Biomarkers of Exposure among Adult Smokeless Tobacco Users in the Population Assessment of Tobacco and Health Study (Wave 1, 2013–2014)

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ABSTRACT

Background: Monitoring population-level toxicant exposures from smokeless tobacco (SLT) use is important for assessing population health risks due to product use. In this study, we assessed tobacco biomarkers of exposure (BOE) among SLT users from the Wave 1 (2013–2014) of the Population Assessment of Tobacco and Health (PATH) Study.

Methods: Urinary biospecimens were collected from adults ages 18 and older. Biomarkers of nicotine, tobacco-specific nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), volatile organic compounds (VOC), metals, and inorganic arsenic were analyzed and reported among exclusive current established SLT users in comparison with exclusive current established cigarette smokers, dual SLT and cigarette users, and never tobacco users.

Results: In general, SLT users ($n = 448$) have significantly higher concentrations of BOE to nicotine, TSNAs, and PAHs compared with never tobacco users; significant dose–response relationships between frequency of SLT use and biomarker concentrations were also reported among exclusive SLT daily users. Exclusive SLT daily users have higher geometric mean concentrations of total nicotine equivalent-2 (TNE2) and TSNAs than exclusive cigarette daily smokers. In contrast, geometric mean concentrations of PAHs and VOCs were substantially lower among exclusive SLT daily users than exclusive cigarette daily smokers.

Conclusions: Our study produced a comprehensive assessment of SLT product use and 52 biomarkers of tobacco exposure. Compared with cigarette smokers, SLT users experience greater concentrations of some tobacco toxicants, including nicotine and TSNAs.

Impact: Our data add information on the risk assessment of exposure to SLT-related toxicants. High levels of harmful constituents in SLT remain a health concern.

Introduction

U.S. smokeless tobacco (SLT) use prevalence has remained relatively stable (~3.5% among people aged 12 and older) over the past decade (1). However, SLT products are known to contain several harmful constituents, including known carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and polycyclic aromatic hydrocarbons (PAH). SLT use is also causally associated with several adverse health outcomes, including cancer (oral, pancreatic, esophageal), gum disease, and cardiovascular diseases (2–4).

SLT users have varying patterns of use by type, frequency, and exclusive or polytobacco use (5). Furthermore, a study using 1999–2012 National Health and Nutrition Examination Survey (NHANES) data assessed tobacco biomarkers of exposure (BOE) and reported elevated concentrations of serum cotinine and urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol (NNAL), a metabolite of NNK, in exclusive SLT users compared with exclusive cigarette smokers (6). However, the U.S. SLT market is changing rapidly, with new products being introduced and growth in SLT consumption during 2000–2015 (7). Chemical analyses of new and traditional SLT products show large variations in toxicants and carcinogens (8). Monitoring population-level SLT toxicant exposures is important for assessing population health risks, especially because SLT toxicant levels can vary widely due to factors such as tobacco blend, growing practices, manufacturing, and storage conditions (9–11).

This study provides a comprehensive assessment of SLT toxicant exposure using biospecimens collected from participants in PATH Study Wave 1 (2013–2014); concentrations of 52 BOEs among exclusive current SLT users were compared with those among exclusive current cigarette smokers, to users of both SLT and cigarettes (dual users), and to never tobacco users. We also assessed BOEs in these user groups by use frequency (daily/nondaily use; ref. 12). Results provide population-level BOEs for a broad range of SLT toxicants in the U.S. population.

Materials and Methods

Study population

Data were collected during PATH Study Wave 1 (2013–2014). The PATH Study is a nationally representative, longitudinal cohort study

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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of 45,971 U.S. adults and youth (ages 12 and older) and includes a detailed assessment of participants' use of several tobacco products. Urine samples were requested from consenting adults ages 18 and older ($N = 32,320$). Among the 21,801 subjects who completed the Wave 1 adult interview and who provided a urine specimen, a stratified probability sample of 11,522 adults was selected for analyses from six tobacco product use groups, including (i) current exclusive established users of cigarettes (ii), current established users of one or more tobacco products other than cigarettes (who may also be current established users of cigarettes or experimental users of other products, including cigarettes), (iii) current experimental users of any tobacco products, (iv) former established users of any tobacco product (last use within the past 12 months), (v) never users of any tobacco products and (vi) current established users of cigarettes who are experimental users of at least one other tobacco products. Details regarding PATH Study design and methods are published elsewhere (12). Details on survey interview procedures, questionnaires, sampling, and data access instructions are available at http://www.icpsr.umich.edu/icpsrweb/NAHDAP/series/00606. Westat's Institutional Review Board approved the study design and data collection protocol. All respondents ages 18 and older provided informed consent, with youth respondents ages 12 to 17 providing assent whereas each one's parent/legal guardian provided consent. Westat's IRB operates in accordance with the regulations set forth by the Office for Human Research Protections (OHRP) within the U.S. Department of Health and Human Services (HHS) under 45 CFR Part 46, the Common Rule.

Tobacco use categories

For each tobacco product, participants were asked whether they ever used the product, even one or two times/puffs; whether they now use/smoke the product daily, nondaily, or not at all; whether they ever used the product "fairly regularly"; and how much of the product they used in their lifetime. Participants were also asked whether they used nicotine replacement therapy (NRT) in the past three days. On the basis of responses, we defined the following four mutually exclusive tobacco use categories for participants who provided urine samples within the normal range of creatinine levels (10–370 mg/dl) and did not report NRT use in the past 3 days: (i) "exclusive current established SLT users" ($N = 448$), who have used SLT (loose snus, moist snuff, dip, spit, chewing tobacco, pouched snus) fairly regularly (established use), now use daily or nondaily, and currently do not use other tobacco (cigarette, e-cigarette, cigar, hookah, pipe, dissolvable tobacco) in addition to SLT; (ii) "exclusive current established cigarette smokers" ($N = 2,427$), who have smoked at least 100 cigarettes in their lifetime (established cigarette use), now smoke daily or nondaily, and currently do not use other tobacco in addition to cigarettes; (iii) "current established dual cigarette and SLT users" ($N = 140$), who are current established users of cigarettes and SLT but do not use other tobacco products; and (iv) never tobacco users ($N = 1,655$), who have never used any tobacco products, even one or two times. For each of the first three groups, we categorized users into daily and nondaily users. Former tobacco users (adults who have ever used but currently did not use any of the tobacco products) were excluded from the analyses.

