Research Article

Nicotine and Toxicant Exposure among U.S. Smokeless Tobacco Users: Results from 1999 to 2012 National Health and Nutrition Examination Survey Data

Brian L. Rostron[1], Cindy M. Chang[1], Dana M. van Bemmel[1], Yang Xia[2], and Benjamin C. Blount[2]

Abstract

Background: It has been suggested that smokeless tobacco users have high nicotine and toxicant exposure, but studies with nationally representative data have been limited.

Methods: We analyzed biomarkers of tobacco exposure for 23,684 adult participants from the National Health and Nutrition Examination Survey from 1999 to 2012. The biomarkers analyzed were serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), blood lead, blood cadmium, blood mercury, urinary arsenic, and urinary N-acetyl-S-(2-cyanoethyl)-L-cysteine. We calculated geometric mean concentrations for each biomarker by tobacco use category and geometric mean ratios adjusting for demographic factors.

Results: Exclusive smokeless tobacco users had higher geometric mean concentrations of serum cotinine [178.9 ng/mL, 95% confidence interval (CI), 145.5–220.0] and NNAL (583.0 pg/mg creatinine, 95% CI, 445.2–763.5) than exclusive cigarette smokers (130.6 ng/mL, 95% CI, 122.3–139.6 and 217.6 pg/mg creatinine,

95% CI, 193.0–245.2, respectively). Smokeless tobacco users also had higher concentrations of blood lead compared with nontobacco users (adjusted geometric mean ratio = 1.30, 95% CI, 1.21–1.38). Based on limited sample sizes, NNAL concentrations for smokeless tobacco users appear to have declined from 2007 to 2008 (geometric mean = 1013.7 pg/mg creatinine, 95% CI, 738.9–1390.8) to 2011 to 2012 (geometric mean = 325.7 pg/mg creatinine, 95% CI, 159.6–664.9).

Conclusions: Exclusive smokeless tobacco users have higher observed levels of exposure to nicotine and carcinogenic tobacco-specific nitrosamines, as measured by cotinine and NNAL biomarker concentrations, than exclusive cigarette smokers. These patterns in NNAL levels for smokeless tobacco users may be changing over time.

Impact: High exposure to harmful constituents among smokeless tobacco users is a continuing health issue. *Cancer Epidemiol Biomarkers Prev; 24(12); 1829–37. ©2015 AACR.*

Introduction

Use of smokeless tobacco products is attracting increasing attention from the public health community (1, 2). According to the National Adult Tobacco Survey, 7.1% of U.S. adult males were current users of chewing tobacco, snuff, dip, snus, or dissolvable tobacco products in 2012 to 2013, making smokeless tobacco the most commonly used tobacco product among men after cigarettes and cigars (3). Smokeless tobacco use is particularly common among young people. Among U.S. high school students, 9.6% of males were current users of chewing tobacco, snuff, or dip and 2.7% were current users of snus in 2013 according to the National Youth Tobacco Survey (NYTS; ref. 4), again making smokeless tobacco the third most commonly used tobacco product in this group. Smokeless tobacco use prevalence

among U.S. youth has also remained relatively consistent over time since 2000 according to the NYTS data (1), even as cigarette smoking prevalence continued to decline among U.S. youth during this period (5).

Biomarkers of tobacco exposure have previously been analyzed for cigarette smokers (6–8) and, to some extent, for cigar smokers (9), but less is known about biomarker levels among smokeless tobacco users. It is known that tobacco-specific nitrosamine (TSNA) levels in smokeless tobacco can vary due to a variety of factors, including tobacco type, growing conditions, curing and fermentation processes, and storage conditions (10, 11), and that TSNA levels in smokeless tobacco products can vary widely (12–14). It has also been suggested that levels of some biomarkers can be as high or higher among smokeless tobacco users as among cigarette smokers. For example, Hecht and colleagues (15) analyzed concentrations of urinary cotinine, a metabolite of nicotine, and the TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) for 182 smokeless tobacco users and 420 cigarette smokers and found that smokeless tobacco users had significantly higher cotinine and NNAL concentrations compared with smokers. Hecht and colleagues (16) subsequently found that cotinine and NNAL concentrations were significantly associated with years of use among smokeless tobacco users. Naufal and colleagues (17), on the other hand, analyzed biomarkers from U.S. National Health and Nutrition Examination Survey (NHANES) data from

[1]Office of Science, Center for Tobacco Products, Food and Drug Administration, Silver Spring, Maryland. [2]Tobacco and Volatiles Branch, Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, Georgia.

Corresponding Author: Brian L. Rostron, Food and Drug Administration, 10903 New Hampshire Avenue, Building 75, Room 4404, Silver Spring, MD 20993. Phone: 301-796-9360; Fax: 240-276-3655; E-mail: brian.rostron@fda.hhs.gov

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1999 to 2008 and concluded that biomarker concentrations were generally significantly lower among smokeless tobacco users compared with cigarette smokers, with the exception of NNAL and some halogenated aromatic hydrocarbons. They also did not find significant differences between smokeless tobacco users and nontobacco users with the exception of NNAL and some poly-aromatic hydrocarbons.

In this study, we analyzed biomarkers of tobacco exposure in a large nationally representative sample of U.S. tobacco users and nonusers from the NHANES from 1999 to 2012. We selected seven biomarkers for analysis based on their particular relevance to tobacco exposure and health outcomes: cotinine, NNAL, cadmium, lead, mercury, arsenic, and N-Acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA), a biomarker of exposure to tobacco smoke. We estimated geometric mean biomarker concentrations for smokeless tobacco users, cigarette smokers, dual cigarette and smokeless tobacco users, and nontobacco users. We also calculated geometric mean ratios using regression analysis to analyze the association between biomarker concentrations and tobacco use status, with and without adjustment for demographic and socioeconomic factors, such as sex, age, race/ethnicity, and educational attainment.

