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TSNA Exposure: Levels of NNAL among Canadian Tobacco Users

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Declaration of interests
The authors have no competing interests to declare.

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Keywords
Tobacco control, population studies, policy

ABSTRACT

Introduction

Tobacco smoke contains more than 7,000 chemicals, including known carcinogens, such as TSNAs. TSNA levels in cigarettes vary considerably within and across markets; however, the extent to which these different TSNA levels translate into differences in human exposure and risk remains unclear. The current study sought to examine TSNA exposure among Canadian tobacco users.

Methods

Data from the 2007-2009 Canadian Health Measures Survey were used to measure levels of urinary NNAL, a metabolite of the TSNA NNK, among tobacco users (n = 507). Geometric mean concentrations of total urinary NNAL and creatinine-corrected total urinary NNAL were calculated. A linear regression model was used to examine predictors of urinary levels of NNAL.

Results

The mean population level of total urinary NNAL and creatinine-corrected total urinary NNAL were 71.2 pg/mL and 82.0 pg/mg creatinine, respectively. NNAL levels were higher among older respondents ($p = 0.02$), among females ($p = 0.04$), and among those with greater daily cigarette consumption ($p < 0.0001$), greater levels of urinary free cotinine ($p < 0.0001$), and greater levels of urinary creatinine ($p < 0.0001$). Overall, the mean level of urinary total NNAL among Canadian tobacco users was approximately one-fourth that of their US counterparts.

Conclusions

The study findings provide the first nationally representative characterization of TSNA exposure among Canadian tobacco users. Although the findings indicate marked differences in TSNA exposure between Canadian and American populations of tobacco users, it is not known whether these differences in exposure translate into differences in risk.

INTRODUCTION

Tobacco use remains the leading risk factor for preventable disease in Canada (Krueger et al, 2014). Despite substantial declines in smoking prevalence, more than 4 million Canadians continue to smoke (Reid et al, 2013). The economic burden from tobacco use in Canada is estimated at $21.3 billion (Krueger et al, 2014).

Tobacco smoke contains more than 7,000 chemicals, including more than 60 known carcinogens, such as benzene, polycyclic aromatic hydrocarbons (PAHs), heavy metals, and tobacco specific nitrosamines (TSNAs) (International Agency for Research on Cancer [IARC], 2004; US Department of Health and Human Services [USDHHS], 2010). TSNAs are known human carcinogens (IARC, 2004) that have been implicated in the development of human cancers (Hecht, 2002; Gray and Boyle, 2004) and include NNK [4-(methylnitrosamino-1-(3-pyridyl)-1-butanone], NNN [N'-nitrosonornicotine], N-nitrosoanabasine, and N-nitrosoanatabine. Although small quantities of TSNAs are found in freshly harvested or green tobacco, TSNAs are predominantly formed during the curing and processing of tobacco, and through combustion that occurs when cigarettes are smoked (IARC, 2004).

At present, there are wide variations in the TSNA levels in cigarettes across countries, which are largely determined by differing growing conditions, use of different tobacco blends, and differences in manufacturing practices. For instance, the Canadian cigarette market consists almost exclusively of Virginia flue-cured tobacco, while the market in the United States (US) is dominated by American blend cigarettes, which consist of a mixture of flue-cured tobacco, air-cured or Burley tobacco, sun-cured or Oriental tobacco, and reconstituted tobacco (IARC, 2004). Due to the fact that Virginia flue-cured tobacco has substantially lower levels of TSNAs compared to the tobacco in American blend cigarettes, the majority of Canadian cigarette brands

contain lower levels of these carcinogens. An analysis of the Canadian tobacco market reported substantially higher nitrosamine levels in imported brands compared to domestic brands. Specifically, total TSNA levels were more than 2.5 times higher, while levels of NNN were more than six times higher in imported brands, which are primarily from the United States (Hammond & O'Connor, 2008). Changes in tobacco curing methods also have an important influence on TSNA levels. For instance, use of heat-exchange methods in indirect-fired barns for Virginia flue-cured tobacco has been shown to significantly reduce TSNA formation compared to the use of propane gas heaters used in direct-fired barns or kilns (Rickert et al, 2008).

