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Biomarkers of Secondhand Smoke Exposure in Automobiles

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Abstract

Objectives—The objectives of this study were: (1) to characterize the exposure of nonsmokers exposed to secondhand smoke (SHS) in a vehicle using biomarkers, (2) to describe the time-course of the biomarkers over 24 h, and (3) to examine the relationship between tobacco biomarkers and airborne concentrations of SHS markers.

Methods—Eight nonsmokers were individually exposed to SHS in cars with fully open front windows and closed back windows over an hour from a smoker who smoked 3 cigarettes at 20 min intervals. The nonsmokers sat in the backseat-passenger side, while the smoker sat in the driver's seat. Plasma cotinine and urine cotinine, 3-hydroxycotinine (3HC), and 4-(methylnitrosoamino)-(3-pyridyl)-1-butanol (NNAL) were compared in samples taken at baseline and several time-points after exposure. Nicotine, particulate matter (PM_{2.5}), and carbon monoxide (CO) were measured inside and outside the vehicle and ventilation rates in the cars were measured.

Results—Average plasma cotinine and the molar sum of urine cotinine and 3HC (COT+3HC) increased 4-fold, urine cotinine increased 6-fold, and urine NNAL increased ~27 times compared to baseline biomarker levels. Plasma cotinine, urine COT+3HC and NNAL peaked at 4–8 hours post-exposure while urine cotinine peaked within 4 hours. Plasma cotinine was significantly correlated to PM_{2.5} (Spearman correlation (r_s) = 0.94) and CO (r_s = 0.76) but not to air nicotine. The correlations between urine biomarkers, cotinine, COT+3HC, and NNAL and air nicotine, PM_{2.5}, and CO were moderate but non-significant (r_s range, 0.31 – 0.60).

Conclusion—Brief SHS exposure in cars resulted in substantial increases in levels of tobacco biomarkers in nonsmokers. For optimal characterization of SHS exposure, tobacco biomarkers should be measured within 4–8 h post-exposure. Additional studies are needed to better describe the relationship between tobacco biomarkers and environmental markers of SHS.

Keywords

Cigarettes; Secondhand smoke; passive smoking; automobiles; motor vehicles; biomarkers; cotinine; tobacco-specific nitrosamines

INTRODUCTION

Secondhand smoke (SHS) exposure is associated with an increased risk of respiratory infection, otitis media and asthma in children as well as acute myocardial infarction and lung cancer in adults¹⁻³. In the U.S. and many other countries around the world there has been substantial progress in limiting SHS exposure in public places, such as restaurants and bars⁴. However, in most places there is no protection for nonsmokers from SHS exposure in motor vehicles.

On average, people spend more than an hour a day in motor vehicles⁵. Smokers commonly smoke while driving or riding in automobiles and passengers are often adult nonsmokers and children⁶. An automobile represents a small, often fully enclosed space if windows are closed, in which concentrations of secondhand smoke can be quite high, potentially posing a health threat for non-smokers who are passengers. Several researchers have measured concentrations of SHS constituents in the air of motor vehicles – including total particulate matter and nicotine⁷⁻⁹. These levels are comparable to or higher than those reported in restaurants and bars in which smoking is permitted^{10,11}. Measuring air concentrations of pollutants is important in determining exposure but may be imprecise as a measure of systemic exposure or dose in epidemiological studies of SHS and disease risk.

Systemic exposure to tobacco smoke is best estimated by using biomarkers of exposure such as nicotine, its metabolites and 4-(methylnitrosoamino)-(3-pyridyl)1-butanol (NNAL) - a metabolite of the tobacco-specific lung carcinogen, 4-(methylnitrosoamino)-(3-pyridyl)1-butanone (NNK)^{12,13}. We are aware of only one study in which biomarkers of exposure to SHS in motor vehicles has been reported. This was a study of urine cotinine concentrations after heavy exposure of non-smokers to SHS for two hours in a tour bus¹⁴.

