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Tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers in the united states: NHANES 2007–2008

Yang Xia[1*], John T. Bernert[1], Ram B. Jain[1], David L. Ashley[2], and James L. Pirkle[1]

[1]*Division of Laboratory Science, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and* [2]*Office of Science, Center for Tobacco Products, Food and Drug Administration, 9200 Corporate Boulevard, Rockville, MD 20850-3229, USA*

Abstract

The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of the tobacco-specific nitrosamine (TSNA) 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), has been measured in urine samples from all participants aged 6 years and older from the National Health and Nutrition Examination Survey 2007–2008. Participants with a serum cotinine concentration of ≥10 ng/mL were identified as tobacco users, primarily cigarette smokers. Regression models were developed to calculate geometric mean NNAL concentrations adjusted for serum cotinine, urinary creatinine, cigarettes per day, and Federal Trade Commission tar values of the cigarettes smoked. Significant differences were found by gender ($p = 0.003$) and race/ethnicity ($p = 0.022$ for non-Hispanic white versus non-Hispanic black smokers), but not by menthol type of the cigarettes. Females and non-Hispanic white smokers had the highest adjusted means for urinary NNAL (353 and 336 pg/mL, respectively). The results from this study demonstrated significant relationships between NNAL concentrations and serum cotinine ($p < 0.001$) and urine creatinine ($p < 0.001$). The joint effect of linear and quadratic terms for number of cigarettes smoked per day was also statistically significant ($p = 0.001$). In addition to addressing current NNK exposure levels, these results will form a baseline for future estimates of tobacco users' exposure to this carcinogen.

Key words: *NNAL; NNK; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; TSNA; NHANES; smoking; tobacco*

Introduction

Health risks associated with active tobacco use have been extensively documented over many years, and tobacco use continues to be the leading preventable cause of death and disability in the United States (U.S. Department of Health and Human Services [USDHHS], 2004; World Health Organization, 1997). Globally, tobacco and tobacco smoke are responsible for ~30% of all cancer deaths in developed countries. In the United States, tobacco use accounts for nearly 1 of 5 deaths; an estimated 443,600 premature deaths occurred every year between 2000 and 2004 (American Cancer Society, 2009). Although the annual prevalence of current smoking among U.S. adults aged 18 and older declined from 42% in 1965 to 21% in 2004, the rate has remained essentially unchanged in recent years (Pleis, Lucas, & Ward, 2009). As recently as 2008, an estimated 46 million U.S. adults were current cigarette smokers (Centers for Disease Control and Prevention [CDC], 2009).

The Surgeon General's report on the health consequences of smoking concluded that smoking harms every organ of the body and reduces the health of smokers in general; smoking is by far the largest cause of lung cancer (USDHHS, 2004). However, smoking is also believed to contribute to many other health problems including abdominal aortic aneurysms, acute myeloid leukemia, cataracts, and cervical, kidney, colorectal,

Address for Correspondence: Yang Xia, Division of Laboratory Science, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway MS F47, Atlanta, GA 30341. Phone: 770-488-4212. Email: yxia@cdc.gov

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pancreatic and stomach cancers (IARC, 1986; Iodice, Gandini, Maisonneuve, & Lowenfels, 2008; Liang, Chen, & Giovannucci, 2009). More than 80 carcinogens, including benzene, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, tobacco specific nitrosamines (TSNAs), acrylamide, acrylonitrile, several inorganic compounds such as cadmium, and lead and polonium-210, have been identified in tobacco and cigarette smoke (Hoffmann & Hoffmann, 1997; IARC, 2004; Smith, Perfetti, Garg & Hansch, 2003).