Our study builds upon previous research in presenting estimates from a large and nationally representative study population for smokeless tobacco users for cotinine, which was not included in the previous analysis by Naufal and colleagues, and NNAL, which was only available in this previous study for 2007 to 2008 NHANES participants, as well as the other selected biomarkers. As such, we present estimates not only of biomarker concentrations by tobacco use status, but also of biomarker concentrations over time, thus allowing us to investigate whether differences in product characteristics or product use patterns have contributed to changes in biomarker exposure for tobacco users in recent years.

Materials and Methods

Study population and tobacco use status

We analyzed biomarker concentrations by tobacco use for adult NHANES participants from 1999 to 2012. NHANES is a health and nutrition examination survey that uses a complex multistage design to obtain a nationally representative sample of the U.S. civilian noninstitutionalized population (18). NHANES has been conducted on a continuous basis by the National Center for Health Statistics since 1999 and surveys approximately 10,000 participants of all ages in each 2-year cycle. Survey participants complete health interviews in their homes that include a cigarette smoking history questionnaire for adults aged 20 years and older. Participants then complete an additional questionnaire on recent tobacco use including smokeless tobacco in a Mobile Examination Center (MEC), where they also receive a medical examination that includes the collection of biospecimens, such as urine and blood.

We analyzed biomarker concentrations among the 38,024 adults aged 20 years and older who participated in NHANES between 1999 and 2012. We excluded 736 survey participants who reported use of tobacco or nicotine products other than cigarettes, chewing tobacco, or snuff (i.e., cigars, pipes, or nicotine replacement therapy products) during the past 5 days as well as 5,318 participants who did not provide information on past 5-day tobacco use. We then categorized study participants into four mutually exclusive groups based on their self-reported cigarette and smokeless tobacco use: (1) 16,313 "nontobacco users" reported having smoked fewer than 100 cigarettes in their lives and not having used cigarettes, chewing tobacco, or snuff in the past 5 days, (2) 488 "smokeless tobacco users" reported using chewing tobacco or snuff in the past 5 days and currently not using cigarettes at all [228 smokeless tobacco users who reported being former cigarette smokers, having smoked at least 100 cigarettes but currently not smoking at all, were excluded from the analysis for cadmium due to its long half-life, which can be upwards of 10 years (19)], (3) 6,791 "cigarette smokers" reported having smoked at least 100 cigarettes in their lives and currently smoking every day or some days and not having used chewing tobacco or snuff in the past 5 days (these smokers did not have to have smoked cigarettes in the past 5 days), and (4) 92 "dual cigarette and smokeless tobacco users" reported having smoked at least 100 cigarettes in their lives, currently smoking every day or some days, and having used chewing tobacco or snuff in the past 5 days. Among the smokeless tobacco users, 309 individuals reported using chewing tobacco, 175 reported using snuff, and 4 reported using both chewing tobacco and snuff in the past 5 days. We did not include former cigarette smokers who had not used chewing tobacco or snuff in the past 5 days in the analysis. The analysis included a total of 23,684 participants.

Biomarkers of exposure

The biomarkers included in this analysis were selected due to their relevance to tobacco exposure and health outcomes. Cotinine is the primary proximate metabolite of nicotine (20, 21). NNAL is a metabolite of the TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The International Agency for Research on Cancer has determined that there is sufficient evidence in experimental animals for the carcinogenicity of NNK and NNAL and has categorized NNK as carcinogenic to humans (22, 23). NNK itself is formed from the nitrosation of nicotine (24). Lead, cadmium, mercury, and arsenic are elements of public health concern due to their toxicity and tendency to accumulate in the body. These and other metals can be found in tobacco products as well as in other environmental sources (25–28). CYMA is a metabolite of acrylonitrile and a selective biomarker of exposure to tobacco smoke (29). Urinary arsenic concentrations were available for 2003 to 2012 NHANES participants, urinary NNAL concentrations were available for 2007 to 2012 NHANES participants, and urinary CYMA concentrations were available for 2005 to 2006 and 2011 to 2012 survey participants. Blood lead, cadmium, and mercury and serum cotinine data were available from 1999 to 2012. Biomarker availability in NHANES data from these years for the individuals selected for the analysis was highest for lead and cadmium (both 96.1%), cotinine (94.7%), and NNAL (93.3%) and lower for CYMA (47.5%) and arsenic (32.6%), which were analyzed for special subsamples.

The analytical methods used to obtain these data are available in NHANES documentation (18). Serum cotinine was measured by an isotope dilution-liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry process. The half-life of cotinine is 15 to 20 hours, and its availability in blood, urine, and saliva makes it a commonly used biomarker of recent nicotine exposure (21, 30). Urinary total NNAL was measured using liquid chromatography linked to tandem mass spectrometry. The half-life of NNAL has been estimated to be 10 to 18 days (31). Blood cadmium, lead, and total mercury were measured using inductively coupled plasma mass spectrometry. Urinary

total arsenic was measured using liquid chromatography coupled to plasma dynamic reaction cell mass spectrometry. Urinary CYMA was measured using liquid chromatography coupled with electrospray tandem mass spectrometry. For concentrations below the limit of detection (LOD), a value equal to the LOD divided by the square root of two was used in the analysis.

