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Arsenic exposure and tobacco consumption: Biomarkers and risk assessment

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ABSTRACT

Arsenic is measurable in tobacco and cigarette mainstream smoke (MSS). Whether arsenic has an independent role in diseases associated with tobacco consumption is not known. Epidemiology and biomonitoring data and probabilistic risk assessment (PRA) methods were used to investigate this potential association. Analysis of data from the National Health and Nutrition Examination Survey (NHANES) showed that urine arsenic concentrations in tobacco consumers were not different or were lower than levels in non-consumers of tobacco. Additionally, urine arsenic levels from NHANES tobacco consumers were five-times or more lower than levels reported in epidemiology studies to be associated with adverse health effects. Results of PRA indicated that mean non-cancer hazard estimates and mean incremental lifetime cancer risk estimates were within accepted ranges. Taken together, these results suggest that arsenic may not be independently associated with tobacco consumption or diseases related to tobacco consumption.

1. Introduction

Arsenic is a naturally-occurring and widely-distributed element in the earth and environment. Arsenic is found in rocks, as well as soil, water, and air as a result of natural environmental processes (e.g., volcanic activity, volatilization), and human activities (e.g., non-ferrous metal smelting, pesticide application) (ATSDR, 2007; IPCS, 2001). Like other crops, tobacco can absorb arsenic from the soil (Lugon-Moulin et al., 2008). Accordingly, arsenic is measurable in tobacco and cigarette mainstream smoke (MSS), although concentrations are relatively smaller than those of other metals, such as cadmium and lead (Counts et al., 2005; Pappas et al., 2008). Arsenic has been detected in cured or processed tobacco leaves at concentrations of approximately 400 ng/g of dry tobacco (Lugon-Moulin et al., 2008), in certain smokeless tobacco (SLT) products at concentrations ranging between approximately 130 and 360 ng/g of dry tobacco (Pappas et al., 2008), and in cigarette MSS, depending on cigarette design and machine smoking regimen, at approximately 1.6–24.9 ng/cigarette (Counts et al., 2005; IARC, 2004a). Actual human exposure to constituents in tobacco varies with type of product (e.g., combustible versus non-combustible products) as well as product usage (e.g., intensity, frequency, and duration of use). For most individuals, the greatest source of arsenic exposure is from the diet and the largest dietary source of arsenic is seafood (ATSDR, 2007).

Biomarkers are the best measure for the assessment of exposure, as they provide direct evidence that both contact and uptake into the body have occurred (Sexton, 2006). In humans, urinary arsenic concentration is considered to be a reliable biomarker of arsenic dose (i.e., recently absorbed arsenic) (CDC, 2009). Determining internal arsenic dose is best accomplished via measurement of "total" and speciated arsenic in urine (IPCS, 2001). A measure of "total" arsenic in urine includes all inorganic and organic species (ATSDR, 2007).

Long term exposure to inorganic arsenic is associated with adverse dermal (e.g., keratosis, hyperpigmentation), cardiovascular (e.g., hypertension, Blackfoot disease), and respiratory non-cancer effects in humans (ATSDR, 2007). Additionally, inorganic arsenic is considered to be a known human carcinogen; arsenic inhalation is associated with lung cancer, and exposure to arsenic in drinking water is associated with cancers of the skin, lung, and bladder (IARC, 2004b; NTP, 2011; Straif et al., 2009; USEPA, 1998).

Draft guidance issued by the United States (US) Food and Drug Administration identified arsenic on the abbreviated list of harmful and potentially harmful constituents in roll-your-own tobacco and cigarette filler as well as smokeless tobacco (USDHHS, 2012). Additionally, an analysis by Cox (2009) suggested, based on potential mechanisms of arsenic carcinogenicity, that removal of arsenic from cigarette MSS might reduce human health risks. In an attempt to investigate if the arsenic exposure from tobacco consumption may be related to the adverse health effects associated with tobacco consumption, epidemiology studies were reviewed, arsenic biomarker concentrations in a population representative of the US

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were evaluated, and a probabilistic risk assessment (PRA) was undertaken. First, epidemiology data were reviewed to identify disease endpoints in common between arsenic exposure alone and exposure to tobacco. Arsenic concentrations associated with adverse health effects were identified. Urinary arsenic concentrations in cigarette smokers, SLT consumers, and non-consumers of tobacco were then determined from the National Health and Nutrition Examination Survey (NHANES). Finally, a probabilistic risk assessment (PRA), including incremental lifetime cancer risk (ILCR) and hazard quotient (HQ) calculations specific to arsenic exposures from cigarette smoking and SLT consumption, was performed.

