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

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Running Title: Urinary NNAL concentrations in the U.S. population.

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ABSTRACT

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

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 LOD, the 50^{th} through 95^{th} percentiles, and in some cases geometric means for groups classified by age, gender and race/ethnicity.

RESULTS: Total urinary NNAL was measureable 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 aged ≥20 years ($p < 0.001$).

CONCLUSIONS: Among NHANES participants, total NNAL was found at measurable levels in the urine of 41% of nonsmokers, and in 87.5% of those with substantial SHS exposure (with serum cotinine concentrations of 0.1 to 10 ng/mL). Children aged 6–11 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 both smokers and nonsmokers. NNAL was detected in 41% of all nonsmokers.

INTRODUCTION

The health risks of smoking are well-established; it has been estimated that cigarette smoking accounts for approximately 438,000 deaths (20% of all deaths) in the United States each year (1-3). Smoking is an important contributor to both cardiovascular and respiratory diseases and has long been associated with a significantly increased risk of 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 the majority 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 approximately 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 (MS) and sidestream (SS) smoke exist, the carcinogens in MS smoke inhaled by active smokers are largely the same as those in SHS (a mixture of MS and SS smoke) to which both smokers and nonsmokers may be exposed. These carcinogens include benzene, a variety of polycyclic aromatic hydrocarbons (PAHs), aromatic amines, tobacco-specific nitrosamines (TSNAs), aldehydes, inorganics such as cadmium and polonium-210, and many additional compounds (6,7). Several N-heterocyclic amines also have been identified at low concentrations in smoke from non-filtered 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 (PhIP) 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 TSNAs and PAHs (8,9).

Since SHS is a mixture of SS and (exhaled) MS tobacco smoke with a composition that constantly changes during aging, it can be difficult to compare relative concentrations of toxicants in MS and SHS. However, the ratios of selected constituents in fresh SS and MS smoke from unfiltered cigarettes have been reported. These ratios for several carcinogens in tobacco smoke including benzene, cadmium, aromatic amines such as 4-aminobiphenyl, TSNAs and PAHs are commonly greater than 1, and may range as high as 10 (6,7,10), with an estimated SS/MS for the TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) of approximately 1-4 (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 – 29.3 ng/mm^3 (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 TSNAs, 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 TSNAs N-nitrosonornicotine (NNN) 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 both smokers and nonsmokers exposed to SHS by measuring 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (the 2^o 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 both smokers and nonsmokers exposed to SHS (9,12). This biomarker has been quantified in urine samples from

both 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 prior 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 to the shorter estimated 16–18 hour half-life of cotinine (6).

We have measured serum cotinine in each National Health and Nutrition Examination Survey (NHANES) beginning with NHANES III 1988–1992 and continuing in subsequent two-year surveys. Starting with NHANES 2007–2008, we also began analyzing total urinary NNAL in the majority of NHANES participants. Specifically, serum cotinine was measured in NHANES participants aged ≥3 years if sufficient serum was available, and NNAL assays were conducted on all available urine samples from participants aged ≥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 manuscript will address the exposure of active tobacco users participating in NHANES to this TSNA.

SUBJECTS AND METHODS

Study Design. The National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) conducts NHANES. This survey is designed to assess the health and nutrition status of the civilian, non-institutionalized 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 or 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 2007–2008 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). CDC's Institutional Review Board reviewed and approved the study protocol, and all study participants provided informed, written consent.

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

1. The NNAL concentrations in this study are total NNAL values which represent the actual measured amount of NNAL in pg/mL 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 concerning the study participants were self-reported. Race or ethnicity was derived from questionnaire data; categories included non-Hispanic white, non-Hispanic black, Mexican American, and "Other." Age groupings were set to 6–11, 12–19, and ≥20 years (adults).

Laboratory Methods. Total urinary NNAL was measured by high-performance liquid chromatography atmospheric-pressure ionization tandem mass spectrometry (HPLC API MS/MS) using a method previously described (17), but with modifications. Briefly, the urine aliquot (5 mL) was spiked with the [$^{13}C_6$]-NNAL internal standard and hydrolyzed overnight with ß-glucuronidase. The sample was then applied to a Chem Elute column (Varian, Walnut Creek, CA), 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 (MIP) column custom designed for NNAL analyses using the approach previously described (17). The MIP columns used in these analyses were purchased from Supleco (Bellefonte, PA).

The instrumental analysis was also modified from our previous procedure by adding a third HPLC pump that added acetonitrile (0.6 mL/min) post-column immediately prior to the source, and by substituting a Sciex API 5000 tandem mass spectrometer (ABI, Foster City, CA) 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 (LOD) 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 more than 4 years at –70 °C (18).