The TSNAs are of particular concern and are believed to play a significant role in carcinogenesis among persons who either use tobacco products or are exposed to tobacco smoke (Hecht, 2002). Major TSNAs include 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosonornicotine (NNN), N-nitrosoanabasine (NAB), and N-nitrosoanatabine (NAT). These TSNAs are formed from their alkaloid precursors in reaction with nitrite or nitrate, predominantly during tobacco curing, fermenting, and aging (Andersen & Kemp, 1985). Among all TSNAs, NNK is believed to be the most prevalent systemic lung carcinogen, causing lung cancer and possibly other forms of cancer in smokers (Hecht, 1999). The International Agency for Research on Cancer has classified NNK as a Group 1 (known human) carcinogen (IARC, 2007). In humans, NNK is rapidly reduced to its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), with a variable portion of NNAL present in the glucuronide forms (both N- and O-glucuronides) (Carmella, Le, Upadhyaya, and Hecht, 2002). NNAL is known to be a potent lung carcinogen in rodents (Hecht, 1998), and it is believed to contribute to lung cancer in humans who smoke as well (Hecht & Hoffmann, 1988; Preston-Martin, 1987).

NNAL can be measured in urine as a biomarker of exposure to NNK. Hecht et al. (Carmella, Akerkar, & Hecht, 1993; Carmella, Akerkar, Richie, & Hecht, 1995; Carmella, et al., 2003; Richie et al., 1997) have studied NNAL extensively in both smokers and non-smokers exposed to secondhand smoke (SHS) by using a method based on gas chromatography (GC) with thermal energy analysis detection. We have measured cotinine, the primary proximate metabolite of nicotine, in participants aged 3 years and older in all National Health and Nutrition Examination Surveys (NHANES) beginning with NHANES III in 1988. Starting with NHANES 2007–2008, we also began analyzing total urinary NNAL in all NHANES participants aged 6 years and older. In this report we describe urinary NNAL and serum cotinine concentrations measured in active tobacco users. Concentrations of urinary NNAL measured in nonsmokers exposed to SHS in this population have been reported previously (Bernert et al. 2010).

Methods

Study design

NHANES is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). This survey is designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations (CDC, National Health and Nutrition Examination Survey [NHANES] 2007–2008). The sampling design for NHANES is based on a complex, multistage probability strategy that includes selection of primary sampling units (counties), household segments within the counties, and sample patients from selected households. Data are collected through household interviews and standardized physical examinations, which are conducted in a mobile examination center. In NHANES 2007–2008, a new sampling method resulted in oversampling of all Hispanic, not just Mexican American, populations (CDC, NHANES, 2007–2008 Sampling Methodology). The total number of participants was 9,762 in this survey period, and urine specimens were collected from each participant aged 6 years or older to analyze urinary total NNAL (free NNAL plus NNAL-glucuronide). The study protocol was reviewed and approved by CDC's institutional review board, and informed, written consent was obtained from all participants in the study.

Demographic variables

Sociodemographic data including age, gender, and race/ethnicity were derived from self-reported questionnaire data. In this report we have focused on smokers in age groups of 12–19, 20–44, 45–64, and ≥65 years. The race/ethnicity variable also was categorized into four groups including non-Hispanic white (NHW), non-Hispanic black (NHB), Mexican American (MA), and other (OTH) participants.

Laboratory methods

During the physical examinations, urine specimens were collected from participants, aliquoted, and stored frozen until shipped to the CDC's National Center for Environmental Health. We measured total NNAL using a previously described method (Xia et al., 2005), with additional modifications (Xia & Bernert, 2010). Briefly, 5-mL urine samples were spiked with $^{13}C_6$-labeled NNAL internal standard, and hydrolyzed overnight with β-glucuronidase. The hydrolysate was then further processed and analyzed by high-performance liquid chromatography atmospheric-pressure ionization tandem mass spectrometry (HPLC-API MS/MS) (Xia et al., 2005;

Xia & Bernert, 2010). NNAL was quantified based on the peak area ratio of analyte to isotope-labeled internal standard.

