH Study, adult tobacco users, young adults ages 18–24, and African Americans were over-sampled relative to their proportion in the population. Further details regarding PATH Study design and methods have been described by Hyland et al.[15] Details on survey interview procedures, questionnaires, sampling, weighting, and information on accessing the data are available on the PATH Study website at https://doi.org/10.3886/Series606. Westat's institutional review board approved the study design and data collection protocol.

Biospecimens collected from a subset of all adult respondents were sent to the laboratory for analyses. Detailed sample collection procedures are described in the Supplementary Materials. A stratified probability sample of 11 522 adults who completed the W1 adult interview and who provided a urine specimen were selected for laboratory analyses. The sample was selected to ensure respondents represented diverse tobacco product use patterns, including users of multiple tobacco products, and never users of any tobacco product. Given that not all respondents agreed to provide biospecimens, the resulting biospecimen assay data represent a subsample, and specific urine weights are needed to account for potential differences between the full set of adult interview respondents

in the specified tobacco product user groups and the set of adults with analyzed biospecimens. These weighted estimates are representative of never, current, and recent former (within 12 months) users of tobacco products in the US civilian, noninstitutionalized adult population at the time of Wave 1. These weighting procedures are outlined in the Biomarker Restricted Use Files User Guide (found here, https://doi.org/10.3886/Series606).

Tobacco Use Categories

Participants were asked a series of questions about each tobacco product, including whether they ever used the product, even if only one or two puffs/times; whether they now smoke or use the product every day, some days, or not at all; whether they ever used the product "fairly regularly"; and how much of the product they have used in their lifetime. Based on these responses, we defined the following tobacco use categories for analyses: exclusive combustible users, exclusive e-cigarette users, exclusive SLT users, and poly users. Each category was subdivided into every day established vs. intermittent users. Nonusers were similarly subcategorized as former tobacco (former) users or never tobacco (never) users. We defined a total of eight groups among all tobacco users, and two subgroups among nonusers as described in Table 1.

Laboratory and Statistical Analysis

The TSNAs were analyzed by liquid chromatography–tandem mass spectrometry using procedures which have been previously described.[18,19] Total urinary nicotine metabolites were measured by the method of Wei et al.[20] Further descriptions of the methodology used in this work are provided in the Supplementary Materials.

Except for Supplementary Table S1, all other statistical analyses were weighted (see weighting procedures outlined in the Biomarker Restricted Use Files User Guide (http://doi.org/10.3886/ICPSR36231) and performed using version 9.4 statistical software application (SAS Institute Inc, Cary, NC) and SUDAAN version 11.0.0 (Research Triangle Institute, Research Triangle Park, Cary, NC). The variance estimate was a balanced repeated replication obtained using Fay's method with the adjustment factor 0.3.[21] We calculated the weighted frequency of detection for each analyte. We also calculated the geometric mean (GM) and 95% confidence interval (CI) for volume-based and creatinine-corrected concentration. To minimize the influence of urine dilution, the volume-based concentrations of TSNA (pg/mL) were normalized by urinary creatinine and presented here as creatinine-corrected TSNA concentrations (ng/g creatinine).[22] For the final dataset, we excluded participants with overly dilute (<10 mg/dL

Table 1. Tobacco User Groups

Tobacco user groups	Definition
All tobacco users (*n* = 8882)	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* = 5576)	
Every day (*n* = 3176)	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* = 2400)	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)	
Every day (*n* = 355)	Current users who are both established and every day users of only smokeless, snus pouches, and/or dissolvable products; not using any other tobacco products.
Intermittent (*n* = 121)	Current experimental or someday users of only smokeless, snus pouches, and/or dissolvable tobacco; not using any other tobacco products.
Exclusive e-cigarette user (*n* = 258)	
Every day (*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.
Poly user (*n* = 2572)	
Every day (*n* = 1983)	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 nonusers (*n* = 2122)	All former users and never users.
Former (*n* = 559)	Former established or experimental cigarette, cigar, cigarillo, filtered cigar, pipe, hookah, smokeless tobacco, snus pouches, dissolvable, and/or e-cigarette users or former NRT users.
Never (*n* = 1563)	Never used any tobacco product (cigarette, cigar, cigarillo, filtered cigar, pipe, hookah, smokeless tobacco, snus pouches, dissolvable, and/or e-cigarette) or NRT.

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; PATH = Population Assessment of Tobacco and Health.

[a]Established cigarette user: used more than 100 cigarettes in lifetime; established user of product other than cigarettes: used product fairly regularly.

[b]All "user" categories (except for former user) exclude pharmaceutical nicotine sources such as nicotine replacement therapy (NRT).

[c]All "user" categories (except for never user) can include former users of any tobacco products or pharmaceutical nicotine sources.

[d]All retained subjects had urine creatinine levels (10 < UCREAT < 370) to eliminate abnormally dilute or concentrated samples.

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

[f]All retained subjects included NNAL measurements including those with nondetectable levels (such as nonsmokers).