Demographic variables

We incorporated several demographic variables, including age categories (ages 18–24, 25–34, 35–54, and $\geq$55 years based on imputed age values), sex, race/ethnicity [non-Hispanic white, other non-Hispanic groups (including multi-race), and Hispanics], and education [less than high school diploma; General Education Development (GED) diploma; high school diploma; some college/associate degree; and completed college or more]. Imputed age was calculated on the basis of date of birth or age in years (when date of birth is not available) provided in the interview or based on age in years provided in the household screener if interview data are missing.

Biomarkers of exposure

We report concentrations of 52 urinary BOEs associated with tobacco, divided into six major biomarker panels by each of the four user groups (Supplementary Table S1). These include: nicotine metabolites ($n = 9$), tobacco-specific nitrosamines (TSNA; $n = 4$), PAHs ($n = 7$), volatile organic compounds (VOC; $n = 20$), inorganic arsenics ($n = 4$), and metals ($n = 8$). Total nicotine equivalent-2 (TNE-2) was calculated for all samples by taking the molar sum of cotinine and trans-3′-hydroxycotinine. Total inorganic arsenic was calculated by taking the sum of the arsenous acid, arsenic acid, dimethylarsinic acid, and monomethylarsonic acid levels in each urine sample. A representative set of biomarkers from these panels based on biomarker association with tobacco exposure or adverse health outcomes was presented, including BOE concentrations by use frequency (daily/nondaily) and intensity of use (0–4, 5–9, 10+ times/day) among exclusive daily SLT users (excluding pouched snus due to limited sample size).

Laboratory measurements of biomarkers

All adult interview respondents were asked to provide biospecimens, and full-void urine specimens were self-collected from 21,801 (67.5%) consenting participants in a 500 mL polypropylene container (PN 6542, Globe Scientific), immediately placed in a Crēdo Cube shipper (Series 4-496, Minnesota Thermal Science). All containers, pipet tips, and vials that came in direct contact with the urine sample were prescreened and determined not to have amounts of metal contamination that would adversely influence the analytical measurements. For more information on the aliquots created from the urine biospecimens, please see the PATH Study W1 Biospecimen Urine Collection Procedures (https://doi.org/10.3886/Series606).

Urine specimens were shipped overnight on dry ice to the laboratories at the Centers for Disease Control and Prevention (CDC; Atlanta, GA), where they were stored at −80°C until ready for laboratory analyses. All biomarker results reported by CDC met the rigorous accuracy and precision requirements of the quality control/quality assurance program of the CDC (13). Total urinary nicotine metabolites, including the free and glucuronide conjugated forms, were measured by two separate isotope dilution high performance liquid chromatography/tandem mass spectrometric (HPLC-MS/MS) methods based on the cotinine cutoff value of 20 ng/mL. For samples with cotinine concentrations $\geq$20 ng/mL, anatabine, anabasine, and nicotine plus its six major metabolites were measured; for samples with cotinine concentration <20 ng/mL, only cotinine and trans-3′-hydroxycotinine were measured (14, 15). TSNAs were measured by isotope dilution HPLC-MS/MS using a modified version of the method of Xia and colleagues (16). PAHs were measured using enzymatic hydrolysis, on-line solid phase extraction, and isotope dilution liquid chromatography tandem mass spectrometry (17). VOCs were measured [e.g., 2-methlyhippuric acid, N-acetyl-S-(N-methylcarbamoyl)-L-cysteine] using isotope dilution UPLC-MS/MS as described by Alwis and colleagues (18) and modified by Alwis and colleagues (19). Metals were measured using inductively coupled plasma mass spectrometry (ICP-MS; refs. 20, 21). Inorganic arsenic species and their metabolites were measured by high performance liquid chromatography/inductively coupled plasma dynamic reaction cell mass spectrometry (HPLC-ICP-DRC-MS; refs. 22, 23). Individual

analytes available in each panel and their limits of detection (LOD) are summarized in Supplementary Table S1. Urinary creatinine was measured by an enzymatic assay on a commercial automated clinical chemistry analyzer (LOD = 1.1 mg/dL) to be adjusted for variable urine dilution because of variable hydration state of study participants in the analyses. In addition, blinded replicate subject samples are also included in shipments to monitor within-run and between run analysis variability.

Statistical analysis

Geometric mean concentrations were estimated using sampling weights to represent never, current, and recent former (within 12 months) tobacco product users in the U.S. civilian, noninstitutionalized adult population at the time of Wave 1. Variances were estimated using the balanced repeated replication method (24) with Fay's adjustment = 0.3 to increase estimate stability (25). Analyses were conducted using SUDAAN statistical software release 11.0.1 (RTI, Research Triangle Park, NC). Analyses were conducted using PATH Study biomarker sample weights with balanced repeated replicate methods to account for complex survey design. Weighting procedures are outlined in the Biospecimen Restricted Use Files User Guide (https://doi.org/10.3886/ICPSR36840).