2. Methods

2.1. Determination of relevant human health endpoints

Publicly available documents from scientific and public health groups were reviewed to identify generally accepted adverse human health effects associated with both arsenic exposure and tobacco consumption (i.e., cigarette smoking and SLT consumption). For health effects associated with arsenic alone, the Agency for Toxic Substances and Disease Registry toxicological profile (ATSDR, 2007) was reviewed followed by a review of the relevant primary peer-reviewed publications identified by ATSDR. ATSDR is a US federal public health agency that provides summaries of toxicological data for certain hazardous substances via peer reviewed toxicological profiles. For adverse effects, the lowest biologically relevant (i.e., urine) total arsenic concentrations associated with these endpoints were identified from ATSDR. External exposure concentrations were converted to internal urinary concentrations using Eq. (1) for inhalation exposures and (2) for drinking water exposures (ATSDR, 2007).

$$Y = 0.304 * X \quad (1)$$

where X = urinary arsenic (μg/L), and Y = airborne arsenic (μg/m^3) (Pinto et al., 1976);

$$Y = 10^{-2.57} * X^{0.63} \quad (2)$$

where X = arsenic in drinking water (μg/L), and Y = total arsenic (μg)/mg creatinine (Calderon et al., 1999).

For health outcomes causally associated with cigarette smoking, the US Surgeon General 2004 report was reviewed (USDHHS, 2004). For the identification of health endpoints associated with SLT consumption, consensus of several scientific organizations (IARC, 2007; LSRO, 2008; SCENIHR, 2008; USDHHS, 1986) were consulted and a literature search was conducted in order to identify relevant primary literature published more recently.

2.2. Biomonitoring data

Data collected in the NHANES Mobile Examination Centers (MEC) from 2003 to 2008 for individuals aged 20 years and older were used to evaluate urinary arsenic (i.e., total and speciated) in cigarette smokers (n = 991), SLT consumers (n = 90), and non-consumers of tobacco (n = 3385). NHANES is conducted by the National Center for Health Statistics (NCHS) of the US Centers for Disease Control and Prevention and is designed to annually assess the health and nutritional status of adults and children in the US. Data are publicly available and are representative of the civilian, non-institutionalized US population. Detailed survey methodology is available (CDC, 2006).

The categories for tobacco consumption (i.e., snuff, chewing tobacco, and cigarettes) and non-consumption 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 small sample sizes. Within each tobacco category, only exclusive (single product use) tobacco consumers were included (i.e., individuals reporting the consumption of multiple tobacco products or pipes, cigars, or nicotine replacement therapy were excluded). Self-reported non-consumers of tobacco with a serum cotinine value >15 ng/mL (NCI, 1999) were excluded from the analysis. Individuals were also excluded if data regarding tobacco consumption were missing or if a response was refused or reported as "do not know."

All statistical methods were performed using the appropriate 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® v.9.2 (SAS Institute Inc., Cary, NC) and the survey package available for the R v.2.10.1 software (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses (Lumley, 2010).

Data collection and analysis for total and speciated arsenic in urine began in the NHANES 2003–2004 survey period. Arsenic analytes measured included: arsenic, total; arsenic (V) acid; arsenobetaine; arsenocholine; arsenous (III) acid; dimethylarsinic acid (DMA); monomethylarsonic acid (MMA); and trimethylarsine oxide (TMAO). For arsenic species where the proportion of measurements below the limit of detection (LOD) was greater than forty percent in all three categories (i.e., cigarette smokers, SLT consumers, non-consumers of tobacco), geometric means were not deemed reliable and therefore were not computed (CDC, 2009). In addition, regression analyses were not performed for these analytes.

Multiple linear regression models for the natural log-transformed urinary arsenic values were used to compare the urinary arsenic concentrations measured in cigarette smokers, SLT consumers, and non-consumers of tobacco. The regression analysis included adjustment for age (six categories: 20–29, 30–39, 40–49, 50–59, 60–69, $\geqslant$70 years), gender (two categories: male, female), race/ethnicity (four categories: non-Hispanic Black, non-Hispanic White, Hispanic, Other), body mass index (BMI, four categories: $\leqslant$22.7, 22.8–26.1, 26.2–30.2, $\geqslant$30.3), urinary creatinine, urinary arsenobetaine (measure of seafood consumption), survey year (three categories: 2003–2004, 2005–2006, 2007–2008), and tobacco consumption category. The data were log-transformed to better meet the distribution assumptions for modeling (i.e., normality). Adjusted geometric means (i.e., geometric least squares means) were computed by taking the exponential of the least squares means from the regression models for the log-transformed urinary arsenic values. Least squares means were computed using SAS v.9.2 (SAS Institute, Cary, NC), and represent within-group means adjusted for the covariates included in the model (i.e., age, gender, race/ethnicity, BMI, urinary creatinine, urinary arsenobetaine, survey year, and tobacco consumption category). Multiplicative factors (and 95% confidence intervals), comparing covariate subgroups by arsenic species, were computed by taking the exponential of each regression coefficient and the corresponding confidence intervals.

