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RESEARCH ARTICLE

Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999–2008

Ziad S. Naufal, Kristin M. Marano, Steven J. Kathman, and Cody L. Wilson

R.J. Reynolds Tobacco Company, Winston-Salem, NC

Abstract

Assessment of biomarkers is an appropriate way to estimate exposure to cigarette mainstream smoke and smokeless tobacco (SLT) constituents in tobacco consumers. Using the US National Health and Nutrition Examination Survey (NHANES, 1999–2008), biomarkers of volatile organic compounds, halogenated aromatic hydrocarbons (HAHs), polycyclic aromatic hydrocarbons (PAHs), acrylamide, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and metals were evaluated. In general, biomarker levels in SLT consumers were significantly lower than in smokers (excluding NNK and some HAHs) and were not significantly different compared with nonconsumers (excluding NNK and some PAHs). These results provide useful information for science-based risk assessment and regulation of tobacco products.

Keywords: NHANES, mainstream smoke, snuff, chewing tobacco

Introduction

Estimating the exposure to chemicals in adult cigarette smokers and smokeless tobacco (SLT) consumers is a challenge in the health-risk assessment of tobacco products. The extent of individual consumer exposure to mainstream cigarette smoke (MSS) and SLT constituents depends primarily on the chemical composition of MSS or SLT and consumption behavior (e.g., amount consumed, frequency, and duration of consumption). Exposure estimates are further complicated by the concentration range of individual chemical constituents in SLT and MSS, which vary by product type and large inter- and intraindividual variability in consumption patterns.

Broadly, human biomonitoring is the measurement of specific chemicals in human cells, tissues, and body fluids (i.e., biomarkers) to assess chemical exposure and potential biological effect (Scherer, 2005). Exposure biomarkers are considered the "gold standard" for exposure assessment, as they provide direct evidence that both exposure and uptake into the body have occurred (Sexton, 2006). In the case of SLT and MSS exposure, exposure biomarkers are typically measured in urine, although blood, saliva, and breath samples have previously been used to measure exposure to some chemicals (IARC, 2004).

Biomarkers can potentially inform all phases of the human health-risk assessment process. In particular, exposure biomarkers play an important role in informing and/or validating the exposure assessment, generally considered the risk assessment phase with the greatest epistemic uncertainty (Santamaria et al., 2006). Specifically, using quantified, direct measurements of exposure and corporeal uptake has the potential to reduce uncertainty associated with the use of some surrogate measures of exposure such as those sometimes used in epidemiological or occupational studies, for example, geographical location, job titles (Santamaria et al., 2006). Data from biological monitoring of human populations, such as those provided by the National Health and Nutrition Examination Survey (NHANES), are useful for estimating the exposure to chemicals of interest in risk assessment.

Address for Correspondence: Ziad Naufal, Product Integrity, Research and Development, R.J. Reynolds Tobacco Company, Bowman Gray Technical Center, 950 Reynolds Blvd, Winston-Salem, NC 27105 USA. E-mail: naufalz@rjrt.com

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More than 8000 compounds have been identified in tobacco or tobacco smoke, and MSS consists of a complex mixture of more than 5300 chemicals in dynamic gaseous and particulate phases (Rodgman and Perfetti, 2009). In recent years, research activities have focused on the exposure of adult tobacco consumers to tobacco and MSS constituents that are known or hypothesized to have toxicological activity. Many of these chemicals have been extensively characterized in environmental matrices such as food, drinking water, and air.

The chemicals evaluated in this report consist of selected compounds identified in SLT and/or MSS for which exposure biomarker data were also available in the NHANES database. These chemicals include acrylamide (AA), volatile organic compounds (VOCs), halogenated aromatic hydrocarbons (HAHs), polycyclic aromatic hydrocarbons (PAHs), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and metals. Among the PAHs included in the NHANES analysis are pyrene, fluorene, phenanthrene, and naphthalene. Data on several other PAHs, including benzo-[*a*]-pyrene, were removed from the NHANES database in July 2009 due to methodological problems (Centers for Disease Control and Prevention (CDC), 2009a). The VOCs included benzene, toluene, ethylbenzene, xylenes, and styrene; HAHs consisted of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs); and metals comprised arsenic (As), beryllium (Be), cadmium (Cd), cobalt (Co), lead (Pb), mercury (Hg), and selenium (Se). A list of the chemicals evaluated in this study with their corresponding exposure biomarker(s) is presented in Table 1. These chemicals were detected in cigarette smoke (Diekmann et al., 2008; Wilson et al., 2008; Rodgman and Perfetti, 2009), and with the exception of the VOCs, HAHs, and mercury, these chemicals were similarly identified in certain SLT products (Hoffmann et al., 1987; Maier et al., 1989; Pérez and Osterman-Golkar, 2003; IARC, 2007; Stepanov et al., 2010).

Whether these exposure biomarkers are associated with tobacco consumption was investigated using serum cotinine as a surrogate for tobacco exposure. Cotinine measured in serum is the only metabolite of nicotine available in the NHANES database and has been reported to be correlated with the number of cigarettes smoked per day or the number of cans/pouches used per week for moist snuff and chewing tobacco (Ebbert et al., 2004).

The objective of this study was to compare the levels of exposure biomarkers of the chemicals of interest, between cigarette smokers ("smokers"), snuff or chewing tobacco consumers ("SLT consumers"), and nonconsumers of tobacco or nicotine replacement therapy (NRT) products ("nonconsumers"), using 10 years of NHANES data. In addition, the correlation between these biomarkers of exposure and cotinine concentrations in serum from smokers and SLT consumers was investigated.

Methods

Data collected in the NHANES Mobile Examination Centers (MEC) from 1999 to 2008 for individuals 20 years of age or older were used in this analysis. NHANES is conducted by the National Center for Health Statistics (NCHS) of the CDC and is designed to annually assess the health and nutritional status of adults and children in the United States (US). Data are publicly available and are representative of the civilian, noninstitutionalized US population. Detailed survey methodology has been published (CDC, 2010a).

The categories for tobacco consumption (snuff, chewing tobacco, or cigarettes) or nonconsumption were determined by an individual indicating on the MEC questionnaire that a particular tobacco product (category) was consumed (or not consumed) in the last 5 days. Self-reported snuff (from the questionnaire: "such as Skoal, Skoal Bandits, or Copenhagen") and chewing tobacco (from the questionnaire: "such as Redman, Levi Garrett, or Beechnut") consumers were combined into one SLT category due to sample size limitations. Only exclusive consumers ($n=5408$) were included (i.e., individuals reporting the consumption of multiple tobacco products or pipes, cigars, or NRT were excluded [$n=534$]). Self-reported nonconsumers with a serum cotinine value greater than 15 ng/mL (National Cancer Institute (NCI), 1999) were excluded ($n=297$). Individuals were also excluded if data regarding tobacco consumption in the last 5 days was missing or if a response was refused or reported as "do not know" ($n=2011$). The number of subjects available for each chemical varied based on the number of years for which the data are available and the proportion of the total sample for which the chemical was measured. Details are available in the NHANES laboratory documentation (CDC, 2010b).

All statistical methods were performed using the appropriate statistical weights and design parameters (i.e., Masked Variance Unit Pseudo-PSU variable and Masked Variance Unit Pseudo-Stratum variable for variance estimation) provided by NCHS. The weights were adjusted to allow for combining multiple years according to the analytical guidelines provided by the NCHS. The survey procedures available in SAS version v.9.2 (SAS Institute Inc., Cary, NC) and the survey package available for the R software version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses (Lumley, 2010).