[g]Exclusions were made for: creatinine <10 or >370 = 250; no measurement of NNAL available = 22; lack of information on product use group = 132; use of NRT = 111; dropping out of the PATH study participants = 3. Wave 1 received subjects (11 522) – exclusions (518) = sample size of 11 004.

urinary creatinine) or hyperconcentrated (>370 mg/dL urinary creatinine) urine samples (250 participants)[23,24]; missing tobacco user categories (132 participants); current nicotine replacement therapy users (111 participants); as well as those with missing TSNA measurements, NNAL (22 participants); missing NNN (251 participants), missing NAT 85 participants), missing NAB (8 participants). Additionally, three participants dropped out of the PATH Study. NNAL was analyzed in 11 004 PATH Study W1 participants. A total of 10 753 NNN results, 10 919 NAT results, and 10 996 NAB results were also analyzed.

We produced linear regression models with urinary NNAL (natural log-transformed) as the dependent variable and gender, age, race/ethnicity, education, tobacco user group, and creatinine concentration as predictors. All urinary NNAL concentrations were natural log-transformed to reduce skewed distributions. We also evaluated pairwise differences in least square means of urinary NNAL between tobacco user groups and adjusted the significance level with Bonferroni correction to control the false positive rate arising from multiple testing.

Results

We used Restricted Use Files (RUF) and Biomarker Restricted Use Files (BRUF) from the PATH Study to categorize all tobacco users (eight subgroups) and nonusers (two subgroups) (Table 1). Supplementary Table S1 presents the demographic counts (sex, age, race/ethnicity, and education) of the study individuals for NNAL data categorized by 10 different tobacco use categories.

Table 2 summarizes the creatinine-corrected GMs, 95% CIs, and detection rates for TSNAs classified by self-reported tobacco use status, and Table 3 provides the data divided by different demographic and user groups. As shown in Table 2, the detection rates for NNAL, NNN, NAT, and NAB among all tobacco users were 96%, 68%, 75%, and 72%, respectively. For NNAL, we observed a high detection rate (>85%) even for intermittent users. Among established every day tobacco product users, the GM of urinary NNAL was highest for exclusive SLT users (993.3, 95% CI: [839.2, 1147.3] ng/g creatinine), followed by exclusive cigarette users (300.9, 95% CI: [277.8, 324.0] ng/g creatinine); exclusive combustible users (285.4, 95% CI: [267.9, 303.0] ng/g creatinine); exclusive poly users (278.6, 95% CI: [254.9, 302.2] ng/g creatinine); and exclusive e-cigarette users (6.3, 95% CI: [4.7, 7.9] ng/g creatinine). Exclusive combustible users include users of all combustible products including cigarettes. However, because most exclusive combustible users are exclusive cigarette users, the latter category is also presented separately for reference in Table 2.

Linear regression model estimates for NNAL concentration by demographic and user groups are presented in Table 4. After adjusting for demographic variables, compared with never users, NNAL levels were 64.5 times higher in exclusive combustible users (Table 2 and 4, p value <.0001) and 3.7 times higher in exclusive e-cigarette users (Table 2 and 4, p value <.0001). The highest levels of each of the four urinary TSNAs were in every day exclusive SLT users (Supplementary Figure S1, Table 2 and Supplementary Table S2B; p value <.0001). Among all four TSNAs, concentrations of NNAL were highest, followed by NAT, NAB, and NNN, in descending order. NNN, NAT, and NAB followed a similar pattern among different tobacco product users with the highest concentrations in SLT users and the lowest concentrations in e-cigarette users (Supplementary Tables S4–S6). These three lower-concentration TSNAs also had similar relative mean concentrations among combustible users, cigarette users, and poly users (Table 2).

Among all nonusers, the NNAL GM was 1.0 ng/g creatinine. The NNN, NAT, and NAB detection rates for nonusers, including both former and never users, were less than 10%. Because those detection rates were too low for reliable mean calculations, the GMs for NNN, NAT, and NAB in all nonusers are flagged in Table 2 to indicate their greater uncertainty.

The creatinine-corrected NNAL GM and 95% CI are listed in Table 3 for different demographic and user groups. Female tobacco users had consistently higher NNAL concentrations than male users. Mean NNAL concentrations also consistently increased with age for all user categories, except e-cigarette users. Among the different racial categories, non-Hispanic whites had the highest mean NNAL concentrations and Hispanics had the lowest concentrations across most of tobacco user groups. When classified by education, tobacco users with bachelor's and graduate degrees had consistently lower mean concentrations of NNAL than the other educational groups, although every day poly users were an exception, and showed only limited variation by educational attainment (Tables 3 and 4).

The corresponding volume-based concentration (pg/mL) NNAL GMs and 95% CI among these groups of tobacco users without creatinine correction are given in Supplementary Table S3. Comparing creatinine-corrected estimates in Table 3 and uncorrected estimates in Supplementary Table S3, the NNAL GMs among gender, age, race/ethnicity, and education groups were similar using either creatinine-corrected or uncorrected data. The creatinine-corrected NNN, NAT, and NAB urinary GM and 95% CI for demographic groups are listed in Supplementary Tables S4–S6. The patterns observed for those TSNAs among demographic groups were all similar to the patterns found for NNAL.

