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Original Investigation

Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers

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Abstract

Introduction: There are about 4,800 different chemical constituents in cigarette smoke. Therefore, the total systemic exposure evaluation of the population of smokers to cigarette smoke is challenging. Measurement of biomarkers as surrogates of cigarette smoke constituents is a realistic approach to assess exposure.

Objective: To estimate cigarette smoke exposure of the U.S. smoker population.

Methods: Stratified, cross-sectional, multicenter design (39 sites in 31 states); 3,585 adult cigarette smokers and 1,077 nonsmokers. Biomarkers were determined from 24-hr urine collections or blood samples. Population estimates were generated by weighting sample data with weights from a large U.S. probability sample (Behavioral Risk Factor Surveillance System).

Results: The adult smoker population estimates for tobacco-specific biomarkers were nicotine equivalents 13.3 mg/24 hr (*SE* 0.14), serum cotinine 184 ng/ml (1.8), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol 439 ng/24 hr (5.5). The population estimates for smokers and nonsmokers for nontobacco-specific biomarkers were 1-hydroxypyrene 317 (6.8) and 110 (7.1) ng/24 hr, 4-aminobiphenyl Hb adducts 43.1 (1.04) and 11.4 (1.5) pg/g Hb, carboxyhemoglobin 5.26(0.04) in percent of hemoglobin saturation and 1.45(0.02), 3-hydroxypropylmercapturic acid 2,030 (24) and 458 (17) μg/24 hr, monohydroxy-butenyl-mercapturic acid 3.61 (0.1) and 0.30 (0.02) μg/24 hr, and dihydroxy-butyl-mercapturic acid 556 (4.9) and 391 (5.5) μg/24 hr. On average, young adult smokers had lower exposure than older smokers; female smokers had lower exposure than males, and Black smokers had lower exposure than Whites.

Discussion: This study estimated the population exposure to cigarette smoke constituents in adult U.S. smokers and identified significant differences between subpopulations. The data may serve as a reference for monitoring the impact of changes in cigarette consumption and the introduction of potentially reduced exposure cigarettes.

Introduction

The U.S. cigarette market shows a large number of cigarette brands with different designs and a wide range of tar levels. The exposure to cigarette smoke has not been well characterized in the U.S. adult smoker population. It is estimated that there are more than 4,800 different chemical constituents present in cigarette smoke (Green & Rodgman, 1996); therefore, the total systemic evaluation of human exposure to these chemicals is almost impossible. Few population studies of cigarette smoke exposure in adult smokers have been reported in the peer-reviewed literature (Blackford et al., 2006; Byrd, Davis, Caldwell, Robinson, & DeBethizy, 1998; Caraballo et al., 1998; Jarvis, Boreham, Primatesta, Feyerabend, & Bryant, 2001; Maron & Fortmann, 1987; O'Connor et al., 2006; Rosa et al., 1992; Woodward, Tunstall-Pedoe, Smith, & Tavendale, 1991). Most studies were of small sample size and assessed only one or two biomarkers of cigarette smoke exposure, and self-report of daily cigarette consumption was used as a measure of cigarettes smoked per day. Nearly all studies lacked a control group of nonsmokers, which is particularly important for those smoke constituents that are not tobacco specific.

The purpose of this Total Exposure Study (TES) was to estimate exposure to a range of selected cigarette smoke constituents in a population of U.S. adult smokers; investigate if there

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were differences between subpopulations in relation to age, gender, body mass index (BMI), and race; and establish a population baseline for risk assessment of future potentially reduced exposure cigarette products.

We selected biomarkers for cigarette smoke constituents or metabolites of cigarette smoke constituents that are representative of the particulate phase (PP) and gas/vapor phase (GVP) of cigarette smoke. These smoke constituents were selected primarily based on the toxicological relevance and their usefulness as surrogates for chemical classes of smoke constituents as well as availability of validated analytical methods.

Nicotine

Nicotine is a major tobacco and cigarette smoke constituent. Nicotine and five major metabolites (cotinine and cotinine-*N*-glucuronide, *trans*-3′-hydroxycotinine and *trans*-3′-hydroxycotinine-*O*-glucuronide, and nicotine-*N*-glucuronide) are excreted in urine, and the 24-hr urine excretion of nicotine and these five metabolites reflects ~90% of the daily nicotine uptake (Feng, Kapur et al., 2007; St. Charles, Krautter, Dixon, & Mariner, 2006). The molar sum of nicotine and its five major metabolites is expressed as nicotine equivalents (NE; Roethig, Kinser, Lau, Walk, & Wang, 2005).