For chemicals where the limit of detection (LOD) varied across survey years, the data were adjusted to have an LOD equal to the largest value. All data points below the largest LOD were set equal to the largest LOD divided by the square root of two (CDC, 2009b). Percentage of samples below detection was also based on the largest LOD.

Multiple linear regression models for the natural log transformed biomarker data were used to compare the chemical concentrations measured in smokers, SLT

Table 1. Chemicals evaluated and corresponding exposure biomarkers.

Chemical	Exposure biomarker	Biological matrix
Metals		
Arsenic	Total arsenic	Urine
Beryllium	Beryllium	Urine
Cadmium	Cadmium	Blood and urine
Cobalt	Cobalt	Urine
Lead	Lead	Blood and urine
Mercury	Total mercury	Blood and urine
Selenium	Selenium	Serum
Volatile organic compounds		
Benzene	Benzene	Blood
Ethylbenzene	Ethylbenzene	Blood
Styrene	Styrene	Blood
Toluene	Toluene	Blood
Xylenes	m/p, o-Xylene	Blood
Halogenated aromatic hydrocarbons		
Polychlorinated dibenzo-*p*-dioxins	1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	Serum
	1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	
	1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	
	1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)	
	1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)	
	1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	
	2,3,7,8,-Tetrachlorodibenzo-*p*-dioxin (TCDD)	
Polychlorinated dibenzofurans	1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	Serum
	1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	
	1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	
	1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	
	1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	
	2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	
	1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)	
	1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	
	2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	
	2,3,7,8-Tetrachlorodibenzofuran (TCDF)	
Polycyclic aromatic hydrocarbons		
Pyrene	1-Hydroxypyrene	Urine
Naphthalene	1- and 2-Hydroxynaphthalene	Urine
Phenanthrene	1-, 2-, 3-, and 4-Hydroxyphenanthrene	Urine
Fluorene	2-, 3-, and 9-Hydroxyfluorene	Urine
Other		
Acrylamide	Acrylamide and glycidamide hemoglobin adducts	Blood
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	Urine

consumers, and nonconsumers. The data were log-transformed to better meet the assumptions for modeling (i.e., normality). The regression analysis included adjustments for gender, race/ethnicity, age (five categories), body mass index (BMI, four categories), urinary creatinine (for urinary biomarkers), poverty income ratio (PIR, a ratio of income to the family's appropriate poverty threshold, as determined by the US Census Bureau) (three categories) as an indicator of socioeconomic status, survey year (five categories), and tobacco-consumption category. The categories are defined in Table 2. Race/ethnicity was categorized as non-Hispanic White, non-Hispanic-Black, Hispanic, and Other. The Hispanic category included those classified in NHANES as Mexican American and Other Hispanic.

All covariates were included in the regression analysis for each of the chemicals considered here, regardless of whether they achieved statistical significance. Interactions were not assessed due to sample size limitations, especially for interactions involving the tobacco-consumption category, because of the relatively small sample size for the SLT consumption category.

Multiplicative factors for individual chemicals were computed by exponentiating the regression coefficients associated with the tobacco categories. Multiplicative factors corresponding to a unit increase in cotinine were similarly constructed separately for smokers and SLT consumers. Geometric means (GMs) were not computed and regression analyses were not performed for

Table 2. Demographic frequencies, survey year, serum cotinine, and urinary creatinine of the study population by tobacco-consumption category.

	Cigarette smokers	Smokeless tobacco consumers	Nonconsumers of tobacco/NRT
	$n = 5{,}040$	$n = 368$	$n = 16{,}443$
Gender (%)			
Male	54.1	93.3	43.4
Female	45.9	6.7	56.6
Race/ethnicity (%)			
Hispanic[†]	11.8	3.0	13.2
Non-Hispanic Black	12.1	5.9	10.0
Non-Hispanic White	71.2	88.4	71.8
Other	4.9	2.7	5.0
Age (years) (%)			
20–29	25.6	15.8	16.0
30–39	23.3	28.2	18.1
40–49	23.9	23.8	20.6
50–59	16.1	15.7	17.5
60–69	7.6	7.4	12.6
≥70	3.5	9.1	15.2
BMI (%)			
≤22.7	24.8	8.7	17.3
22.8–26.1	25.3	18.4	22.9
26.2–30.2	23.5	30.6	27.6
≥30.3	26.4	42.3	32.2
PIR (%)			
Low (≤1.7)	42.9	29.3	29.0
Medium (1.8–3.9)	32.2	31.7	31.9
High (>3.9)	24.9	39.0	39.1
Survey year (%)			
1999–2000	18.9	17.1	18.3
2001–2002	20.8	18.8	20.7
2003–2004	20.1	25.8	19.3
2005–2006	20.1	18.8	20.6
2007–2008	20.1	19.5	21.1
Serum cotinine (ng/mL)	$n = 4{,}790$	$n = 359$	$n = 15{,}532$
Geometric mean (95% CI)	127.7 (119.1, 135.6)	188.7 (152.9, 235.1)	0.050 (0.047, 0.054)
Urinary creatinine (mg/dL)	$n = 4{,}961$	$n = 357$	$n = 16{,}141$
Geometric mean (95% CI)	106.7 (102.5, 109.9)	124.0 (113.3, 137.0)	96.0 (94.2, 97.7)

NRT, nicotine replacement therapy; BMI, body mass index; PIR, poverty income ratio; CI, confidence interval.
[†]Includes Mexican American and Other Hispanic.

chemicals with the proportion of measurements below the LOD greater than 40% in all tobacco-consumption categories (CDC, 2009b).

Results

Table 2 presents the 1999–2008 NHANES sample population demographics by tobacco-consumption category for subjects 20 years of age and older. Smokers comprised the largest group of tobacco consumers ($n = 5040$ smokers, $n = 368$ SLT consumers). For smokers and SLT consumers, the proportion of males exceeded the number of females (54% and 93%, respectively), and non-Hispanic White subjects comprised the majority of smokers and SLT consumers (71% and 88%, respectively). In addition, Table 2 presents urinary creatinine and serum cotinine levels for smokers, SLT consumers, and nonconsumers. The GMs for urinary creatinine and serum cotinine concentrations were significantly higher in SLT consumers than in smokers and nonconsumers. For all three categories of tobacco consumption, a roughly equal proportion of subjects were sampled from each of the five, 2-year survey cycles.

Tables 3 and 4 present the unadjusted GMs for the biomonitoring analytes evaluated in blood and urine, respectively, by tobacco category. Also included are the years of NHANES for which the data on each analyte were collected and are currently available, the combined number of subjects from all NHANES for whom data are currently available for each analyte, and the percentage of the sample with analyte concentration below the LOD. The number of subjects with self-reported tobacco consumption available for analysis varies by analyte, likely due to most analytes being collected only on one-third, one-quarter, or smaller subsamples of the study population.

Table 3. Unadjusted geometric means, percent nondetect, and sample size for analytes measured in blood or serum by tobacco category for available National Health and Nutrition Examination Survey (NHANES) years.

