

Urine Concentrations of a Tobacco-Specific Nitrosamine Carcinogen in the U.S. Population from Secondhand Smoke Exposure

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Abstract

Background: The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its reduction product in the body, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), are potent pulmonary carcinogens. We have measured total NNAL in the U.S. population of tobacco users and nonsmokers exposed to secondhand smoke.

Methods: We measured total urinary NNAL (free NNAL plus its glucuronides following hydrolysis) by using a sensitive and specific high-performance liquid chromatography/tandem mass spectrometry method. We calculated the percentage above the limit of detection, the 50th through 95th percentiles, and in some cases, geometric means for groups classified by age, gender, and race/ethnicity.

Results: Total urinary NNAL was measurable at or above its limit of detection (0.6 pg/mL) in 55% of the study participants, including 41% of nonsmokers. The population distribution of urinary NNAL included smoker and nonsmoker regions similar to the bimodal distribution of serum cotinine, and serum cotinine and total urinary NNAL were strongly correlated ($r = 0.92$; $P < 0.001$). Among nonsmokers, children had significantly higher concentrations of NNAL than did adults with the age of ≥ 20 years ($P < 0.001$).

Conclusions: Among National Health and Nutrition Examination Survey participants, total NNAL was found at measurable levels in the urine of 41% of nonsmokers and in 87.5% of those with substantial secondhand-smoke exposure (with serum cotinine concentrations of 0.1-10 ng/mL). Children with the age of 6 to 11 years had the highest NNAL concentrations among all nonsmokers.

Impact: We describe for the first time the distribution of total urinary NNAL in the entire U.S. population, including smokers and nonsmokers. NNAL was detected in 41% of all nonsmokers. *Cancer Epidemiol Biomarkers Prev*; 19(11); 2969–77. ©2010 AACR.

Introduction

The health risks for smoking are well established; it has been estimated that cigarette smoking accounts for ~438,000 deaths (20% of all deaths) in the United States each year (1-3). Smoking is an important contributor to cardiovascular and respiratory diseases and has long been associated with a significantly increased risk for cancer, especially lung cancer. Smoking is the proximate cause of lung cancer in 90% of men and nearly 80% of women in whom that illness develops (3). However, the risk is not limited to lung cancer; smoking has been identified as causative for as many as 19 forms of cancer, including bladder, esophageal, kidney, cervical, pancreatic,

head and neck, and stomach cancer (3-5). Thus, exposure to carcinogens in tobacco smoke is a crucial concern for the 21% of the U.S. population that continues to smoke cigarettes. Furthermore, this risk may extend to include most of the population who are nonsmokers. Although the relation remains uncertain between nonsmokers' exposure to secondhand smoke (SHS) and most cancers, sufficient evidence exists to conclude that exposure to SHS can cause lung cancer in nonsmokers (6). An estimated 30% of lung cancer among nonsmokers, causing ~3,000 deaths per year in the United States, has been attributed to exposure to SHS. SHS exposure is regarded as the third leading cause of lung cancer, after active smoking and exposure to radon. Although differences in the production and concentrations of chemical compounds in mainstream and sidestream smoke exist, the carcinogens in mainstream smoke inhaled by active smokers are largely the same as those in SHS (a mixture of mainstream and sidestream smoke) to which both smokers and nonsmokers may be exposed. These carcinogens include benzene, a variety of polycyclic aromatic hydrocarbons (PAH), aromatic amines, tobacco-specific nitrosamines, aldehydes, inorganic compounds such as cadmium

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and polonium-210, and many additional compounds (6, 7). Several *N*-heterocyclic amines also have been identified at low concentrations in smoke from nonfiltered cigarettes (8). These are important carcinogens in cooked foods, but to date, only metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine have been measured in urine, and no effect of smoking on its levels have been detected (9). Of the known compounds in tobacco smoke, the most critical pulmonary carcinogens are believed to be the tobacco-specific nitrosamines and PAHs (8, 9).

Because SHS is a mixture of sidestream and (exhaled) mainstream tobacco smoke with a composition that constantly changes during aging, it can be difficult to compare relative concentrations of toxicants in mainstream and SHS. However, the ratios of selected constituents in fresh sidestream and mainstream smoke from unfiltered cigarettes have been reported. These ratios for several carcinogens in tobacco smoke, including benzene, cadmium, aromatic amines such as 4-aminobiphenyl, tobacco-specific nitrosamines, and PAHs, are commonly >1 and may range as high as 10 (6, 7, 10), with an estimated sidestream/mainstream for the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) of ~1 to 4 (7, 10). With dilution in room air, the final concentration of NNK in SHS has been reported to be in the range of 0.2 to 29.3 ng/mm³ (6).