Serum Cotinine

Serum cotinine has been widely used in a number of clinical studies (Benowitz, 2001). Serum cotinine has a relatively long half-life (16–18 hr; St. Charles et al., 2006) and is at an apparent steady state in smokers who have a set routine of smoking behaviors. However, intersubject variability in nicotine metabolism related to gender, age, BMI, and race as well as other genetic variants may limit its usefulness as a biomarker of exposure (Benowitz, 2001; Tricker, 2003).

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Several tobacco-specific nitrosamines are found in tobacco and cigarette smoke; of these, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has recently been classified as a Group 1 carcinogen (World Health Organization [WHO], 2007). NNK exposure can be measured by its metabolites 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and NNAL glucuronides, which are excreted in urine (Hecht, 2002; Hecht et al., 1993). NNAL has an elimination half-life of 4 days (Roethig et al., 2007).

Polycyclic aromatic hydrocarbons

There are hundreds of polycyclic aromatic hydrocarbons (PAHs) and their isomers in the PP of cigarette smoke (Pelkonen & Nebert, 1982; Rodgman, Smith, & Perfetti, 2000). In contrast to nicotine and tobacco-specific nitrosamines, exposure to PAHs arises also from food and other sources (Jongeneelen, 1994, 2001; Strickland & Kang, 1999). A number of PAHs have been classified as carcinogens (WHO, 2007). Pyrene, although not carcinogenic, is one of the PAHs found in cigarette smoke and is often used as an indicator of total PAH exposure (Brandt and Watson, 2003; Jongeneelen, 1994). It is primarily (~90%) metabolized to 1-hydroxypyrene (1-OHP) by CYP1A enzymes and is renally eliminated as glucuronide and sulfate conjugates (total 1-OHP) with a half-life of about 20 hr (Jacob et al., 2007; Jongeneelen, 1994; Pelkonen & Nebert, 1982).

Aromatic amines

Aromatic amines are a group of compounds found in the PP of cigarette smoke that have been associated with bladder cancer (Bartsch, Malaveille, Friesen, Kadlubar, & Vineis, 1993; Vineis & Martone, 1996). Of the numerous aromatic amines in cigarette smoke, 4-aminobiphenyl (4-ABP) has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen (WHO, 2007). Human exposure to 4-ABP occurs from cigarette smoke (Bryant, Skipper, Tannenbaum, & Maclure, 1987) as well as other sources, including dietary and environmental exposure (Hammond et al. 1993; Sarkar et al. 2006; Ward et al. 1996). 4-ABP exposure can be measured by 4-ABP hemoglobin (Hb) adducts (Sarkar et al.), which correlate with 4-ABP–DNA adduct levels (Dallinga et al. 1998).

Carbon monoxide

Carbon monoxide (CO) is a combustion product found in the gas phase of cigarette smoke (Green & Rodgman, 1996). CO exposure can most accurately be measured as carboxyhemoglobin in percent of hemoglobin saturation (COHb %sat.; Benowitz, Jacob, Kozlowski, & Yu, 1986; Roethig et al., 2007).

Acrolein

Acrolein is one of the most abundant aldehydes in the GVP of cigarette smoke (Newsome, Norman, & Keith, 1965). Acrolein has been shown to cause DNA damage (Feng et al., 2006) and to interfere with the repair response of bronchial epithelial cells (Wang et al., 2001). In addition to cigarette smoke exposure, there is also exposure from environmental sources, and acrolein is also formed endogenously as a product of lipid peroxidation. Acrolein appears to be involved in the atherosclerotic lesions of human vasculature (Shao et al., 2005; Tamamizu-Kato et al., 2007). Urinary excretion of 3-hydroxypropylmercapturic acid (3-HPMA), a metabolite of acrolein, has been shown to be a biomarker of acrolein exposure (Mascher, Mascher, Scherer, & Schmid, 2001; Roethig et al., 2007).

1,3-Butadiene

Exposure to the gaseous olefin 1,3-butadiene (BD), a major industrial chemical that has been classified as a Group 2A carcinogen (WHO, 2007), can be occupational (Fajen, Lunsford, & Roberts, 1993) or through automobile emissions (Pelz, Dempster, & Shore, 1990) or cigarette smoke (Brunnemann, Kagan, Cox, & Hoffmann, 1989). Although BD effects have been extensively studied for occupational exposure, there are limited data on BD exposure in cigarette smokers. Biomonitoring in occupationally exposed humans has been carried out by measuring either protein and DNA adducts of BD or urinary excretion of mercapturic acid metabolites dihydroxy-butyl-mercapturic acid (DHBMA) and monohydroxy-butenyl-mercapturic acid (MHBMA; Albertini et al., 2001; Boogaard, Van Sittert, & Megens, 2001; Swenberg et al., 2001).