A blank and two quality-control pools were analyzed in each analytical run with unknown samples. Reported results met the accuracy and precision specifications of the quality control/quality assurance program of the Division of Laboratory Sciences, National Center for Environmental Health, CDC (Caudill, Schleicher, & Pirkle, 2008). This method for measuring NNAL has a limit of detection (LOD) of 0.6 pg/mL, based on the variance from the repetitive analysis of a low spiked urine sample (2 pg/mL). We have confirmed that NNAL remains stable in urine for at least several years during long-term storage at –70°C (Xia & Bernert, 2010). Serum cotinine was also measured by HPLC-API MS/MS in all NHANES participants aged 3 years and older. Urinary creatinine concentrations of all participants aged 6 years and older were determined using an enzymatic (creatinase) method implemented on a Beckman Synchron CX3 Clinical Analyzer. Details of both methods are available at the NHANES Web site (CDC, NHANES 2007–2008).

Statistical analysis

For univariate analyses, we calculated geometric mean concentrations of urinary total NNAL among smokers, including subcategories such as gender, race/ethnicity and age. Smokers were separated from non-smokers based on a serum cotinine concentration of 10 ng/mL or higher (Pirkle et al., 1996). Both urinary total NNAL and serum cotinine were log-transformed to reduce the skewness in their distributions. A preliminary analysis suggested a non-linear relationship between NNAL and cigarettes per day (CPD) for smokers (see Figure 1 and 2). For this reason, both a linear and a quadratic term for CPD were included in the model. A backward-elimination approach was used to decide upon the final model. This model had the log of urinary NNAL as the dependent variable, and race (NHW, NHB, MA, and OTH), gender (males, females), log of serum cotinine, and urinary creatinine as the independent variables. Menthol or non-menthol cigarette type, CPD, CPD^2 and Federal Trade Commission (FTC) tar value were also included, although neither menthol nor FTC values were significant predictors of NNAL. The initial model also included age as a continuous variable, but age was not found to be a significant predictor ($p = 0.23$) and it was excluded from the final model. All statistical analyses were performed using SUDAAN (release 10.0) Proc DESCRIPT and Proc REGRESS from RTI (Research Triangle Park, NC), with graphical analyses performed by using SAS (version 9.2, SAS Institute, Cary, NC). Analyses incorporated sampling weights that adjusted for unequal probabilities of selection.

Results

In NHANES 2007–2008, a total of 9,762 participants were examined, of which 8,132 were aged 6 years or older. We found 1,382 participants with serum cotinine of 10 ng/mL or higher. After users of the nicotine patch, gum, or other non-tobacco nicotine products were excluded based on self-reported questionnaires, 1,373 participants were classified as tobacco users. Most of these tobacco users were cigarette smokers (N = 1053), and some participants used more than one form of tobacco products during this time. For example, among 75 chewing-tobacco users, 14

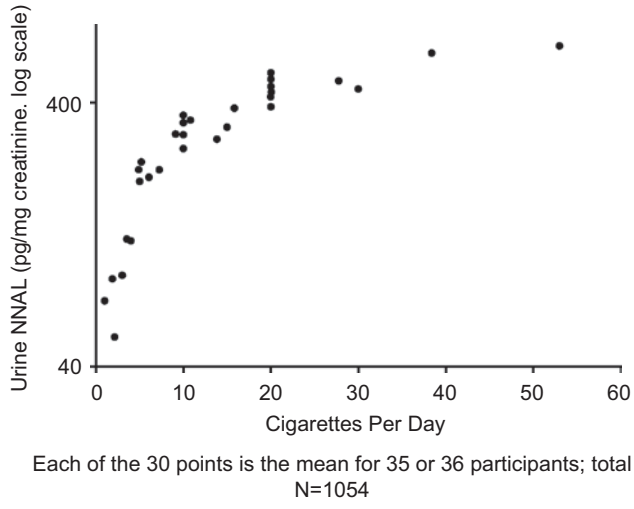

Figure 1. Concentrations of total urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (pg/mg creatinine) and cigarettes per day in 1054 National Health and Nutrition Examination Survey 2007–2008 smokers.