Several biomarkers exist that can help document exposure to carcinogens in tobacco smoke. However, exposure to many of these key carcinogens may result from sources in addition to tobacco, which complicates monitoring of the tobacco-associated carcinogen risk among nonsmokers. Exceptions are the tobacco-specific nitrosamines,

which as their name indicates, are considered completely specific to tobacco (9). *N*-nitrosamines encompass a large group of compounds that are known to be carcinogenic to many animal species and are believed to be carcinogenic to humans as well. Thus, the tobacco-specific nitrosamines *N*-nitrosanornicotine and NNK are of special significance because they combine an inherent potent pulmonary carcinogenic potential with a high degree of tobacco-exposure specificity (6, 9, 11). Exposure to NNK can be readily measured in smokers and nonsmokers exposed to SHS by measuring 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; the secondary alcohol reduction product of NNK) in urine samples (6, 9, 12, 13).

NNAL, which may also form *N*- and *O*-glucuronides, has been measured previously in the urine of smokers and nonsmokers exposed to SHS (9, 12). This biomarker has been quantified in urine samples from adult nonsmokers and in children with SHS exposure (14); because of its specificity, NNAL is the most suitable carcinogen marker of SHS exposure available. Cotinine, the primary proximate metabolite of nicotine, is generally regarded as the most useful general biomarker of tobacco exposure from either active smoking or exposure to SHS (6, 15, 16) because of its specificity, relative abundance, and ease of measurement. However, cotinine assays indicate only previous exposure to nicotine and provide information by inference on exposures to other toxicants of interest, including carcinogens. By contrast, urinary NNAL measurements provide a direct index of exposure to a potent tobacco-specific pulmonary carcinogen and are therefore intrinsically valuable to monitor within the population. An additional advantage to urinary NNAL measurements is the longer terminal half-life of this compound, estimated to range from 26 to 45 days in tobacco users (9) compared with the shorter estimated 16 to 18 hour half-life of cotinine (6).

We have measured serum cotinine in each National Health and Nutrition Examination Survey (NHANES) beginning with NHANES III 1988 to 1992 and continuing in subsequent 2-year surveys. Starting with NHANES 2007 to 2008, we also began analyzing total urinary NNAL in most NHANES participants. Specifically, serum cotinine was measured in NHANES participants with the age of ≥3 years if sufficient serum was available, and NNAL assays were conducted on all available urine samples from participants with the age of ≥6 years. These measurements enable us, for the first time, to describe the distribution of this tobacco-specific biomarker in the entire U.S. population, as well as in selected subsets of nonsmokers. In this article, we have focused primarily on the exposure of nonsmokers to NNK. A subsequent article will address the exposure of active tobacco users participating in NHANES to this tobacco-specific nitrosamine.

Materials and Methods

Study design

The National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) conducts

Table 1. Unweighted sample sizes for participants in NHANES 2007 to 2008

	All NHANES participants, <i>n</i>	Nonsmokers* only, <i>n</i>
Total	6,599	5,206
Males	3,314	2,459
Females	3,285	2,747
Non-Hispanic Whites	2,755	2,046
Non-Hispanic Blacks	1,411	1,056
Mexican Americans	1,353	1,205
Other race or ethnicity	1,080	899
6-11 y	879	875
12-19 y	960	840
≥20 y	4,760	3,491

*Nonsmokers are defined as participants with a serum cotinine concentration <10 ng/mL.