Analyte	NHANES years	Tobacco category[†]	Sample size	Percent nondetect	Unadjusted geometric mean (95% CI)	Units
Cadmium (Cd)	99–08	Smokers	4830	5.8	0.90 (0.87, 0.93)	μg/L
		SLT Cons.	360	49.1	0.28 (0.25, 0.30)	
		Non-Cons.	15811	43.4	0.30 (0.29, 0.31)	
Lead (Pb)	99–08	Smokers	4830	0.1	1.86 (1.80, 1.92)	μg/dL
		SLT Cons.	360	0.3	1.92 (1.76, 2.09)	
		Non-Cons.	15811	0.6	1.39 (1.36, 1.43)	
Mercury (Total) (Hg)	03–08	Smokers	2999	15.5	0.81 (0.75, 0.87)	μg/L
		SLT Cons.	231	18.5	0.78 (0.69, 0.87)	
		Non-Cons.	9628	10.1	1.06 (0.99, 1.13)	
Selenium (Se)	03–04	Smokers	506	0.0[‡]	130.3 (129.0, 133.0)	μg/L
		SLT Cons.	52	0.0	137.0 (130.3, 145.5)	
		Non-Cons.	2062	0.0	137.0 (134.3, 139.8)	
Benzene	99–04	Smokers	646	6.8	0.12 (0.11, 0.14)	ng/mL
		SLT Cons.	44	47.7	0.031 (0.029, 0.033)	
		Non-Cons.	1520	51.9	0.031 (0.027, 0.035)	
Toluene	99–04	Smokers	683	0.0	0.34 (0.31, 0.39)	ng/mL
		SLT Cons.	50	2.0	0.12 (0.09, 0.17)	
		Non-Cons.	1579	5.7	0.10 (0.09, 0.11)	
Styrene	99–04	Smokers	659	17.2	0.067 (0.059, 0.076)	ng/mL
		SLT Cons.	46	52.6	0.033 (0.028, 0.040)	
		Non-Cons.	1498	58.4	0.031 (0.028, 0.033)	
m/p-Xylene	99–04	Smokers	680	0.3	0.22 (0.20, 0.25)	ng/mL
		SLT Cons.	49	2.6	0.15 (0.13, 0.17)	
		Non-Cons.	1583	3.9	0.13 (0.11, 0.14)	
Ethylbenzene (Ethylbenz)	99–04	Smokers	637	10.3	0.063 (0.056, 0.069)	ng/mL
		SLT Cons.	44	34.6	0.029 (0.025, 0.035)	
		Non-Cons.	1485	40.0	0.028 (0.026, 0.031)	
Acrylamide (AA) (Hb adducts)	03–04	Smokers	833	0.0	122.7 (112.2, 134.3)	pmoL/g Hb
		SLT Cons.	70	0.0	53.5 (40.4, 70.8)	
		Non-Cons.	2728	0.06	47.9 (46.1, 49.9)	
Glycidamide (GA) (Hb adducts)	03–04	Smokers	854	0.4	101.5 (92.8, 111.1)	pmoL/g Hb
		SLT Cons.	72	1.4	50.4 (38.9, 65.4)	
		Non-Cons.	2755	2.3	47.5 (45.2, 49.4)	
1,2,3,4,5,6,7,8-Octa-chlorodibenzo-p-dioxin (OCDD)	99–04	Smokers	751	27.3	200.3 (186.8, 214.9)	pg/g lipid
		SLT Cons.	62	15.7	308.0 (254.7, 372.4)	
		Non-Cons.	2519	15.0	298.9 (278.7, 320.5)	
1,2,3,4,6,7,8-Hepta-chlorodibenzo-p-dioxin (HpCDD)	99–04	Smokers	760	25.3	18.5 (17.3, 20.1)	pg/g lipid
		SLT Cons.	63	7.7	45.6 (36.2, 56.8)	
		Non-Cons.	2547	7.1	42.1 (39.3, 56.8)	
1,2,3,6,7,8-Hexa-chlorodibenzo-p-dioxin (HxCDD)	99–04	Smokers	764	27.0	17.5 (15.6, 19.3)	pg/g lipid
		SLT Cons.	64	18.1	24.0 (16.4, 35.5)	
		Non-Cons.	2559	21.9	22.4 (20.5, 24.8)	
1,2,3,4,6,7,8-Hepta-chlorodibenzofuran (HpCDF)	99–04	Smokers	731	24.6	7.17 (6.69, 7.77)	pg/g lipid
		SLT Cons.	59	15.4	10.6 (8.25, 13.5)	
		Non-Cons.	2456	23.2	7.39 (6.96, 7.85)	
1,2,3,4,7,8-Hexa-chlorodibenzofuran (HxCDF)	99–04	Smokers	764	36.8	4.18 (3.94, 4.44)	pg/g lipid
		SLT Cons.	64	32.2	5.00 (4.31, 5.75)	
		Non-Cons.	2542	32.3	4.71 (4.44, 5.00)	
2,3,4,7,8-Penta-chlorodibenzofuran (PeCDF)	99–04	Smokers	760	40.9	4.18 (3.93, 4.43)	pg/g lipid
		SLT Cons.	65	29.6	5.47 (4.57, 6.49)	
		Non-Cons.	2556	32.6	5.21 (4.90, 5.53)	

Hb, hemoglobin.
[†]Smokers, cigarette smokers; SLT Cons., smokeless tobacco consumers; Non-Cons., nonconsumer of tobacco or NRT products.
[‡]Based on a method detection limit of 5.7 μg/L (personal communication).

Table 4. Unadjusted geometric means, percent nondetect, and sample size for analytes measured in urine by tobacco category for available National Health and Nutrition Examination Survey (NHANES) years.