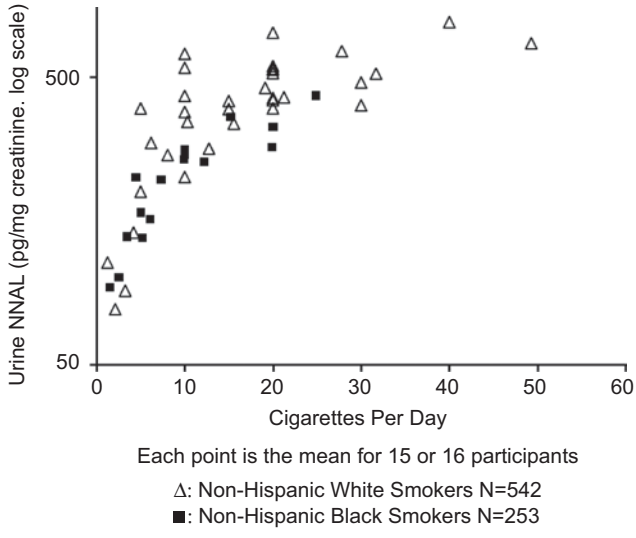

Figure 2. Concentrations of total urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (pg/mg creatinine) and cigarettes per day (CPD) in Non-Hispanic White and Non-Hispanic Black smokers.

reported they also smoked cigarettes; 3 of the 75 smoked both cigarettes and cigars along with using chewing tobacco. One participant reported the use of cigarettes, cigars, chewing tobacco, and pipes. Because all forms of tobacco use (cigarettes, pipes, cigars, chewing tobacco, and snuff) are sources of TSNAs exposure, all 1,373 participants were included in the calculation of geometric means of NNAL.

Geometric means and selected percentiles of total NNAL concentrations in the NHANES urine samples from tobacco users are given in Table 1 (uncorrected, in pg/mL) and in Table 2 (creatinine corrected, in pg/mg creatinine). Geometric means and selected percentiles are presented for the total population of tobacco users, as well as subsets defined by gender, race/ethnicity and age. As shown in Table 1, on average, age group 12–19 had lower NNAL concentration than older tobacco users. Female tobacco users had lower NNK exposure than males, and NHB had lower exposure than NHW. These observations are consistent with the report from Roethig et al. (Roethig et al., 2009) in which the analysis was performed based on 24 hour urine samples. Comparing Tables 1 and 2, the difference in mean concentrations among race/ethnicity groups and among the age groups were similar using either corrected or uncorrected data. However, creatinine correction had a strong impact on gender differences.

The creatinine-corrected geometric mean of urinary NNAL for all smokers in the study was 285 pg/mg creatinine (95% CI = 236–346), whereas the uncorrected geometric mean of urinary NNAL was 299 pg/mL (95% CI = 253–353). For unadjusted data (i.e., without adjustment for other covariates), the geometric mean NNAL concentrations among males was higher than among females when not corrected for creatinine (318 versus 269 pg/mL) although the difference was not statistically significant ($p = 0.162$). However, after correcting for creatinine concentrations, the NNAL concentrations among females was significantly higher than among male smokers (314 versus 268 pg/mg; $p = 0.035$). Among the four race/ethnicity groups, NHW consistently had the highest and MA had the lowest total NNAL concentrations, either creatinine-corrected or uncorrected.

Among all 1,373 tobacco users, four participants between the ages of 6 and 11 years were identified.

Table 1. Geometric means and selected percentiles of urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pg/mL) among tobacco users* in National Health and Nutrition Examination Survey 2007–2008.