We present results for a subset of the PATH Study Wave 1 population (i.e., never users of tobacco, current SLT users, cigarette smokers, and dual users). We report weighted percentages and 95% confidence intervals (95% CI) for demographic variables, including age, race/ethnicity, education, and sex, stratified by the four tobacco use categories described above (**Table 1**). We calculated the weighted geometric mean concentration of each BOE by tobacco use category. Biomarker concentration values less than the LOD were replaced with imputed values using the convention LOD/sqrt 2 (26). Creatinine-corrected values were calculated for urinary BOE for samples within the normal creatinine range (10–370 mg/dL) to avoid the confounding effects of overly diluted or hyper-concentrated urine due to differing renal clearance rates (27). We calculated the creatinine-corrected values by dividing biomarker mass (U/mL) by creatinine mass (g/mL) to produce biomarker mass/g of creatinine. Concentrations of creatinine-adjusted biomarkers were log-transformed before analysis to minimize the effect of skewness, and geometric means and 95% CIs are reported by tobacco use category, use frequency (daily/nondaily), and times/day (0–4, 5–9, 10+). TNE2 was calculated for all samples by taking the molar sum of the two most abundant nicotine metabolites: Cotinine and trans-3′-hydroxycotinine. Total inorganic arsenic was calculated by adding the arsenous acid, arsenic acid, dimethylarsinic acid, and monomethylarsonic acid concentrations in each urine sample.

To calculate the statistical significance of differences in BOE geometric mean concentrations by tobacco use category (see **Tables 2** and **3**), we performed multivariable linear regression, where the non-creatinine corrected biomarker value (log-transformed) was the dependent variable and tobacco user categories (dummy variables) were independent variables with never tobacco users as the reference category. Statistical significance was assessed using *P* values (for differences in geometric means) for each user group compared with never users after adjusting for age, sex, race/ethnicity, educational attainment, and urinary creatinine. To test for the significance of the dose–response relationship between daily SLT use frequency and biomarker concentrations, *P* values for trend were obtained by multivariable linear regression using non-creatinine–corrected biomarker values (log-transformed) as the dependent variable and times/day as

Table 1. Demographics of PATH Study Wave 1 adults with urinary biospecimen, by tobacco user groups.

	Exclusive current established cigarette smokers (*n* = 2,427)		Exclusive current established SLT users (*n* = 448)		Dual current established cigarette and SLT users (*n* = 140)	Never tobacco users (*n* = 1,655)
	Daily (*n* = 1,990)	Nondaily (*n* = 437)	Daily (*n* = 351)	Nondaily (*n* = 97)		
	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)
Age, mean (y)	45.2 (44.0–46.3)	38.5 (36.6–40.3)	46.7 (44.9–48.6)	42.6 (38.2–47.0)	36.3 (33.6–39.0)	44.7 (43.8–45.6)
Age group (y; %)						
18–24	7.9 (6.2–10)	15 (11.1–20)	8.2 (6–11.1)	13.3 (8.1–21)	17.9 (11.1–27.6)	16.1 (14.5–17.9)
25–34	20.8 (18.3–23.6)	33 (24.7–42.6)	16.8 (12.3–22.4)	31 (20.4–44.2)	34.4 (28–41.4)	17.2 (14.6–20.1)
35–54	43 (39.3–46.8)	36.6 (28.6–45.3)	46.3 (40.7–52)	30.7 (20.7–42.9)	39 (29.4–49.7)	36 (32.5–39.6)
55+	28.2 (24.6–32.1)	15.4 (11.4–20.4)	28.7 (23.5–34.5)	25 (16.2–36.4)	8.7 (4.0–18.0)[a]	30.7 (27.6–34.1)
Sex (%)						
Male	46.3 (42.9–49.6)	49.8 (41.8–57.8)	95.4 (91.3–97.6)	95.9 (90.8–98.2)	95 (90.3–97.5)	37.5 (34.9–40.1)
Female	53.7 (50.4–57.1)	50.2 (42.2–58.2)	4.6 (2.4–8.7)[a]	4.1 (1.8–9.2)[a]	5 (2.5–9.7)[a]	62.5 (59.9–65.1)
Race/ethnicity (%)						
White, non-Hispanic	73.0 (69.0–76.7)	54.1 (44.4–63.6)	89.1 (84.9–92.2)	81.4 (73.0–87.7)	90.9 (84.9–94.6)	56.5 (52.7–60.3)
Others, non-Hispanic	17.6 (14.5–21.2)	21.4 (16.0–28.0)	8.2 (5.5–12.0)	12.9 (7.5–21.1)	5.8 (3.0–11.1)[a]	22.4 (19.8–25.1)
Hispanic	9.4 (7.4–11.8)	24.5 (14.7–37.9)	2.7 (1.5–4.9)[a]	5.7 (2.2–14.2)[a]	3.3 (1.3–8.1)[a]	21.1 (18.3–24.2)
Education (%)						
Less than high school diploma	17.3 (14.6–20.4)	18.1 (10.7–28.8)	19.9 (15.4–25.3)	8.7 (4.9–15)	10.0 (5.8–16.9)	13.3 (11.4–15.3)
GED	11.5 (9.2–14.2)	6.3 (3.9–9.9)	6.9 (4.6–10.2)	4.6 (1.7–11.7)[a]	16.7 (12.4–22)	2.8 (1.8–4.2)
High school diploma	32.8 (29.6–36.2)	14 (11.1–17.6)	31 (25.6–36.9)	29 (20.3–39.6)	29.2 (21.4–38.5)	25.5 (22–29.4)
Some college/associate degree	31.6 (28.4–35)	38 (29.6–47.2)	29.5 (24.2–35.4)	28.1 (19.3–39)	32.6 (24–42.4)	27.4 (24.3–30.7)
Completed college or more	6.8 (5.5–8.2)	23.6 (16.5–32.6)	12.7 (9–17.7)	29.6 (18.4–43.8)	11.5 (6.6–19.5)	31.0 (27.2–35.2)

[a]Estimates were flagged if relative standard error (RSE) was >30% or sample size <50; for dichotomous variables, estimates were flagged if RSE for proportion or (1-proportion) was >30%. Estimate should be interpreted with caution because it has low precision.

the independent variable, adjusting for age, sex, race/ethnicity, educational attainment, and urinary creatinine. For multivariable analyses, a sensitively analysis was also performed by restricting the analyses to non-Hispanic male respondents only and adjusted for age, educational attainment, and urinary creatinine.