Demographic variables

NHANES participants reported information on sex, age, race/ethnicity, and educational attainment. Race/ethnicity was subsequently categorized as non-Hispanic white, non-Hispanic black, Mexican-American, other Hispanic, and other race including multiracial. Educational attainment was categorized as less than high school graduate or equivalent, high school graduate or equivalent, and more than high school graduate or equivalent. Body mass index (BMI) for survey participants was calculated as kg/m^2 from their measured height and weight as a continuous variable.

Statistical analysis

Demographic and tobacco use variables were characterized using mean for continuous variables and percentages for categorical variables. Biomarker concentrations were log-transformed for the analysis to minimize the effects of skewness in the data on estimates, and geometric means of observed biomarker concentrations by tobacco use category were calculated. Univariate and multivariate linear regression analysis were also used to analyze the relationship between biomarkers of exposure and tobacco use category, adjusting for sex, age, race/ethnicity, educational attainment, and BMI, with nontobacco users as the reference category.

Geometric mean ratios and 95% confidence intervals (CI), from these analyses were calculated by exponentiating the estimated coefficients and their standard errors. Results for analyses for lead were robust to the inclusion of ratio of family income to poverty threshold and survey year as covariates. Geometric mean biomarker concentrations were also calculated for cotinine and NNAL by 2-year NHANES survey cycle, and trend tests by time were conducted using regression analysis. Box plots were also created to show the unweighted distribution of cotinine and NNAL concentrations for smokeless tobacco users by days reported using chewing tobacco or snuff in the past 5 days. All analyses were conducted using the SAS version 9.3 (SAS Institute), and all figures were constructed using the R version 3.0.2 (R Core Team). Analyses were conducted using the MEC sample weights with the exception of analyses for arsenic, which were conducted with environmental subsample weights, and analyses for CYMA, which were conducted with the 2005 to 2006 volatile organic compounds subsample weights and 2011 to 2102 smoking subsample weights. Analyses were conducted taking into account the NHANES complex survey design.

Results

Characteristics of the study population by tobacco use status

Table 1 presents weighted demographic and tobacco use information for the NHANES study participants according to tobacco use status. Smokeless tobacco and dual users were overwhelmingly male, at 94.7% (95% CI, 92.1%–97.2%) and 99.4% (95% CI, 98.6%–100.0%), respectively. Dual users tended to be younger than members of other tobacco use groups with a mean age of 33.1 years (95% CI, 30.3–35.8). Smokeless tobacco and dual

Table 1. Characteristics of NHANES participants by tobacco use status: United States 1999 to 2012

Characteristics	N	Nontobacco users (N = 16,313)	Exclusive smokeless tobacco users (N = 488)	Exclusive cigarette smokers (N = 6,791)	Dual cigarette and smokeless tobacco users (N = 92)
Age (years)	23,684	45.9 (45.3–46.5)[a]	44.2 (42.7–45.7)	42.0 (41.5–42.4)	33.1 (30.3–35.8)
Sex					
Male	10,571	40.1% (39.2%–41.0%)	94.7% (92.1%–97.2%)	53.9% (52.5%–55.4%)	99.4% (98.6%–100.0%)
Female	13,113	59.9% (59.0%–60.8%)	5.3% (2.8%–7.9%)	46.1% (44.6%–47.5%)	0.6% (0.0%–1.4%)
Race/ethnicity					
Mexican-American	4,583	9.0% (7.6%–10.3%)	2.5% (1.3%–3.7%)	6.8% (5.6%–8.0%)	2.1% (0.0%–4.2%)
Other Hispanic	1,765	5.9% (4.7%–7.2%)	0.7% (0.0%–1.5%)	4.9% (3.4%–6.4%)	1.4% (0.0%–4.3%)
Non-Hispanic white	10,780	66.8% (64.2%–69.4%)	88.7% (85.3%–92.1%)	70.8% (67.9%–73.7%)	94.2% (90.1%–98.2%)
Non-Hispanic black	5,095	11.7% (10.3%–13.2%)	5.7% (3.5%–7.8%)	12.3% (10.7%–13.9%)	1.1% (0.0%–2.4%)
Other	1,461	6.6% (5.7%–7.4%)	2.5% (0.9%–4.1%)	5.1% (4.2%–6.0%)	1.2% (0.0%–3.3%)
Education					
<High school graduate	6,936	15.4% (14.4%–16.4%)	20.3% (15.9%–24.6%)	27.7% (26.1%–29.3%)	15.1% (7.6%–22.6%)
High school graduate	5,587	21.1% (20.1%–22.2%)	34.2% (28.8%–39.7%)	31.5% (30.0%–32.9%)	42.7% (30.0%–55.4%)
>High school graduate	11,134	63.5% (62.0%–65.0%)	45.5% (40.0%–51.0%)	40.9% (39.0%–42.8%)	42.3% (30.6%–54.0%)
BMI, kg/m^2	23,311	28.7 (28.5–28.8)	30.0 (29.3–30.8)	27.5 (27.3–27.6)	26.7 (25.5–27.9)
Past 5-day cigarette/smokeless tobacco use					
Number of days smoked cigarettes				4.4 (4.4–4.5)	3.2 (2.7–3.7)
Number of cigarettes smoked per day on days smoking cigarettes				14.8 (14.4–15.3)	11.9 (8.6–15.2)
Number of days used chewing tobacco			4.2 (4.1–4.4)		3.7 (3.3–4.2)
Number of days used snuff			4.3 (4.1–4.5)		3.5 (3.0–4.0)