2.3. Arsenic concentration in cigarette MSS

Arsenic concentrations in tobacco were determined for input into the PRA model. Thirty-four cigarette brands representative of the 2005–2006 US cigarette market were selected for analysis.

Seven brands were in the 'tar' category of 0.9–6.4 mg/cigarette, seventeen brands were in the 'tar' category of 8.6–11.7 mg/cigarette, and ten brands were in the 'tar' category of 13.1–19.0 mg/cigarette, with 'tar' category determined by the Cambridge Filter Method (previously known as the Federal Trade Commission method, where a 35 cc puff is taken every 60 s for a 2 s duration with no cigarette ventilation holes blocked) (FTC, 1967; Pillsbury et al., 1968). Cigarettes were purchased from two locations in the US, and data were collected under an intense machine smoking regimen, i.e., 60 cc puff, twice every 60 s, for a two second duration with 50% blocking of filter ventilation. Cigarettes were conditioned and smoked at 60% relative humidity at 75 °F to a butt length of tipping plus 3 mm according to the Federal Trade Commission method (FTC, 1967; Pillsbury et al., 1968). Smoke condensate was collected by electrostatic precipitation using a 20 port rotary smoking machine. The samples were extracted with methanol and concentrated under nitrogen followed by closed vessel digestion with nitric acid in a microwave digestion system. For determination of arsenic content, the resulting solutions were analyzed in triplicate using the Health Canada method T-109 (Canada, 1999), with the following exceptions: only nitric acid was used in the sample digestion and inductively coupled mass spectrometry was used as the detector system.

2.4. Arsenic concentration in SLT products

As part of a survey of the chemical composition of SLT products sold in the US in 2008, arsenic concentrations were determined in fourteen moist snuff and three chewing tobacco products. Determination of arsenic content was according to Health Canada method T-306 (Canada, 2000) and triplicate analyses were conducted by Labstat International ULC (Kitchener, Ontario, Canada).

2.5. Probabilistic risk assessment

2.5.1. Lifetime average daily intake (LADI)

Exposure to arsenic following both inhalation and oral exposures (lifetime average daily intake, $LADI_i$ and $LADI_o$, respectively) were estimated using Eqs. (3) and (4) from the US Environmental Protection Agency (USEPA, 1989), modified for tobacco consumption:

$$LADI_i(\mu g/m^3) = \frac{C_i * DTC_i * ED * EF}{DIR * BW * AT * CF_i} \quad (3)$$

$$LADI_o(mg/kg - d) = \frac{C_o * DTC_o * ED * EF * CF_o}{BW * AT} \quad (4)$$

where lifetime average daily intake (LADI) was calculated for each exposure route, inhalation in the case of cigarette smokers ($LADI_i$) and oral in the case of SLT consumers ($LADI_o$); C_i is arsenic concentration in μg/cigarette; C_o is arsenic concentration in μg/g dry weight tobacco; DTC_i is daily tobacco consumption in cigarettes per day; DTC_o is daily tobacco consumption in moisture-adjusted g of tobacco per day; ED is exposure duration (years); EF is exposure frequency (days/year); DIR is daily inhalation rate (L/kg-day); BW is body weight (kg); AT is averaging time (70 years × 365 days/year for ILCR calculations and ED × 365 days/year for HQ calculations); CF_i is a conversion factor of 10^{-3} m^3/L; CF_o is a conversion factor of 10^{-6} mg/ng.

To account for variability in LADI values and provide a more meaningful estimate of the probable range of exposures, a probabilistic approach using Monte Carlo simulation was incorporated to establish distributions around exposure inputs (Eqs. (3) and (4)). The simulation was run in Crystal Ball version 11.2.1 (Denver, Colorado, USA) with 10,000 iterations. Independent simulations at 1,000, 5,000, 10,000, and 100,000 iterations were conducted to test the convergence and stability of the numerical output. The results showed that 10,000 iterations were sufficient to ensure the stability of the output distributions.