Estimates were flagged if relative standard error (RSE) >30% or sample size <50; for dichotomous variables, estimates were flagged if RSE for proportion or (1-proportion) was >30%. Flagged estimates should be interpreted with caution due to low statistical precision. In addition, biomarker geometric means were flagged if >40% of samples had biomarker values <LOD.

Results

Characteristics of study participants by use category

In the PATH Study Wave 1 study population, 2.9% of adult participants were current established SLT users and 18.1% were current established cigarette smokers (5, 28). **Table 1** and **Fig. 1** present the weighted frequency of demographic characteristics and product use, respectively, for each user group. Exclusive current established SLT users were predominantly male (95.4% of daily users, 95.9% of nondaily users) and non-Hispanic white (89.1% of daily users, 81.4% of nondaily users). Current established dual users were also overwhelmingly male (95%) and non-Hispanic white (90.9%). In addition, dual users were much younger (mean age = 36.3) than other user groups, with a higher proportion of young adults ages 18–24 (17.9%) and 25–34 (34.4%) than among exclusive current established daily SLT users or cigarette smokers (**Table 1**). In addition, 83.6% (95% CI, 80.4%–86.3%) of exclusive cigarette smokers and 78.0% (95% CI, 72.4%–82.8%) of exclusive SLT users reported daily product use. The most common dual use frequency of use combination was daily cigarette/nondaily SLT use (40.9%; 95% CI, 33.2%–48.9%), followed by daily cigarette/daily SLT use (25.0%; 95% CI, 16.9%–35.2%), nondaily cigarette/daily SLT use (24.0%; 95% CI, 17.0%–32.9%), and nondaily use of both products (10.1%; 95% CI, 5.1%–19.2%; **Fig. 1**).

Concentrations of BOEs by product type

Table 2 presents geometric mean concentrations and 95% CI of selected members of each biomarker panel for nicotine metabolites, TSNAs, PAHs, VOCs, metals, and arsenic by current tobacco use categories; all values were compared with never tobacco users. We also present statistical differences using cigarette smokers as the reference group. These biomarkers are selected for discussion due to their relevance to tobacco exposures and health outcomes. Results of all 52 BOEs analyzed are reported in Supplementary Tables S2–S7.

For nicotine metabolites, mean urinary TNE2 concentrations were highest among exclusive daily SLT users (68.6 μmol/g creatinine), followed by exclusive daily cigarette users (46.3 μmol/g creatinine) and dual users (44.2 μmol/g creatinine). Exclusive SLT users and exclusive cigarette smokers reporting daily use had substantially higher TNE2 concentrations than nondaily users. Similar patterns were observed for other nicotine metabolites (Supplementary Table S2).

Mean urinary TSNA concentrations were highest among exclusive daily SLT users (NNAL: 996.7 ng/g; N'-nitrosonornicotine (NNN): 33.9 ng/g) and dual users (NNAL: 456.6 ng/g; NNN: 17.8 ng/g), followed by exclusive daily cigarette smokers (NNAL: 298.3ng/g; NNN: 14.9 ng/g). Exclusive SLT users and exclusive cigarette users reporting daily use also had substantially higher TSNA concentrations than nondaily users. Similar trends were observed for NAB and NAT (Supplementary Table S3).

In contrast with TSNAs, PAH and VOC urinary biomarker concentrations were highest among exclusive daily cigarette smokers,

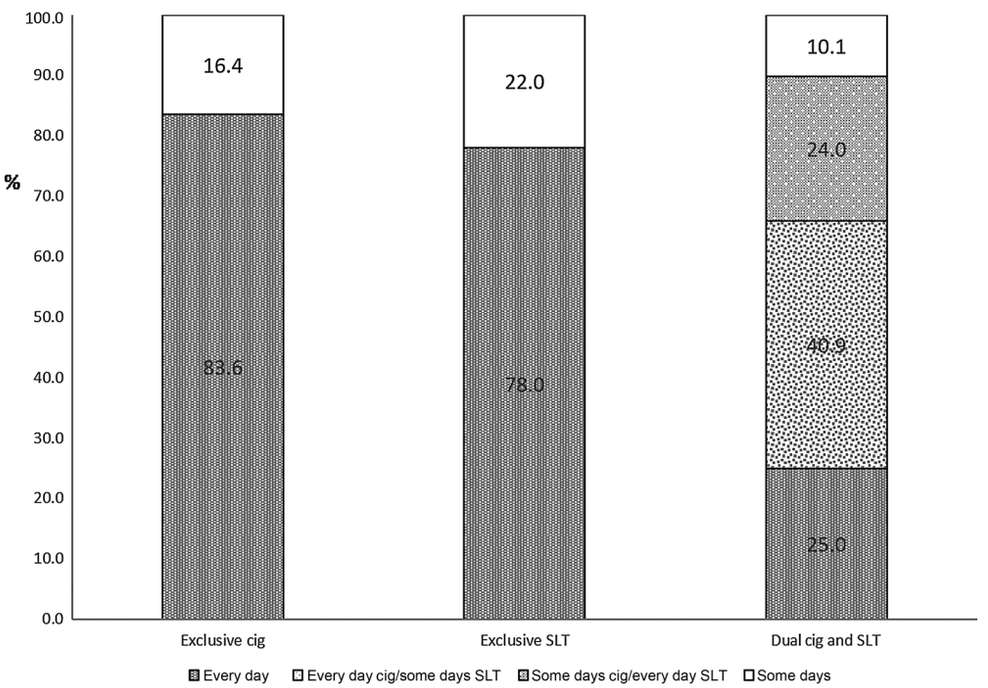

Figure 1.
Weighted frequency of product use (daily vs. nondaily use) by tobacco user groups. cig, cigarette.