The aim of the present study was to examine multiple biomarkers of SHS exposure, including plasma and urine cotinine and urine NNAL over time after an experimental exposure to SHS in a stationary automobile with open front windows. Air levels of environmental markers of SHS were also measured to examine the relationships between environmental and biological markers of SHS exposure. Since the biomarkers of exposure are tobacco smoke specific, no control condition was deemed necessary.

METHODS

Overview

The study was conducted in the Clinical Research Center at San Francisco General Hospital (SFGH) and in an automobile parked in the hospital parking lot. Non-smoking participants sat in the back seat of the car with the front windows fully open and the back windows closed, while a smoker sat in the front seat and smoked three cigarettes over the course of an hour. Blood, urine and air samples were collected to measure cigarette smoke exposure in the nonsmokers. The study was approved by the Committee on Human Research at the University of California, San Francisco. Written, informed consent was obtained from each participant and all participants were financially compensated for their time.

Subjects

There were 10 participants, including 8 nonsmokers and 2 active smokers. The active smokers' involvement in the study was limited to smoking cigarettes in the car during the 1-hour SHS exposure period. The nonsmokers included 4 men and 4 women who were all healthy and had prior histories of SHS exposure. A sample size of 8 was judged to be adequate to characterize typical biomarker changes in nonsmokers. Prior exposure was required to ensure that we were not exposing subjects to an unfamiliar risk. Nonsmoking status was determined by self-report and confirmed by plasma cotinine concentrations. Exclusion criteria included a history of recent respiratory illness, history of major medical or psychiatric conditions, body mass index (BMI) >30, pregnancy or lactation, current illicit drug or alcohol abuse, inability to speak English or a history of fainting.

Study Procedures

The nonsmoking participants arrived at the Clinical Research Center by 7 A.M. An intravenous (IV) line for blood sampling was placed and baseline blood and urine samples were collected. Between 8 and 9 A.M., the participant was escorted to the hospital parking lot and asked to sit in the back seat, passenger side, of the car, while the smoker sat in the driver's seat. Air monitoring equipment was placed in the back seat next to the nonsmoker. The front windows were fully open, while the back windows were closed for the duration of the SHS exposure session. The same brand of cigarettes, Marlboro Regulars, was smoked at each exposure session. Three cigarettes in total were smoked at 20 minute intervals, starting at time 0. The nonsmoker exited the car 60 minutes after the lighting of the first cigarette, and then immediately returned to the research ward for a 24 hour stay.

The Automobile

Rental cars that permitted smoking were obtained for the study. While the make and model of rental cars used varied, we selected cars with comparable interior volumes. The vehicles included a 2007 Ford Taurus, a 2007 Pontiac Grand Prix and 2007, 2008 and 2009 Chevrolet Malibus. The volume of the passenger compartment ranged from 2.75 to 2.92 m³ for these vehicles ¹⁵.

Air Measurements

Air in the cars was sampled for nicotine, carbon monoxide (CO) and particulate matter less than 2.5 μ m in aerodynamic diameter (PM_{2.5}). Monitors were collocated in the middle of the backseat and tube inlets were placed at the approximate breathing height of the seating nonsmoking participant (~0.5 m from the seat). We simultaneously conducted ambient air monitoring for background concentrations of nicotine, CO, and PM_{2.5} using monitors collocated ten meters away from the automobile.

Time-integrated vehicle and ambient nicotine were actively sampled on 37 mm Pall EMFAB filters impregnated with sodium bisulfate using TSI SidePak pumps (TSI Incorporated, Shoreview, MN) with the flow rate set at 2 L/min. Vehicle and ambient real-time CO was measured using Hobo CO Data Loggers, model H11-001 (Onset Computer Corp, Bourne, MA), which were programmed to record CO levels continuously every second starting 15 minutes before the first cigarette was smoked and during the 60 minute exposure period. Two additional CO monitors were used in the car, one centered on the dashboard and the other centered on the shelf behind the backseat. These monitors were used to measure CO concentration and decay – a measure used for determining average air changes per hour (ACH).