The variable distributions incorporated included C, DTC, EF, BW, and ED. Parameter distributions, values, and sources are provided in Table S-1 (Supplementary data). Distributions were fit using the data from the 34 cigarette brands for C_i (see Section 2.3) and the 14 moist snuff and 3 chewing tobacco products for C_o (see Section 2.4) in combination with the distribution fitting function in Crystal Ball version 11.1.2. The C distributions were truncated at the minimum and maximum analytical data points. Goodness-of-fit tests provided by Crystal Ball included the Kolmogorov–Smirnov, Chi-Square, and Anderson–Darling. If the three tests were not in agreement, the Anderson–Darling test was preferred. DTC_o was constructed as an empirical distribution based on risk maximizing SLT consumption assumptions of one to five cans or pouches per day, 34 g/can of moist snuff, and 85 g/pouch for chewing tobacco. DTC_i was constructed as an empirical distribution of daily cigarette consumption based on 1999–2006 NHANES data (CDC, 2006). Distributions of EF and BW were also based on data from the 1999–2006 NHANES data (CDC, 2006). EF was constructed as a discrete distribution with three possible values: 219, 292, and 365 days/year, corresponding to consumption of tobacco products for 3 days or less, 4 days, and 5 days out of the last 5 days, respectively. The ED distribution was based on US life expectancy data (Arias, 2006); values were calculated as the sum of age (i.e., 18 to 100 years, a uniform discrete distribution) and life expectancy subtracting 18 years, the minimum legal age for tobacco purchase in the US. ED and BW data were subsequently combined into a single bivariate distribution to ensure that age and BW were paired.

2.5.2. Cancer risk: incremental lifetime cancer risk (ILCR)

$ILCR_i$ and $ILCR_o$ were calculated by multiplying the exposure route specific LADI by the cancer inhalation unit risk (IUR, $[(\mu g/m^3)^{-1}]$) (Eq. (5)) or cancer slope factor (CSF, $[(mg/kg\text{-}d)^{-1}]$) (Eq. (6)), respectively (USEPA, 1989). For $ILCR_o$, the moist snuff and chewing tobacco ILCRs were combined into one ILCR weighted by the number of products included in each category. Mean, 5th percentile, and 95th percentile for $ILCR_i$ and $ILCR_o$ were obtained from the outcome distributions.

$$ILCR_i = IUR * LADI_i \quad (5)$$

$$ILCR_o = CSF * LADI_o \quad (6)$$

An IUR of 4.3E-3/μg/m^3 was derived by the USEPA (1998) based on epidemiology studies of males exposed to arsenic via inhalation in the workplace with subsequent deaths due to lung cancer (Brown and Chu, 1983a,b,c; Enterline and Marsh, 1982; Higgins, 1982; Lee-Feldstein, 1982). A CSF of 1.5/mg/kg body weight-day was derived by the USEPA (1998) based on epidemiology studies of Taiwanese individuals exposed to arsenic via drinking water and prevalent skin cancer (Tseng et al., 1968; Tseng, 1977). For cancer risk, a commonly referenced benchmark for the protection of public health used by the USEPA is an excess risk in the range of 10^{-6}–10^{-4} (i.e., the probability of 1 in 1,000,000 to 1 in 10,000 that an individual may develop cancer when exposed to a carcinogen for a lifetime).

2.5.3. Non-cancer hazard: hazard quotient (HQ)

Non-cancer hazards (hazard quotient, HQ) were assessed by comparing average daily intake (ADI_i) with the chronic reference exposure level (REL, μg/m^3) for inhalation exposure (Eq. (7)) and comparing the ADI_o with the reference dose (RfD, mg/kg-d) for oral exposure (Eq. (8)) (USEPA, 1989). For HQ_o, the moist snuff and chewing tobacco HQs were combined into one HQ weighted by

the number of products included in each category. Mean, 5th percentile, and 95th percentile for HQ_i and HQ_o were obtained from the outcome distributions.

$$HQ_i = \frac{ADI_i}{REL} \quad (7)$$

$$HQ_o = \frac{ADI_o}{RfD} \quad (8)$$

REL and RfD values represent an exposure level below which adverse effects would not be expected to occur following chronic exposure. These toxicity values incorporate margin of safety factors to account for variability in human response (i.e., sensitive sub-populations). The chronic arsenic REL of 0.015 μg/m^3 was derived by CalEPA (2008) based on a drinking water study in children with a decrease in intellectual function and adverse effects on neurobehavioral development identified as the critical effects (Wasserman et al., 2004). The RfD of 0.3 μg/kg body weight-day was derived by the USEPA (1993) based on chronic oral exposure studies via drinking water in humans (Tseng et al., 1968; Tseng, 1977) in which the critical effect was "hyperpigmentation, keratosis and possible vascular complications" (USEPA, 1993). HQ is not a direct measure of risk of non-cancer health effects, but rather a ratio of actual exposure to an established threshold exposure value. If the actual arsenic exposure (i.e., the ADI) is lower than the corresponding threshold exposure (e.g., RfD), then HQ < 1 and the hazard is not expected to be a threat to public health. If the arsenic exposure exceeds the corresponding threshold exposure, then HQ > 1 and potential non-cancer effects may exist (USEPA, 1989).