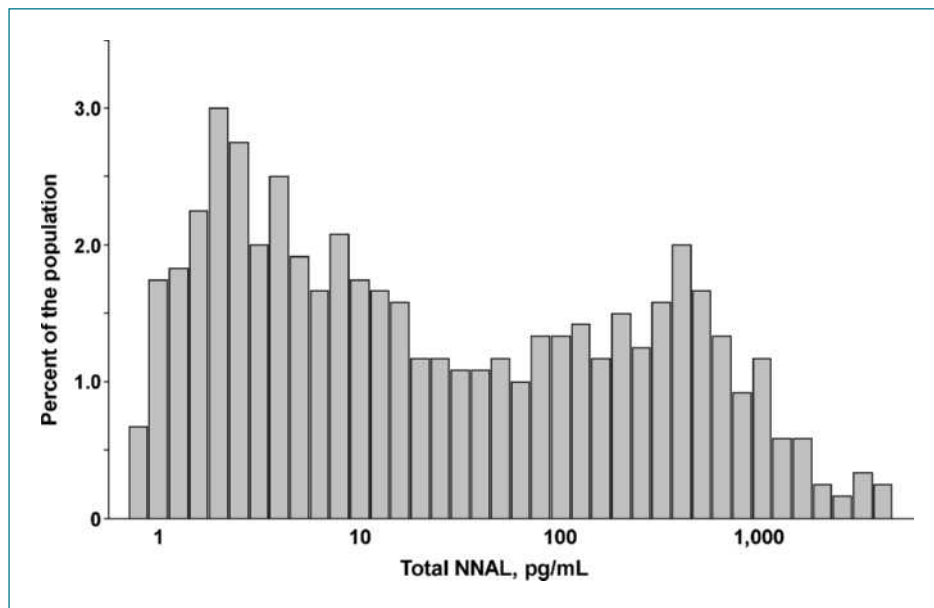


Figure 1. Distribution of urinary total NNAL concentrations in the U.S. population with the age of ≥ 6 years from the 2007 to 2008 NHANES.

NHANES. This survey is designed to assess the health and nutrition status of the civilian noninstitutionalized U.S. population. NHANES uses a complex multistage probability sampling design to provide results that are representative of the U.S. population based on age, sex, and race/ethnicity. Data reported here are based on interviews and standardized physical examinations, including blood and urine tests, which were conducted in mobile examination centers. NHANES 2007 to 2008 was conducted in 30 locations (Primary Sampling Units) selected from a frame of all U.S. counties. In this survey, Hispanics (not just Mexican Americans) were oversampled, and the total number of participants was 9,762. A full description of the NHANES survey is available on the NHANES Web site (2). The Institutional Review Board of CDC reviewed and approved the study protocol, and all study participants provided informed written consent.

Total urinary NNAL (that is, free NNAL plus NNAL liberated by the hydrolysis of NNAL-glucuronides)¹ was measured in all NHANES 2007 to 2008 participants with the age of ≥ 6 years. NNAL was measured in 6,599 samples in this study. Table 1 shows unweighted sample sizes by gender, race/ethnicity, and age for the total population examined, specifically for nonsmokers.

¹ The NNAL concentrations in this study are total NNAL values that represent the actual measured amount of NNAL in picogram per milliliter after hydrolysis. These values on a weight basis will be lower than total NNAL values calculated by summing free NNAL and NNAL-glucuronide measured separately, when the latter includes the weight of the carbohydrate moiety.

Demographic variables

Sociodemographic data about the study participants were self-reported. Race and ethnicity were derived from questionnaire data; categories included non-Hispanic White, non-Hispanic Black, Mexican American, and other. Age groupings were set to 6 to 11, 12 to 19, and ≥ 20 years (adults).

Laboratory methods

Total urinary NNAL was measured by high performance liquid chromatography atmospheric-pressure ionization tandem mass spectrometry using a method previously described (17) but with modifications. Briefly, the urine aliquot (5 mL) was spiked with the [¹³C₆]-NNAL internal standard and hydrolyzed overnight with β -glucuronidase. The sample was then applied to a Chem Elute column (Varian), eluted with methylene chloride, and back extracted into 0.1 N HCl. The latter solution was neutralized, buffered, and processed on a Molecular Imprinted Polymer column custom designed for NNAL analyses using the approach previously described (17). The Molecular Imprinted Polymer columns used in these analyses were purchased from Supleco.

The instrumental analysis was also modified from our previous procedure by adding a third high performance liquid chromatography pump that added acetonitrile (0.6 mL/min) postcolumn immediately before the source and by substituting a Sciex API 5000 tandem mass spectrometer (ABI) for the API 4000 instrument used previously. Both changes were made to enhance the sensitivity of the analysis and have been described in more detail elsewhere (18). This method provided an estimated limit of detection of 0.6 pg/mL based on a 5-mL sample volume, which was calculated from the variance measured

in the repetitive analysis of a low concentration (2 pg/mL) fortified urine sample. Accelerated stability studies conducted at various temperatures have shown that total NNAL values remain stable in urine samples during long-term storage for >4 years at -70°C (18).