Table 2. Weighted geometric mean of biomarker of tobacco exposures, by six tobacco use categories, PATH Study Wave 1 (2013–2014).

Urinary biomarker (creatinine-corrected)	Exclusive current est. cig users, daily		Exclusive current est. cig users, nondaily		Exclusive current est. SLT users, daily		Exclusive current est. SLT users, nondaily		Dual current est. cig + SLT users ($n = 140$)		Never tobacco users ($N = 1,655$)	
	n	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)
Nicotine metabolites												
TNE2, µmol/g	1,988	46.3 (43.2–49.6)[a]	434	2.1 (1.3–3.4)[a,b,c]	351	68.6 (60.2–78.2)[a,b]	97	5.4 (2.6–11.1)[a,b]	140	44.2 (35.2–55.5)[a]	1,633	0 (0–0)[b]
TSNA												
NNAL, ng/g	1,988	298.3 (276.3–322.1)[a]	436	27.1 (21.7–33.9)[a,b]	351	996.7 (852.1–1,165.9)[a,b]	97	117.5 (69.8–197.8)[a,b]	140	456.6 (372.1–560.3)[a,b]	1,653	0.9 (0.8–1)[b,c,d]
NNN, ng/g	1,898	14.9 (13.8–16) [a]	428	3.4 (2.8–4)[a,b,d]	335	33.9 (29.6–38.8)[a,b]	94	7.4 (5–10.9)[a,b,d]	138	17.8 (13.1–24.2)[a,b]	1,647	1.9 (1.8–2.0)[b,d]
PAH												
1-PYR, ng/g	1,990	333.9 (316.9–351.9)[a]	437	173.4 (155.5–193.4)[a,b]	351	173.1 (158.3–189.2)[a,b]	97	151.8 (125.3–184)[b]	140	263.5 (224.9–308.8)[a]	1,655	128.1 (120.7–136)[b]
2-FLU, ng/g	1,990	1,228.2 (1,172.2—1,286.8)[a]	437	334.4 (295.7–378.2)[a,b]	351	324.1 (299.5–350.7)[a,b]	96	213.8 (176.7–258.7)[a,b]	140	781.1 (671.3–908.9)[a,b]	1,655	167.2 (158.1–176.9)[b]
VOC												
HPMA, µg/g	1,962	1,387.6 (1,300.7–1,480.3)[a]	427	402.5 (345.1–469.4)[a,b]	342	250.1 (230.6–271.3)[b]	97	267.5 (229.4–312.0)[b]	135	738.5 (509.0–895.6)[a,b]	1,653	261.7 (247.2–277.1)[b]
CYMA, µg/g	1,989	176.0 (164.7–188.2)[a]	437	17.5 (13.1–23.3)[a,b]	349	1.8 (1.5–2.0)[a,b]	97	1.5 (1.2–1.9)[b]	139	66.3 (48.3–91.1)[a,b]	1,653	1.3 (1.2–1.4)[b]
MHB3, µg/g	1,989	34.5 (32.6–36.4)[a]	437	8.9 (7.5–10.5)[a,b]	348	4.3 (4.0–4.7)[b]	97	4.0 (3.4–4.7)[b]	139	17.9 (14.6–22.0)[a,b]	1,653	4.4 (4.2–4.6)[b]
Metals												
Cadmium, µg/g	1,982	0.3 (0.3–0.3)[a]	437	0.1 (0.1–0.2)[a,b]	351	0.1 (0.1–0.1)[a,b]	97	0.1 (0.1–0.1)[b]	140	0.1 (0.1–0.2)[a,b]	1,652	0.1 (0.1–0.2)[b]
Lead, µg/g	1,982	0.5 (0.5–0.5)[a]	437	0.4 (0.3–0.4)[a,b]	351	0.4 (0.4–0.5)[a,b]	97	0.4 (0.3–0.4)[b]	140	0.4 (0.4–0.5)[a]	1,653	0.4 (0.3–0.4)[b]
Total inorganic arsenic, µg/g	1,988	4.7 (4.5–4.9)[a]	437	5.1 (4.7–5.6)[b]	351	4.5 (4.2–4.8)	97	4.9 (4.3–5.6)	140	4.5 (4.1–5)[b]	1,653	5.4 (5.1–5.7)[b]

Abbreviations: 1-PYR, 1-hydroxypyrene; 2-FLU: 2-hydroxyfluorene; CI, confidence interval; cig, cigarette; CYMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine; GM, geometric mean; HPMA, N-Acetyl-S-(3-hydroxypropyl)-L-cysteine; MHB3, N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine; NNN, N′-nitrosonornicotine.

[a]Biomarker levels are significantly ($P < 0.05$) different from those in never tobacco users; adjusted for age, sex, race/ethnicity, education attainment, and log-transformed creatinine levels.

[b]Biomarker levels are significantly ($P < 0.05$) different from those in daily cigarettes smokers; adjusted for age, sex, race/ethnicity, education attainment, and log-transformed creatinine levels.

[c]Estimate should be interpreted with caution because it has low precision. It is based on a sample size of less than 50, or the coefficient of variation of the estimate is larger than 30%.