3. Results

3.1. Epidemiology data

3.1.1. Arsenic

A review of the available epidemiology data showed that some adverse health outcomes associated with occupational and/or environmental arsenic exposure may also be associated with tobacco consumption. It is generally accepted that arsenic exposure is associated with lung and bladder cancers, decreased respiratory function, and adverse cardiovascular effects (ATSDR, 2007; IARC, 2004b). The lowest identified arsenic exposures (ATSDR, 2007) and accompanying urinary total arsenic concentrations (Eqs. (1) and (2)) associated with adverse health outcomes in humans are presented in Table S-2 (Supplementary data) for inhalation exposures and in Table S-3 (Supplementary data) for oral exposures. Not all outcomes are considered to be associated with tobacco consumption (see Section 3.1.2) but were included in this analysis for completeness. Of all adverse effects, the lowest associated urine total arsenic level was determined to be 39.1 μg/g creatinine for lung cancer and arsenic exposure via drinking water (Ferreccio et al., 1998). For inhalation exposures, the lowest associated urine total arsenic level was determined to be 164.5 μg/L, also for lung cancer (Järup et al., 1989).

3.1.2. Cigarette smoking and SLT consumption

Of the health effects related to arsenic exposure, it is generally accepted that cigarette smoking is also associated with increased risk of non-cancer respiratory disease, lung and bladder cancers and cardiovascular disease (USDHHS, 2004). For SLT consumers, the American Heart Association has stated that there is evidence that long-term SLT consumption may be associated with increased risk of cardiovascular mortality, specifically stroke and myocardial infarction (Piano et al., 2010).

3.2. Biomonitoring data

Characteristics of the sample of the NHANES participants for urinary arsenic data are provided in Table S-4 (Supplementary data). The sample size for SLT consumers was relatively small ($n = 90$, compared with $n = 991$ cigarette smokers and $n = 3385$ non-consumers of tobacco). The SLT group was mostly male (96.5%, compared with 55.5% cigarette smokers and 42.7% non-consumers of tobacco), was mostly White, non-Hispanic (92.9%, compared with 74.3% cigarette smokers and 72.0% non-consumers of tobacco), and a larger proportion had BMI values >30.2 (40.4%, compared with 27.8% cigarette smokers and 32.1% non-consumers of tobacco). Mean concentrations of urinary creatinine were statistically significantly greater in SLT consumers compared with non-consumers of tobacco (120.3 [95% CI: 104.6, 138.4] and 92.8 [95% CI: 90.0, 96.5] mg/dL, respectively).

Adjusted geometric means for urine total arsenic, DMA, and arsenobetaine in cigarette smokers, SLT consumers, and non-consumers of tobacco are presented in Table 1. Tenth, 25th, 50th, 75th, and 90th percentiles of these data are provided in S-5 (Supplementary data). MMA, arsenous acid, arsenic acid, arsenocholine, and TMSO were all below the LOD in more than 40% of the population sample in each of the three exposure categories (i.e., cigarette smokers, SLT consumers, non-consumers of tobacco) (Table S-6, Supplementary data) and accordingly, further analyses of these analytes was not conducted (CDC, 2009).

Urinary total arsenic, DMA, and arsenobetaine concentrations were similar among the three consumer groups, although consistently highest in non-consumers of tobacco and lowest in SLT consumers (Table 1). Box plots of the natural log of urine total arsenic (μg/L) in cigarette smokers, SLT consumers, and non-consumers of tobacco are presented in Fig. 1, graphically illustrating the similarity in urine total arsenic concentrations among the three groups.

Urinary total arsenic concentrations of cigarette smokers, SLT consumers, and non-consumers of tobacco from NHANES 2003–2008 (this analysis), and urinary total arsenic concentrations calculated from the lowest identified arsenic levels associated with adverse human health effects from epidemiology studies are presented in Figs. 2 and 3. As previously noted, not all outcomes are considered to be associated with tobacco consumption but were included in this analysis for completeness. For oral exposures, urine total arsenic levels associated with adverse health outcomes are at least 5 times greater than levels observed in tobacco consumers (Fig. 2). For inhalation exposures (Fig. 3), urine total arsenic levels associated with adverse health outcomes are at least 20 times greater than levels observed in tobacco consumers.

3.3. Arsenic concentrations in cigarette MSS and SLT products

Arsenic concentrations by tobacco category are presented in Table 2. The mean (range) arsenic concentration in machine-generated MSS of the 34 cigarette products analyzed was determined to be 12.7 (4.8–18.4) ng/cigarette. The mean (range) arsenic concentration in the 14 moist snuff products and 3 chewing tobacco products evaluated were 189 (80.2–300) and 155 (131–184) ng/g dry weight, respectively. These measures in cigarette MSS and SLT were consistent with previous reports (Counts et al., 2005; Pappas et al., 2008; IARC, 2004a).