PM_{2.5} in the cars was measured using a Grimm Series 1.108 Aerosol Spectrometer (Grimm Technologies, Inc., Douglasville, GA) set to record PM_{2.5} concentrations every 6 seconds at

1.2 L/min. A personal DataRAM (pDR-1200) monitor (Thermo Electron Corp., Franklin, MA), in conjunction with a Pall 2.0 μm pore 37 mm PTFE filter and TSI SidePak SP350 pump (5 L/min), was used to record ambient $\text{PM}_{2.5}$ concentrations. The pDR-1200 monitor measures both real-time (continuous) $\text{PM}_{2.5}$ using the data-logger and time-integrated (gravimetric) $\text{PM}_{2.5}$ on the PTFE filter. Each PTFE filter was weighed before and after sampling using a Cahn 29 microbalance. We corrected the real-time $\text{PM}_{2.5}$ levels reported by the Grimm and pDR aerosol monitors by the gravimetric readings obtained from analysis of $\text{PM}_{2.5}$ on the pDR filters collected during the exposures.. Details of air sampling measurement methods are described in more detail elsewhere ¹⁶.

Biomarker Measurements

Blood samples were taken immediately before entering the vehicle (baseline) and 15, 30, 45, 60 and 90 minutes, and 2, 3, 4, 6, 8, 12, 16 and 24 hours after exiting the vehicle; and plasma was analyzed for concentrations of nicotine and cotinine. Urine was collected at baseline and then in blocks of 0–4, 4–8, 8–12 and 12–24 hours. Urine was analyzed for concentrations of cotinine, and trans-3'-hydroxycotinine (3HC), NNAL and creatinine.

Analytical Chemistry

Air nicotine concentrations were measured through a chemical extraction and gas chromatography with a capillary column and nitrogen-phosphorus detection quantification as described previously ¹⁷. The limit of quantification (LOQ) for airborne nicotine mass and concentration were 0.005 μg and 0.038 $\mu\text{g}/\text{m}^3$, respectively. Plasma nicotine was measured by gas chromatography-mass spectrometry with a lower limit of quantitation (LLOQ) of 0.2 ng/mL, as described previously ¹⁸. Plasma cotinine was measured by liquid chromatography – tandem mass spectrometry (LC-MS/MS) and the LLOQ was 0.02 ng/mL ¹⁸. Urine total (free plus conjugated) cotinine and 3-HC were measured using LC-MS/MS and had LLOQ of 0.05 and 0.10 ng/mL, respectively ¹⁸. Urine total NNAL was measured by LC-MS/MS as previously described and had an LLOQ of 0.25 pg/mL ¹⁹.

Statistical Analysis

Data analysis of air measurements are described in detail elsewhere ¹⁶. In brief, the time-integrated concentrations of analytes in ambient air were subtracted from concentrations of these analytes in the vehicles to control for ambient levels. Ventilation rates in air changes per hour were estimated using the CO levels as a tracer, and calculated by determining the slope of the semi-log plot of the natural log of carbon monoxide concentration (averaged over 15 seconds) over time following the extinction of each cigarette smoked ²⁰. The air changes per hour (ACH) for each location (front, middle, back) was determined using the final cigarette smoked in each exposure period.

Descriptive statistics were computed for SHS constituents in the vehicles and biomarkers of SHS exposure among the nonsmokers. Descriptive statistics for biomarkers were computed for baseline concentrations, maximum concentration (C_{max}) following SHS exposure and time of maximum concentration, and maximum change (Δ_{max}). Biomarker concentrations below the limit of quantitation (LOQ) were replaced with $\text{LOQ}/2$. Because of the small sample size ($n = 8$), Spearman correlation coefficients were used to examine relationships between 1-hr time-integrated air measurements and exposure biomarkers.

RESULTS

Eight nonsmokers were enrolled in the study were equally distributed by sex, included 2 Hispanic whites, 2 non-Hispanic whites, 3 Asians, and 1 mixed race, and had the following

characteristics (as mean and range): age, 26 years (18–34); weight, 69 kg (48.2–82.4); and BMI, 24.1 (18.8–27.5).