Bench and blind (that is, unknown to the analyst) quality control pools were prepared by fortifying blank nonsmoker urine pools with known amounts of NNAL, and aliquots of each type of quality control pool plus a blank urine sample were included with each analytic run. All final reported data were from runs confirmed to be within statistical control limits using the multirule quality control system implemented in the Division of Laboratory Sciences, CDC, which has been described previously (19).

Serum cotinine was measured in all participants with the age of ≥ 3 years by liquid chromatography atmospheric-pressure ionization tandem mass spectrometry, and urinary creatinine was measured in all participants with the age of ≥ 6 years by an enzymatic (creatinase) method implemented on a Roche ModP clinical analyzer. Details of both methods are available at the NHANES Web site (2).

Statistical analysis

Nonsmokers were defined as persons who had a serum cotinine concentration of <10 ng/mL (20). Because the detection rate for NNAL among nonsmokers was $<60\%$ we did not compute means for all nonsmokers but rather calculated total urinary NNAL concentrations by percentiles in this group, including subcategories based on age, sex, and race/ethnicity, and included median values for those subgroups with detection rates

above 50%. In addition, geometric means were calculated for a group of nonsmokers defined as having relatively substantial SHS exposure based on their serum cotinine concentrations. This nonsmoker subgroup had an NNAL detection rate of $>87\%$. Urinary total NNAL and serum cotinine were log transformed to reduce the skewness in their distributions. All statistical analyses were done using Proc DESCRIPT and Proc REGRESS in SUDAAN (version 10.0; RTI), with graphical analyses done using SAS (version 9.2; SAS Institute). SEs also were calculated using SUDAAN, a program that adjusted for the complex sample design when calculating variance estimates. Analyses incorporated sampling weights that adjusted for unequal probabilities of selection.

Results

Urinary total NNAL was detected in 54.8% of the total population of smokers and nonsmokers in NHANES 2007 to 2008. Figure 1 is a log scale distribution plot for urinary total NNAL concentrations measured in this study for the entire population. Note that the plot excludes the substantial number of samples with a nominal concentration of 0, which could not be plotted on a log basis but which would form a large bar on the left of the figure. The association of NNAL concentrations with tobacco exposure was supported by the regression of total urinary NNAL concentrations on serum cotinine in the same persons from the entire population, which indicated that serum cotinine concentrations were strongly predictive of total urinary NNAL levels ($r = 0.92$; $P < 0.001$). Figure 2 shows this close association between

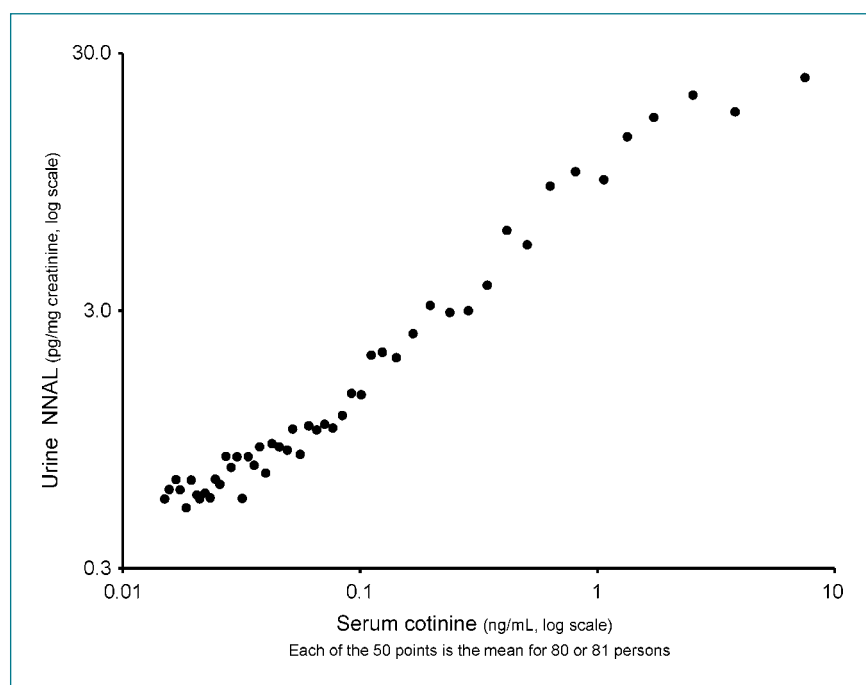


Figure 2. Relationship between concentrations of total urinary NNAL (picogram per milligram creatinine) and serum cotinine (nanogram per milliliter) in 4,035 NHANES participants.