NOTE: Nontobacco users reported not having smoked at least 100 cigarettes in their lives and not having used cigarettes, chewing tobacco, or snuff in the past 5 days. Smokeless tobacco users reported having used chewing tobacco or snuff in the past 5 days and currently not smoking cigarettes at all. Cigarette smokers reported having smoked at least 100 cigarettes in their lives and currently smoking every day or some days and not having used chewing tobacco or snuff in the past 5 days. Dual cigarette and smokeless tobacco users reported having smoked at least 100 cigarettes in their lives, currently smoking every day or some days, and having used chewing tobacco or snuff in the past 5 days. Survey participants were excluded from each group if they reported having used cigars, pipes, or nicotine gum, patches, or other nicotine products in the past 5 days.
[a]95% CIs for mean and percentages are shown in parentheses.

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users were also more likely to be non-Hispanic whites than members of other tobacco use groups at 88.7% (95% CI, 85.3%–92.1%) and 94.2% (95% CI, 90.1%–98.2%), respectively. The estimated mean number of cigarettes that dual users smoked on days that they smoked cigarettes in the past 5 days was less than the estimated mean for cigarette smokers, at 11.9 compared with 14.8 cigarettes, but the difference was not statistically significant ($P = 0.071$). Dual users smoked cigarettes on fewer of the past 5 days on average than exclusive cigarette smokers ($P = 0.042$), and dual users who used chewing tobacco or snuff in the past 5 days used on fewer days than exclusive smokeless tobacco users who used these products ($P < 0.0001$ for both chewing tobacco and snuff). Smokeless tobacco users tended to have consistently used smokeless tobacco in the past 5 days, with an average of 4.2 days (95% CI, 4.1–4.4) having used chewing tobacco for chewing tobacco users and an average of 4.3 days (95% CI, 4.1–4.5) having used snuff for snuff users.

Analysis of biomarkers of exposure by tobacco use status

Table 2 presents geometric mean biomarker concentrations by tobacco use status. Mean serum cotinine concentrations were higher for smokeless tobacco users (178.9 ng/mL, 95% CI, 145.5–220.0) than for cigarette smokers (130.6 ng/mL, 95% CI, 122.3–139.6). Cotinine concentrations for dual users (184.1 ng/mL, 95% CI, 132.4–256.0) were similar to concentrations for smokeless tobacco users. Mean urinary NNAL concentrations were higher for smokeless tobacco users (583.0 pg/mg creatinine, 95% CI, 445.2–763.5) and dual users (430.3 pg/mg creatinine, 95% CI, 284.8–650.1) than for cigarette smokers (217.6 pg/mg creatinine, 95% CI, 193.0–245.2). Mean NNAL concentrations were generally comparable for exclusive chewing tobacco (564.1 pg/mg creatinine, 95% CI, 391.0–813.9) and snuff (631.3 pg/mg creatinine, 95% CI, 378.1–1054.2) users. Exclusion of the relatively small proportion of current cigarette smokers who reported not having smoked cigarettes in the past 5 days ($n = 301$ of 6,791)

in the sensitivity analysis produced similar results. For example, the geometric mean concentration of cotinine for the remaining smokers was 156.7 ng/mL (95% CI, 150.3–163.4) and the mean concentration for NNAL was 247.3 pg/mg creatinine (95% CI, 225.4–271.3).

Mean concentrations of blood lead were higher among smokeless tobacco users (1.76 µg/L, 95% CI, 1.62–1.91), dual users (1.76 µg/L, 95% CI, 1.55–2.00), and cigarette smokers (1.76 µg/L, 95% CI, 1.71–1.81) compared with nontobacco users (1.18 µg/L, 95% CI, 1.16–1.21). Mean concentrations of blood cadmium, blood mercury, and urinary arsenic were not elevated among smokeless tobacco users compared with nontobacco users.

Mean CYMA concentrations were higher among cigarette smokers (117.3 ng/mg creatinine, 95% CI, 103.1–133.4) and dual users (35.4 ng/mg creatinine, 95% CI, 2.1–606.8) but not among smokeless tobacco users (2.21 ng/mg creatinine, 95% CI, 1.11–4.39) compared with nontobacco users (1.47 ng/mg creatinine, 95% CI, 1.37–1.58).

Associations between biomarkers of exposure and tobacco use status

Table 3 presents results from multivariate regression analyses conducted to analyze whether tobacco use status was associated with higher biomarker concentrations, adjusting for demographic and socioeconomic factors.

After adjustment for age, sex, race/ethnicity, education, and BMI, smokeless tobacco users, cigarette smokers, and dual users had increased geometric mean ratios for serum cotinine compared with nontobacco users. Smokeless tobacco users also had increased geometric mean ratios compared with cigarette smokers ($P = 0.039$). Smokeless tobacco users, cigarette smokers, and dual users also had increased geometric mean ratios for urinary NNAL compared with nontobacco users, and smokeless tobacco users and dual users had increased geometric mean ratios compared with cigarette smokers.