Air measurements and ventilation

Time-integrated air concentrations of nicotine, PM_{2.5}, and CO and average ventilation rates over the 60-min exposure period for each subject are shown in Table 1. Controlling for ambient concentrations as described before, average vehicle concentrations of SHS constituents over the 1-hr exposure period were as follows (mean $\pm$ SD): nicotine, 9.5 ± 5.4 $\mu\text{g}/\text{m}^3$; PM_{2.5}, 746 ± 281 $\mu\text{g}/\text{m}^3$; and CO, 1.4 ± 0.7 ppm. Air changes in the vehicles averaged 7.5 (range 4.7–10.6) air changes per hour (ACH) in the front, 2.7 (1.8–3.6) ACH in the middle, and 1.9 (1.3–3.2) ACH in the back.

Biomarker levels

Table 2 presents individual and average data on plasma cotinine, urine cotinine, molar sum of urine cotinine and 3-hydroxycotinine (COT+3HC), and urine total NNAL, including concentrations at baseline before SHS exposure, the maximum concentration (C_{max}) after exposure to SHS, and the maximum change (Δ_{max}) in the concentration from baseline to peak... We analyzed the sum of urine cotinine and 3HC because it has been shown to correlate better with systemic nicotine dose than cotinine alone ²¹.

Plasma nicotine concentrations did not change after SHS exposure. Plasma cotinine increased from an average of 0.04 ± 0.03 ng/mL at baseline to an average maximum concentration of 0.17 ± 0.05 ng/mL, a 4-fold increase, occurring at a median of 4.5 h post exposure (range, 3 – 9 h). The time course of plasma cotinine from baseline up to 24 h post SHS exposure is presented in Figure 1. Average baseline urine cotinine was 0.38 ± 0.25 ng/mg creatinine and increased 6-fold to an average maximum concentration of 2.4 ng/mg creatinine. Peak urine cotinine concentrations were measured in the 0–4 h post-exposure urine samples (Figure 2A). Urine COT+3HC increased from 0.006 ± 0.005 nmol/mg creatinine at baseline to a maximum concentration of 0.025 ± 0.020 nmol/mg creatinine, a 4-fold increase. The peak urine COT+3HC concentrations were measured in the 4–8 h post-exposure urine samples (Figure 2B). Urine NNAL increased from an average of 0.10 ± 0.19 pg/mg creatinine at baseline to an average maximum concentration of 2.68 ± 1.36 pg/mg creatinine, a 26.8-fold increase. The peak urine NNAL concentrations were also measured in the 4–8 h post SHS exposure urine samples (Figure 2C).

Correlations between environmental markers and biomarkers

Spearman correlation coefficients between airborne concentrations of SHS and biomarkers of SHS exposure are presented in Table 3. Time-integrated PM_{2.5} measured in the cars was significantly correlated to the maximum change in plasma cotinine ($r_s = 0.94$), had moderate to high but non-significant correlations with CO, urine cotinine, COT+3HC, and NNAL, and was poorly correlated with air nicotine. Air nicotine was poorly correlated to air measurements of SHS and plasma cotinine but moderately correlated to urine cotinine, COT+3HC, and NNAL (these correlations were non-significant). Urine NNAL was not significantly correlated to plasma cotinine, urine cotinine or COT+3HC. Spearman correlations between air changes (ACH) measured in the backseat and environmental markers and biomarkers of SHS were as follows: PM_{2.5}, $r_s = 0.71$ (*ns*) (*ns* = nonsignificant); air nicotine, $r_s = 0.38$ (*ns*); CO, $r_s = 0.34$ (*ns*); plasma cotinine, $r_s = 0.32$ (*ns*); urine COT, $r_s = 0.57$ (*ns*); urine COT+3HC, $r_s = 0.54$ (*ns*); and, urine NNAL, $r_s = 0.76$, $p = 0.049$.