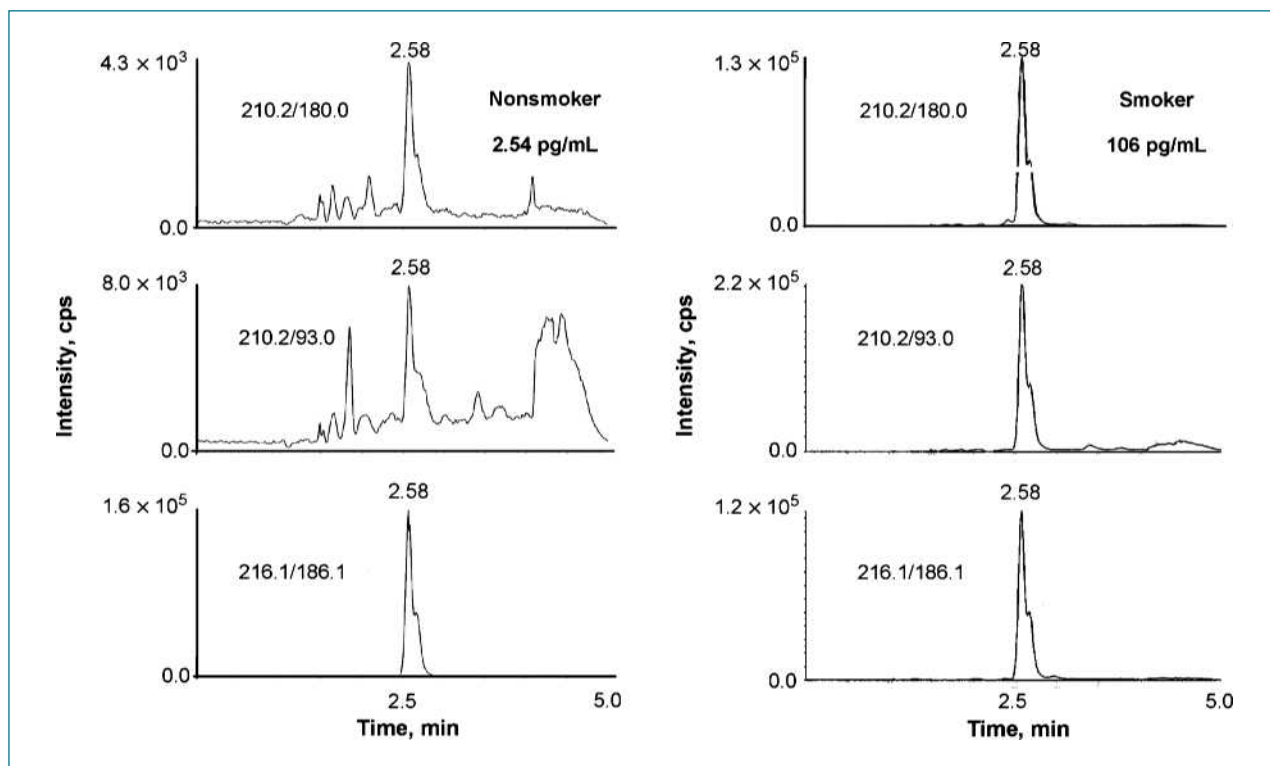


Figure 3. Multiple reaction monitoring ion chromatograms for the measurement of NNAL in urine samples from a nonsmoker and a smoker. NNAL elutes at ~2.58 min. The transition ion at m/z 210.2→180.0 was used for quantification, and the ion at m/z 210.2→93.0 was used as a qualifier. The transition ion at m/z 216.1→186.1 is the internal standard. The calculated NNAL concentration for each sample is indicated on the figure. cps, counts per second.

serum cotinine and urinary NNAL concentrations observed among nonsmokers.