Table 2. Geometric mean biomarker concentrations by tobacco use status, NHANES 1999 to 2012

Biomarkers of exposure	Nontobacco users	Exclusive smokeless tobacco users	Exclusive cigarette smokers	Dual cigarette and smokeless tobacco users
Serum cotinine, ng/mL				
Number of observations	15,424	476	6,439	90
Mean (95% CI)	0.043 (0.041–0.046)	179.6 (145.8–221.1)	130.6 (122.3–139.6)	184.1 (132.4–256.0)
Urinary NNAL, pg/mg creatinine				
Number of observations	7,243	210	2,952	43
Mean (95% CI)	0.98 (0.92–1.04)	583.0 (445.2–763.5)	217.6 (193.0–245.2)	430.3 (284.8–650.1)
Blood cadmium, µg/L				
Number of observations	15,687	254	6,509	90
Mean (95% CI)	0.268 (0.262–0.273)	0.220 (0.201–0.240)	0.941 (0.916–0.968)	0.644 (0.515–0.806)
Blood lead, µg/L				
Number of observations	15,687	477	6,509	90
Mean (95% CI)	1.18 (1.16–1.21)	1.76 (1.62–1.91)	1.76 (1.71–1.81)	1.76 (1.55–2.00)
Blood mercury, µg/L				
Number of observations	12,997	357	5,258	64
Mean (95% CI)	1.02 (0.97–1.06)	0.82 (0.73–0.93)	0.77 (0.73–0.81)	0.63 (0.49–0.80)
Urinary arsenic, ng/mg creatinine				
Number of observations	3,905	119	1,538	27
Mean (95% CI)	9.53 (8.98–10.11)	6.43 (5.36–7.71)	7.65 (7.05–8.30)	6.73 (4.84–9.37)
Urinary CYMA, ng/mg creatinine				
Number of observations	1,883	41	1,202	14
Mean (95% CI)	1.47 (1.37–1.58)	2.21 (1.11–4.39)	117.3 (103.1–133.4)	35.4 (2.1–606.8)

NOTE: Urinary NNAL, arsenic, and CYMA concentrations were adjusted for creatinine. NNAL data were available for 2007 to 2012 NHANES participants, arsenic data were available for 2003 to 2012 NHANES participants, and CYMA data were available for 2005 to 2006 and 2011 to 2012 NHANES participants. Former cigarette smokers were excluded from smokeless tobacco users for the analysis for cadmium.

Table 3. Geometric mean ratios for biomarkers of exposure by tobacco use status, NHANES 1999 to 2012

Biomarker	Tobacco use category	Unadjusted geometric mean ratio (95% CI)	Adjusted geometric mean ratio (95% CI)
Serum cotinine	Exclusive smokeless tobacco users	4,160 (3,406–5,081)	3,194 (2,623–3,888)
	Exclusive cigarette smokers	3,027 (2,801–3,270)	2,439 (2,240–2,655)
	Dual cigarette/smokeless tobacco users	4,265 (3,064–5,936)	3,009 (2,174–4,164)
	Nontobacco users (Ref)	1	1
Urinary NNAL	Exclusive smokeless tobacco users	760 (574–1006)	587 (451–764)
	Exclusive cigarette smokers	229 (205–255)	190 (171–210)
	Dual cigarette/smokeless tobacco users	541 (313–935)	393 (252–614)
	Nontobacco users (Ref)	1	1
Blood cadmium	Exclusive smokeless tobacco users	0.82 (0.75–0.90)	1.00 (0.93–1.08)
	Exclusive cigarette smokers	3.52 (3.41–3.63)	3.69 (3.57–3.81)
	Dual cigarette/smokeless tobacco users	2.41 (1.93–3.00)	3.10 (2.50–3.85)
	Nontobacco users (Ref)	1	1
Blood lead	Exclusive smokeless tobacco users	1.49 (1.37–1.61)	1.30 (1.21–1.38)
	Exclusive cigarette smokers	1.48 (1.44–1.53)	1.46 (1.42–1.49)
	Dual cigarette/smokeless tobacco users	1.49 (1.31–1.70)	1.50 (1.34–1.67)
	Nontobacco users (Ref)	1	1
Blood mercury	Exclusive smokeless tobacco users	0.81 (0.71–0.92)	0.86 (0.75–0.98)
	Exclusive cigarette smokers	0.76 (0.72–0.80)	0.83 (0.79–0.87)
	Dual cigarette/smokeless tobacco users	0.62 (0.48–0.79)	0.70 (0.55–0.89)
	Nontobacco users (Ref)	1	1
Urinary arsenic	Exclusive smokeless tobacco users	0.91 (0.75–1.09)	0.86 (0.73–1.02)
	Exclusive cigarette only smokers	0.83 (0.76–0.92)	0.87 (0.80–0.94)
	Dual cigarette/smokeless tobacco users	0.83 (0.45–1.55)	0.81 (0.59–1.10)
	Nontobacco users (Ref)	1	1
Urinary CYMA	Exclusive smokeless tobacco users	2.04 (1.04–4.01)	1.62 (0.83–3.18)
	Exclusive cigarette only smokers	84.9 (72.5–99.3)	75.3 (65.2–87.1)
	Dual cigarette/smokeless tobacco users	33.5 (0.8–1398.5)	18.4 (0.7–463.5)
	Nontobacco users (Ref)	1	1

NOTE: NNAL data were available for 2007 to 2012 NHANES participants, arsenic data were available for 2003 to 2012 NHANES participants, and CYMA data were available for 2005 to 2006 and 2011 to 2012 NHANES participants. The adjusted geometric mean ratios control for age, sex, race/ethnicity, educational attainment, and body mass index. For urinary arsenic, CYMA, and NNAL, the adjusted ratios further control for urinary creatinine.

Smokeless tobacco users, along with cigarette smokers and dual users, had increased geometric mean ratios for blood lead compared with nontobacco users. Smokeless tobacco users did not have increased geometric mean ratios for any of the other biomarkers.