DISCUSSION

We present novel data from nonsmokers exposed individually to SHS over a 1-h period in a car with open front windows and closed back windows including (1) the uptake of tobacco-specific compounds, including the carcinogen NNK, (2) the time course of SHS biomarkers in the nonsmokers, and (3) the relationships between air concentrations of the SHS constituents and SHS biomarkers in these participants. Plasma cotinine and urine COT+3HC increased 4-fold, urine cotinine increased 6-fold, and urine NNAL increased by an average of ~27 times compared to baseline pre-exposure concentrations of these biomarkers. Plasma cotinine, urine COT+3HC, and urine NNAL peaked at 4–8 hours after exposure ended while urine cotinine peaked within about 4 hours after exposure. The findings of this study are useful for exposure estimates and health risk assessment of SHS exposure in cars as well as identifying optimal sampling times to capture maximum changes in plasma and urine tobacco biomarkers following SHS exposure in future studies.

Cotinine, 3-hydroxycotinine, and NNAL are tobacco-specific biomarkers that have been used to characterize systemic exposure to tobacco smoke constituents in both active and passive smokers. Cotinine, the proximate metabolite of nicotine, has on average a half-life of 16 h, and is eliminated from the body within 3–4 days following the last exposure¹². We measured cotinine in both plasma and urine in this study. We showed that the average maximum change in plasma cotinine following 1-hour SHS exposure was 0.13 ng/mL. Plasma cotinine has not been previously reported after exposure to SHS in vehicles. Serum cotinine concentrations in nonsmokers exposed to aged and diluted sidestream smoke in an environmental chamber have been reported²². In that study the air nicotine and serum cotinine levels were more than 10 times higher than what we observed in our study. Plasma cotinine values reported here were also about 10 times lower than changes in saliva cotinine measured in nonsmokers exposed to 3 hours of SHS inside bars that permitted smoking²³ and also lower than changes in saliva cotinine of casino employees exposed to SHS at work²⁴. Studies have shown that plasma and saliva cotinine levels are highly correlated with a range of 1.1 to 1.4 saliva-to-blood ratio²⁵, hence our direct comparison of the two matrices. On the other hand, the average change in plasma cotinine we report here were very similar to changes in saliva cotinine observed in nonsmokers exposed to SHS for 3 to 6 hours outside bars with relatively heavy outdoor smoking^{26,27}.

Urine cotinine increased 6-fold and urine COT+3HC increased 4-fold compared to baseline. Following exposure to SHS from 78 smoked cigarettes over 2 hours in a closed bus, Willers and colleagues reported maximum urine cotinine levels (~70 nmol/mL) that were several orders of magnitude higher than what we report in this study¹⁴. Air nicotine measured in the bus by Willers and colleagues was ~10 times higher than what we report here (110 µg/m³ vs. 9.5 µg/m³). However, our exposure scenario, i.e. exposure to 3 smoked cigarettes over 1 hour, is a more likely exposure scenario for children and nonsmoking adults riding in an automobile with a person who is smoking, thus making our data more useful for risk characterization than the previous study.

For 6 of 8 nonsmoking participants, the baseline urine NNAL level was below the LOQ. After SHS exposure, urine NNAL increased an average of ~27-fold among all participants. NNAL is useful as a specific marker of tobacco smoke exposure, and because both NNAL and its parent compound, NNK, are potent pulmonary carcinogens, NNAL provides direct evidence of carcinogen exposure^{2,13}. Since there is no known safe level of exposure to SHS, measurement of NNAL after brief exposure to SHS in a motor vehicle is important for health risk assessment. The half-life of NNAL is 10–16 days²⁸. While urine NNAL has not previously been reported after SHS exposure in cars, studies of indoor exposure to SHS among nonsmokers have previously shown urine NNAL changes ranging from 3.8 pg/mg

creatinine to 12.3 pg/mL^{29,30}, and similar to changes following a 3-hour exposure to SHS outside a bar with heavy outdoor smoking²⁷. Much higher increases in urine NNAL were observed in nonsmokers exposed to sidestream smoke in an environmental chamber²². Together with the plasma cotinine and urine cotinine and COT+3HC data presented here, our measurement of increased NNAL excretion in these participants over time indicates significant systemic exposure to tobacco toxicants after SHS exposure over a relatively short period of time in cars.