Methods

Study design, subjects, and study conduct

This study employed a cross-sectional, observational, multicenter ambulatory design. Adult males and females, 21 years of age and older, who were in generally good health were enrolled from 31 states (39 investigative sites across the United States) into one of five parallel groups: four tar delivery categories (i.e., ≤2.9, 3.0–6.9, 7.0–12.9, and ≥13 mg tar) and a nonsmoking group. Sampling of each of these five groups was stratified by gender, age (21–34, 35–49, and ≥50 years), and BMI (<25 and ≥25 kg/m^2), resulting in 12 stratification cells for each of the five groups.

Smoking status was defined as regular cigarette consumption of a minimum of one manufactured cigarette per day over the last 12-month period. Eligible subjects did not use a different brand of cigarette outside their regular brand's tar delivery category for more than 10% of their daily cigarette consumption nor used any nicotine-containing products other than the manufactured cigarettes during the 3 months prior to the study. Nonsmoking status was defined as no use of tobacco or nicotine-containing products during the 5 years prior to the study. Pregnant or nursing women were excluded.

The study was approved by the Human Institutional Review Board (IRB) for each site and conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. Volunteers were recruited using IRB-approved advertising that solicited "adult men and women who smoke at least 1 cigarette a day" or "who do not use tobacco products." Interested adults gave written informed consent and were paid for their participation. A call center prequalified interested volunteers and referred each to an investigative site.

At Visit 1, medical history and concomitant medications were recorded, and a urine pregnancy test was performed for females. Each participant then collected—in his or her normal life setting—a 24-hr urine sample which was kept in a cooler with a refrigerant gel pack until returned to the site at Visit 2. Visit 2 occurred within 3 days after Visit 1, between 7 a.m. and 3 p.m. A blood sample was taken for the COHb and serum cotinine. A check for the completeness of the 24-hr urine collections was performed using 24-hr creatinine excretion. Each adult smoker also collected the butts of all cigarettes smoked during the urine collection interval as a measure for cigarettes smoked.

Biomarker sampling and analytical methods

The total volume of urine was measured and recorded prior to removal of any aliquots. Aliquots were removed and stored frozen at −20 °C until analysis. All urinary biomarkers were measured by liquid chromatography/tandem mass spectrometry methods validated according to the U.S. Food and Drug Administration Guidance for Industry (U.S. DHSS FDA, 2001) as described previously (Roethig et al., 2005, 2007). COHb and serum cotinine were measured spectrophotometrically and by enzyme immunoassay, respectively (Roethig et al., 2005). Adducts of 4-ABP Hb were measured as described previously (Sarkar et al., 2006). MHBMA and DHBMA methods are described below. A summary of the bioanalytical methods, lower limits of quantification, and precision is presented in Table 1.

Analysis of MHBMA in urine. After the addition of the internal standard (d_6-MHBMA), each urine sample (0.5 ml) was acidified before separation on an Oasis HLB solid phase extraction cartridge. The samples were eluted with 5% ammonium hydroxide in acetone before evaporation. The samples were reconstituted with acetonitrile and then injected onto a Thermo

Table 1. Biomarkers and bionalytical methods

Biomarker	Smoke constituent	Smoke phase	Matrix	Method	Lower limit of quantitation	Precision CV (%)
Nicotine equivalents	Nicotine	PP	Urine			
Nicotine[a]			Urine	LC-MS/MS	1 ng/ml	3.3–10.8
Cotinine[a]			Urine	LC-MS/MS	1 ng/ml	2.3–10.9
trans-3′-Hydroxycotinine[a]			Urine	LC-MS/MS	1 ng/ml	2.7–11.7
Cotinine[b]	Nicotine	PP	Serum	Immunoassay	10 ng/ml	6.3–17.5
Total NNAL[c]	NNK	PP	Urine	LC-MS/MS	5.0 pg/ml	2.1–3.3
Total 1-OHP[d]	Pyrene	PP	Urine	LC-MS/MS	10 pg/ml	6.9–11.0
3-HPMA[d]	Acrolein	GVP	Urine	LC-MS/MS	35 ng/ml	5.3–7.5
4-ABP Hb adducts[a]	4-ABP	PP	Blood	GC-MS	0.5 pg/g Hb	4.3–11.7
Carboxyhemoglobin[b]	Carbon monoxide	GVP	Blood	Spectrophotometry	0.30%	0.4–4.7
MHBMA[c]	1,3-butadiene	GVP	Urine	LC-MS/MS	0.100 ng/ml	1.8–5.5
DHBMA[c]	1,3-butadiene	GVP	Urine	LC-MS/MS	10.1 ng/ml	3.0–4.9