TSNA levels for different tobacco user groups are displayed in bar graphs in Supplementary Figure S1. These plots clearly demonstrate that NNAL, NNN, NAT, and NAB had similar patterns across the different tobacco user groups, and that urine concentrations for NNAL were highest, followed by NAT, NAB, and NNN, respectively. When compared by category, all TSNA GMs were higher in every day users than in intermittent users.

Supplementary Figure S2 displays the distribution of the natural log of urinary NNAL for every day vs. intermittent users among product use categories. Across all product use categories, the distribution of urinary NNAL measurements for every day users in Supplementary Figure S2 resembled the log-normal. Similarly, the distributions appeared log-normal for combustible, cigarette, and poly user categories, whereas exclusive SLT user distributions were shifted to the right, and exclusive e-cigarette user distributions were displaced to the left. However, the overlap among all categories was such that a clear distinction could not be drawn based on concentration alone. For intermittent users, the NNAL distributions were broader and showed considerable overlap with every day users.

Supplementary Figure S3 is a scatter plot matrix of the four TSNAs, total cotinine (COTT) and total nicotine equivalent-2 (TNE2) for all tobacco users. The concentrations in this figure are not creatinine corrected because they involved multiple measurements within the same urine samples. All available results greater than limit of detection were used for this plot. Concentrations of COTT were strongly correlated with TNE2, and concentrations of NAT were strongly correlated with NAB. Correlations of NNAL with all of the other biomarkers, except NNN, were consistently strong, ranging from 0.70 to 0.73. The correlation coefficient

Table 2. Weighted TSNA Geometric Mean (GM) Concentrations (ng/g Creatinine) and Detection Rate (DR) by Tobacco Use Status in PATH Study Wave 1

Analyte		NNAL			NNN			NAT			NAB		
		N	GM [95% CI]	DR (%)	*N*	GM [95% CI]	DR (%)	*N*	GM [95% CI]	DR (%)	*N*	GM [95% CI]	DR (%)
All users		8882	93.0 [85.9, 100.2]	96	8642	8.0 [7.7, 8.4]	68	8805	48.5 [45.1, 51.9]	75	8871	9.2 [8.7, 9.7]	72
Every day	Combustible	3176	285.4 [267.9, 303.0]	100	3060	13.8 [13.0, 14.6]	91	3140	125.2 [116.2, 134.1]	96	3167	19.6 [18.2, 21.0]	93
	Smokeless	355	993.3 [839.2, 1147.3]	100	339	33.8 [29.3, 38.4]	94	349	452.6 [376.0, 529.2]	97	355	41.6 [34.7, 48.4]	96
	E-Cig	152	6.3 [4.7, 7.9]	93	148	5.2 [4.3, 6.0]	57	152	4.5 [3.8, 5.1]	17[a]	152	1.7 [1.4, 1.9]	22[a]
	Poly	1983	278.6 [254.9, 302.2]	100	1945	12.9 [12.1, 13.8]	88	1964	130.3 [118.4, 142.2]	93	1979	20.5 [18.8, 22.2]	92
Intermittent	Combustible	2400	13.0 [11.4, 14.6]	89	2352	3.1 [2.9, 3.3]	29[a]	2388	9.1 [8.2, 9.9]	41	2403	2.5 [2.3, 2.7]	38[a]
	Smokeless	121	100.9 [40.7, 161.2][b]	95	118	7.4 [4.4, 10.4]	53	121	39.1 [17.3, 60.9]	65	121	6.3 [3.6, 9.1]	63
	E-Cig	106	3.8 [2.3, 5.2]	85	104	2.2 [1.9, 2.6]	13[a]	105	3.4 [2.7, 4.1]	11[a]	106	1.2 [1.0, 1.4]	10[a]
	Poly	589	22.1 [16.6, 27.7]	95	576	3.3 [2.9, 3.7]	35[a]	586	12.8 [10.1, 15.5]	49	588	3.1 [2.6, 3.6]	44
Nonusers		2122	1.0 [0.9, 1.1]	62	2111	2.0 [1.9, 2.1]	3[a]	2114	3.0 [2.8, 3.2]	4[a]	2125	1.1 [1.0, 1.2]	5[a]
Former		559	2.1 [1.6, 2.6]	83	554	2.2 [1.9, 2.5]	7[a]	556	3.7 [3.2, 4.3]	11[a]	560	1.3 [1.1, 1.5]	12[a]
Never		1563	0.9 [0.8, 1.0]	54	1557	1.9 [1.8, 2.0]	1[a]	1558	2.9 [2.7, 3.1]	2[a]	1565	1.1 [1.0, 1.1]	3[a]
Every day exclusive cigarette users[c]		2037	300.9 [277.8, 324.0]	100	1942	14.9 [13.8, 16.0]	93	2015	141.0 [128.4, 153.6]	98	2030	21.3 [19.4, 23.2]	95
Intermittent exclusive cigarette users[c]		838	49.2 [39.7, 58.6]	97	809	5.0 [4.4, 5.5]	50	834	22.4 [18.4, 26.4]	64	840	4.9 [4.2, 5.6]	60

CI = confidence interval; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = *N*′-nitrosonornicotine; PATH = Population Assessment of Tobacco and Health; TSNA = tobacco-specific nitrosamine. DR: percentage of results greater than or equal to limit of detection (LOD). Every day established exclusive cigarette users: current users who are both established and every day users of only cigarettes; not using any other tobacco products. Intermittent exclusive cigarette users: current experimental/some day users of only cigarettes; not using any other tobacco products.