Despite the existing variation in TSNA levels in cigarettes within and across markets, the extent to which this variation translates into differences in human exposure and risk remains unclear. Firstly, variability exists with respect to the quantity of constituents "transferred" from whole tobacco to tobacco smoke, which is influenced by cigarette design parameters, such as filter ventilation (Hammond & O'Connor, 2008). For instance, in the analysis of domestic versus imported cigarette brands in the Canadian market noted earlier, NNK levels were similar in the unburnt "whole" tobacco, but twice as high in the smoke emissions of imported brands as measured using the standard ISO regimen (Hammond & O'Connor, 2008). Secondly, standardized laboratory-based machine measures of tobacco smoke constituents fail to take into consideration "smoking topography" or human adjustments in puffing behaviour, which enable smokers to consistently acquire a desired level of nicotine (Jarvis et al, 2001; US Department of Health and Human Services, 2001; Hammond, Fong, Cummings & Hyland, 2005; Hammond & O'Connor, 2008). For example, it is well established that machine-based measures of nicotine emissions in smoke do not predict biomarkers of nicotine uptake among smokers (Jarvis et al,

2001). Therefore, differences in the TSNA levels in either unburnt tobacco or tobacco smoke may not necessarily translate into differences in human exposure.

Human TSNA exposure is typically measured using biomarkers, such as NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol], a metabolite of NNK (Hecht, 2002). A recent industry-sponsored review of NNAL levels among US cigarette smokers indicated a downward trend in urinary NNAL levels over an 18-year period; however, the studies did not include probability-based samples (Appleton et al, 2014). In order to assess actual levels of human TSNA exposure, robust population-level data are needed. Xia et al (2011) provided the only population-based description of TSNA exposure among American smokers through use of measures collected through the National Health and Nutrition Examination Survey (NHANES) in 2007-2008. Mean urinary total NNAL among tobacco users was found to be 299 pg/mL, with significantly higher rates among females and non-Hispanic white smokers. In addition, serum cotinine and urinary creatinine were found to be significant predictors of NNAL.

Human TSNA exposure data for the Canadian context remains very limited. To date, only one study has examined exposure of Canadian smokers to NNK, alongside smokers from Australia, the United Kingdom, and the United States (Ashley et al, 2010). The study calculated mouth-level exposure of NNN and NNK from used cigarette butts for adult daily smokers. Compared to Canadian smokers, American smokers had 10-12 times and 2-3 times the mouth-level of exposure of NNN and NNK, respectively (Ashley et al, 2010). The study findings also indicated that subjects with higher levels of mouth-level exposure of NNK in smoke had higher levels of NNAL in their urine. In addition, the results reflected patterns across cigarette markets, with significantly lower levels of urinary NNAL found in Canada and Australia, in contrast to the United States, as per established differences in TSNA levels in respective cigarettes (Ashley

et al, 2010). However, the study findings are limited in their generalizability due to a relatively small sample size, meaning actual levels of human exposure at the population level remain unquantified.

The current study sought to examine TSNA exposure at the population level in Canada by assessing nationally representative measures of urinary NNAL. The primary objectives of the study were to: 1) characterize levels of NNAL in Canadian tobacco users; 2) examine whether sociodemographic variables and tobacco use are associated with NNAL levels; and 3) examine associations between NNAL levels and nicotine metabolites.

METHODS

Study Design

Data source

The Canadian Health Measures Survey (CHMS) is a nationally representative, cross-sectional survey administered biennially to the Canadian household population aged 6-79 years. Persons living on Indian Reserves or Crown lands, residents of institutions, full-time members of the Canadian Forces, and residents of certain remote regions were excluded from the survey. Overall, a total of 5,604 participants were examined, representing approximately 97% of the Canadian population.

The survey was designed to provide national-level estimates for five age groups (6-11, 12-19, 20-39, 40-59, and 60-79 years) for each sex. To achieve this goal, a multi-stage sampling strategy was used. The area frame of Canada's Labour Force Survey (LFS) was used to create collection sites for the CHMS. The LFS geographic units were also grouped with respect to provincial and census metropolitan-area boundaries and population density criteria. In total, 15

data collection sites were included, allocated by region: Atlantic (1), Quebec (4), Ontario (6), Prairies (2), and British Columbia (2). Within each region, sites were sorted according to the size of their population and whether or not they belonged to a census metropolitan area. Sites were randomly selected using a systematic sampling method with probability proportional to the size of each site's population. Approximately 350 reporting units per site participated in the survey. Within each of the 15 selected sites, the list of the Census 2006 dwellings were used as a frame. Dwellings were stratified according to the five CHMS age groups using the date of birth of household members present at the time of the Census. The sample was allocated in each stratum in such a manner as to obtain an equal number of respondents by age group. Participants were selected at random from within each household. A full description of the survey methodology is available online (Statistics Canada, 2011).