							Urinary creatinine-corrected	
Analyte	NHANES years	Tobacco category[†]	Sample size	Percent nondetect	Unadjusted geometric mean (95% CI)	Units	Unadjusted geometric mean (95% CI)	Units
1-Hydroxypyrene (1-OH-Pyr)	01-04	Smokers	654	0.1	145.5 (130.3, 162.4)	ng/L	122.7 (111.1, 134.3)	ng/g creatinine
		SLT Cons.	45	2.8	83.9 (58.0, 122.7)		67.4 (55.7, 81.5)	
		Non-Cons.	2046	2.1	43.4 (39.6, 47.5)		43.8 (40.4, 47.5)	
2-Hydroxyfluorene (2-OH-Fluor)	01-04	Smokers	653	0.0	1141 (1043, 1261)	ng/L	962.9 (880.1, 1053.6)	ng/g creatinine
		SLT Cons.	45	0.0	379.9 (259.8, 550.0)		301.9 (237.5, 387.6)	
		Non-Cons.	2047	0.0	194.4 (175.9, 214.9)		196.4 (181.3, 212.7)	
3-Hydroxyfluorene (3-OH-Fluor)	01-04	Smokers	652	0.1	658.5 (589.9, 735.1)	ng/L	555.6 (502.7, 614.0)	ng/g creatinine
		SLT Cons.	45	0.0	170.7 (113.3, 254.7)		135.6 (98.5, 186.8)	
		Non-Cons.	2039	0.7	70.8 (64.1, 77.5)		71.5 (66.0, 77.5)	
9-Hydroxyfluorene (9-OH-Fluor)	03-04	Smokers	330	0.0	487.8 (419.9, 566.8)	ng/L	411.6 (368.7, 464.1)	ng/g creatinine
		SLT Cons.	24	0.0	607.9 (432.7, 854.1)		387.6 (235.1, 632.7)	
		Non-Cons.	947	0.0	206.4 (177.7, 239.8)		214.9 (192.5, 237.5)	
1-Hydroxyphenanthrene (1-OH-Phen)	01-04	Smokers	653	0.1	223.6 (206.4, 244.7)	ng/L	190.6 (175.9, 204.4)	ng/g creatinine
		SLT Cons.	45	0.0	184.9 (133.0, 257.2)		148.4 (120.3, 181.3)	
		Non-Cons.	2041	0.5	126.5 (116.7, 138.4)		129.0 (120.3, 138.4)	
2-Hydroxyphenanthrene (2-OH-Phen)	01-04	Smokers	651	0.6	101.5 (90.9, 113.3)	ng/L	85.6 (79.0, 92.8)	ng/g creatinine
		SLT Cons.	45	0.0	76.7 (55.7, 104.6)		60.9 (50.4, 73.7)	
		Non-Cons.	2040	4.5	46.5 (42.1, 51.4)		47.0 (42.9, 51.4)	
3-Hydroxyphenanthrene (3-OH-Phen)	01-04	Smokers	640	0.3	210.6 (190.6, 232.8)	ng/L	177.7 (160.8, 196.4)	ng/g creatinine
		SLT Cons.	45	0.0	137.0 (97.5, 192.5)		108.9 (83.1, 142.6)	
		Non-Cons.	2006	1.3	82.3 (75.2, 90.0)		83.1 (76.7, 90.0)	
4-Hydroxyphenanthrene (4-OH-Phen)	03-04	Smokers	325	1.7	47.0 (40.0, 55.1)	ng/L	39.6 (35.5, 44.7)	ng/g creatinine
		SLT Cons.	23	0.0	30.3 (24.3, 37.7)		19.1 (10.8, 33.8)	
		Non-Cons.	923	14.2	18.9 (16.3, 22.0)		19.3 (16.9, 21.8)	
1-Hydroxynaphthalene (1-OH-Naph)	01-04	Smokers	659	0.0	8518 (7631, 9509)	ng/L	7187 (6438, 8022)	ng/g creatinine
		SLT Cons.	45	2.8	1686 (1300, 2165)		1339 (1012, 1772)	
		Non-Cons.	2790	0.1	1619 (1466, 1772)		1636 (1510, 1772)	
2-Hydroxyaphthalene (2-OH-Naph)	01-04	Smokers	656	0.0	10615 (9605, 11731)	ng/L	8955 (8184, 9897)	ng/g creatinine
		SLT Cons.	45	0.0	2368 (1808, 3072)		1881 (1603, 2208)	
		Non-Cons.	2779	0.1	1790 (1620, 1978)		1808 (1669, 1959)	
Arsenic (Total) (As)	03-08	Smokers	958	2.5	8.08 (7.03, 9.30)	µg/L	8.08 (7.17, 9.12)	µg/g creatinine
		SLT Cons.	87	1.4	7.54 (6.30, 9.03)		6.17 (5.05, 7.46)	
		Non-Cons.	3263	0.8	8.90 (8.28, 9.56)		9.58 (8.94, 10.28)	
Cadmium	99-08	Smokers	1574	3.1	0.36 (0.34, 0.39)	µg/L	0.34 (0.32, 0.37)	µg/g creatinine
		SLT Cons.	122	8.1	0.21 (0.16, 0.26)		0.16 (0.13, 0.20)	
		Non-Cons.	5282	5.3	0.23 (0.22, 0.24)		0.24 (0.23, 0.25)	

Table 4. continued on next page

Table 4. Continued.

Analyte	NHANES years	Tobacco category[†]	Sample size	Percent nondetect	Unadjusted geometric mean (95% CI)	Units	Urinary creatinine-corrected: Unadjusted geometric mean (95% CI)	Urinary creatinine-corrected: Units
Cobalt (Co)	99-08	Smokers	1574	3.8	0.34 (0.32, 0.36)	µg/L	0.33 (0.31, 0.34)	µg/g creatinine
		SLT Cons.	122	4.5	0.34 (0.29, 0.39)		0.26 (0.24, 0.28)	
		Non-Cons.	5282	3.6	0.33 (0.31, 0.34)		0.34 (0.33, 0.35)	
Lead	99-08	Smokers	1574	1.4	0.75 (0.71, 0.79)	µg/L	0.72 (0.69, 0.75)	µg/g creatinine
		SLT Cons.	122	1.3	0.75 (0.65, 0.87)		0.58 (0.50, 0.68)	
		Non-Cons.	5282	3.0	0.57 (0.55, 0.59)		0.59 (0.58, 0.61)	
Mercury (Total)	03-08	Smokers	973	14.8	0.42 (0.38, 0.47)	mg/L	0.42 (0.38, 0.46)	µg/g creatinine
		SLT Cons.	88	12.6	0.43 (0.34, 0.54)		0.36 (0.30, 0.43)	
		Non-Cons.	3307	10.7	0.51 (0.48, 0.54)		0.54 (0.52, 0.57)	
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	07-08	Smokers	1098	1.8	0.21 (0.16, 0.27)	µg/L	0.21 (0.16, 0.27)	µg/g creatinine
		SLT Cons.	80	0.0	1.26 (0.96, 1.64)		0.99 (0.72, 1.38)	
		Non-Cons.	3346	64.7	0.00093 (0.00085, 0.0010)		0.0010 (0.00090, 0.0011)	

[†]Smokers, cigarette smokers; SLT Cons., smokeless tobacco consumers; Non-Cons., Nonconsumer of tobacco or NRT products.