[a]DR is less than 40%.

[b]Relative standard error (RSE) is more than 30%. The RSE is calculated by 100 × standard error (estimate)/estimate.

[c]Cigarette user groups are just for reference groups which are not included in other tables.

Table 3. Weighted NNAL Geometric Mean (GM) With 95% CI (ng/g Creatinine) by Tobacco Use Status in PATH Study Wave 1

	Tobacco users									Nonusers		
		Combustible		Smokeless		E-Cig		Poly				
	All users	Every day	Intermittent	Every day	Intermittent	Every day	Intermittent	Every day	Intermittent	All non	Former	Never
All adults	93.0 [85.9, 100.2]	285.4 [267.9, 303.0]	13.0 [11.4, 14.6]	993.3 [839.2, 1147.3]	100.9 [40.7, 161.2][b]	6.3 [4.7, 7.9]	3.8 [2.3, 5.2]	278.6 [254.9, 302.2]	22.1 [16.6, 27.7]	1.0 [0.9, 1.1]	2.1 [1.6, 2.6]	0.9 [0.8, 1.0]
Sex												
Female	112.3 [98.7, 125.9]	329.7 [299.3, 360.0]	16.9 [13.5, 20.2]	3832.7 [−1866.6, 9532.0][a,b]	96.4 [1.3, 191.4][a]	7.2 [5.1, 9.3]	4.2 [2.5, 5.9]	307.1 [274.0, 340.2]	33.4 [20.1, 46.7]	1.1 [0.9, 1.2]	2.3 [1.6, 3.0]	1.0 [0.8, 1.1]
Male	81.4 [74.3, 88.5]	248.6 [229.8, 267.5]	11.0 [9.2, 12.9]	930.9 [817.2, 1044.5]	101.1 [39.5, 162.7][b]	5.2 [3.1, 7.3]	3.2 [0.9, 5.5] [a,b]	262.7 [233.9, 291.5]	17.6 [12.7, 22.4]	1.0 [0.8, 1.1]	1.8 [1.1, 2.6]	0.9 [0.7, 1.0]
Age												
18–24	23.2 [20.2, 26.3]	124.4 [106.9, 141.8]	5.1 [4.3, 5.8]	287.7 [165.3, 410.1]	20.1 [6.3, 33.9][a,b]	7.3 [−0.6, 15.2][a,b]	3.2 [1.1, 5.4][a,b]	139.1 [116.3, 161.9]	8.8 [6.3, 11.4]	1.1 [0.9, 1.3]	2.2 [1.6, 2.7]	1.0 [0.8, 1.1]
25–34	65.3 [54.2, 76.4]	231.8 [205.5, 258.2]	8.1 [6.1, 10.0]	649.9 [479.4, 820.4]	54.9 [−21.5, 131.3][a,b]	5.8 [2.6, 9.1][a]	4.1 [0.6, 7.6][a,b]	226.1 [187.4, 264.7]	14.0 [8.2, 19.8]	1.1 [0.9, 1.3]	2.4 [1.3, 3.5]	0.9 [0.7, 1.1]
35–54	153.7 [136.2, 171.3]	336.0 [308.2, 363.7]	21.4 [16.1, 26.6]	1107.4 [946.5, 1268.3]	171.8 [43.8, 299.8][a,b]	5.8 [3.5, 8.1]	2.1 [1.1, 3.0][a]	388.1 [336.1, 440.1]	49.5 [22.4, 76.6]	1.0 [0.8, 1.2]	2.3 [1.1, 3.5]	0.9 [0.7, 1.1]