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

Serum cotinine was measured in all participants aged ≥3 years by LC API MS/MS, and urinary creatinine was

measured in all participants aged ≥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). Since the detection rate for NNAL among nonsmokers was less than 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%. Both urinary total NNAL and serum cotinine were log-transformed to reduce the skewness in their distributions. All statistical analyses were performed using Proc DESCRIPT and Proc REGRESS in SUDAAN (version 10.0, RTI, Research Triangle Park, NC), with graphical analyses performed by using SAS (version 9.2, SAS Institute, Cary, NC). Standard errors 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 both smokers and nonsmokers in NHANES 2007–2008. 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 serum cotinine and urinary NNAL concentrations observed among nonsmokers.

Table 2 presents medians and selected percentiles with their 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). Since the detection rate for urinary NNAL among nonsmokers was approximately 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 aged 12-19 had concentrations approximately twice as high as adults, whereas the concentrations in the youngest children aged 6-11 were nearly 4-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 greater than or equal to 0.1 ng/mL, but less than 10 ng/mL. In this group, the detection rate for urinary NNAL was 87.5%, thus geometric means were calculated and both 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 both 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 (5.95 vs. 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 aged 6-11 and adults in which both adjusted and unadjusted concentrations were significantly greater in the children ($p< 0.001$ in either case). Young children also had higher concentrations than older children aged 12-19 in both unadjusted and adjusted data (p=0.004 and $p<0.001$, respectively), and children aged 12-19 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 the U.S. population and have documented the widespread nature of these exposures. When a cigarette burns, the TSNAs partition into both mainstream and sidestream smoke and can be measured in the resulting SHS. Thus nonsmokers are also exposed to TSNA. Consequently, beginning with NHANES 2007–2008, we began measuring total urinary NNAL in all participants aged ≥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 greater than 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 urinary NNAL concentration was approximately 5.6 pg/mL (n = 1,489). This corresponds to approximately 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 about 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 aged 6–11 years were the group most at risk of 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 of 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 20–80 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 TSNA contents, such as seen in Canada and Australia, were included in the analysis or if the TSNA content of cigarettes was reduced in the future. Continued monitoring of both 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, non-institutionalized population, including more than 5,000 nonsmokers. Thus, our results provide an estimate that is representative of the overall exposure of U.S. nonsmokers to this TSNA. 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 approximately 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 aged ≥6 years in these assays and were therefore unable to measure concentrations in younger children, who are also believed to be at significant risk of exposure based on cotinine measurements.

Many health risks are known to be associated with exposure to SHS including an increased risk of 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.

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FIGURE LEGENDS

Figure 1. Distribution of urinary total NNAL concentrations in the U.S. population aged ≥6 years from the 2007–2008 National Health and Nutrition Examination Survey (NHANES).

Figure 2. Relationship between concentrations of total urinary NNAL (pg/mg creatinine) and serum cotinine (ng/mL) 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 approximately 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.

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

	All NHANES* Participants Sample Size (N)	**Nonsmokers Only**** Sample Size (N)
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
Others	1,080	899
Aged 6–11years	879	875
Aged 12–19 years	960	840
Aged ≥20 years	4,760	3,491

* National Health and Nutrition Examination Survey

** Nonsmokers defined as participants with a serum cotinine concentrations <10 ng/mL

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

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

*4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

**Based on a serum cotinine concentration of 10 ng/mL or above denoting a smoker

Table 3. Geometric means and selected percentiles among nonsmokers* in NHANES** 2007–2008.

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

*Nonsmokers defined as having serum cotinine concentrations <10 ng/mL
**National Health and Nutrition Examination Survey
***4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. The LOD was 0.060 pg/mL.

Table 4. Selected percentiles among nonsmokers* in NHANES** 2007–2008.

Total Urinary NNAL***, pg/mg 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 2.4–3.7	10.5 7.5–12.9	20.9 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
Aged 6–11 years	2.1	10.6	38.3	60.9
	1.4–3.2	5.9–17.9	21.2–56.3	46.4–72.1
Aged 12–19 years	1.1 0.7–1.7	4.4 2.6–7.4	14.2 10.5–23.3	29.1 15.6–41.9
Aged ≥20 years	---	2.3 2.0–2.7	6.8 5.5–8.7	15.1 11.8–17.7

*Nonsmokers defined as having serum cotinine concentrations <10 ng/mL
**National Health and Nutrition Examination Survey
***4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

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

		Geometric Mean (95% CI)	
	N	**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)
Aged 6–11 years	320	11.3 (9.2 – 13.9)	13.9 (11.3 – 17.1)
Aged 12–19 years	315	7.32 (5.8 – 9.2)	5.81 (4.8 – 7.1)
Aged ≥20 years	854	4.48 (3.8 – 5.4)	4.20 (3.5 – 5.0)

* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
** Nonsmokers with higher exposure to SHS are defined as having serum cotinine greater than or equal to 0.1 ng/mL and less than 10 ng/mL
*** No results are listed for the race-ethnicity category of "Other".