3.4. Probabilistic risk assessment

Mean, 5th percentile, and 95th percentile HQ_i, HQ_o, $ILCR_i$, and $ILCR_o$ for arsenic from cigarette smoking and SLT consumption are presented in Table 3. The mean HQ_i of 0.82 and the mean HQ_o of 0.07 indicate that on average, non-cancer hazard estimates fall within US regulatory guidelines (HQ < 1) (USEPA, 1989) for

Table 1
Adjusted[a] geometric mean (95% confidence interval) of urinary arsenic[b] concentrations, by tobacco category, NHANES 2003–2008.

	Cigarette smokers $n = 991$	SLT consumers $n = 90$	Non-consumers of tobacco $n = 3385$
Urine arsenic (μg/L)			
Total arsenic	8.04 (6.97, 9.28)	7.55 (6.12, 9.31)	8.93 (8.31, 9.60)
DMA	3.55 (3.26, 3.86)	3.23 (2.81, 3.72)	3.76 (3.58, 3.95)
Arsenobetaine	1.51 (1.25, 1.83)	1.32 (0.91, 1.93)	1.84 (1.65, 2.05)
Urine arsenic (μg/g creatinine)			
Total arsenic	7.98 (7.08, 9.00)	6.14 (4.86, 7.74)	9.56 (8.92, 10.27)
DMA	3.53 (3.28, 3.80)	2.68 (2.22, 3.23)	4.01 (3.82, 4.22)
Arsenobetaine	1.50 (1.26, 1.78)	1.10 (0.74, 1.62)	1.96 (1.75, 2.19)

DMA = dimethylarsinic acid, SLT = smokeless tobacco.
[a] Age, gender, race/ethnicity, body mass index, urinary arsenobetaine, urinary creatinine, survey year, tobacco category.
[b] Only includes analytes with <40% of sample below the limit of detection.

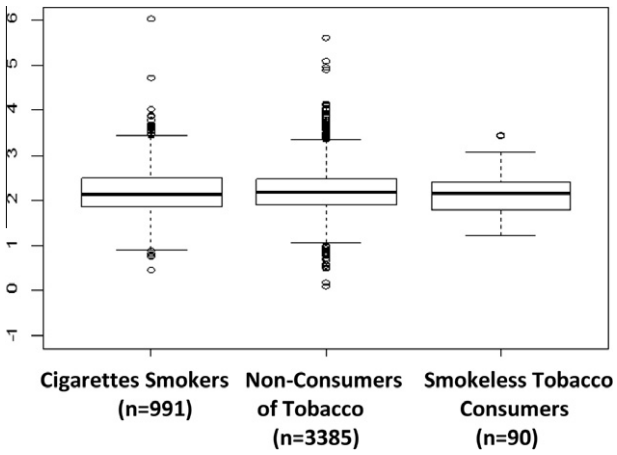

Fig. 1. Box plot of the natural log (ln) of urine total arsenic (μg/L) by tobacco category, NHANES 2003–2008.

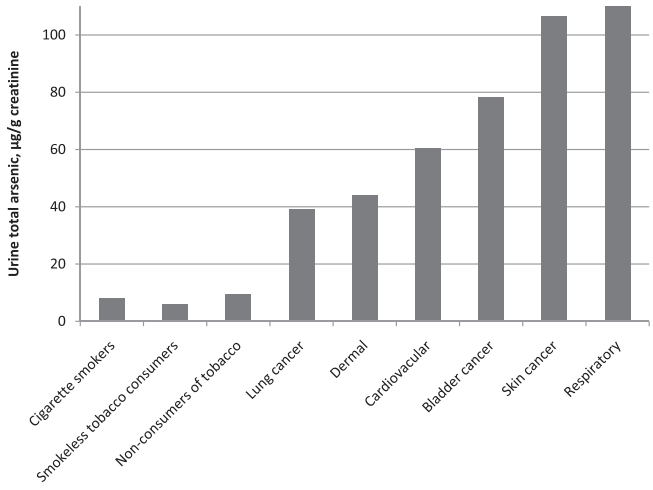

Fig. 2. Urine total arsenic concentrations (μg/g creatinine) in cigarette smokers ($n = 991$), smokeless tobacco consumers ($n = 90$), and non-consumers of tobacco ($n = 3385$) (NHANES 2003–2008) and urine total arsenic concentrations (μg/g creatinine) calculated from the lowest arsenic oral exposures associated with specific adverse health outcomes in humans (ATSDR, 2007).