55+	189.2 [163.5, 214.9]	372.1 [325.6, 418.6]	36.7 [23.5, 49.8]	1523.8 [943.4, 2104.3]	278.2 [−48.4, 604.9][a,b]	7.7 [4.8, 10.5][a]	9.6 [−0.7, 20.0][a,b]	433.5 [384.6, 482.3]	172.0 [85.8, 258.2][a]	1.0 [0.8, 1.1]	1.6 [0.6, 2.6][b]	0.9 [0.8, 1.1]
Race/ethnicity												
HA	28.6 [23.4, 33.8]	184.3 [143.0, 225.6]	9.9 [7.5, 12.3]	910.0 [527.1, 1292.8][a]	3.6 [−13.3, 20.5][a,b]	1.2 [0.1, 2.2][a,b]	0.9 [0.4, 1.3][a]	119.8 [85.2, 154.4]	9.1 [5.3, 12.8]	1.0 [0.8, 1.1]	2.2 [0.9, 3.5]	0.9 [0.8, 1.0]
NHB	97.8 [79.9, 115.7]	204.4 [175.8, 233.1]	38.7 [26.7, 50.6]	1126.3 [−1164.6, 3417.2][a,b]	42.4 [−114.3, 199.1][a,b]	5.8 [1.0, 10.6][a,b]	8.0 [0.4, 15.6][a,b]	183.6 [150.3, 217.0]	48.1 [2.5, 93.6][b]	1.3 [1.0, 1.6]	2.1 [0.5, 3.7][b]	1.2 [0.9, 1.5]
NHW	125.8 [113.4, 138.2]	335.0 [310.8, 359.1]	11.1 [9.0, 13.2]	1002.7 [848.5, 1156.9]	155.6 [70.5, 240.6]	7.1 [5.1, 9.1]	3.9 [2.4, 5.3]	320.1 [290.8, 349.5]	29.6 [19.4, 39.8]	1.0 [0.8, 1.2]	2.2 [1.5, 2.8]	0.9 [0.7, 1.1]
OTH	40.9 [25.8, 56.0]	217.4 [166.1, 268.7]	6.2 [3.4, 8.9]	749.3 [295.8, 1202.8] [a,b]	60.7 [−88.0, 209.5][a,b]	4.4 [−1.4, 10.1][a,b]	5.9 [−9.3, 21.2][a,b]	203.4 [144.1, 262.7]	13.3 [1.8, 24.8][b]	0.9 [0.6, 1.1]	1.5 [0.5, 2.5][b]	0.8 [0.5, 1.1]
Education												
<HS/GED	189.5 [167.6, 211.4]	310.7 [281.0, 340.4]	58.9 [43.4, 74.3]	1407.8 [819.4, 1996.2]	410.7 [105.8, 715.6] [a]	9.3 [4.0, 14.5][a]	5.6 [1.8, 9.4][a,b]	332.6 [275.4, 389.8]	37.6 [20.0, 55.2]	1.3 [1.1, 1.6]	2.8 [0.6, 5.0][b]	1.2 [1.0, 1.4]
HS	143.7 [128.2, 159.2]	303.5 [273.9, 333.0]	30.1 [23.1, 37.2]	929.8 [733.6, 1125.9]	79.9 [−30.9, 190.6][a,b]	6.9 [3.9, 10.0][a]	2.2 [0.5, 3.9][a,b]	280.4 [241.4, 319.4]	29.1 [14.0, 44.2]	1.5 [1.1, 1.9]	2.7 [1.3, 4.0]	1.3 [0.9, 1.7]
<4 college/ associate	75.4 [67.3, 83.5]	261.3 [232.5, 290.2]	8.2 [6.7, 9.8]	856.2 [663.8, 1048.7]	79.4 [−16.4, 175.2][a,b]	6.3 [3.7, 9.0]	4.4 [1.7, 7.1][a,b]	242.1 [213.8, 270.4]	22.2 [13.4, 31.0]	1.1 [0.9, 1.2]	2.6 [1.7, 3.5]	0.9 [0.8, 1.0]
Bachelors/ advanced	19.8 [15.5, 24.0]	228.8 [185.4, 272.2]	3.7 [2.8, 4.5]	804.9 [588.3, 1021.5][a]	65.1 [−8.6, 138.9][a,b]	3.5 [2.0, 5.1][a]	2.9 [0.1, 5.7][a,b]	290.6 [210.6, 370.5]	7.5 [3.2, 11.8]	0.6 [0.5, 0.7]	0.9 [0.5, 1.4]	0.6 [0.5, 0.7]