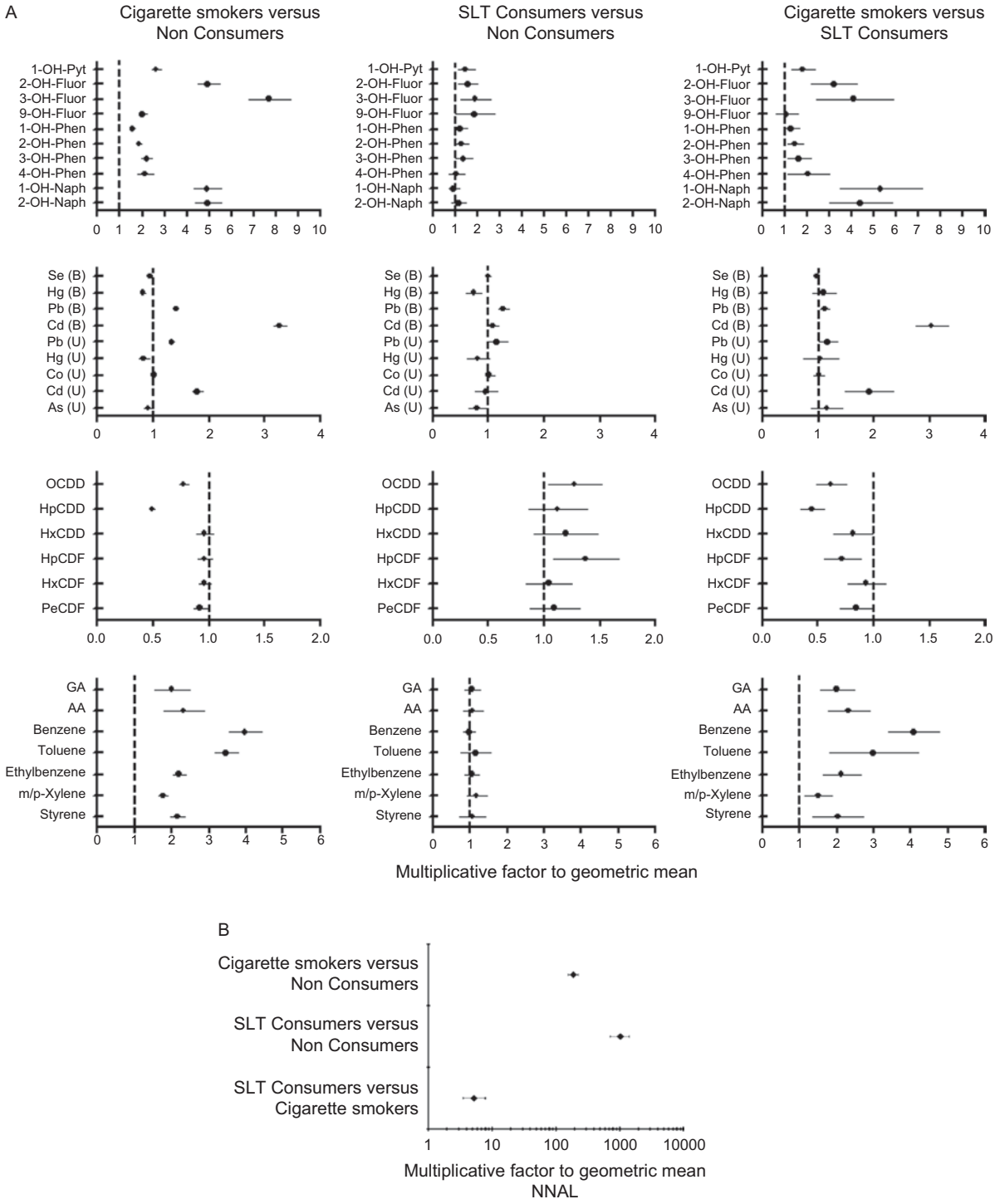

Figure 1. Multiplicative factor to the geometric mean and 95% confidence interval for each chemical from regression models adjusted for gender, race/ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine (for urinary analytes) and tobacco-consumption category (A). NNAL is on the logarithmic scale (B). Dotted line (1.0) corresponds to no difference. B, blood; U, urine; see Tables 3 and 4 for other abbreviations.

Out of a total of 46 exposure biomarkers evaluated, 13 (1,2,3,4,6,7,8,9-octachlorodibenzofuran, 1,2,3,4,7,8,9-heptachlorodibenzofuran,1,2,3,4,7,8-hexachlorodibenzo-p-dioxin, 1,2,3,6,7,8-hexachlorodibenzofuran, 1,2,3,7,8,9-hexachlorodibenzofuran, 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin, 1,2,3,7,8-pentachlorodibenzofuran, 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 2,3,4,6,7,8-hexachlorodibenzofuran, 2,3,7,8-tetrachlorodibenzofuran, 2,3,7,8-tetrachlorodibenzo-p-dioxin, Be, o-xylene) were excluded, as more than 40% of the samples were below the LOD for all three categories (data not shown). Three analytes (benzene, blood Cd, and styrene) for which the percentage of samples with values below detection was more than 40% for one or two of the three categories (Table 3) were included in the analysis.

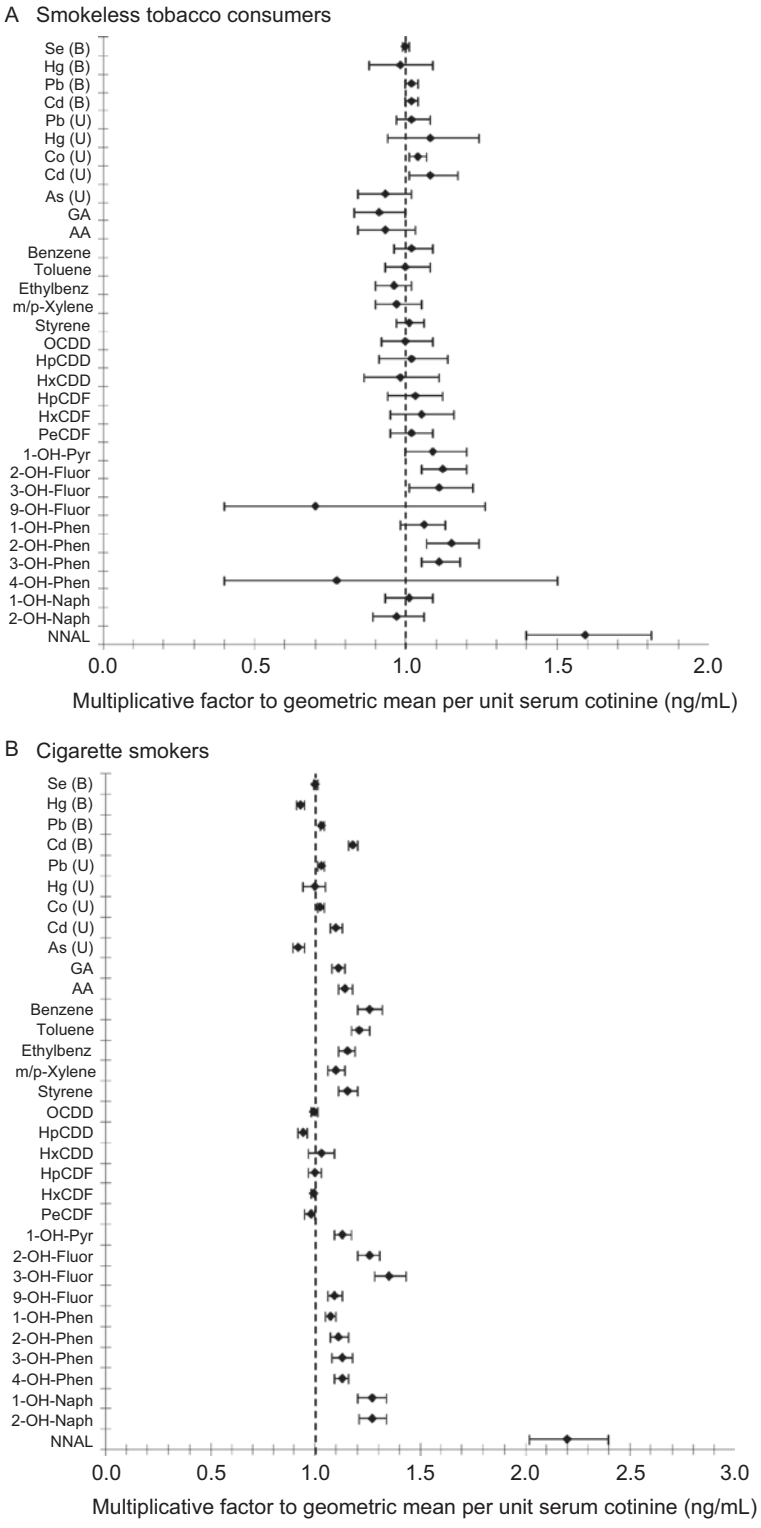

Figure 2. Multiplicative factor to the geometric mean and 95% confidence interval for each chemical per unit serum cotinine (ng/mL) based on regression models adjusted for gender, race/ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine (for urinary analytes) and tobacco-consumption category for cigarette smokers (A) and smokeless tobacco consumers (B). Dotted line (1.0) corresponds to no difference. B, blood; U, urine; see Tables 3 and 4 for other abbreviations.