We present correlations between air constituents of SHS and biomarkers of SHS. PM_{2.5} and CO were significantly correlated with plasma cotinine while air nicotine had moderate but non-significant correlations with urine cotinine, COT+3HC, and NNAL, and poor correlation with plasma cotinine. The non-significant correlations between air nicotine and plasma and urine nicotine metabolites is most likely related to the small number of subjects, the relatively narrow range of exposures, and the large intrinsic inter-individual variability observed in biomarker levels. A larger number of subjects with a wider range of exposures need to be studied to get a better estimate of how biomarkers relate to air exposure levels.

While it is not known what the precise health risks associated with the biomarker data presented here represent, asthmatic adults with low level outdoor SHS exposure measured using a nicotine badge (average nicotine, 0.03 µg/m³) showed increased risk of respiratory symptoms and additional bronchodilator use³¹. Average air nicotine measured in our study (9.5 µg/m³) was much higher than 0.03 µg/m³, indicating that SHS exposure in cars may reach levels associated with significant health risks, particularly among children with asthma and other respiratory problems. Studies have also shown large immediate cardiovascular effects of acute low level SHS exposure among adults^{3,32}.

Our study provides benchmark data for assessing biomarkers of exposure to SHS. We find that a plasma sample for cotinine concentration obtained at 4–5 hours or a urine sample for cotinine, COT+3HC and NNAL obtained within 4 to 8 hours after exposure demonstrated the maximum concentration for most participants. Willers and colleagues had previously concluded that the time of sampling is not very critical for urine cotinine¹⁴. While this may be true for high SHS exposure conditions, such as in Willers and colleagues' study, exposure to lower-level SHS can be underestimated based on time of sampling. Hence, the optimal sampling times presented here are valuable to obtaining more accurate estimates of systemic exposure and health risk assessments.

In addition to the small sample size, our study's generalizability is limited because we studied SHS exposure in a stationary vehicle. The ventilation in our vehicle ranged from an average of 7.5 ACH in the front and 1.9 ACH in the back. In contrast, in a vehicle driving at 20 miles per hour with windows closed the ACH is 13. Nonetheless, the air concentrations of PM_{2.5}, CO, and nicotine that we measured are consistent with other studies of PM_{2.5}, CO, and air nicotine after a cigarette is smoked and at various ventilation conditions^{8,33–35}. Air concentrations of SHS constituents in vehicles are lower at higher ventilation rates, which are influenced by window configurations and vehicle driving speed.

In conclusion, we show that brief exposure to secondhand smoke in a motor vehicle results in substantial increases in plasma cotinine and urine cotinine, COT+3HC and NNAL in nonsmokers. Such measurements are best done within 4 to 8 hours after exposure, when peak levels are observed. These measurements can be used in field studies to document exposures and to conduct risk assessments that can be used to strengthen public health policies directed at protecting nonsmokers, especially children, from the harmful effects of SHS exposure.

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WHAT THIS PAPER ADDS

The paper provides the first description of levels and time course of biomarkers of tobacco toxicant exposure in nonsmokers after a well-characterized brief exposure to secondhand smoke in an automobile. These data will be useful for field studies of exposure and associated risk from SHS exposure that can be used to strengthen public health policies to protect nonsmokers.

FIGURE 1.

Time course of plasma cotinine among 8 nonsmokers exposed to 1 hour of secondhand smoke in a car. Time 0 h represents end of SHS exposure and the beginning of post-exposure monitoring.

FIGURE 2.

Measurement of cotinine (ng/mg creatinine) (A); molar sum of cotinine and 3-hydroxycotinine (COT+3HC) (nmol/mg creatinine) (B); and 4-(methylnitrosoamino)-(3-pyridyl)1-butanol (NNAL) (pg/mg creatinine) (C) in urine at baseline (BL) before exposure, and in four sampling intervals over 24 h after exposure.