CI = confidence interval; GED = General Educational Development; HA = Hispanic; HS = high school; NHB = non-Hispanic Black; NHW = non-Hispanic White; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; OTH = Other Hispanic/Other Race/Multiracial; PATH = Population Assessment of Tobacco and Health.

[a]Sample size is less than 50.

[b]Relative standard error (RSE) is more than 30%. The RSE is calculated by 100 × standard error (estimate)/estimate.

Table 4. Weighted Linear Regression Model for NNAL (ng/mL) (Natural Log-Transformed, $N = 11\,004$) With Tobacco User Group and Demographic Predictors and Pairwise Comparisons for Tobacco User Group Least Square Means (LSMs)

Regression results						Tobacco users group LSM differences and ratios				
Predictor	Level	*N*	Coefficient [95% CI]	Exponentiated coefficient [95% CI]	*p*	Group A	Group B	A–B [95% CI] (ln)	Ratio A/B [95% CI]	Adjusted *p*[a]
Tobacco user group	Combustible	5576	4.17 [4.02, 4.31]	64.5 [55.7, 74.6]	<.0001	Combustible	Never	4.17 [3.94, 4.39]	64.5 [51.5, 80.7]	<.0001
	E-Cig	258	1.32 [1.05, 1.58]	3.73 [2.87, 4.85]	<.0001	E-Cig	Never	1.32 [0.913, 1.72]	3.73 [2.49, 5.58]	<.0001
	Smokeless	476	5.94 [5.69, 6.20]	382 [296, 491]	<.0001	Former	Never	0.727 [0.348, 1.10]	2.07 [1.42, 3.02]	<.0001
	Poly	2572	4.88 [4.73, 5.02]	131 [114, 151]	<.0001	Poly	Never	4.88 [4.66, 5.10]	131 [105, 163]	<.0001
	Former	559	0.727 [0.477, 0.976]	2.07 [1.62, 2.65]	<.0001	Smokeless	Never	5.94 [5.56, 6.33]	382 [259, 562]	<.0001
	Never	1563	Ref.			Combustible	Former	3.44 [3.05, 3.83]	31.2 [21.1, 46.1]	<.0001
Sex	Male	5953	Ref.			E-Cig	Former	0.589 [0.0702, 1.11]	1.80 [1.07, 3.03]	.0139
	Female	5051	0.145 [0.0316, 0.258]	1.16 [1.03, 1.29]	.0126	Combustible	Smokeless	−1.78 [−2.10, −1.45]	0.169 [0.122, 0.234]	<.0001
Age group	18–24	3541	−0.649 [−0.796, −0.502]	0.523 [0.452, 0.604]	<.0001	E-Cig	Smokeless	−4.63 [−5.12, −4.14]	9.77E−03 [5.98E−03, 0.0160]	<.0001
	25–34	2341	Ref.			Former	Smokeless	−5.22 [−5.73, −4.71]	5.42E−03 [3.26E−03, 9.01E−03]	<.0001
	35–54	3352	0.315 [0.137, 0.493]	1.37 [1.15, 1.63]	.0007	Poly	Smokeless	−1.07 [−1.39, −0.747]	0.344 [0.250, 0.474]	<.0001
	55+	1770	0.143 [−0.0249, 0.310]	1.15 [0.977, 1.36]	.0944	Combustible	Poly	−0.711 [−0.856, −0.566]	0.491 [0.425, 0.568]	<.0001
Race/ethnicity	HA	1845	−0.866 [−1.04, −0.693]	0.421 [0.354, 0.499]	<.0001	E-Cig	Poly	−3.56 [−3.95, −3.17]	0.0284 [0.0193, 0.0418]	<.0001
	NHB	1667	0.0494 [−0.136, 0.235]	1.05 [0.874, 1.26]	.599	Former	Poly	−4.15 [−4.57, −3.73]	0.0158 [0.0104, 0.0239]	<.0001
	NHW	6608	Ref			Combustible	E-Cig	2.85 [2.48, 3.22]	17.3 [11.9, 25.1]	<.0001
	OTH	884	−0.235 [−0.475, 3.57E−03]	0.790 [0.624, 1.00]	.0535					
Education	<HS/GED	2717	0.225 [0.0650, 0.386]	1.25 [1.07, 1.47]	.0064					
	HS	2735	Ref.							
	<4 y college/associate	4084	−0.486 [−0.640, −0.332]	0.615 [0.529, 0.716]	<.0001					
	Bachelors	1025	−1.41 [−1.62, −1.21]	0.244 [0.199, 0.298]	<.0001					
	Advanced	443	−1.61 [−1.86, −1.36]	0.199 [0.156, 0.255]	<.0001					
Creatinine, urine (Ln)	Continuous		0.515 [0.438, 0.592]	1.67 [1.55, 1.81]	<.0001					

CI = confidence interval; GED = General Educational Development; HA = Hispanic; HS = high school; LSM = Least Square Mean; NHB = non-Hispanic Black; NHW = non-Hispanic White; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; OTH = Other Hispanic/Other Race/Multiracial.

Combustible: combustible exclusive user. E-Cig: e-cigarette exclusive user. Smokeless: smokeless exclusive user. Poly: poly user. Former: former user. Never: never user.

[a]Bonferroni corrected for multiple comparisons.

between NNAL and NNN was 0.60. Supplementary Table S7 shows NNAL, COTT, and TNE2 correlation coefficients for every day exclusive SLT users, every day exclusive e-cigarette users, every day exclusive combustible users, and every day exclusive cigarette users. These product-stratified data show similarly strong correlations among NNAL, COTT, and TNE2 for every day SLT, every day combustible, and every day cigarette users. Conversely, NNAL, COTT, and TNE2 correlations were weaker in urine collected from every day e-cigarette users.

Discussion

The PATH Study measured and analyzed urinary TSNAs (NNAL, NNN, NAT, and NAB) in the W1 adult participants of the PATH Study (2013–2014). To our knowledge, this is the first report to include all four TSNAs measured in a large, population-representative study of tobacco users and nonusers. Because the PATH Study focuses specifically on tobacco-related exposures, these results characterize TSNA exposures among users of many types of tobacco products, including SLT, cigarettes, cigars, e-cigarettes, hookah tobacco, and others.[15,17] While NHANES has also examined US population exposure resulting from tobacco product use, it lacked the large number of non-cigarette tobacco users included in the PATH Study.[12]

SLT users have been reported to be at particular risk of exposure from TSNAs.[5,6,25,26] We found that among established every day exclusive tobacco product users, the GM for urinary NNAL in SLT users was the highest observed for any group. This observation is consistent with previous studies reporting the highest level of NNAL in SLT users.[27] Thus PATH Study results for every day exclusive SLT users (NNAL GM: 993.3 ng/g creatinine (95% CI: [839.2, 1147.3])) are similar to the results for SLT users from NHANES 2007–2008 (NNAL GM: 1013.7 ng/g creatinine (95% CI: [738.9, 1390.8])).[27] The PATH Study results for every day exclusive cigarette users (NNAL GM: 300.9 ng/g creatinine (95% CI: [277.8, 324.0])) also agreed well with NHANES 2007–2008 results (NNAL GM:285 ng/g creatinine (95% CI: [236, 346])).[8,9]