Figure 1 (A and B) presents the results of the regression models examined for each of the 33 analytes evaluated. Performing the analyses with age, BMI, and PIR as continuous variables did not change the final results (data not shown).

Smokers versus nonconsumers

The data presented in Figure 1 suggest that 22 of the 33 analytes examined were significantly higher in smokers compared with nonconsumers. Six analytes were significantly lower in smokers than in

nonconsumers (1,2,3,4,5,6,7,8-octachlorodibenzo-*p*-dioxin (OCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), blood and urine Hg, and Se). The levels of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF), Co, and As were not significantly different between smokers and nonconsumers.

SLT consumers versus nonconsumers

For most analytes evaluated, levels in SLT consumers were not significantly different than in nonconsumers. The analytes with levels significantly higher in SLT consumers compared with nonconsumers were 1-hydroxypyrene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluoranthene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, blood Pb, OCDD, HpCDF, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, exposure biomarker of NNK) (Figure 1A and 1B). In contrast, blood Hg and urinary As were significantly lower in SLT consumers compared with nonconsumers.

Smokers versus SLT consumers

A comparison of analyte levels in smokers with levels in SLT consumers (Figure 1A and 1B) suggests that 18 (2-hydroxynaphthalene, 1-hydroxynaphthalene, 4-hydroxyphenanthrene, 3-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyfluorene, 2-hydroxyfluorene, 1-hydroxypyrene, urine Cd, AA, glycidamide (GA), styrene, m/p-xylene, ethylbenzene, toluene, benzene, blood Pb, blood Cd) of the 33 analytes examined were significantly lower in SLT consumers compared with smokers. Ten of 33 analytes (urine Pb, Co, As, urine and blood Hg, 9-hydroxyfluorene, 1-hydroxyphenanthrene, PeCDF, HxCDF, and Se) were not different. Analytes such as OCDD, HpCDD, HxCDD, HpCDF, and NNAL were all lower in smokers compared with SLT consumers.

Overall, these results suggest that for most analytes examined in this study, SLT consumers have exposure biomarker levels lower than smokers and not different than nonconsumers.

Correlation of analytes with serum cotinine

To evaluate the association between each analyte and serum cotinine, a multiplicative factor was calculated for each analyte and by category. The multiplicative factor for a particular analyte is a measure of the relative association of the analyte for each unit increase in serum cotinine. The estimated multiplicative association of each analyte with serum cotinine ranges from 0.70 to 1.59 for SLT consumers and 0.92 to 2.20 for smokers. A value below 1.0, as with As in smokers, represents a negative association and indicates that higher levels of serum cotinine are associated with lower average values of the analyte. Of the 33 analytes evaluated, only seven (Co, urine Cd, 2-hydroxyfluorene, 3-hydroxyfluorene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, NNAL) were significantly positively associated with serum cotinine in SLT consumers (Figure 2A) compared with 23 (blood and urine Pb, blood and urine Cd, Co, GA, AA, all VOCs, all PAHs, NNAL) in smokers (Figure 2B). Data for seven (Co, urine Cd, 2-hydroxyfluorene, 3-hydroxyfluorene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, NNAL) analytes showed a statistically significant, positive association with serum cotinine in both smokers and SLT consumers.

Of the analytes with a significant, positive association with serum cotinine among both SLT consumers and smokers, only NNAL was higher in SLT consumers (Figure 1B).

Discussion

To better understand the differences in exposure associated with the use of various tobacco products, differences in the exposure biomarkers of certain chemical constituents in tobacco and MSS may be of interest. This study compared levels of exposure biomarkers for certain chemical constituents of SLT and MSS in a large sample representative of the US population.

Overall, the results observed in this study are consistent with available and previously published findings from the US. Although publications of exposure biomarkers data in SLT consumers are largely missing from the peer-reviewed literature, relevant data comparing smokers with nonsmokers are more widely available. Previous publications regarding exposure biomarkers of certain PAHs and VOCs, Cd, Pb, and AA/GA have reported increased concentrations in smokers compared with nonsmokers, consistent with this analysis (Hajimiragha et al., 1989; Willers et al., 1992; Heudorf and Angerer, 2001; Nan et al., 2001; Hecht, 2002; Scherer, 2005; McKelvey et al., 2007; Lin et al., 2008; Richter et al., 2009; Roethig et al., 2009; Vesper et al., 2010). Although of note, three previous publications reported no significant difference in certain PAH biomarkers levels between smokers and nonsmokers (Jacob et al., 1999; Alexandrie et al., 2000; Apostoli et al., 2003). Also consistent with the current findings, evidence from previous publications support urinary As and Co may not be different in cigarette smokers and nonconsumers of tobacco (Gebel et al., 1998; Heck et al., 2009; Richter et al., 2009), and serum Se (Wei et al., 2001; Kafai and Ganji, 2003) and Hg concentrations may not be different or may be lower in smokers compared with nonconsumers (McKelvey et al., 2007; Richter et al., 2009). There is evidence that the findings for Se and Hg may be a result of dietary differences among tobacco-consumption groups (Kafai and Ganji, 2003; Smith et al., 2009). For Se, Kafai and Ganji (2003) provided evidence to support lower dietary intakes of Se in smokers. For Hg, results from a study of women NHANES participants 1999–2002 (Smith et al., 2009) indicated that increased fish consumption

was associated with increases in blood Hg concentrations, yet decreased levels of serum cotinine. Previous studies found similar levels of total NNAL in urine of SLT consumers and smokers (Kresty et al., 1996; Hecht et al., 2002). However, results from a larger study (Hecht et al., 2007) indicated that SLT consumers showed significantly higher levels of NNAL in their urine when compared with smokers, consistent with findings from this analysis.

Regarding the relationship between serum cotinine and tobacco-relevant chemical exposures, the overall results of this analysis are similar to previously reported results. Biomarkers of nicotine in NHANES are limited to cotinine measured in serum. In the literature, chemical associations with cotinine measured in urine are more widely available; however, any trend observed may still be relevant to these results. Consistent with these results, certain PAHs, benzene, AA, Cd, Pb, and NNAL have been previously reported to be positively associated with cotinine in populations of US and non-US smokers (Willers et al., 1992; Shaham et al., 1996; Yang et al., 1999; Buratti et al., 2000; Scherer et al., 2000; Hecht et al., 2002, 2007; Mannino et al., 2005; Manini et al., 2006, 2008; Vesper et al., 2007). Different from the results presented here, others have reported no significant association for 1-OHP, toluene, ethylbenzene, and xylenes with cotinine (Murphy et al., 2004; Manini et al., 2008). In addition, Se has been reported to be negatively associated with cotinine (Kafai and Ganji, 2003; Ellingsen et al., 2009), whereas in this analysis, no significant association was observed. The significant inverse association of HpCDD in smokers reported here has been observed for PCDDs previously (Lee et al., 2008). As these compounds tend to accumulate in adipose tissue, such a finding could possibly be due to a generally lower BMI observed in smokers compared with nonconsumers. The significant positive association between NNAL and SLT consumers observed here has also been reported previously (Hecht et al., 2002, 2007).