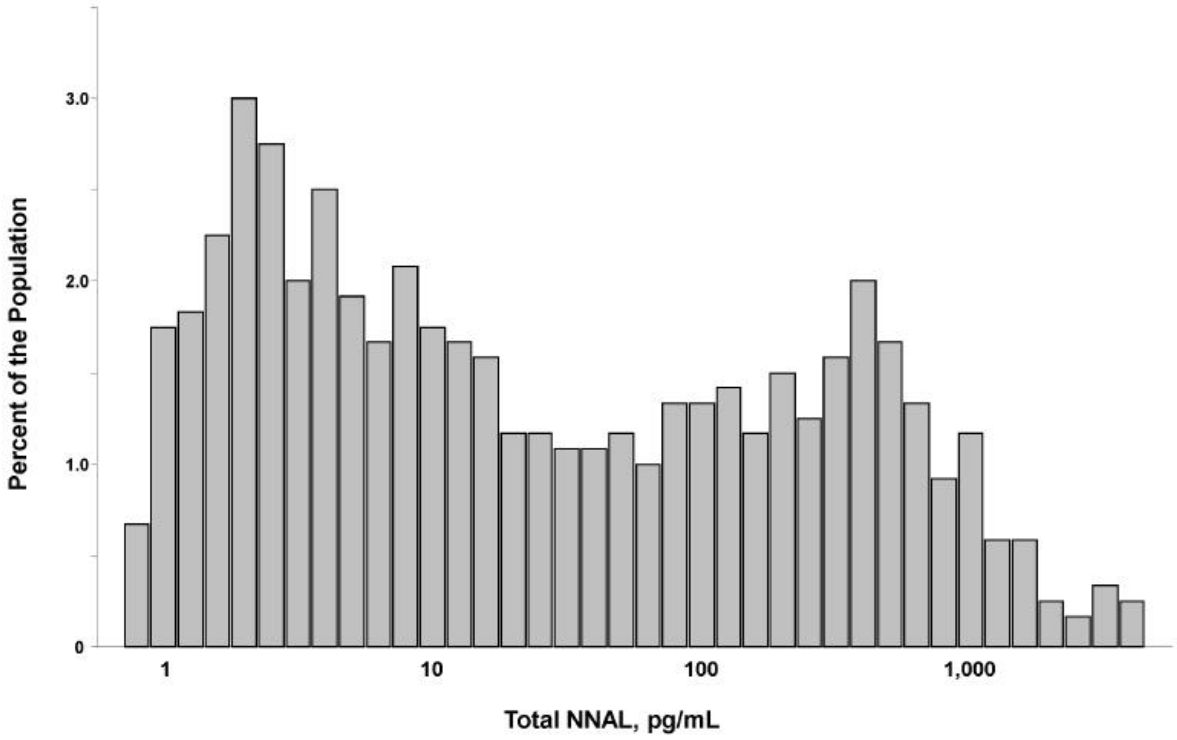
Percent of the Population
3.0
2.0
1.0
0
1
10
100
1,000
Total NNAL, pg/mL

Figure 1

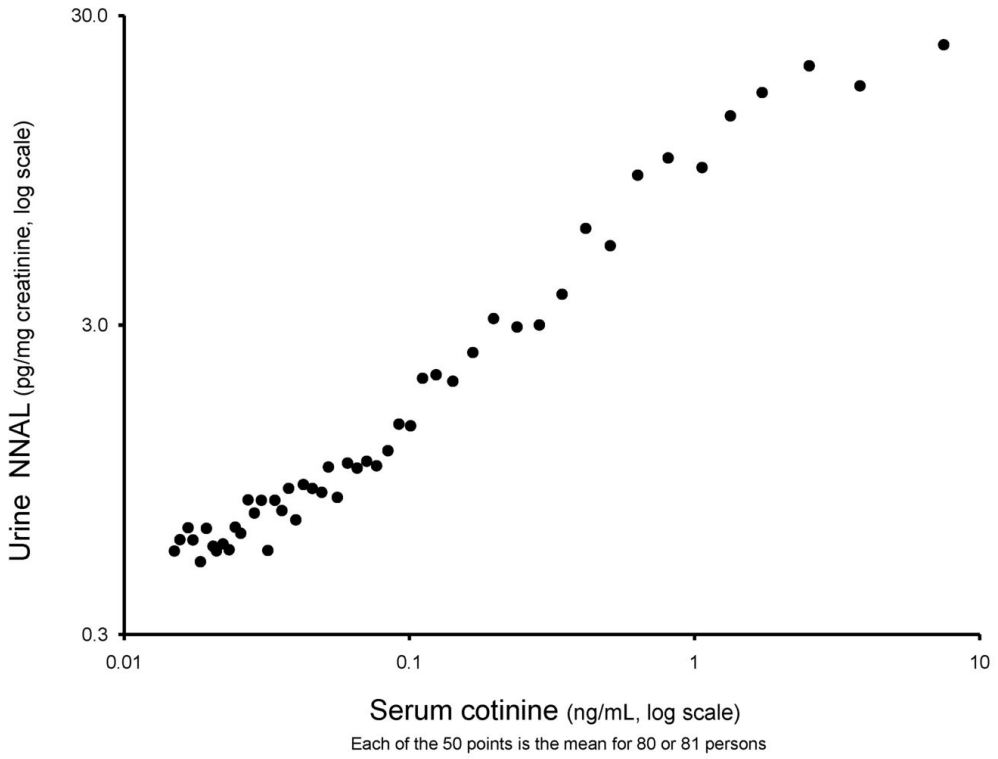
30.0
3.0
0.3
Urine NNAL (pg/mg creatinine, log scale)
0.01
0.1
1
10
Serum cotinine (ng/mL, log scale)
Each of the 50 points is the mean for 80 or 81 persons