Table 2 presents medians and selected percentiles with their 95% confidence interval (95% CI) for total urinary NNAL concentrations among smokers and nonsmokers in the population. Benowitz et al. (21) recommended using a serum cotinine cutoff of 3 ng/mL to separate smokers from nonsmokers in national population data. We repeated the analyses in Table 2 using this cutoff and found little difference in the results (data not shown). Because the detection rate for urinary NNAL among nonsmokers was ~41% overall, which is a detection rate too low for reliable means calculations, geometric means for all nonsmokers are not reported. However, this detection rate was adequate in all cases for estimates of the 75th, 90th, and 95th percentiles.

Table 3 provides selected percentiles for NNAL concentrations in U.S. nonsmokers. The variations observed based on either gender or race/ethnicity were relatively minor, although concentrations in Mexican Americans were consistently lower than those in non-Hispanic Whites or non-Hispanic Blacks. However, a substantial inverse association was seen by age. When examined by age, children with the age of 12 to 19 had concentrations approximately twice as high as adults, whereas the concentrations in the youngest children with the age of 6 to 11 years were nearly four times

greater than those of adults. Differences based on gender or race/ethnicity were generally even smaller in the creatinine-corrected data (Table 4), but the age differences were maintained.

On the basis of exposure levels measured as part of NHANES III, a target nonsmoker cotinine concentration of <0.1 ng/mL was established as a Healthy People 2010 objective (22). For this analysis, we defined a group of nonsmokers with higher SHS exposure levels by selecting all participants with serum cotinine concentrations ≥ 0.1 ng/mL but <10 ng/mL. In this group, the detection rate for urinary NNAL was 87.5%, thus geometric means were calculated and uncorrected and creatinine-corrected results are reported in Table 5.

In this group, males had significantly higher unadjusted NNAL concentrations than females ($P = 0.04$), but following adjustment for creatinine the concentrations were slightly higher in females and the difference by gender was not significant ($P = 0.67$). Non-Hispanic whites had consistently higher concentrations than either non-Hispanic blacks or Mexican Americans. The concentration difference between non-Hispanic whites and Mexican Americans was significant before and after adjustment for creatinine ($P = 0.03$ and $P = 0.02$, respectively), whereas the NNAL concentrations in non-Hispanic Whites was only significantly higher than non-Hispanic blacks when the data were adjusted for creatinine concentrations

Table 2. Selected percentiles for the population according to smoking status

	<i>n</i>	Total urinary NNAL, pg/mL			
		Percentiles (95% CI)			
		50th	75th	90th	95th
Nonsmoker*	5,206	—	2.7 2.2-3.6	11.0 8.7-14.1	24.4 17.8-30.3
Smoker	1,393	329 276-386	643 530-782	1,260 1,080-1,540	1,800 1,580-2,710

*Based on a serum cotinine concentration of ≥ 10 ng/mL denoting a smoker.

(5.95 versus 4.01 pg/mg creatinine; $P = 0.01$). No significant difference was found in either adjusted or unadjusted data between non-Hispanic black and Mexican-American nonsmokers.

Among these more exposed nonsmokers, the differences among geometric mean concentrations calculated by age groups were all significant. The greatest differences as indicated in Table 5 were between young children with the age of 6 to 11 years and adults, in which adjusted and unadjusted concentrations were significantly greater in the children ($P < 0.001$ in either case). Young children also had higher concentrations than older chil-

dren with the age of 12 to 19 years in unadjusted and adjusted data ($P = 0.004$ and $P < 0.001$, respectively), and children with the age of 12 to 19 years also had higher unadjusted and adjusted NNAL concentrations than adults ($P = 0.003$ and $P = 0.005$, respectively).

Discussion

Despite improvements in recent years, exposure to SHS remains a significant health risk and public health concern (6). Earlier NHANES evaluations have measured serum cotinine as an index of SHS exposure in

Table 3. Selected percentiles among nonsmokers in NHANES 2007 to 2008

		Total urinary NNAL,* pg/mL			
		Percentiles (95% CI)			
	Percentage above the LOD	50th	75th	90th	95th
All	41.2%	—	2.7	11.0	24.4
			2.2-3.6	8.7-14.1	17.8-30.3
Males	46.2	—	3.7	12.5	26.9
			2.9-4.4	10.3-14.7	18.3-37.1
Females	37.0	—	2.0	9.6	21.1
			1.5-2.8	7.1-13.4	16.6-28.0
Non-Hispanic White	40.2	—	2.8	11.8	27.4
			1.8-4.0	8.1-17.1	19.6-35.4
Non-Hispanic Black	51.4	0.7	4.8	13.9	25.7
		0.4-1.8	3.6-6.3	11.6-16.9	18.7-33.2
Mexican American	39.9	—	1.9	6.8	13.9
			1.1-2.9	4.9-8.8	10.1-17.2
6-11 y	56.7	1.2	8.3	31.4	60.6
		0.6-2.6	4.7-13.8	17.2-49.8	37.1-75.5
12-19 y	58.5	1.1	4.9	19.7	38.9
		0.5-2.0	3.8-9.2	11.9-27.1	20.6-60.9
≥20 y	36.3	—	2.0	8.1	16.4
			1.7-2.5	6.9-9.8	13.9-20.7