Trends in tobacco-specific biomarkers over time

Figure 1 presents geometric mean serum cotinine and urinary NNAL concentrations for cigarette smokers and smokeless tobacco users over time. Cotinine concentrations for smokers and smokeless users were relatively consistent over time, although estimates for the smaller number of smokeless tobacco users showed more variability. Tests of trend for cotinine concentrations produced P values of 0.895 for smokers and 0.403 for smokeless tobacco users. Mean NNAL concentrations for smokers were relatively consistent from 2007 to 2008 to 2011 to 2012 but declined dramatically for smokeless tobacco users from a geometric mean of 1013.7 pg/mg creatinine (95% CI, 738.9–1390.8, $n = 81$) in 2007 to 2008 to 328.6 pg/mg creatinine (95% CI, 164.7–655.6, $n = 53$) in 2011 to 2012. Tests of trend for NNAL concentrations produced P values of 0.943 for smokers and 0.003 for smokeless tobacco users. NNAL concentrations were higher for smokeless tobacco users than for cigarette smokers in 2007 to 2008 and 2009 to 2010 ($P < 0.001$) but not necessarily in 2011 to 2012 ($P = 0.297$).

Analysis of dose–response relationship for tobacco-specific biomarkers

Figure 2 presents boxplots showing the distribution of cotinine and NNAL concentrations for chewing tobacco and snuff users by frequency of use in terms of the number of days that they had used the product in the past 5 days. The figure shows that concentrations consistently increased with number of days of use. Tests of trend for the association between biomarker concentrations and days using the product produced P values less than 0.001 for chewing tobacco and snuff for cotinine and equal to 0.003 for chewing tobacco and 0.031 for snuff for NNAL.

Discussion

In this study, we have analyzed important tobacco-related biomarkers for over 23,000 NHANES participants from 1999 to 2012. To our knowledge, this work provides the first estimates from a large, nationally representative U.S. health survey population that compare cotinine and NNAL concentrations for smokeless tobacco and cigarette users and presents trends in NNAL concentrations over time. We have found higher cotinine concentrations and much higher NNAL concentrations for smokeless tobacco users compared with cigarette smokers as well as higher NNAL concentrations for dual users compared with smokers. We have also found evidence that NNAL concentrations among smokeless tobacco users are declining over time, although the sample sizes for this analysis were limited due to the introduction of analysis of NNAL with the 2007 to 2008 NHANES cycle. We also found that smokeless tobacco users have higher concentrations of lead, but not cadmium, mercury, arsenic, or CYMA, compared with nontobacco users.

The results for NNAL in this study are rather striking, both in terms of the magnitude of overall exposure for smokeless tobacco

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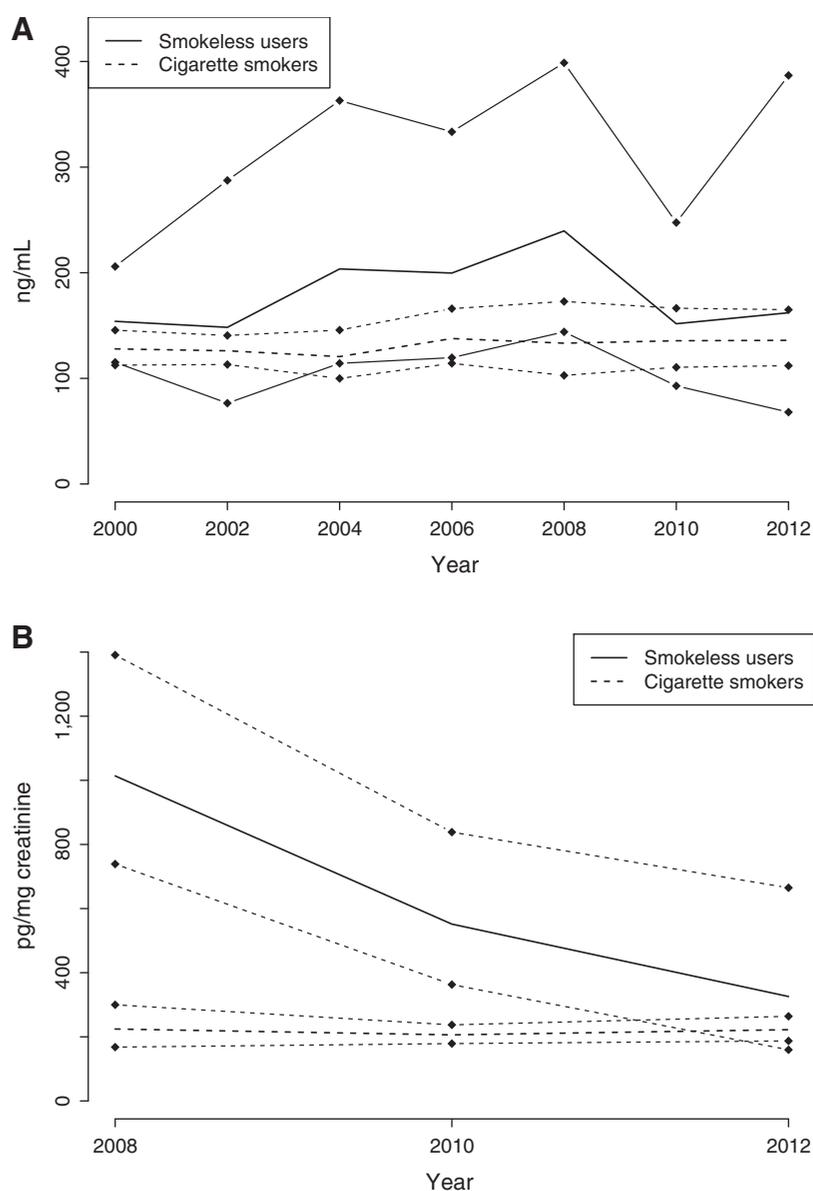
Figure 1.
Geometric mean biomarker concentrations by tobacco use status by year for (A) serum cotinine and (B) urinary total NNAL. Lines with diamonds indicate 95% CIs.