The PATH Study every day exclusive e-cigarette users NNAL GM is 6.3 ng/g creatinine (95% CI: [4.7, 7.9]). The much lower TSNAs levels in e-cigarette users compared with other tobacco users are consistent with lower levels of TSNAs in e-liquids compared with cigarettes and SLT.[28] These PATH Study TSNA data are both qualitatively and quantitatively similar to previously published results.[29–31] Because NHANES lacked adequate data on exclusive e-cigarette users, we compared our results with those from a small study by Shahab et al.[29] In that study, exclusive e-cigarette users unweighted NNAL GM was 2.5 ng/g creatinine (95% CI: [1.5, 4.2]). The slightly higher NNAL levels found in PATH Study e-cigarette users may result from higher cigarette smoke exposures than in the narrowly defined e-cigarette user category used by Shahab et al. Further discussion of product-specific exposure patterns can be found in additional reports specifically focused on SLT, e-cigarettes, cigars, and hookah.[32–34]

TSNA concentrations were consistently higher in every day users than in intermittent users (Supplementary Figures S1 and S2 and Table 2). NNAL was significantly higher in every day users of combustible (p value <.0001), SLT (p value <.0001), and poly users (p value <.0001) compared with intermediate users (Supplementary Table S2B). These findings were generally consistent across all TSNAs despite the fact that NNAL has a much longer physiological half-life (16–18 days). Every day users are more likely to use more product than intermittent users and their exposure to these known carcinogens reflects this more intense use. The distribution of urinary NNAL appears to be wider for intermittent users compared with every day users across all product types studied (Supplementary Figure S2), perhaps because of differing degrees of frequency and intensity of product use.

Current, established every day poly users had urinary NNAL GM (278.6 ng/g creatinine (95% CI: [254.9, 302.2])) similar to every day, established exclusive combustible product users (285.4 ng/g creatinine (95% CI: [267.9, 303.0])) and to every day, established exclusive cigarette users (300.9 ng/g creatinine (95% CI: [277.8, 324.0])). This finding agrees with previous studies,[12,35–37] and suggests that every day poly user exposure does not significantly differ from every day combustible users (p value = .424) (Supplementary Table S2B) when one of the products used is ether a combustible or SLT product. Further investigation can better characterize TSNA exposures resulting from poly users.

TSNAs correlate well with each other, with correlation coefficients ranging from 0.60 to 0.95. NAT and NAB are tightly correlated in the PATH Study data, likely because they both form from nitrosation of minor tobacco alkaloids that are themselves closely correlated.[38–40] All TSNAs also correlate well with urinary cotinine, underscoring the importance of tobacco as the specific exposure source. NNAL is also a valuable biomarker because it has a relatively long half-life (~16–18 days), and a low limit of detection, resulting in a detection rate (62%) among nonusers that was much higher than that of other TSNAs (<10%) for nonusers. Additionally, more of the NNK dose is excreted in the urine as NNAL than the amount of the NNN dose that is excreted in urine as NNN.[4] Therefore, urinary NNAL levels are consistently higher than urinary NNN levels, despite many tobacco products having higher concentrations of NNN than NNK.

Urinary TSNA levels were primarily related to the frequency and type of tobacco products used; however, demographic variables (eg, sex, race, and education) also contributed modest differences in TSNA exposures. Among users of a single product, females consistently had higher urinary TSNAs than males, after creatinine correction (Table 3 and Supplementary Tables S4–S6). These patterns were consistent with previous NHANES results. Findings presented here showed that female tobacco users have a 1.38-fold higher urinary NNAL GM than male tobacco users (Tables 3). In NHANES (2007–2008), female smokers had a 1.20-fold higher urinary NNAL GM than male smoker.[8] However, the results for females in Supplementary Table S3 are generally either very similar to or lower than in males. The higher results for females were seen in the creatinine-corrected data of Table 3 and the linear model of Table 4 which includes creatinine as a covariate. Since creatinine is generally higher in males than in females, these results might incorporate a possible bias. Genotypic and phenotypic differences in cytochrome P450 enzymes between females and males could affect NNK metabolism as well. However, the detailed influence of sex and smoking on NNK metabolism remains unclear and future research can inform this matter.

Urinary NNAL concentrations increased with increasing age for users of all product types except e-cigarettes (Table 3). Established users of tobacco products tend to increase use and dependence with time,[41,42] resulting in higher TSNA exposures. The positive correlation with age observed in PATH Study data is also evident in NHANES tobacco users.[8,9] Conversely, age was not correlated with

TSNA exposures in PATH Study nonusers; urinary TSNA levels were similar across all age groups studied for both former and never users (Table 3). Previous studies have found similar results in adult nonusers,[8,9] although children tend to have higher urinary NNAL because of higher secondhand smoke exposure compared with adults.[43]