[d]Proportion of samples lower than LOD are >40%.

followed by dual users, and were substantially lower among exclusive SLT users who reported either daily or nondaily use. PAH concentrations (e.g., 1-hydroxypyrene (1-PYR): 173.1ng/g; 2-hydroxyfluorene (2-FLU): 324.1ng/g) in exclusive daily SLT users were substantially lower than those in exclusive daily smokers (**Table 2**). VOC biomarker concentrations were similar for exclusive SLT users and never tobacco users (**Table 2** and Supplementary Tables S4 and S5). In contrast with nicotine metabolites and TSNAs, biomarkers of PAHs and VOCs were higher among smokers than SLT users even when accounting for use frequency (**Table 2**).

For metals, urinary biomarker concentrations were generally similar across user groups, with slightly elevated concentrations observed in daily cigarette smokers (e.g., cadmium: 0.3 μg/g; lead 0.5 μg/g) than other user groups. Other metal exposures and total inorganic arsenic concentrations were also similar across user groups (**Table 2** and Supplementary Tables S6 and S7).

Concentrations of BOEs among dual SLT and cigarette users by use frequency

Table 3 summarizes BOE geometric mean concentrations by use frequency among current established dual users of SLT and cigarettes. Concentrations of TNE2 were highest among daily dual users (60.7 μmol/g), followed by users reporting daily SLT use/nondaily cigarette use and daily cigarette use/nondaily SLT use (53.6, 47.8μmol/g, respectively), and lowest among nondaily dual users (9.3 μmol/g). Similarly, TSNA biomarker concentrations differed by tobacco use frequency. Nondaily cigarette/daily SLT users also had higher NNAL (geometric mean = 680.2; 95% CI, 479.4–965.3) than daily cigarette/nondaily SLT users (geometric mean = 358.0; 95% CI, 298.7–429.1).

In contrast, urinary concentrations of PAH and VOC biomarkers were greatest among cigarette smokers. Dual users reporting daily smoking had substantially higher PAH and VOC concentrations (e.g., 1-PYR: 289.3ng/g; N-Acetyl-S-(3-hydroxypropyl)-L-cysteine (HPMA): 1,031.2μg/g) than dual users who reported nondaily smoking, regardless of whether they used SLT daily or nondaily.

Dose–response relationship between BOE concentrations and use frequency in SLT users

We analyzed mean BOE concentrations according to SLT use frequency (0–4, 5–9, 10+ times/day) among exclusive daily SLT users (excluding pouched snus; **Fig. 2**). In general, a positive dose–response relationship was observed for nicotine metabolites (TNE2: $P < 0.0001$), TSNAs (NNAL: $P = 0.007$; NNN: $P = 0.04$), and PAHs (1-PYR: $P = 0.02$); however, neither the VOC metabolites (e.g., N-Acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA): $P = 0.22$) nor the metals or inorganic arsenic metabolites (e.g., cadmium = 0.60) differed by increasing use frequency. When restricting the analyses to non-Hispanic males only (sensitivity analyses), the P value for the positive association between SLT use frequency and TNE2, NNAL, NNN remained significant ($P < 0.05$) whereas the association with 1-PYR was borderline significant ($P = 0.06$).

Discussion

Using urinary biospecimens collected from adult participants in PATH Study Wave 1, we provide population-level estimates of exposures to a broad range of tobacco product toxicants. A major strength

Table 3. Weighted geometric mean of biomarker of tobacco exposures among current established dual cigarette and SLT users, by frequency of use, PATH Study Wave 1 (2013–2014).

Urinary biomarker (creatinine-corrected)	Daily cig, daily SLT		Daily cig, nondaily SLT		Nondaily cig, daily SLT		Nondaily cig, nondaily SLT	
	n	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)	*n*	GM (95% CI)
Nicotine metabolites								
TNE2, μmol/g	33	60.7 (33.7–109.3)[a,b]	56	47.8 (41.3–55.4)[a]	35	53.6 (39.9–72)[a,b,c]	16	9.3 (3.9–22.5)[a,b,c]
TSNA								
NNAL, ng/g	33	863.1 (634.6–1,173.9)[a,b,c]	56	358 (298.7–429.1)[a,c]	35	680.2 (479.4–965.3)[a,b,c]	16	98.4 (54.3–178.1)[a,b,c]
NNN, ng/g	33	33.6 (18.3–61.6)[a,b,c]	55	16.9 (13.7–21)[a,c]	34	18.1 (11.6–28.3)[a,b,c]	16	4.4 (2.4–8.1)[a,b,c]
PAH								
1-PYR, ng/g	33	289.3 (229.7–364.3)[a,b,c]	56	343.6 (239.3–493.3)[a]	35	181.7 (145.8–226.3)[a,b,c]	16	173.5 (119.7–251.6)[a,b,c]
2-FLU, ng/g	33	872.8 (635–1,199.7)[a,b,c]	56	1,194 (940.7–1,515.5)[a]	35	436.9 (347.3–549.5)[a,b,c]	16	426.2 (298.6–608.3)[a,b,c]
VOC								
HPMA, μg/g	32	1,031.2 (620.3–1,714.1)[a,b]	53	1,211.5 (988.6–1,484.7)[a]	34	289.0 (228.9–365.0)[b,c]	16	383.9 (286.6–514.3)[a,b,c]
CYMA, μg/g	32	99.7 (45.0–221.0)[a,b]	56	153.8 (121.6–194.5)[a]	35	14.1 (8.1–24.4)[a,b,c]	16	33.3 (17.2–64.2)[a,b,c]
MHB3, μg/g	32	25.0 (16.2–38.5)[a,b]	56	29.7 (24.8–35.6)[a]	35	6.9 (5.2–9.1)[a,b,c]	16	10.3 (6.8–15.8)[a,b,c]
Metals								
Cadmium, μg/g	33	0.2 (0.2–0.3)[a,b]	56	0.2 (0.1–0.2)[a]	35	0.1 (0.1–0.1)[b,c]	16	0.1 (0–0.1)[b,c]
Lead, μg/g	33	0.5 (0.4–0.6)[a,b]	56	0.4 (0.4–0.5)[a,c]	35	0.3 (0.3–0.4)[a,b]	16	0.3 (0.2–0.5)[a,b]
Total inorganic arsenic, μg/g	33	4.9 (3.7–6.7)[b]	56	4.3 (3.9–4.8)	35	4.8 (3.8–6)[b,c]	16	4 (3–5.2)[b]