The strengths of the evaluation presented here include the use of data from NHANES, a well-established biomonitoring program in the US. NHANES data provide a large sample, representative of the US population, and individual level data are available to account for potential confounders including, age, gender, race/ethnicity, BMI, PIR, and urinary creatinine. In addition, survey participants reported consumption of tobacco products under actual and typical conditions, as opposed to an experimental study, in which participation may be altered by self-selection or inclusion/exclusion criteria, and tobacco product type or frequency of consumption may be restricted. That individual level data are available by tobacco use category (i.e., smokers, SLT consumers, and nonconsumers) is useful for comparison of exposure biomarkers across these groups.

Using NHANES data also presents limitations. Tobacco consumers are a minority of the NHANES participants, and of tobacco consumers, the majority are cigarette smokers. Accordingly, for most analytes, the sample sizes for SLT consumers were relatively small (<100). Thus, some of the results from this analysis should be interpreted with caution. An additional limitation of this data set is that tobacco-consumption groups were based on self-reporting of tobacco consumption (or nonconsumption) in the previous 5 days before the survey, which may not be representative of chronic tobacco consumption or nonconsumption (e.g., the nonconsumption group may include recent quitters and consumer groups may include occasional tobacco consumers). However, nonconsumers were confirmed using a serum cotinine cut off of ≤15 ng/mL (NCI, 1999), which likely reduces some potential misclassification. The misclassification rate in this analysis was 1.9% (i.e., 297/15829), lower than some previously reported (NCI, 1999). Certain potential confounders such as dietary or occupational exposures for individual chemicals were not included in analyte-specific analyses.

The data in the analyses presented here represent information relevant to exposure and absorbed dose of certain chemicals of potential toxicological concern in smokers and SLT consumers. These biomarker data represent total exposure, whereby the contribution of individual and likely multiple sources of exposure cannot be determined. In addition, the effects of varying routes of exposure and accompanying potential differences in metabolism among participants cannot be evaluated using these data (Albertini et al., 2006). For example, the increased levels of NNAL in the urine of SLT consumers compared with smokers could, at least partially, be explained by those effects (Benowitz et al., 1989; Ebbert et al., 2004; Hecht et al., 2007). Previous studies indicated that NNK was metabolized to NNAL to a greater extent (approximately three- to four-fold) in SLT consumers when compared with smokers (Hecht et al., 2008; Stepanov et al., 2008).

Finally, differences in exposure levels for several chemicals between smokers and SLT consumers were presented. As a whole, the results suggest a lower level of exposure among SLT consumers when compared with smokers. These results are potentially useful in informing the health-risk assessment and regulatory processes related to tobacco products.

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Declaration of interest

All four authors are R.J. Reynolds Tobacco Company employees. The authors declare no conflict of interest.

References

Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L, Zenick H. (2006). The use of biomonitoring data in exposure and human health risk assessments. *Environ Health Perspect* 114:1755–1762.

Alexandrie AK, Warholm M, Carstensen U, Axmon A, Hagmar L, Levin JO, Ostman C, Rannug A. (2000). CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. *Carcinogenesis* 21:669–676.

Apostoli P, Neri G, Lucas D, Manno M, Berthou F. (2003). Influence of genetic polymorphisms of CYP1A1 and GSTM1 on the urinary levels of 1-hydroxypyrene. *Toxicol Lett* 144:27–34.

Benowitz NL, Jacob P 3rd, Yu L. (1989). Daily use of smokeless tobacco: systemic effects. *Ann Intern Med* 111:112–116.

Buratti M, Pellegrino O, Brambilla G, Colombi A. (2000). Urinary excretion of 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons from different sources. *Biomarkers* 5:368–381.

CDC (Centers for Disease Control and Prevention). (2009a). *National Health and Nutrition Examination Survey, What's New Archive, July 2009*. Available at: http://www.cdc.gov/nchs/nhanes/archive_new_nhanes.htm [accessed May 2010]

CDC (Centers for Disease Control and Prevention). (2009b). Fourth national report on human exposure to environmental chemicals. Department of Health and Human Services. Available at: http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf [accessed May 2010]

CDC (Centers for Disease Control and Prevention). (2010a). *National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data*. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Available at: http://www.cdc.gov/nchs/nhanes.htm [accessed May 2010]

CDC (Centers for Disease Control and Prevention). (2010b). *National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Questionnaire (or Examination Protocol, or Laboratory Protocol)*. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Available: http://www.cdc.gov/nchs/nhanes.htm [accessed May 2010]

Diekmann J, Wittig A, Stabbert R. (2008). Gas chromatographic-mass spectrometric analysis of acrylamide and acetamide in cigarette mainstream smoke after on-column injection. *J Chromatogr Sci* 46:659–663.

Ebbert JO, Dale LC, Nirelli LM, Schroeder DR, Moyer TP, Hurt RD. (2004). Cotinine as a biomarker of systemic nicotine exposure in spit tobacco users. *Addict Behav* 29:349–355.

Ellingsen DG, Thomassen Y, Rustad P, Molander P, Aaseth J. (2009). The time-trend and the relation between smoking and circulating selenium concentrations in Norway. *J Trace Elem Med Biol* 23:107–115.

Gebel TW, Suchenwirth RH, Bolten C, Dunkelberg HH. (1998). Human biomonitoring of arsenic and antimony in case of an elevated geogenic exposure. *Environ Health Perspect* 106:33–39.

Hajimiragha H, Ewers U, Brockhaus A, Boettger A. (1989). Levels of benzene and other volatile aromatic compounds in the blood of non-smokers and smokers. *Int Arch Occup Environ Health* 61:513–518.

Hecht SS. (2002). Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 23: 907–922.

Hecht SS, Carmella SG, Murphy SE, Riley WT, Le C, Luo X, Mooney M, Hatsukami DK. (2007). Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. *Cancer Epidemiol Biomarkers Prev* 16:1567–1572.

Hecht SS, Carmella SG, Stepanov I, Jensen J, Anderson A, Hatsukami DK. (2008). Metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to its biomarker total NNAL in smokeless tobacco users. *Cancer Epidemiol Biomarkers Prev* 17:732–735.

Hecht SS, Carmella SG, Ye M, Le KA, Jensen JA, Zimmerman CL, Hatsukami DK. (2002). Quantitation of metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use. *Cancer Res* 62:129–134.

Heck JE, Andrew AS, Onega T, Rigas JR, Jackson BP, Karagas MR, Duell EJ. (2009). Lung cancer in a U.S. population with low to moderate arsenic exposure. *Environ Health Perspect* 117:1718–1723.

Heudorf U, Angerer J. (2001). Urinary monohydroxylated phenanthrenes and hydroxypyrene–the effects of smoking habits and changes induced by smoking on monooxygenase-mediated metabolism. *Int Arch Occup Environ Health* 74:177–183.

Hoffmann D, Adams JD, Lisk D, Fisenne I, Brunnemann KD. (1987). Toxic and carcinogenic agents in dry and moist snuff. *J Natl Cancer Inst* 79:1281–1286.

IARC. (2004). Tobacco and Involuntary Smoking. *IARC Monogr Eval Carcinog Risks Hum* 83:1452.

IARC. (2007). Smokeless tobacco and some tobacco-specific N-nitrosamines. *IARC Monogr Eval Carcinog Risks Hum* 89:626.

Jacob J, Grimmer G, Dettbarn G. (1999). Profile of urinary phenanthrene metabolites in smokers and non-smokers. *Biomarkers* 4:319–327.

Kafai MR, Ganji V. (2003). Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988-1994. *J Trace Elem Med Biol* 17:13–18.