		Geometric Mean	Percentiles			
	N	95% CI	50th	75th	90th	95th
All	1373	299 (253–353)	333 (282–387)	648 (532–794)	1290 (1080–1540)	1860 (1580–2710)
Male	844	318 (258–393)	345 (298–417)	752 (587–887)	1540 (1220–1700)	2240 (1700–3100)
Female	533	269 (231–314)	303 (241–375)	529 (461–622)	1030 (765–1150)	1510 (1100–1990)
Non-Hispanic White	703	334 (277–403)	373 (324–418)	741 (558–882)	1510 (1150–1710)	2010 (1540–3640)
Non-Hispanic Black	349	277 (243–315)	284 (222–375)	554 (486–617)	922 (794–1150)	1350 (1000–2050)
Mexican-American	145	129 (102–162)	128 (91–174)	327 (192–486)	670 (458–887)	887 (638–1030)
Other Race	180	223 (174–285)	230 (186–314)	457 (351–642)	1070 (675–1240)	1630 (1040–2900)
Ages 12–19 years	117	137 (103–181)	151 (84–226)	295 (170–420)	462 (367–646)	636 (420–1390)
Ages 20–44 years	626	292 (223–383)	343 (245–424)	696 (503–994)	1469 (1070–1720)	2070 (1590–2920)
Ages 45–64 years	472	346 (294–408)	375 (321–447)	662 (577–769)	1210 (1040–1530)	1640 (1440–1990)
Ages ≥65 years	158	368 (285–476)	303 (246–396)	556 (404–991)	1790 (782–4540)	4540 (1700–10500)

*Tobacco users defined as having serum cotinine concentrations >10 ng/mL.

Table 2. Geometric means and selected percentiles of creatinine-corrected urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pg/mg creatinine) among tobacco users* in National Health and Nutrition Examination Survey 2007–2008.

		Geometric Mean	Percentiles			
	N	95% CI	50th	75th	90th	95th
All	1373	285 (236–346)	324 (261–402)	625 (515–754)	1120 (870–1368)	1707 (1308–2179)
Male	844	268 (213–337)	301 (232–388)	612 (495–751)	1120 (813–1471)	1727 (1241–2571)
Female	533	314 (267–369)	348 (289–431)	663 (517–838)	1090 (882–1383)	1707 (1308- 2300)
Non-Hispanic White	703	345 (283–421)	402 (332–478)	725 (612–841)	1230 (1007–1607)	1920 (1368–2606)
Non-Hispanic Black	349	193 (168–221)	206 (170–239)	348 (313–408)	571 (495–699)	840 (641–2120)
Mexican-American	145	115 (91–145)	115 (84–135)	310 (180–391)	559 (391–737)	770 (484–1248)
Other Race	180	195 (154–245)	211 (139–255)	447 (283–647)	805 (647–1014)	1120 (805–1413)
Ages 12–19 years	117	92 (74–115)	103 (67–138)	178 (143–207)	251 (193- 536)	536 (253–816)
Ages 20–44 years	626	246 (187–323)	279 (211–389)	595 (447–764)	1078 (790–1317)	1497 (1090–1895)
Ages 45–64 years	472	402 (335–483)	431 (358–500)	740 (612–850)	1250 (953–1951)	1951 (1325–2917)
Ages ≥65 years	158	474 (362–620)	400 (317–431)	687 (476–1672)	2020 (849–6394)	5147 (1800–17845)

*Tobacco users defined as having serum cotinine concentrations >10 ng.

Because of this small number, these four participants were excluded from analyses that included age. For uncorrected and creatinine-corrected data, the geometric mean for the age group 12–19 years was significantly lower (p <0.001) than for all other age groups. Geometric mean for the age group 20–44 years was also significantly lower (p <0.001) than for the age groups 45–64 years and 65 years or older, but only for creatinine-corrected data.