Note. CV = coefficient of variation; PP = particulate phase; GVP = gas/vapor phase; LC-MS/MS = liquid chromatography/tandem mass spectrometry; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; 3-HPMA = 3-hydroxypropylmercapturic acid; 1-OHP = 1-hydroxypyrene; 4-ABP = 4-aminobiphenyl; DHBMA = dihydroxy-butyl-mercapturic acid; and MHBMA = monohydroxy-butenyl-mercapturic acid.

[a]Samples were analyzed at Covance, Harrogate, Covance Laboratories Limited, Harrogate, North Yorkshire, England.

[b]Samples were analyzed at Covance Central Laboratory Services, Indianapolis, IN.

[c]Samples were analyzed at MDS Pharma Services, Lincoln, NE.

[d]Samples were analyzed at MDS Pharma Services, Saint-Laurent, Montreal (Quebec), Canada.

Hypersil-Keystone BioBasic AX column (150 × 4.6 mm, 5 μ) with a guard column and eluted with 60:40 acetonitrile:50 mM ammonium acetate, pH 3.5 mobile phase into an AB/MDS Sciex API 4000, using electrospray ionization (ESI). The flow rate was 1.0 ml/min. Negative ions were monitored in the multiple reaction monitoring (MRM) mode, and the following ion transitions were monitored: *m/z*: 232.0 → 103.2 for MHBMA and 238.0 → 109.1 for d_6-MHBMA.

Analysis of DHBMA in urine. After the addition of the internal standard (d_7-DHBMA), each urine sample (0.2 ml) was diluted threefold before being injected onto a Thermo Hypersil-Keystone BioBasic AX column (50 × 4.6 mm, 5 μ) with a guard column and eluted with 90:10 acetonitrile:50 mM ammonium acetate, pH 3.5 mobile phase into an AB/MDS Sciex API 4000, using ESI. The flow rate was 1.5 ml/min. Negative ions were monitored in the MRM mode, and the following ion transitions were monitored: *m/z*: 250.4 → 121.2 for DHBMA and 257.0 → 128.1 for d_7-DHBMA.

Statistical methods

The TES survey was stratified by age (three categories), gender (two categories), and BMI (two categories) for both smoking and nonsmoking populations and by tar delivery (four categories) for smoking population only. In the absence of reliable population proportions by tar delivery for the smoker population, we used age, gender, BMI, and smoker status (smoker and nonsmoker) as cross-classification variables to form weighting adjustment cells. The population proportions from the Behavioral Risk Factor Surveillance System (BRFSS), a national heath survey system (Center for Disease Control and Prevention [CDC], 2001a, 2001b), were used to obtain weighting adjustments for the cells. To ensure correct variance estimation of the population, the survey design features of TES were taken into account, and the study was treated as a stratified simple random design. The estimate of variance was the aggregation of the variance estimates of the strata. Weighted sample mean and sample *SE* were calculated based on the approaches described in Lohr (1999).

A Wilcoxon rank-sum test was used to test for differences in biomarkers of exposure between smokers and nonsmokers and between gender, BMI, and race groups. A Kruskal–Wallis test was used to test for differences between age groups. Linear regression analysis was used to examine the relationship between NE (independent variable) and each of the other biomarkers of exposure (dependent variable).

Urine collections with 24-hr creatinine excretion less than the 75th percentile of the lower limit of the gender-specific reference range (<750 mg for males or <500 mg for females) were considered incomplete and excluded from the statistical analyses of 24-hr biomarker excretion. Values below the lower limit of quantification were replaced by half the lower limit of quantification.

Results

Subjects' demographics and cigarette consumption

A total of 4,706 subjects were enrolled between August 2002 and October 2003 (Table 2). A total of 4,662 subjects were evaluable, 3,585 adult smokers and 1,077 adult nonsmokers. A total of 44 subjects were nonevaluable due to missing information. A total of 57.4% of the adult smokers and 59.3% of the nonsmokers were females. The mean age was 41.7 years for smokers and 43.3 years for nonsmokers. Smokers had an average BMI of 27.8 kg/m^2 compared with 28.11 kg/m^2 in nonsmokers. A total of 76% of the smokers and 79% of the nonsmokers were Whites; Blacks comprised 17% of the smokers and 14% of the nonsmokers. In total, 30% of the smokers had an annual household income of <$20,000 compared with 16% of nonsmokers; 37% of smokers and 54% of nonsmokers had an annual household income of $40,000 or more. About 91% of adult smokers and 96% of nonsmokers had high school or higher education.