Abbreviations: 1-PYR, 1-hydroxypyrene; 2-FLU, 2-hydroxyfluorene; CI, confidence interval; cig, cigarette; CYMA, N-Acetyl-S-(2-cyanoethyl)-L-cysteine; GM, geometric mean; HPMA, N-Acetyl-S-(3-hydroxypropyl)-L-cysteine; MHB3, N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine; NNN, N′-nitrosonornicotine.

[a]Biomarker levels are significantly ($P < 0.05$) different from those in never tobacco users; adjusted for age, sex, race/ethnicity, education attainment, and log-transformed creatinine levels.

[b]Estimate should be interpreted with caution because it has low precision. It is based on a sample size of less than 50, or the coefficient of variation of the estimate is larger than 30%.

[c]Biomarker levels are significantly ($P < 0.05$) different from those in daily cigarette smokers; adjusted for age, sex, race/ethnicity, education attainment, and log-transformed creatinine levels.

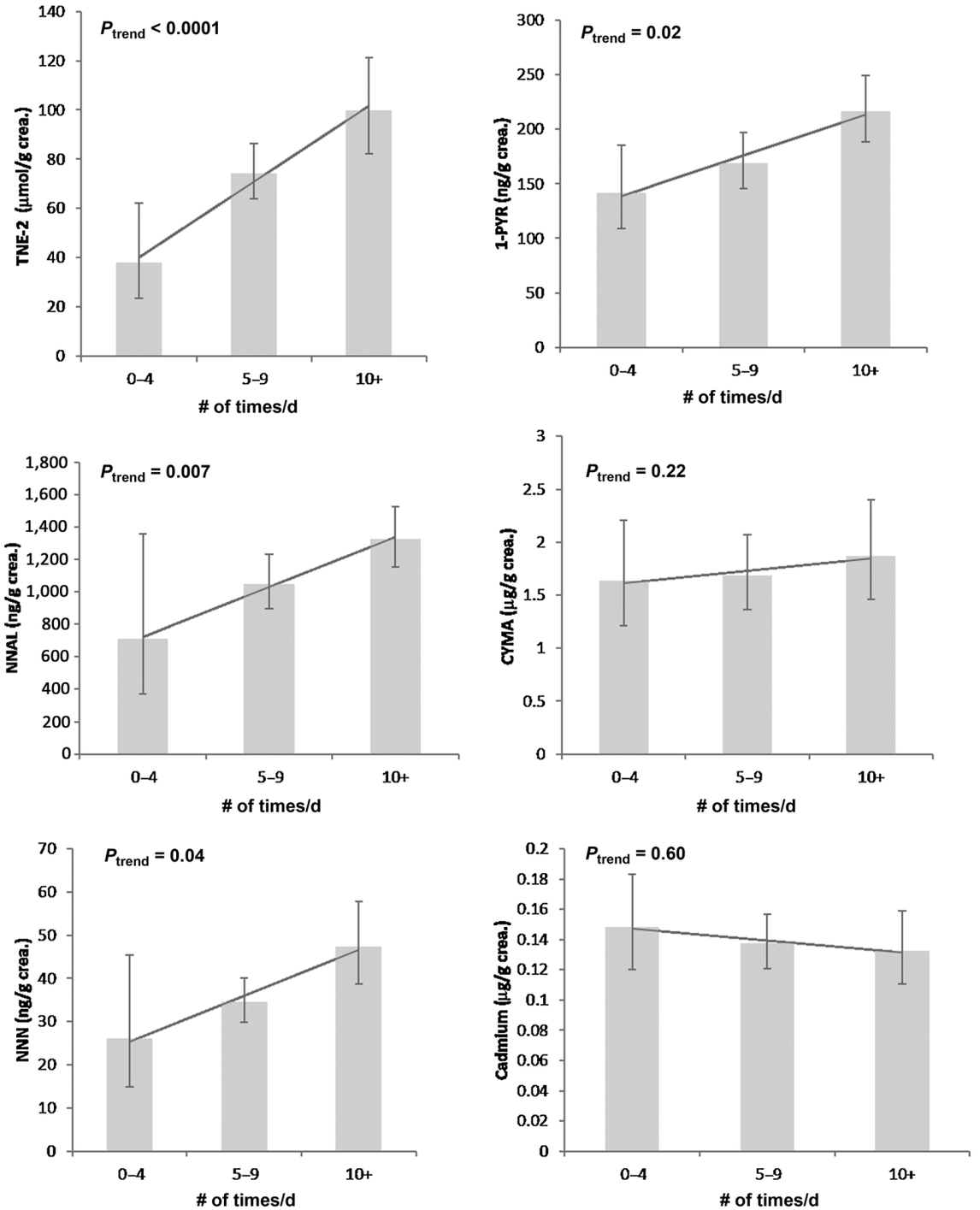

Figure 2.
Dose–response relationship between weighted biomarker levels and number of times used per day in exclusive daily SLT users. crea, creatinine.

of our study is the nationally representative sample of never and current established SLT users, cigarette users, and dual users in the U.S. adult population. To our knowledge, our study provides a more detailed and comprehensive assessment of tobacco BOEs than has been previously published (6). We included 52 metabolites of nicotine, TSNAs, PAHs, VOCs, metals and speciated arsenic. In addition, PATH Study Wave 1 includes a detailed assessment of tobacco product use frequency, which enhanced our ability to characterize SLT use, including daily and nondaily tobacco use. Several BOEs analyzed, including TSNAs such as NNAL, a metabolite of NNK, and NNN,

PAHs and inorganic arsenic compounds (4), are known human carcinogens; SLT is considered causally associated with oral cavity and pancreatic cancers by the International Agency for Research on Cancer (IARC; ref. 4). Our data indicated that several known harmful carcinogens, including NNAL and PAHs, are more elevated among SLT users than non-tobacco users, providing further information to help our understanding of the health risk associated with SLT use.