Kresty LA, Carmella SG, Borukhova A, Akerkar SA, Gopalakrishnan R, Harris RE, Stoner GD, Hecht SS. (1996). Metabolites of a tobacco-specific nitrosamine, 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK), in the urine of smokeless tobacco users: relationship between urinary biomarkers and oral leukoplakia. *Cancer Epidemiol Biomarkers Prev* 5:521–525.

Lee DH, Jacobs DR, Kocher T. (2008). Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. *Environ Health Perspect* 116:1558–1562.

Lin YS, Egeghy PP, Rappaport SM. (2008). Relationships between levels of volatile organic compounds in air and blood from the general population. *J Expo Sci Environ Epidemiol* 18:421–429.

Lumley T.(2010). *R package version 3.22-1. Survey: analysis of complex survey samples*. Available at: http://faculty.washington.edu/tlumley/survey/ [accessed March 2010]

Maier RH, Bray JT, Pories WJ. (1989). Trace element status of some commercial smokeless tobaccos. *J Toxicol Environ Health* 28:171–181.

Manini P, De Palma G, Andreoli R, Poli D, Mozzoni P, Folesani G, Mutti A, Apostoli P. (2006). Environmental and biological monitoring of benzene exposure in a cohort of Italian taxi drivers. *Toxicol Lett* 167:142–151.

Manini P, De Palma G, Andreoli R, Poli D, Petyx M, Corradi M, Mutti A, Apostoli P. (2008). Biological monitoring of low benzene exposure in Italian traffic policemen. *Toxicol Lett* 181:25–30.

Mannino DM, Homa DM, Matte T, Hernandez-Avila M. (2005). Active and passive smoking and blood lead levels in U.S. adults: data from the Third National Health and Nutrition Examination Survey. *Nicotine Tob Res* 7:557–564.

McKelvey W, Gwynn RC, Jeffery N, Kass D, Thorpe LE, Garg RK, Palmer CD, Parsons PJ. (2007). A biomonitoring study of lead, cadmium, and mercury in the blood of New York city adults. *Environ Health Perspect* 115:1435–1441.

Murphy SE, Link CA, Jensen J, Le C, Puumala SS, Hecht SS, Carmella SG, Losey L, Hatsukami DK. (2004). A comparison of urinary biomarkers of tobacco and carcinogen exposure in smokers. *Cancer Epidemiol Biomarkers Prev* 13:1617–1623.

Nan HM, Kim H, Lim HS, Choi JK, Kawamoto T, Kang JW, Lee CH, Kim YD, Kwon EH. (2001). Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. *Carcinogenesis* 22:787–793.

NCI (National Cancer Institute). (1999). Health effects of exposure to environmental tobacco smoke (Smoking and Tobacco Control Monograph no. 10). Bethesda, MD: National Cancer

Institute. Available at: http://cancercontrol.cancer.gov/tcrb/monographs/10/ [accessed May 2010]

Pappas RS, Stanfill SB, Watson CH, Ashley DL. (2008). Analysis of toxic metals in commercial moist snuff and Alaskan iqmik. *J Anal Toxicol* 32:281–291.

Pérez HL, Osterman-Golkar S. (2003). A sensitive gas chromatographic-tandem mass spectrometric method for detection of alkylating agents in water: application to acrylamide in drinking water, coffee and snuff. *Analyst* 128:1033–1036.

Richter PA, Bishop EE, Wang J, Swahn MH. (2009). Tobacco smoke exposure and levels of urinary metals in the U.S. youth and adult population: the National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Int J Environ Res Public Health* 6:1930–1946.

Rodgman A, Perfetti TA.(2009). *The chemical components of tobacco and tobacco smoke*. Boca Raton, FL: Taylor & Francis.

Roethig HJ, Munjal S, Feng S, Liang Q, Sarkar M, Walk RA, Mendes PE. (2009). Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers. *Nicotine Tob Res* 11:1216–1225.

Santamaria A, Ferriby L, Harris M, Paustenbach D. (2006). Use of Biomarkers in Health Risk Assessment. In: DeCaprio AP, editor. *Toxicologic biomarkers*. New York: Taylor & Francis, xii, 295 p.

Scherer G. (2005). Biomonitoring of inhaled complex mixtures–ambient air, diesel exhaust and cigarette smoke. *Exp Toxicol Pathol* 57 Suppl 1:75–110.

Scherer G, Frank S, Riedel K, Meger-Kossien I, Renner T. (2000). Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. *Cancer Epidemiol Biomarkers Prev* 9:373–380.

Sexton K. (2006). Biomarkers of Toxicant Exposure. In: DeCaprio AP, editor. *Toxicologic biomarkers*. New York: Taylor & Francis, xii, 295 p.

Shaham J, Meltzer A, Ashkenazi R, Ribak J. (1996). Biological monitoring of exposure to cadmium, a human carcinogen, as a result of active and passive smoking. *J Occup Environ Med* 38:1220–1228.

Smith KM, Barraj LM, Kantor M, Sahyoun NR. (2009). Relationship between fish intake, n-3 fatty acids, mercury and risk markers of CHD (National Health and Nutrition Examination Survey 1999-2002). *Public Health Nutr* 12:1261–1269.

Stepanov I, Upadhyaya P, Carmella SG, Feuer R, Jensen J, Hatsukami DK, Hecht SS. (2008). Extensive metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers. *Cancer Epidemiol Biomarkers Prev* 17:1764–1773.

Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami D, Hecht SS. (2010). Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry. *Chem Res Toxicol* 23:66–73.

Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, Myers GL. (2007). Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. *Cancer Epidemiol Biomarkers Prev* 16:2471–2478.

Vesper HW, Caudill SP, Osterloh JD, Meyers T, Scott D, Myers GL. (2010). Exposure of the U.S. population to acrylamide in the National Health and Nutrition Examination Survey 2003-2004. *Environ Health Perspect* 118:278–283.

Wei W, Kim Y, Boudreau N. (2001). Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988-1994. *Am J Public Health* 91:258–264.

Willers S, Attewell R, Bensryd I, Schutz A, Skarping G, Vahter M. (1992). Exposure to environmental tobacco smoke in the household and urinary cotinine excretion, heavy metals retention, and lung function. *Arch Environ Health* 47:357–363.

Wilson CL, Bodnar JA, Brown BG, Morgan WT, Potts RJ, Borgerding MF. (2008). Assessment of dioxin and dioxin-like compounds in mainstream smoke from selected US cigarette brands and reference cigarettes. *Food Chem Toxicol* 46:1721–1733.

Yang M, Koga M, Katoh T, Kawamoto T. (1999). A study for the proper application of urinary naphthols, new biomarkers for airborne polycyclic aromatic hydrocarbons. *Arch Environ Contam Toxicol* 36:99–108.

Erratum

Since the first online publication of this article on 25 February 2011, the following has been corrected:

1) Page 5, line 5 under the heading "*SLT consumers versus nonconsumers*":
2-hydroxyfluoranthene has been replaced with 2-hydroxyfluorene
3-hydroxyfluoranthene has been replaced with 3-hydroxyfluorene

2) Page 5, line 11 under the heading "*Smokers versus SLT consumers*":
9-hydroxyfluoranthene has been replaced with 9-hydroxyfluorene

There has also been a sentence added to the Declaration of interest to read:
All four authors are R.J. Reynolds Tobacco Company employees.