Multivariate regression results for NNAL are presented in Table 3. Both serum cotinine (p <0.0001) and urinary creatinine (p <0.0001) were positively associated with NNAL concentrations. While neither CPD nor CPD^2 were significantly associated (p >0.50) with NNAL, their joint effect was found to be statistically significant (p = 0.001 using Wald Chi-Square). Table 4 provides the geometric means by gender, race/ethnicity, and cigarette menthol levels after adjusting for four covariates (serum cotinine, urinary creatinine, CPD, and FTC tar levels). Females had significantly higher adjusted NNAL concentrations than did males (p = 0.003). Among the race/ethnicity groups, NNAL concentrations among NHW smokers was significantly higher than those among NHB smokers (p = 0.022), but not significantly different from MA smokers (p = 0.134) or OTH smokers (p = 0.303). No statistically significant differences existed between NHB, MA, or OTH smokers. Although NNAL concentrations among menthol smokers were lower than those among non-menthol smokers, the difference did not achieve statistical significance (p = 0.095).

Table 5 displays average CPD during the last 30 days and the last 5 days for cigarette smokers based on two separate questionnaires. CPD during the last 30 days was obtained from the household interview and CPD during the last 5 days was reported by smokers who came to the mobile examination center. In both cases, female smokers, in general, smoked fewer CPD than did males. MA smokers had the lowest CPD among all ethnicity groups and those aged 12–19 years had lower CPD than any other age group.

The positive correlation of NNAL concentrations with CPD was found primarily at lower smoking rates. As indicated in Figure 1, urinary concentrations of NNAL increased only slightly at the highest CPD levels. A similar response was found with both NHW and NHB smokers (Figure 2), although relatively few NHB smokers smoked more than 20–25 CPD.

Table 3. Regression coefficients and standard errors for variables associated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol from a multivariate model for smokers in National Health and Nutrition Examination Survey 2007–2008.

Variable	Beta (SE)	Significance (p value)
Intercept	-2.6284 (0.1931)	< 0.0001
Male	reference	
Female	0.0807 (0.0232)	0.003
Non-Hispanic White	reference	
Non-Hispanic Black	-0.1270 (0.0515)	0.025
Mexican-American	-0.0549 (0.0343)	0.129
Other	-0.0486 (0.0449)	0.295
Menthol	reference	
Non-Menthol	0.0648 (0.0366)	0.095
Log Cotinine	0.7397 (0.0853)	< 0.0001
Urine Creatinine	0.2461 (0.2498)	< 0.0001
Average Cigarettes Per Day	0.0036 (0.0054)	0.513
(Average Cigarettes Per Day)^2	0.00003(0.00008)	0.705
FTC Tar	-0.0007 (0.0042)	0.870

Table 4. Adjusted geometric means in regression model with 95% confidence intervals.

	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, pg/mL	Significance (p value)
Variable	Geometric Mean (95%CI)	
Male	293 (264–325)	reference
Female	353 (324–384)	0.003
Non-Hispanic White	336 (298–379)	reference
Non-Hispanic Black	252 (218–291)	0.025
Mexican-American	297 (263–336)	0.129
Other Race	301 (253–358)	0.295
Menthol	285 (243–334)	reference
Non-Menthol	331 (304–360)	0.095

Discussion

We have measured the tobacco-specific carcinogen NNAL, a metabolite of NNK, in urine samples from all participants of NHANES 2007–2008 aged 6 years and older, who were identified as tobacco users based on their serum cotinine concentrations. These data provide the first description of TSNA exposures among smokers in the U.S. noninstitutionalized population, and will provide a basis for the evaluation of trends in the exposure of U.S. tobacco users to NNK over time in the future.

Table 5. Average cigarettes per day (CPD) during last 30 days and last 5 days for cigarette smokers.