Smoking history characteristics for adult smokers are presented in Table 2. On average, adult smokers had smoked on a regular basis for 22 years. Fewer than 6% of all smokers had smoked for 1–4 years, and 11% had smoked for 40 or more years.

Daily cigarette consumption for adult smokers ranged from 1 to 84 with a mean population estimate of 16.0 (Table 3). Males smoked about 0.6 cigarettes per day more than females, and Blacks smoked 6 cigarettes less per day than Whites (Table 4). Daily consumption was lowest in younger smokers, 21–34 years old, and highest in the 50 years and older smokers.

Population estimates for biomarkers of exposure for adult smokers and nonsmokers

The population estimates for tobacco-specific biomarkers, that is, NE, serum cotinine, and total NNAL, were 13.3 mg/24 hr (*SE* 0.14), 184 ng/ml (*SE* 1.8), and 439 ng/24 hr (*SE* 5.5) for adult smokers (Table 3). The population estimates for NE, serum cotinine, and total NNAL for the nonsmokers were not calculated, as the majority (78%–98%) of values was below the lower limit of quantification. There was a large variability in the tobacco-specific biomarkers of exposure in adult smokers. The coefficient of variation was 60% for NE, 58% for serum cotinine, and 71% for total NNAL. Population estimates of nontobacco-specific biomarkers were significantly higher in adult smokers than in nonsmokers: 1-OHP 2.9 fold, 4-ABP Hb adducts 3.8 fold, COHb 3.6 fold, 3-HPMA 4.4 fold, MHBMA 12 fold, and DHBMA 1.4 fold. The coefficients of variation for the nontobacco-specific biomarkers were high: 4-ABP Hb adducts 80%, 1-OHP 120%, 3-HPMA 67%, MHBMA 95%, and DHBMA 50%.

Population estimates for biomarkers of exposure by age, gender, BMI, and race for adult smokers

The excretion of NE per day in general followed the number of cigarettes smoked per day (Table 4). NE excretion per day, total NNAL per day, 4-ABP Hb adducts, 3-HPMA, MHBMA, and DHBMA were lowest in the youngest age group and highest in the middle age group, while NE per cigarette, total NNAL per cigarette, and total 1-OHP were highest in the middle age group and lowest in the oldest age group. Serum cotinine and COHb were lowest in the youngest age group and highest in the oldest age group. NE per day, NE per cigarette, total NNAL, total NNAL per cigarette, serum cotinine, total 1-OHP, 3-HPMA,

Table 2. Demographics and cigarette consumption

Characteristics	Smokers (n = 3,585)	Nonsmokers (n = 1,077)
Gender, no. (%)		
Female	2,059 (57.4)	639 (59.3)
Male	1,526 (42.6)	438 (40.7)
Age (years)		
M (*SD*)	41.7 (12.71)	43.3 (14.70)
Range (*min, max*)	(*21, 80*)	(*21, 88*)
BMI (kg/m^2)		
M (*SD*)	27.80 (6.69)	28.11 (6.54)
Range (*min, max*)	(*15.0, 70.0*)	(*14.9, 60.2*)
Race, no. (%)		
White	2,727 (76.1)	851 (79.0)
Black	614 (17.1)	151 (14.0)
Other	218 (6.1)	67 (6.2)
n missing	26	8
Annual household income, no. (%)		
<$20,000	1,065 (29.9)	169 (15.8)
$20,000–$39,999	1,193 (33.5)	323 (30.2)
$40,000–$49,999	456 (12.8)	152 (14.2)
$50,000–$74,999	532 (14.9)	234 (21.9)
≥$75,000	316 (8.9)	190 (17.8)
n missing	23	9
Education, no. (%)		
<HS graduate	312 (8.8)	44 (4.1)
HS graduate or some college	2,664 (75.0)	628 (58.9)
College graduate	574 (16.2)	395 (37.0)
n missing	35	10
No. of years smoked, *M* (*SD*) and category, no. (%)		
M (*SD*)	21.9 (12.8)	NA
1–4 years	208 (5.9)	NA
5–9 years	485 (13.8)	NA
10–19 years	838 (23.8)	NA
20–29 years	889 (25.2)	NA
30–39 years	707 (20.1)	NA
≥40 years	398 (11.3)	NA
n missing	60	NA
No. of butts returned per day, *M* (*SD*) and category, no. (%)		
M (*SD*)	16.0 (8.9)	NA
Range (*min, max*)	(*1, 84*)	NA
1–10 butts	1,070 (29.8)	NA
11–20 butts	1,608 (44.9)	NA
21–30 butts	670 (18.7)	NA
>30 butts	231 (6.4)	NA
n missing	6	NA

Note. NA = not applicable; HS = high school.