Educational attainment was inversely associated with urinary TSNAs for all product use categories. Tobacco users with a bachelor's or higher-level degree had significantly lower mean concentrations of NNAL (p value <.0001) than did users with lower educational attainment. More highly educated individuals are more likely to be aware of the potential harm of tobacco product use and thus may use tobacco products less frequently or less intensely compared with users with lower educational attainment.[44,45] Educational attainment also was associated with decreased NNAL exposure in nonusers, perhaps resulting from having less tobacco exposure at work. For example, Wei et al. found that NHANES study participants working in jobs that required less education (eg, food preparation and other blue-collar jobs) had higher secondhand smoke exposure compared with workers whose jobs required more advanced academic credentials.[45] Among all nonusers, the NNAL GM was 1.0 ng/g creatinine. Among these self-reported nonusers, former users had a GM of 2.1 ng/g creatinine while never users had a GM of 0.9 ng/g creatinine (Table 2). When adjusting for other variables, former users had a LSM 2.1 times higher than never users (Table 4, p value <.0001). This might result from a greater continued exposure to secondhand smoke of former users in comparison with never users.

The NNN concentration in tobacco is generally greater than NNK.[37,46,47] However, the metabolism and excretion rates of NNK and NNN are markedly different. NNK is predominantly metabolized to NNAL and excreted in the urine with a half-life of 16–18 days. Conversely, NNN is rapidly and extensively metabolized to nonspecific end-products that cannot be linked to NNN exposure. Thus, only relatively small amounts of unchanged NNN are excreted in urine,[4,6] and other NNN metabolites are not effective exposure biomarkers. These facts likely explain why urinary NNN levels are in the low pg/mL range. An additional technical problem with urinary NNN is the artifactual formation of NNN from nornicotine and nitrate after the urine is collected.[48] Although our analytical method is designed to minimize such false positive results, both the relatively low concentrations and potential artifactual formation of NNN during analysis resulted in relatively lower precision for measurements of NNN in comparison to the other TSNA analytes. For example, in Table 2, every day established exclusive e-cigarette users have 5.2 ng/g creatinine NNN which is nominally higher than NAT (4.5 ng/g creatinine). This pattern in e-cigarette users is different from the TSNA pattern in users of other tobacco products for which NNN is lower than NAT. One possible explanation of this difference is that e-cigarette users may generate less tobacco alkaloid nitrosation compared with users of other products. If so, then the relatively small amount of nornicotine nitrosation that artifactually occurs during sample preparation may account for a larger percentage of the total NNN measured in e-cigarette user urine. For all tobacco product users any artifactual NNN formation will negatively impact the accuracy and precision of urinary NNN measurement. Consequently, cautious interpretation of NNN results is advised.

In addition to biomarkers of exposure to carcinogenic TSNAs, the PATH Study W1 dataset also includes novel baseline measures of exposure to NAT and NAB in never, current, and recent former tobacco users in the US population. NAT and NAB are highly correlated with each other (r = 0.95) across all user groups because they form from the closely correlated minor tobacco alkaloids anatabine and anabasine, respectively. These nitrosated products of nicotine analogues are not known to be carcinogenic, but provide additional information about overall TSNA exposure patterns.

There are some limitations in this study. Tobacco use was self-reported, and thus the tobacco user groups were defined based on questionnaire data only, and might include some self-misclassification. Furthermore, the relative length of time from the most recent use of tobacco to the time of sample collection can affect biomarker concentrations. Lastly, we did not adjust for variations in TSNA concentration that may exist among different brands of tobacco products.

In conclusion, the urinary TSNAs were found to be highly correlated with nicotine biomarkers, and TSNA concentrations were associated with both the choice of tobacco product and frequency of use. Current exclusive established every day SLT users had the highest TSNA concentrations of any group, and every day user TSNA concentrations were consistently higher than TSNA concentrations in intermittent users.

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.

Supplementary data are available at *Nicotine & Tobacco Research* online.

Supplementary Table S1. Unweighted PATH Study Wave 1 demographic counts (N = 11 004) with urinary NNAL data

Supplementary Table S2A. Weighted linear regression model 2 for NNAL (ng/mL) (natural log-transformed, N = 11 004) in different tobacco user groups (subdivided by frequency) and demographic groups

Supplementary Table S2B. Least square mean differences between user groups for NNAL model 2

Supplementary Table S3. Weighted NNAL geometric mean (GM) with 95% CI (pg/mL) by tobacco use status in PATH Study Wave 1

Supplementary Table S4. Weighted NNN geometric mean (GM) with 95% CI (ng/g creatinine) by tobacco use status in PATH Study Wave 1

Supplementary Table S5. Weighted NAT geometric mean (GM) with 95% CI (ng/g creatinine) by tobacco use status in PATH Study Wave 1

Supplementary Table S6. Weighted NAB geometric mean (GM) with 95% CI (ng/g creatinine) by tobacco use status in PATH Study Wave 1

Supplementary Table S7. Weighted NNAL correlation with NNN, NAT, NAB, COTT, and TNE2 for current exclusive established every day users of different tobacco products

Supplementary Figure S1. Weighted TSNAs geometric mean (GM) and 95% confidence interval (CI) bar graph in different tobacco users.

Supplementary Figure S2. Weighted distribution of NNAL among current established every day exclusive users and intermittent exclusive users.

Supplementary Figure S3. Scatter plots for TSNAs, COTT, and TNE2 for all tobacco users.

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