users as well as the apparent decrease in NNAL exposure over time. Our results from a large and nationally representative survey population confirm previous findings from a smaller local study that NNAL and cotinine concentrations are at least as high among smokeless tobacco users as among cigarette smokers (15), with NNAL concentrations for smokeless tobacco users in this study being on average almost three times as high as concentrations for cigarette smokers. The causes of these differences in exposure between cigarette and smokeless tobacco users are not entirely understood. Possible explanations include differences in use of the products including how nicotine and other constituents are absorbed by users (15) as well as differences in constituent levels in cigarette and smokeless tobacco (32) due to factors such as tobacco type and curing and fermentation processes. It has also been previously suggested that higher cotinine concentrations for smokeless tobacco users in urine, but not necessarily in serum, could be related to first pass clearance of swallowed tobacco juice,

whereby constituents could to some extent be metabolized and excreted before they reach the systemic circulatory system (15). Similar issues related to metabolism and clearance of NNK and NNAL could also affect urinary NNAL levels among smokeless tobacco users in this study. Even so, results from this and previous research (15) suggest that nicotine and NNK exposure in smokeless tobacco users is at least as high as, if not higher than, exposure among cigarette smokers.

Although based on limited sample sizes, estimated NNAL concentrations for smokeless users fell by more than two thirds from 2007 to 2008 to 2011 to 2012, even though cotinine concentrations for these users declined much less dramatically during this period. This decrease in NNAL concentrations could be due to a variety of factors, including reductions in the quantity of smokeless tobacco used, although estimates from NHANES do not show a decrease in the number of days that individuals used chewing tobacco or snuff in the past 5 days. For example, chewing
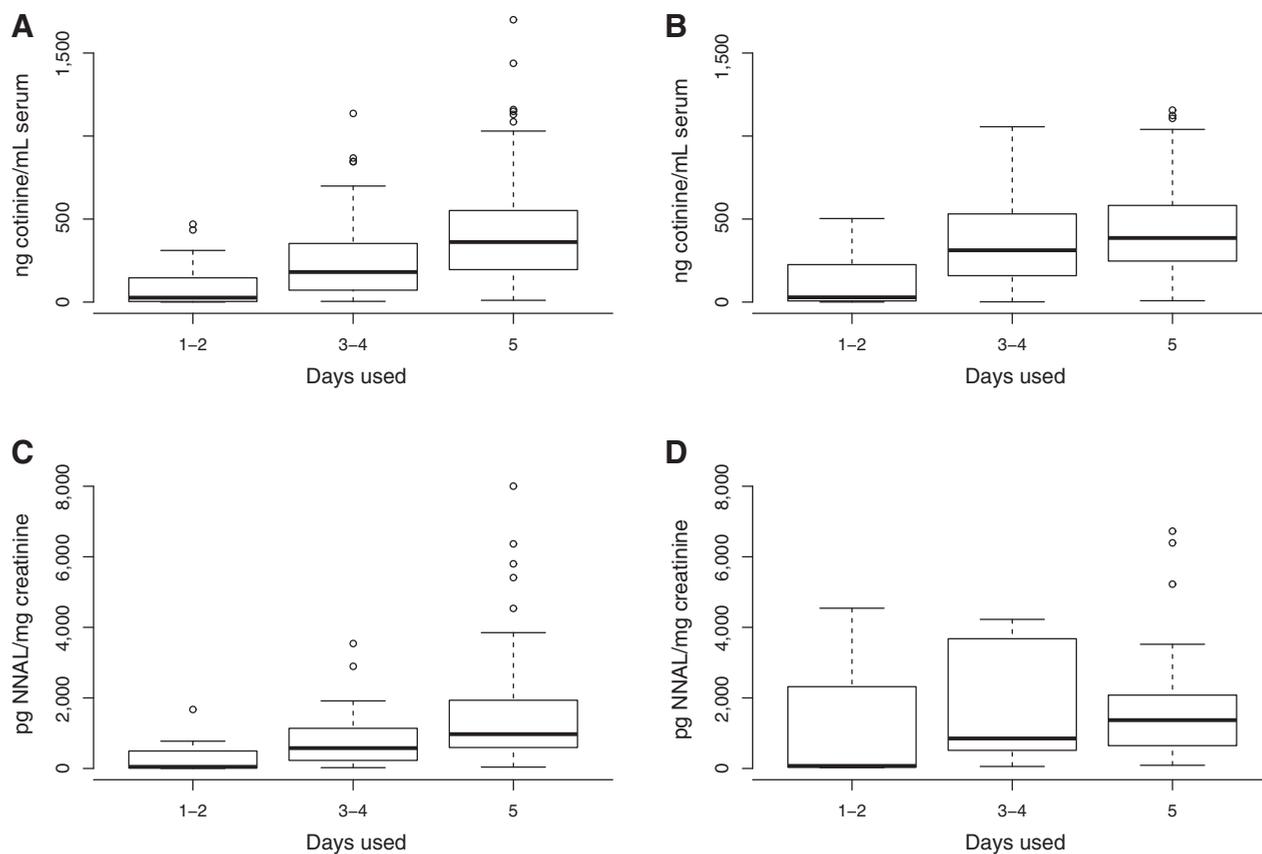
Figure 2.
Biomarker concentrations for smokeless tobacco users by number of days used chewing tobacco or snuff in the past 5 days for (A) serum cotinine for chewing tobacco users, (B) serum cotinine for snuff users, (C) urinary total NNAL for chewing tobacco users, and (D) urinary total NNAL for snuff users.