	Average CPD during last 30 days (N = 1070)	Average CPD during last 5 days (N = 1054)
Variable	Mean (SE)	Mean (SE)
All Male	15.42 (0.962)	13.81 (0.702)
	16.26 (1.163)	14.43 (0.859)
Female	14.30 (1.042)	13.02 (0.660)
Non-Hispanic White	17.39 (0.944)	15.41 (0.684)
Non-Hispanic Black	10.21 (0.435)	9.37 (0.361)
Mexican-American	7.80 (0.932)	6.67 (0.758)
Other Race	12.00 (1.528)	12.03 (1.341)
Ages 12–19 years	7.46 (0.953)	7.65 (0.898)
Ages 20–44 years	14.30 (1.116)	12.55 (0.873)
Ages 45–64 years	18.13 (1.093)	16.42 (0.807)
Ages ≥65 years	16.52 (1.602)	14.76 (1.571)

Creatinine correction had an important effect on the gender differences in the unadjusted data given in Tables 1 and 2, and correction for creatinine also influenced results categorized by either race/ethnicity or age. However, in both the corrected and uncorrected data, NHW had the highest concentrations of urinary NNAL and MA had the lowest. Urinary NNAL concentrations increased with increasing age. The two groups with the lowest urinary total NNAL concentrations were MA and younger smokers aged 12–19 years. In both cases, this may have reflected the lower smoking rates (Table 5) that are prevalent in those groups, which has been noted in previous studies (Caraballo et al., 1998; CDC, 1994; USDHHS, 2003). When CPD was included in the model, the NNAL concentrations of MA smokers were no longer significantly different from other race/ethnicity groups ($p = 0.129$).

Although NHW had a higher geometric mean concentration of total NNAL regardless of creatinine correction status (Table 1 and 2), the mean concentration of NNAL per cigarette smoked was actually lower among NHW because of the greater number of CPD smoked by this group (Table 5). NHW and NHB had NNAL concentrations of 19.2 and 27.1 pg/mL per cigarette smoked respectively, based on CPD during the last 30 days. The corresponding mean concentrations of NNAL in NHW and NHB were 21.7 and 29.6 pg/mL per cigarette smoked, respectively, using self-reported CPD during last 5 days. Muscat et al. (2005) also reported higher mean concentration of total NNAL in Black men than in White men for each cigarette smoked in a community-based cross-sectional study.

Based on multiple regression analyses, significant predictors of NNAL include serum cotinine, urinary creatinine, and CPD and CPD^2 jointly. Serum cotinine is a specific marker of tobacco exposure (Pirkle et al., 1996; USDHHS, 2004), and urine creatinine is influenced by an individual's state of hydration, as well as by race, gender, and age (Barr et al., 2005). After adjusting for these covariates, female smokers had significantly higher urine NNAL concentrations than male ($p = 0.003$), and NHW smokers had significantly higher concentrations than did NHB smokers ($p = 0.022$). We found similar results in a previous, much smaller study of smokers in which the adjusted NNAL concentrations were higher in female and in white smokers compared with black smokers (Bernert et al., 2005). In our previous study in which free and NNAL-glucuronide concentrations were measured separately, only the glucuronides were higher in black smokers; the free NNAL concentrations were similar. In this NHANES study with a large sample size, we assayed only total concentrations by analyzing free NNAL plus the glucuronides together.

The NNAL difference we observed between NHW and NHB smokers may be associated with cigarette choices because smokers of non-menthol cigarettes had higher urinary NNAL concentrations than did menthol smokers, although that difference was not statistically significant ($p = 0.095$). The use of menthol cigarettes is much more prevalent among black smokers compared with whites (Caraballo et al., 1998; Perez-Stable, Herrera, Jacob, & Benowitz, 1998). Heck (2009) also found no significant difference in urinary NNAL concentrations between smokers of menthol and nonmenthol cigarettes. FTC tar is another variable that was not correlated to urinary NNAL concentration ($p = 0.870$). Cigarettes are classified as regular, light, or ultralight based on FTC tar and nicotine values. Our results suggest that smokers' selection of cigarette type (FTC tar) did not make a statistically significant difference in their exposure to carcinogens in the tobacco, which is consistent with several previous findings (Benowitz et al. 2005; Bernert et al., 2005; Hecht et al., 2005;).