MHBMA, and DHBMA were statistically significantly higher in males than females. NE per day, total NNAL per day, 4-ABP Hb adducts, COHb, 3-HPMA, MHBMA, and DHBMA were statistically significantly higher in Whites than in Blacks. NE per cigarette, total NNAL per cigarette, and serum cotinine were statistically significantly higher in Blacks than in Whites. Serum cotinine, 4-ABP Hb adducts, and COHb were higher with lower BMI. Total NNAL, MHBMA, and DHBMA were higher with higher BMI.

Table 3. Population estimates for smokers and nonsmokers

Biomarker	Population estimates[a] Smokers	Nonsmokers
Number of butts returned/24 hr	16.0 (0.2)	NA
NE (mg/24 hr)	13.3 (0.1)	NA
NE (mg/cig)	0.93 (0.01)	NA
Serum cotinine (ng/ml)	184 (1.8)	NA
Total NNAL (ng/24 hr)	439 (5.5)	NA
Total NNAL (ng/cig)	30.2 (0.4)	NA
4-ABP Hb adducts (pg/g Hb)	43.1 (1.04)	11.4 (1.5)
Total 1-OHP (ng/24 hr)	317 (6.8)	110 (7.1)
COHb (%)	5.26 (0.04)	1.45 (0.02)
3-HPMA (μg/24 hr)	2,030 (24)	458 (17)
MHBMA (μg/24 hr)	3.61 (0.1)	0.30 (0.02)
DHBMA (μg/24 hr)	556 (4.9)	391 (5.5)

Note. NA = not applicable; NE = nicotine equivalent; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 4-ABP Hb= 4-aminobiphenyl hemoglobin; 1-OHP = 1-hydroxypyrene; COHb = carboxyhemoglobin; 3-HPMA = 3-hydroxypropylmercapturic acid; DHBMA = dihydroxy-butyl-mercapturic acid; and MHBMA = monohydroxy-butenyl-mercapturic acid.

[a]Weighted mean (*SE*).

Coefficient of determination (R^2) for NE and biomarkers of exposure for adult smokers

Regression analysis between NE and other biomarkers of exposure showed a strong linear relationship of NE with total NNAL (R^2 = .5) and 3-HPMA (R^2 = .48); a moderate correlation with COHb (R^2 = .38), serum cotinine (R^2 = .3), DHBMA (R^2 = .23), and MHBMA (R^2 = .21); and a poor correlation with total 1-OHP (R^2 = .06) and 4-ABP Hb adducts (R^2 = .04).

Discussion

This is the first study in a large sample of the adult American smoker population of any manufactured cigarette in the U.S. retail market, in which the population exposure to several cigarette smoke constituents of the PP and GVP was estimated at the same time. With a goal of representing the adult smoker population, participants from 31 states in the United States were enrolled in the TES with stratification to balance gender, age, and BMI and by applying population weights from a nationally representative survey (BRFSS) to these stratification groups. The BRFSS is the world's largest ongoing health survey system, tracking smoking status of the U.S. population by gender, age, and BMI. The weights from BRFSS have been found to be valid and have been widely used for population estimation (Nelson, Holtzman, Bolen, Stanwyck, & Mack, 2001). Although the sampling in this study was not random, the weighting of this study sample by use of a large probability sample made the data as representative as possible for the U.S. population. As only subjects in general good health were recruited, the data are thus limited to the overtly healthy U.S. population. Another limitation is the potential confounding of having selected

Table 4. Population estimates (*M* [*SE*]) of numbers of cigarettes per day and biomarkers of exposure for smokers by age, gender, BMI, and race and correlation with NE (R^2)