tobacco users reported using the product on an average of 4.5 days (95% CI, 4.4–4.7) in 2007 to 2008 and 4.3 days (95% CI, 3.7–4.9) in 2011 to 2012. Moreover, cotinine concentrations among smokeless tobacco users were relatively consistent during the period. The decrease in NNAL concentrations could result in part from reductions in TSNAs in smokeless tobacco products generally. Borgerding and colleagues (33) analyzed toxicant concentrations in 43 U.S. smokeless tobacco products sold in the United States in 2006 and 2007 and found that TSNA concentrations observed for all of these commercial products were lower than historically reported values. Fisher and colleagues (10) found a decrease in average TSNAs for three commercial moist snuff products from 1997 to 2010, particularly in the period prior to 2005. The decrease in NNAL concentrations among smokeless tobacco users may also reflect a movement among users to smokeless products with lower levels of certain harmful constituents. Stepanov and colleagues (12), for example, analyzed total TSNAs in relatively new smokeless tobacco products such as Taboka, Marlboro Snus, Camel Snus, and Skoal Dry as compared with popular traditional brands of moist snuff such as Copenhagen Snuff, Skoal Long Cut, and Kodiak Wintergreen that were purchased in 2006 to 2007. They found that total TSNAs averaged 1.97 μg/g dry weight tobacco in Taboka, Marlboro Snus, and Camel Snus, 4.54 μg/g dry weight tobacco in Skoal Dry, and 7.42 μg/g in the traditional moist snuff brands. Similar results were

found specifically for NNK, the precursor of NNAL. Changes have also been observed in smokeless tobacco product use over time. Delnevo and colleagues (34) analyzed smokeless tobacco convenience store sales data from 2005 to 2011 and found changes in product market share during this period. Market share for chewing tobacco, for example, decreased from 9.0% to 4.3% during this time, and sales of snus increased from 0.0% to 3.7%. Approximately 90% of smokeless tobacco sold in convenience stores throughout the period was moist snuff, but the market share of portion pouches within this category increased from 5.5% to 14.5% during the period. Trends in NNAL concentrations among smokeless and other tobacco users should continue to be monitored and evaluated over time.

This analysis has also found that blood lead levels in smokeless tobacco users are comparable with those of cigarette smokers and higher than levels for nontobacco users. This result is consistent with previous analysis of NHANES data (17). Further research on this topic is needed to establish that smokeless tobacco is the cause of these elevated lead levels among users and, if so, to identify the aspects of smokeless tobacco production that contribute to these higher levels. CYMA concentrations were also higher among cigarette smokers and dual cigarette and smokeless tobacco users, but not among exclusive smokeless tobacco users when compared with nontobacco users. This result is consistent with expectations, given that CYMA is a biomarker for smoke exposure.

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Results in this study are subject to certain limitations, primarily due to the nature of the data being collected. First, all information on tobacco use comes from self-report by survey participants and may be subject to some misclassification. For example, a majority of NHANES smokeless tobacco users reported that they used chewing tobacco, but market share data indicated that most smokeless tobacco purchased in convenience stores during the period was moist snuff. It may be possible that some snuff users reported themselves as chewing tobacco users. Second, we do not have detailed information on the type of smokeless tobacco product used, such as information on brand or product type, such as snus, apart from chewing tobacco and snuff. Third, we do not have information on the quantity of product used, such as amount used per day, apart from the number of days using the product in the past 5 days. Fourth, we have reported results for all NHANES participants reporting current cigarette or smokeless tobacco use, but results may vary when participants are categorized by groups such as daily or some day users or by race/ethnicity. Finally, NHANES participants were only asked about past 5-day use of certain tobacco products other than cigarettes. We were thus unable to evaluate any effects of duration or former use of smokeless tobacco products. We also have no information on e-cigarette use in NHANES data, but e-cigarette use was minimal during much of the period of this analysis.

Our results have shown that smokeless tobacco users have high levels of known harmful and addictive constituents and that in some cases these levels are higher than those observed among cigarette smokers. This finding is a cause of considerable concern for individual and public health. These results thus demonstrate the need for continuing study of the toxic constituents of smokeless tobacco as well as their health effects on the individuals who use them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The views and opinions expressed in this article are those of the authors only and do not necessarily represent the views, official policy, or position of the US Department of Health and Human Services or any of its affiliated institutions or agencies.

Authors' Contributions

Conception and design: B.L. Rostron, C.M. Chang, D.M. van Bemmel, B.C. Blount
Development of methodology: B.L. Rostron, D.M. van Bemmel, B.C. Blount
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.L. Rostron, Y. Xia, B.C. Blount
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.L. Rostron, C.M. Chang, Y. Xia, B.C. Blount
Writing, review, and/or revision of the manuscript: B.L. Rostron, C.M. Chang, D.M. van Bemmel, Y. Xia, B.C. Blount
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Xia
Study supervision: B.L. Rostron

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Brian L. Rostron, Cindy M. Chang, Dana M. van Bemmel, et al.

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