Abbreviation: LOD, limit of detection.

*The limit of detection was 0.060 pg/mL.

Table 4. Selected percentiles among nonsmokers in NHANES 2007 to 2008 total urinary NNAL (picogram per milligram creatinine)

	Creatinine corrected data, percentiles			
	50th	75th	90th	95th
All	—	2.8	10.3	20.4
		2.3-3.5	7.4-14.0	16.2-27.3
Males	—	3.0	10.5	20.9
		2.4-3.7	7.5-12.9	15.7-28.9
Females	—	2.7	9.8	19.9
		2.1-3.3	6.8-14.7	15.7-27.9
Non-Hispanic White	—	2.9	11.4	23.9
		2.2-4.0	7.2-17.0	17.1-31.8
Non-Hispanic Black	1.0	3.7	11.5	20.4
	0.6-1.4	2.5-5.3	9.2-14.2	14.5-28.8
Mexican American	—	2.1	5.8	13.1
		1.5-3.0	4.3-8.3	8.3-17.9
6-11 y	2.1	10.6	38.3	60.9
	1.4-3.2	5.9-17.9	21.2-56.3	46.4-72.1
12-19 y	1.1	4.4	14.2	29.1
	0.7-1.7	2.6-7.4	10.5-23.3	15.6-41.9
≥20 y	—	2.3	6.8	15.1
		2.0-2.7	5.5-8.7	11.8-17.7

the U.S. population and have documented the widespread nature of these exposures. When a cigarette burns, the tobacco-specific nitrosamines partition into mainstream and sidestream smoke and can be measured in the resulting SHS. Thus, nonsmokers are also exposed to tobacco-specific nitrosamines. Consequently, beginning with NHANES 2007 to 2008, we began measuring total urinary NNAL in all participants with the age of ≥6 years. Although concentrations of this carcinogenic metabolite of the tobacco-specific contaminant

NNK were much lower than the levels of serum cotinine, we measured total urinary NNAL at or above its detection limit in 41% of nonsmokers, including many young children. In a subgrouping of nonsmokers with elevated levels of serum cotinine indicating relatively substantial exposure to SHS, the detection rate for urinary NNAL was >87%.

Among nonsmokers identified as having had higher exposure to SHS (defined as those with serum cotinine concentrations of 0.1-10 ng/mL), the geometric mean total

Table 5. NNAL concentrations among nonsmokers with higher exposure to SHS

	<i>n</i>	Geometric mean (95% CI)	
		Uncorrected NNAL, pg/mL	Corrected NNAL, pg/mg creatinine
All	1,489	5.56 (4.8-6.4)	5.27 (4.5-6.2)
Males	766	6.25 (5.3-7.4)	5.15 (4.3-6.1)
Females	723	4.92 (4.1-5.9)	5.39 (4.4-6.7)
Non-Hispanic White*	607	6.12 (5.3-7.1)	5.95 (5.0-7.0)
Non-Hispanic Black	421	5.12 (4.2-6.3)	4.01 (3.1-5.2)
Mexican American	236	4.61 (3.8-5.6)	4.53 (3.9-5.3)
6-11 y	320	11.3 (9.2-13.9)	13.9 (11.3-17.1)
12-19 y	315	7.32 (5.8-9.2)	5.81 (4.8-7.1)
≥20 y	854	4.48 (3.8-5.4)	4.20 (3.5-5.0)

NOTE: Nonsmokers with higher exposure to SHS are defined as having serum cotinine ≥0.1 ng/mL and <10 ng/mL.