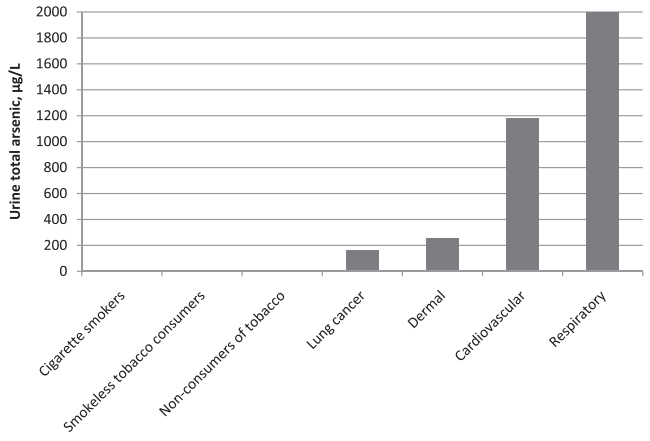

Fig. 3. Urine total arsenic concentrations (μg/L) in cigarette smokers ($n = 991$), smokeless tobacco consumers ($n = 90$), and non-consumers of tobacco ($n = 3385$) (NHANES 2003–2008) and urine total arsenic concentrations (μg/L) calculated from the lowest arsenic inhalation exposures associated with specific adverse health outcomes in humans (ATSDR, 2007).

Table 2
Arsenic concentrations in smokeless tobacco products and machine generated mainstream smoke of cigarettes.

Product category	n	Mean	Standard deviation	Range
Moist snuff, ng/g dry weight	14	189	57	80.2–300
Chewing tobacco, ng/g dry weight	3	155	20	131–184
Cigarettes, ng/cigarette	34	12.7	3.6	4.8–18.4

Each product was measured in triplicate.

arsenic exposures in both cigarette smokers and SLT consumers. The mean calculated $ILCR_i$ for arsenic exposure in cigarette smokers was 5.42E-5, a value within the commonly referenced benchmark range for the protection of human health (i.e., 10^{-6}–10^{-4}). Although the cancers commonly associated with oral arsenic exposure (i.e., lung, skin, and bladder) are not cancers generally associated with SLT consumption, an $ILCR_o$ for arsenic in SLT consumers was calculated. The mean calculated $ILCR_o$ for arsenic in SLT consumers was 3.09E-5, a value within the commonly referenced benchmark range for the protection of human health (i.e., 10^{-6} to 10^{-4}).

4. Discussion

Review of occupational and environmental epidemiology studies (Figs. 2 and 3, Tables S-2 and S-3), analysis of NHANES exposure biomonitoring data (Table 1 and Fig. 1), and PRA (Table 3) suggest that urine total arsenic levels in tobacco consumers: (1) are not different than levels in non-consumers of tobacco; (2) are lower than urine arsenic concentrations associated with adverse health outcomes in epidemiology studies; and (3) do not contribute to calculated non-cancer hazard or cancer risk. Accordingly, tobacco consumption, including cigarette smoking and SLT consumption,

Table 3
Calculated incremental lifetime cancer risk ($ILCR_i$, $ILCR_o$) and hazard quotient (HQ_i, HQ_o) values for arsenic from cigarette smoking (inhalation) and SLT (oral) consumption.

Probabilistic risk assessment	5th Percentile	Mean	95th Percentile
Cancer risk, inhalation ($ILCR_i$)	8.16E-6	5.42E-5	1.42E-4
Cancer risk, oral ($ILCR_o$)	1.12E-5	3.09E-5	1.55E-4
Non-cancer hazard, inhalation (HQ_i)	0.12	0.82	2.30
Non-cancer hazard, oral (HQ_o)	0.03	0.07	0.41

HQ_i = hazard quotient (inhalation), HQ_o = hazard quotient (oral), $ILCR_i$ = incremental lifetime cancer risk (inhalation), $ILCR_o$ = incremental lifetime cancer risk (oral), SLT = smokeless tobacco.

does not appear to contribute to urine arsenic concentrations; and arsenic in tobacco may not be independently associated with increased risk of tobacco consumption related diseases.

The geometric mean concentrations presented in this analysis were consistent with urinary arsenic concentrations previously published for the US population based on NHANES 2003–2004 data, i.e., for individuals 20 years and older, mean total arsenic, DMA, and arsenobetaine were 8.64, 3.79, 1.79 μg/g creatinine, respectively (Caldwell et al., 2009). The results of the current analysis were also consistent with previous reports that have indicated that urinary arsenic concentrations in cigarette smokers and non-consumers of tobacco are not different (Gebel et al., 1998; Heck et al., 2009; Richter et al., 2009) and in cigarette smokers, SLT consumers, and non-consumers of tobacco (Naufal et al., 2011). Additionally, results from Naufal et al. (2011) showed that in cigarette smokers, the relationship between urine total arsenic and serum cotinine (a metabolite of nicotine and marker of tobacco exposure) was negative, and in SLT consumers, urinary total arsenic was not correlated with serum cotinine. This provides additional evidence of no relationship between arsenic and (frequency or intensity of) tobacco consumption. Of note, results from human autopsy studies have additionally indicated no differences in arsenic concentrations in lung tissues of smokers and non-smokers (Gerhardsson and Nordberg, 1993; Kraus et al., 2000; Wester et al., 1981). It is possible that due to the relatively low concentrations of arsenic in tobacco (compared with other metals, for example (Counts et al., 2005; Pappas et al., 2008)), differences in urine arsenic levels in tobacco consumers and non-consumers are not apparent.