Like other published work, our study observed higher concentrations of biomarkers of nicotine metabolites and TSNAs among SLT users compared with cigarette smokers and never smokers (6, 29). BOEs, including most PAH and VOC panel constituents, were, as expected, greater among cigarette smokers than SLT users. The difference between tobacco user groups remained significant when restricting the analyses to non-Hispanic white male respondents in the sensitivity analyses (data not shown). Exposure to metals and arsenic was roughly comparable by tobacco user group and may reflect cumulative exposure from current and former tobacco use (regardless of route of intakes) or other environmental exposures. When restricting our analysis to daily tobacco users, differences by tobacco user group persisted. These results are consistent with previous findings that urinary and serum cotinine concentrations are at least as high among SLT users as among cigarette smokers (6, 30). Dual SLT and cigarette users who reported daily use of at least one product had higher urinary TNE2 and cotinine concentrations than exclusive daily cigarette users. This suggests that dual users may be supplementing their nicotine intake, rather than substituting products to maintain higher nicotine concentrations.

We found that NNAL concentrations among exclusive current established SLT users were about 3-fold higher than among exclusive current established cigarette smokers. Higher levels of NNAL in the urine of SLT users than cigarette smokers were first reported in 2007 (30). These results are also consistent with 1999–2012 NHANES data analyses (6). Differences were even more striking when we restricted the analyses to daily users. Unique to our study, we also observed similar patterns for other TSNA biomarker (e.g., NNN). TSNAs are formed during tobacco growing, curing, fermenting, and aging (3). Elevated concentrations of nicotine metabolites and TSNAs among SLT users could be related to absorption pathways (oral vs. inhalation; ref. 30) differences in tobacco product constituents due to manufacturing processes (curing, fermentation), storage (e.g., TSNA formation through microbial growth), or toxicant metabolism (8–10, 31, 32).

In contrast, PAH and VOC biomarkers were substantially higher among cigarette smokers than SLT users; among dual users, biomarker concentrations were mostly driven by smoking frequency rather than SLT use frequency. These data reflect the noncombustible nature of SLT products and provide evidence of reduced exposure to tobacco combustion toxicants among SLT users compared with smokers. Interestingly, dual product users reporting daily cigarette/nondaily SLT use had higher PAH and VOC concentrations than dual product users reporting daily use of both products. This could be related to the higher number of cigarettes/day (CPD) reported by the former group [median CPD = 18.43 (95% CI, 14.54–22.32) vs. 14.32 (95% CI, 9.12–19.53)]. Nevertheless, SLT users overall were still exposed to higher PAH concentrations than never tobacco users. Stepanov and colleagues (32) previously reported the presence of a variety of PAHs in 40 SLT products; concentrations varied across products. The presence of PAHs in SLT could be due to the fire-curing process, which includes direct contact with wood-burning smoke that contains high levels of PAHs; differences between products may reflect variations in tobacco processing techniques (32). We further demonstrated that PAHs were present in SLT product users, with a suggestive positive dose–response relationship between self-reported frequency of SLT use and PAH concentrations. Unlike PAHs, concentrations of VOC biomarkers (except for CYMA) were similar between SLT users and never tobacco users, consistent with previous findings that VOCs are present in tobacco smoke due to combustion (33–35).

This study has several limitations. First, tobacco use was self-reported. Biomarker concentrations reflect the most recent tobacco product used at the time of questionnaire response. In addition, toxicant concentrations can vary across SLT products (spit-free tobacco pouch vs. moist snuff; ref. 32). Previous research showed that Swedish snus and some newer American-brand snus products containing pasteurized tobacco had lower TSNAs levels than traditional SLT products (8). However, we were unable to assess whether BOEs differed between users of pouched snus versus other SLT products, due to the limited number of subjects using pouched snus exclusively. Finally, PATH Study Wave 1 data are cross-sectional; future PATH Study waves may allow examination of SLT use trajectories, changes in type of SLT used, and associated biomarker concentration changes within individuals over time and in relation to disease. Strengths of this study include its nationally representative sample of tobacco users, contemporary measure of SLT use, and tobacco exposure characterization.

In summary, we demonstrated that exclusive current established SLT users have higher nicotine metabolite and TSNA concentrations but lower PAH and VOC concentrations than exclusive current established cigarette smokers. However, SLT users still present higher biomarker concentrations of a variety of tobacco toxicants, including carcinogenic PAHs, compared with never tobacco users. We observed a strong dose–response relationship between self-reported SLT use frequency and nicotine, TSNA and PAH biomarker concentrations. These data, along with future PATH Study biomarker data, will illuminate tobacco toxicant exposure variations among SLT users resulting from within-product transitions (initiation, cessation) and product switching and inform new tobacco product application review.

Disclosure of Potential Conflicts of Interest

M.L. Goniewicz is a consultant/advisory board member for Johnson & Johnson. No potential conflicts of interest were disclosed by the other authors.

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