	Age (years)	Gender	BMI	Race	R^2
	21–34	Males	<25 kg/m^2	Whites	
	35–49	Females	≥25 kg/m^2	Blacks	
	≥50				
Number of cigarettes per day	13.1 (0.4)**	16.4 (0.5)^{ns}	15.6 (0.4)^{ns}	17.4 (0.3)**	
	16.7 (0.5)	15.8 (0.4)	16.3 (0.4)	11.3 (0.5)	
	18.3 (0.6)				
Biomarkers					
NE (mg/24 hr)	11.3 (0.2)**	14.3 (0.2)**	13.0 (0.2)^{ns}	14.1 (0.2)**	NA
	14.3 (0.2)	12.4 (0.2)	13.5 (0.2)	10.8 (0.3)	
	13.7 (0.2)				
NE (mg/cig)	0.924 (0.02)*	0.995 (0.02)**	0.907 (0.02)^{ns}	0.892 (0.01)**	NA
	0.962 (0.02)	0.865 (0.01)	0.937 (0.02)	1.1 (0.035)	
	0.858 (0.02)				
Serum cotinine (ng/ml)	163 (3.3)**	193 (2.9)**	195 (2.9)**	184 (2.0)*	.3
	193 (2.9)	177 (2.3)	178 (2.4)	196 (5.0)	
	195 (3.3)				
Total NNAL (ng/24 hr)	360 (9.0)**	467 (9.1)**	408 (7.8)*	465 (6.5)**	.5
	481 (9.2)	413 (6.5)	457 (7.4)	343 (10.4)	
	453 (9.2)				
Total NNAL (ng/cig)	29.0 (0.8)**	31.8 (0.7)**	28.3 (0.6)*	29.2 (0.5)*	NA
	32.2 (0.7)	28.8 (0.5)	31.3 (0.6)	34.1 (1.2)	
	28.0 (0.7)				
4-ABP Hb adducts (pg/g Hb)	36.4 (1.2)**	43.0 (0.9)**	48.8 (2.5)**	45.2 (1.3)**	.04
	46.6 (2.0)	43.2 (1.8)	39.7 (0.8)	38.4 (1.2)	
	45.0 (1.6)				
Total 1-OHP (ng/24 hr)	295 (11.6)**	361 (12.1)**	312 (11.0)^{ns}	316 (7.6)^{ns}	.06
	352 (11.0)	278 (7.2)	320 (8.7)	342 (20.1)	
	275 (12.2)				
COHb (%)	4.58 (0.1)**	5.19 (0.1)^{ns}	5.42 (0.1)*	5.43 (0.0)**	.38
	5.50 (0.1)	5.33 (0.1)	5.16 (0.1)	4.74 (0.1)	
	5.62 (0.1)				
3-HPMA (μg/24 hr)	1,662 (37.2)**	2,276 (40.7)**	1,928 (35.1)^{ns}	2,132 (28.2)**	.48
	2,208 (39.0)	1,811 (26.6)	2,090 (31.6)	1,690 (46.7)	
	2,133 (44.7)				
MHBMA (μg/24 hr)	3.10 (0.1)**	4.00 (0.1)**	3.28 (0.1)*	3.86 (0.1)**	.21
	3.93 (0.1)	3.26 (0.1)	3.80 (0.1)	2.72 (0.1)	
	3.62 (0.1)				
DHBMA (μg/24 hr)	523 (7.3)**	634 (7.8)**	497 (6.2)**	566 (5.7)**	.23
	582 (8.6)	487 (6.0)	591 (6.8)	524 (11.2)	
	547 (7.3)				

Note. The estimates were weighted based on population weights from Behavioral Risk Factor Surveillance System. BMI = body mass index; *ns* = not statistically significant; NA = not applicable; NE = nicotine equivalent; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 4-ABP = 4-aminobiphenyl hemoglobin; 1-OHP = 1-hydroxypyrene; COHb = carboxyhemoglobin; 3-HPMA = 3-hydroxypropylmercapturic acid; DHBMA = dihydroxy-butyl-mercapturic acid; and MHBMA = monohydroxy-butenyl-mercapturic acid.

*Statistically significant difference between the demographic groups at $p < .05$ level.

**Statistically significant difference between the demographic groups at $p < .0001$ level.

smokers based on the tar yield that they typically smoked. Adult smokers had to collect cigarette butts from all cigarettes smoked, which is probably more reliable than self-reported numbers of daily cigarette consumption, since self-report of cigarette consumption has significant errors (Lawrence, Aveyard, & Croghan, 2003; Lewis et al., 2003; Murray, Connett, Lauger, & Voelker, 1993). In this study, 24-hr urine was collected using creatinine excretion to exclude samples, which were most likely not complete. Urine and butt collections were done in the subjects' normal life setting and should not have impacted the results, in particular smoking behavior. A large group of nonsmokers was recruited for comparison to allow differentiation between exposures from cigarette smoke and from other sources.