Notwithstanding the fact that urine arsenic levels in non-consumers of tobacco were not different from (or greater than) those levels in consumers of tobacco, review of the available epidemiology data (ATSDR, 2007) indicated that the lowest identified urine arsenic concentration associated with adverse human health effects was 39.1 μg/g creatinine, for lung cancer. This concentration is approximately 5-fold or more higher than the urine concentrations actually observed in cigarette smokers and SLT consumers (and non-consumers). It is possible that detectable adverse health effects in some epidemiology studies may have been limited by sample size and/or duration of follow-up. Additionally, results of the PRA (Table 3) indicated that: (1) average non-cancer hazards due to arsenic from cigarette smoking and SLT consumption would not be expected to be a threat to public health (i.e., HQ < 1); and (2) the mean calculated cancer risk due to arsenic from cigarette smoking and SLT consumption were less than the upper bound of the acceptable cancer risk range, 10^{-4}.

Strengths of the evaluation presented here include incorporating the review of biomonitoring data from NHANES, exposure data from the epidemiology literature, and a PRA analysis. NHANES is a well-established biomonitoring program in the US. This data set provides a large sample, which is designed to be representative of the US population, and individual level data are available by consumption category (i.e., cigarette smokers, SLT consumers, and non-consumers of tobacco). Due to the availability of individual level data, it was possible to control for demographic and other factors (i.e., gender, race/ethnicity, BMI, arsenobetaine, creatinine), which were significantly associated with urine arsenic concentrations, when arsenic levels in the three tobacco exposure groups were estimated (Table S-7). A previous report indicated that urinary total arsenic concentration may also vary according to protein consumption (Heck et al., 2009). In a separate analysis (data not shown), both protein and seafood consumption were included in the regression model, but these dietary covariates did not significantly alter the results and thus were not included in the final model.

PRA is a scientific evidence based analysis that can be used as a decision tool to quantify the potential impact of a defined risk or hazard. To varying degrees, similar risk assessment methodologies have been used to estimate non-cancer hazards and cancer risks of chemicals in occupational and environmental settings (e.g., WHO, 2011), as well as in cigarette MSS and SLT products (e.g., Ayo-Yusuf and Connolly, 2011; Xie et al., 2012). In this application, PRA incorporated a range of potential exposures to arsenic from consumption of tobacco products, as well as a range of human characteristics related to exposure to tobacco products, an improvement over most previously published calculated hazard and risk estimates for tobacco products.

Limitations in the analysis presented here are also recognized. Detailed discussion of arsenic speciation (e.g., potential differential toxicities and concentrations of species) was beyond the scope of this presentation. NHANES data may be subject to sampling and non-sampling error (CDC, 2005). Interview questionnaire data are based on self-report by participants and may be subject to recall bias and/or misunderstanding. Additionally, laboratory data may be subject to measurement variation. This analysis, however, minimized some tobacco category misclassification by confirming non-consumers of tobacco using a serum cotinine cut-off value, and participants reported consumption under actual conditions of use (i.e., as opposed to experimental conditions). Similar to the US population, tobacco consumers are a fraction of NHANES participants, and of tobacco consumers, the majority is cigarette smokers. Therefore, sample sizes for the SLT consumers were relatively small (<100). Snuff and chewing tobacco categories were combined due to small sample sizes, recognizing that differences in these SLT product categories exist (e.g., in product composition).

Whether any individual constituent in SLT or cigarette MSS has a direct and independent effect on the development of tobacco consumption related disease is not clear. Notwithstanding this fact, reducing the levels of known toxicants in tobacco and/or reducing human exposure to known toxicants from consumption of tobacco products may be relevant to the discussion of risk modification of tobacco products (FSPTCA, 2009). Although the potential role of chemical and toxicological interactions between arsenic and other chemical constituents of SLT and cigarette MSS is currently unknown, results of the analysis presented here indicated that arsenic exposure was not impacted by tobacco consumption and it is possible that arsenic may not be independently associated with adverse health effects in tobacco consumers.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2012.07.007.

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