Figure 2

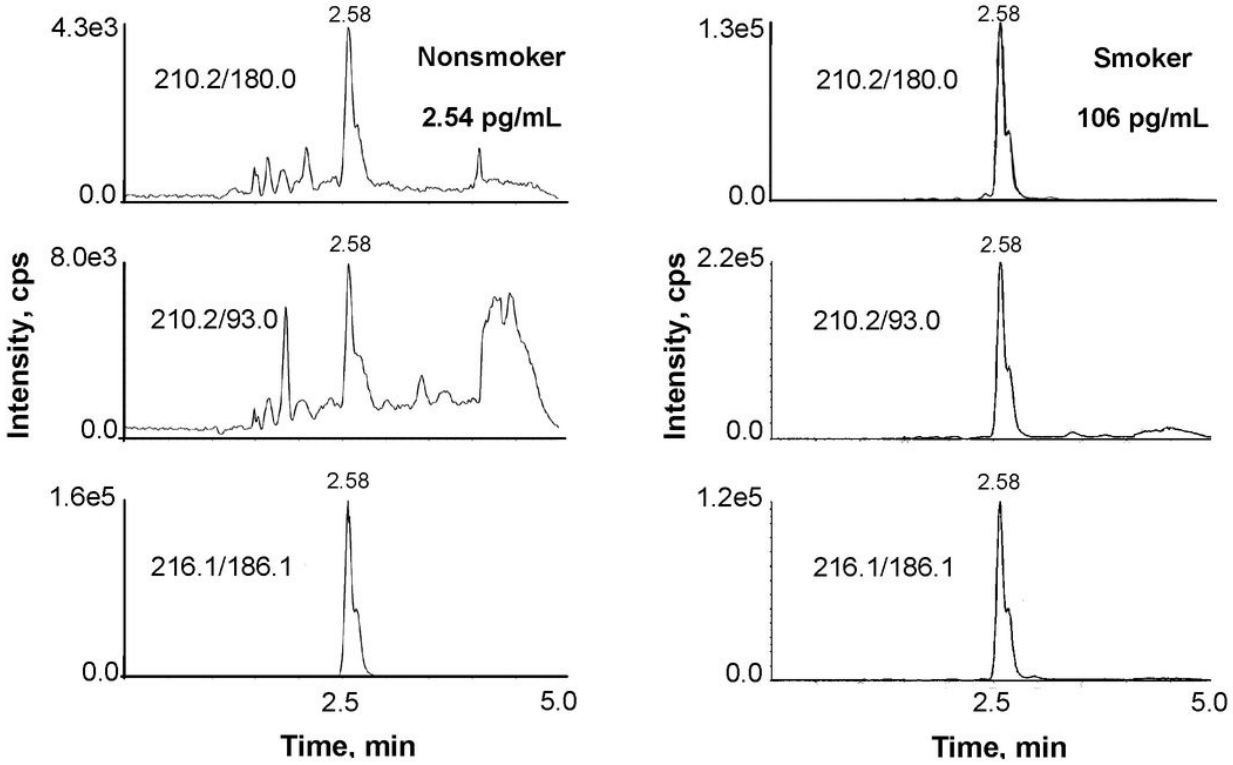
4.3e3
2.58
210.2/180.0
Nonsmoker
2.54 pg/mL
0.0
1.3e5
2.58
210.2/180.0
Smoker
106 pg/mL
0.0
8.0e3
2.58
210.2/93.0
0.0
Intensity, cps
2.2e5
2.58
210.2/93.0
0.0
1.6e5
2.58
216.1/186.1
0.0
1.2e5
2.58
216.1/186.1
0.0
2.5
5.0
Time, min
2.5
5.0
Time, min

Figure 3