On average, a smoker's intake of nicotine from cigarettes per day was about 15 mg, based on NE reflecting about 90% of the nicotine dose absorbed (Feng et al., 2007; St. Charles et al., 2006). The nicotine dose per day was 15% higher for men than for women. The youngest age group smoked fewer cigarettes and had 16% lower exposure to nicotine. Blacks smoked fewer cigarettes than Whites, resulting in a lower daily exposure to nicotine, though they appeared to smoke the cigarettes more intensely, as indicated by higher NE/cig and higher total NNAL/cig.

Serum cotinine followed nicotine exposure pattern concerning age and gender but was higher with lower BMI despite nominally lower daily nicotine exposure. Higher BMI may be associated with higher volume of distribution or higher enzymatic capacity. Effects of obesity on cytochrome P-450 enzymes have been found (Kotlyar & Carson, 1999). Serum cotinine was higher in Blacks than in Whites despite lower nicotine exposure in Blacks. Higher serum cotinine values for Black smokers were found by others, too (Caraballo et al., 1998; Wagenknecht et al., 1990). The majority of nicotine is metabolized by the enzyme cytochrome P-450 2A6 (CYP 2A6) to cotinine and then by the same enzyme to *trans*-3′-hydroxycotinine, the latter being the rate-limiting step (Dempsey et al., 2004). Black race seems to be associated with lower enzymatic capacity for nicotine metabolism. Several genetic variants of CYP 2A6 with lower enzymatic activity have been identified in Blacks (Mwenifumbo et al., 2008).

NNAL followed the pattern of nicotine exposure, in line with being a tobacco-specific biomarker, and showed the strongest correlation with nicotine. It is not surprising that the coefficient of variation was higher for NNAL than for NE, as cigarettes differ in the nitrosamine content depending on the tobacco blend (Counts, Hsu, & Tewes, 2006).

4-ABP followed the exposure pattern of nicotine with the exception that values were higher in women and in smokers with lower BMI. This may be explained by a lower amount of hemoglobin/lower volume of distribution in these circumstances. Lower 4-ABP–DNA carcinogen adducts in lymphocytes were found in subjects with higher BMI (Godschalk, Feldker, Borm, Wouters, & Van Schooten, 2002). COHb showed a similar pattern as 4-ABP Hb. This may be explained similarly by a lower amount of hemoglobin/lower volume of distribution for CO in females and with lower BMI. 1-OHP in general followed the nicotine exposure pattern in the subpopulations except that Black smokers had nominally higher 1-OHP values than Whites. This may be due to different nontobacco-related exposure—for example, dietary differences. Even in highly controlled studies, increases in 1-OHP can be found due to dietary exposure (Roethig et al., 2007). A lack of correlation of 1-OHP with NE in smokers has been observed (Murphy et al., 2004). The highest coefficient of variation of all biomarkers was found for 1-OHP (120%) in smokers, indicative of multiple exposure sources other than smoking cigarettes. 3-HPMA, MHBMA, and DHBMA in general followed the nicotine exposure pattern in the subpopulations. The nontobacco-specific biomarkers were generally several fold higher in smokers compared with nonsmokers, indicating that between 10% (MHBMA) and 30% (1-OHP) of the total exposure were from sources other than smoking. The exception was DHBMA, for which 70% of the total exposure seemed to come from other than cigarette smoke exposure.

It is not surprising that NNAL showed the best correlation with nicotine exposure, as both are tobacco-specific biomarkers and both were measured from 24-hr urine. In contrast, serum cotinine, a metabolite of nicotine, had only a moderate correlation with NE. Whereas NE was from a 24-hr period, serum cotinine was measured at one timepoint but at various timepoints during the day for the different smokers, and thus was influenced by the cigarettes smoked before blood sampling. The same is true for COHb, which also showed only a moderate correlation with NE. Surprisingly, 3-HPMA, a biomarker for the gas phase smoke constituent acrolein, showed a good correlation with nicotine exposure comparable to NNAL. Similar correlations were observed in previous studies (Roethig et al., 2006). Acrolein from cigarette smoke is retained in the respiratory tract comparable to nicotine (Baker & Dixon, 2007). The strong correlation with nicotine may also be caused by lipid peroxidation, and in case of cigarette smoking, this may correlate with nicotine exposure.

The Total Exposure Study for the first time has determined exposure to a number of cigarette smoke constituents in the population of adult smokers and identified significant differences between subpopulations. In addition to behavioral factors influencing exposure to cigarette smoke, physiological differences between the subpopulations have to be taken into account. The TES data may be useful as a reference for monitoring the impact of changes in cigarette consumption and the introduction of potentially reduced exposure cigarette products.

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Declaration of Interest

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