TABLE 1

Constituents of secondhand smoke in air and air changes per hour in stationary cars

Subjects	1-h Time-integrated averages for SHS constituents ^a			
	Nicotine ($\mu\text{g}/\text{m}^3$)	PM _{2.5} ($\mu\text{g}/\text{m}^3$)	Carbon monoxide (ppm)	Air exchanges per hour (h^{-1})
1	4.8	806	2.7	1.4
2	12.2	656	1.2	1.6
3	8.0	1027	1.6	3.2
4	5.1	n/a	0.4	n/a
5	9.1	n/a	1.2	1.8
6	9.7	1090	1.7	2.4
7	21.4	513	1.1	1.8
8	6.0	384	0.9	1.3
Average $\pm$ SD	9.5 $\pm$ 5.4	746 $\pm$ 281	1.4 $\pm$ 0.7	1.9 $\pm$ 0.7

Notes:

^aValues presented are 1-hr time integrated averages corrected for background ambient concentrations. Air exchanges per hour are for backseat measurements.

n/a = not available

TABLE 2

Plasma cotinine, urine cotinine plus 3-hydroxycotinine and urine total NNAL at baseline (BL), maximum concentration (C_{max}), and maximum change (Δ_{max}) following 1-hr exposure to secondhand smoke (SHS) in a vehicle

Subject	Plasma cotinine (ng/mL)			Urine cotinine (ng/mL)			Urine COT+3HC (nmol/mg creat)			Urine total NNAL (pg/mg creat)		
	BL	C_{max}	Δ_{max}	BL	C_{max}	Δ_{max}	BL	C_{max}	Δ_{max}	BL	C_{max}	Δ_{max}
1	0	0.17	0.17	0.09	1.11	1.02	0.002	0.016	0.014	0	1.27	1.27
2	0.07	0.20	0.13	0.19	2.52	2.32	0.003	0.015	0.012	0	2.62	2.62
3	0.06	0.21	0.15	0.88	3.69	2.80	0.016	0.039	0.023	0	4.05	4.05
4	0.05	0.13	0.08	0.21	1.04	0.83	0.004	0.014	0.010	0	1.20	1.20
5	0.06	0.12	0.06	0.21	0.90	0.63	0.002	0.010	0.008	0	4.92	4.92
6	0	0.28	0.28	0.41	2.92	2.51	0.007	0.032	0.024	0.48	3.51	3.03
7	0.06	0.16	0.10	0.52	6.00	5.48	0.008	0.068	0.060	0	2.23	2.23
8	0.04	0.12	0.07	0.48	1.10	0.61	0.006	0.010	0.004	0.32	1.65	1.33
Average	0.04	0.17	0.13	0.38	2.41	2.03	0.006	0.025	0.019	0.10	2.68	2.58
SD	0.03	0.06	0.07	0.25	1.79	1.66	0.005	0.020	0.018	0.19	1.36	1.37

Notes: COT = cotinine; 3HC = 3-hydroxycotinine; NNAL = 4-(methylnitrosoamino)-(3-pyridyl)1-butanol; creat = creatinine

TABLE 3

Spearman correlation coefficients (r_s) between environmental markers and biomarkers of SHS (presented as r_s , p-value)

	PM _{2.5}	Air NIC	CO	Plasma COT ^a	Urine COT ^a	Urine COT+3HC ^a	Urine NNAL ^a
PM _{2.5}	1.00	-0.09 (0.9)	0.77 (0.07)	0.94 (0.005)	0.31 (0.5)	0.43 (0.4)	0.60 (0.2)
Air NIC		1.00	-0.07 (0.9)	0.02 (0.9)	0.55 (0.1)	0.48 (0.2)	0.52 (0.2)
CO			1.00	0.76 (0.028)	0.39 (0.4)	0.45 (0.3)	0.36 (0.4)
Plasma COT				1.00	0.67 (0.07)	0.74 (0.037)	0 (1.0)
Urine COT					1.00	0.95 (<.001)	0.24 (0.6)
Urine COT+3HC						1.00	0.14 (0.7)
Urine NNAL							1.00

Notes: PM_{2.5} = particulate matter 2.5 μ m; NIC = nicotine; CO = carbon monoxide; COT = cotinine; 3HC = 3-hydroxycotinine; NNAL = 4-(methylnitrosoamino)-(3-pyridyl)-1-butanol;

^a maximum concentration minus baseline concentration