Lung cancer involves a histologically and clinically diverse group of malignancies (USDHHS, 2004). Until recently, most cancers were believed to start in the large airways of the lung, but since the 1960s, adenocarcinomas that tend to develop in the peripheral lung (Chen, 2007) have increased notably. This difference may reflect changes in the cigarette manufacturing process over time. Because of increasing health concerns, commercial cigarettes underwent a gradual compositional change beginning in the mid 1950s, resulting in a reduction in yields (as measured by the Federal Trade Commission smoking machine method) of tar and nicotine in cigarette smoke. Over time, these changes in cigarette design and composition led to lower concentrations of nicotine, carbon monoxide, hydrogen cyanide, and PAHs (again as measured by smoking machine methods) in mainstream smoke. These changes were also associated with several manufacturing changes such as the relative proportions of bright (flue-cured) versus burley (air-cured) tobacco. This latter change led to a rise in the nitrate content of tobacco from ~0.3–0.5% to 0.6–1.35% in more recent cigarettes (Hoffmann, & Hoffmann, 1997). These newer compositions resulted in more complete combustion, which might be expected to reduce PAH contributions; however, they also increased the formation of the TSNA (Hoffmann & Hoffmann, 1997). Thus, differences in cigarette manufacturing may influence the TSNA content of the smoke, and these differences can be linked to differences in concentrations of the urinary biomarker NNAL as well (Ashley et al., 2010).

Monitoring possible future changes in cigarette composition and delivery will be important. Currently, potential reduced-exposure products (PREPs) have not been proven to be associated with a reduced risk of disease compared with conventional tobacco use (Institute of Medicine [IOM], 2001). Increasing focus

is being placed on developing newer PREPs, and NNAL measurements in future NHANES will be a valuable biomarker of such trends, reflecting possible difference in cigarette content and, more importantly, measuring differences in the actual exposure of the smoker. For this reason, the Institute of Medicine has explicitly recommended that biomarkers such as NNAL be included in national health surveys such as NHANES (IOM, 2001).

This study has several strengths and a few limitations. The relatively large and representative sample of NHANES and the overall quality of the survey design and execution were among the strengths of the study. We used a validated and specific tandem mass spectrometric method for this assay to help ensure the reliability of the analysis, and maintained our assays within a robust quality control /quality assurance program. Smoker status was confirmed in each case based on serum cotinine concentrations, an established basis for assessing exposure to tobacco. Information on the brands of cigarettes used by smokers in this study, primarily from recorded UPC codes, was also collected as part of NHANES. However, because of the size of the population, we analyzed only total NNAL rather than measuring the free and glucuronide forms specifically. The additional level of information from both free and glucuronide forms could be useful because the two forms may reflect different exposure risks (Hecht, 2002; USDHHS, 2004). Our analyses excluded persons who used nicotine in pharmaceutical form such as nicotine gum or the patch, but it included all others with serum cotinine levels ≥10 ng/mL, and, thus, did not distinguish between cigarette smokers and smokers of cigars or pipes or oral tobacco users. However, based on self-reports, the number of non-cigarette smoking tobacco users was relatively small (~13%), and Hecht et al. (Hatsukami, Benowitz, Rennard, Oncken, & Hecht, 2006; Hecht, et al., 2007) have found similar NNK exposures in smokers and smokeless tobacco users.

Conclusions

These results provide the first characterization of exposure to the major tobacco-specific carcinogen, NNK, in the U.S. smoking population by measuring its metabolite (NNAL) in urine. Expected significant relationships with serum cotinine and CPD were found. These NNAL levels can help guide toxicological research on NNK with levels most relevant to smokers. As tobacco products in the United States come under regulation by the FDA, these results may form a baseline against which smoker's exposure to this carcinogen can be compared in the future.

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Declaration of interests

The authors report no declarations of interest.

***Disclaimer*:** The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services or the Centers for Disease Control and Prevention.

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