*No results are listed for other race or ethnicity.

urinary NNAL concentration was ~ 5.6 pg/mL ($n = 1,489$). This corresponds to $\sim 2\%$ of the geometric mean total urinary NNAL concentration measured in the active smokers in this study (285.2 pg/mL; 95% CI, 241-337; $n = 1,393$). Previous investigations of total urinary NNAL concentrations in nonsmokers with known substantial SHS exposures have reported similar (arithmetic) averages of ~ 10.5 pg/mL (14).

Previous studies have reported the detection of urinary NNAL in young children and even among newborns (14, 23, 24). Among nonsmokers in our study, children with the age of 6 to 11 years were the group most at risk for exposure to NNK as estimated from their urinary NNAL concentrations. These younger children had uncorrected NNAL concentrations in their urine that were 2.5 times as high as in adult nonsmokers, and the difference was even greater when creatinine-adjusted values were calculated. Children have previously been identified as the group most at risk for SHS exposure based on their higher serum cotinine concentrations (20). Part of this difference may reflect their smaller size and differences in respiration, but children also are among the most vulnerable nonsmokers in the home because of the time spent in the home and their limited options to avoid exposure. Our results confirm that the more young children are exposed to tobacco smoke, as reflected in their serum cotinine concentrations, the greater their exposure to one of the more hazardous and carcinogenic components of tobacco smoke.

Gender was not found to be a significant predictor of urinary NNAL concentrations among nonsmokers, but non-Hispanic Whites had consistently higher concentrations than either non-Hispanic Blacks or Mexican Americans. The difference between non-Hispanic whites and Mexican Americans was significant regardless of creatinine adjustment, but the difference between non-Hispanic white and non-Hispanic blacks was significant only in the creatinine-adjusted data. Although urinary NNAL concentrations were consistently higher in non-Hispanic Blacks than in Mexican Americans, the differences were not statistically significant.

When plotted on a log basis, urinary NNAL and serum cotinine concentrations were found to have a significant association ($r = 0.92$; $P < 0.001$), which remained despite the fact that the two analytes were measured in separate assays using two distinct matrices. Several previous studies have reported a statistically significant association between urinary NNAL and urinary cotinine (14, 25, 26) using data from 20 to 80 persons. Serum cotinine is often an indicator of exposure to SHS and, by inference, to the many toxicants associated with it. Our results confirm that these two tobacco-specific analytes provide similar exposure estimates and indicate that serum cotinine measurements provide a useful index of the relative exposure to NNK as well. However, these results might be altered if cigarettes with lower tobacco-specific nitrosamine contents, such as seen in Canada and Australia, were included in the analysis or if the tobacco-specific nitrosamine

content of cigarettes was reduced in the future. Continued monitoring of serum cotinine and urinary NNAL in future NHANES will be important to detect such changes in population exposure resulting from newer types of cigarettes or in reductions in smoking. Specific biomarkers such as urinary NNAL can serve as valuable early sentinels of potential changes in population risk over time.

Our study has several strengths and some limitations. An important advantage is the use of a large national sample of persons who are representative of the U.S. civilian noninstitutionalized population, including $>5,000$ nonsmokers. Thus, our results provide an estimate that is representative of the overall exposure of U.S. nonsmokers to this tobacco-specific nitrosamine. Our measurements provided a sensitive and specific analysis based on a specific isolation procedure and analysis using isotope-dilution tandem mass spectrometry. However, despite the use of a highly sensitive method, we were able to measure this analyte above its detection limit in only $\sim 41\%$ of all nonsmokers, which precluded the calculation of group mean concentrations for all nonsmokers. Geometric means could, however, be calculated for a more heavily exposed subgroup as defined by serum cotinine concentrations. We also were limited to participants with the age of ≥ 6 years in these assays and were therefore unable to measure concentrations in younger children, who are also believed to be at significant risk for exposure based on cotinine measurements.

Many health risks are known to be associated with exposure to SHS, including an increased risk for lung cancer (6). The presence of a tobacco-specific pulmonary carcinogen in the urine of many nonsmokers provides one potential biochemical link between such exposures and adverse health outcomes. The findings reported here provide further evidence of the risks faced by nonsmokers who are regularly exposed to tobacco smoke in their environment. These data also provide a baseline analysis that should be useful in future evaluations. The continued monitoring of the exposure of U.S. nonsmokers to NNK in future NHANES surveys will detect future trends in exposure to this key tobacco carcinogen.

Disclosure of Potential Conflicts of Interest

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. 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 CDC. No potential conflicts of interest were disclosed.

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