Survey weights were assigned to each survey respondent, corresponding to the number of people represented by the respondent in the population as a whole. As mentioned earlier, the CHMS used two sampling frames for selecting its sample: an area frame of geographic units (clusters) for constructing and selecting collection sites, and an area frame of dwellings within each site. In accordance with the weighting strategy, the selection weights for collection sites were multiplied by the selection weights for dwellings (households), adjusted for non-response. Following the conversion of household weights into person weights, the latter were adjusted for non-response at the interview stage and the mobile examination centre stage, and with several other adjustments, this weight became the final person weight. Details of the survey weights are available online (Statistics Canada, 2011).

Protocol

Data were collected from March 2007 through February 2009. Data collection involved a combination of a personal interview using a computer-assisted interviewing method, and a visit to a mobile examination centre (MEC) for collection of physical measures. During the household interview, data regarding sociodemographic characteristics and smoking behavior were collected. Spot mid-stream urine samples (approximately 60 mL) were collected at the MEC. Standardized procedures were developed for the collection of urine specimens, processing and aliquoting and for shipping biospecimens to the testing laboratory at the Centre de toxicologie du Québec of the Institut National de Santé Publique du Québec (INSPQ) for analyses. The INSPQ is accredited under ISO 17025 and followed standardized procedures that were developed for the assays and techniques performed in its laboratory.

Of the households selected for the CHMS, 69.6% agreed to participate. In each responding household, one or two members were selected; 88.3% of selected household members completed the household interview, and 84.9% of the responding household members participated in the subsequent MEC component. The overall response rate, after adjusting for the sampling strategy, was 51.7%. Ethics approval for the CHMS was obtained from the Health Canada Research Ethics Board.

Tobacco Sub-sample

A sub-sample (n = 2,481) of clinic participants aged 12-79 years were randomly selected for the analysis of urinary nicotine, nicotine metabolites, and NNAL. The sub-sample was allocated by CHMS age group and by smoking status derived from the respondents' answers to the household questionnaire. The sub-sample was selected among persons who provided urine and consented to storage of their urine sample for future health studies. Adjustment factors were

applied to respondents in this sub-sample to account for those who did not provide urine, did not consent, or on whom nicotine analysis was not performed. Of the 4,530 participants aged 12-79 years who reported to the CHMS MECs for physical measurement, 4,356 participants provided urine and consented to storage of their urine sample for future health studies. The tobacco sub-sample was selected among these participants, with a combined response rate of 50.1%.

Measures

Demographic and Smoking Behaviour Measures

Self-reported age, gender and ethnicity were measured as follows: age (12-19, 20-44, 45-64, 65-79 years); gender (male, female); and ethnicity (White, Aboriginal, Other). During the household questionnaire, respondents who reported smoking cigarettes were asked to indicate the number of cigarettes smoked per day, and whether they had used alternative tobacco products in the past month (cigars, pipes, snuff, chewing tobacco).

Laboratory Methods and Measures

Urinary cotinine was extracted from urine on solid phase extraction plates with a mixed-mode cation exchange and reversed phase medium using a Janus robotic station. The extract was brought to dryness, taken in the mobile phase and analyzed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS-MS) in the positive MRM mode. For free cotinine, the limit of detection and limit of quantification were 0.59 ng/mL and 2.0 ng/mL, respectively, and reproducibility was 14%.

For analysis of total NNAL (free NNAL plus NNAL-glucuronide), urine was hydrolyzed enzymatically with β-glucuronidase. The sample was extracted on a solid phase extraction cartridge with a supported liquid extraction medium, and then back extracted under acidic

conditions. The extract was then extracted again on a solid phase extraction cartridge with a molecular imprinted polymer medium specifically designed for the selective extraction of NNAL. The extracts were brought to dryness, taken in the recovery solvent and analyzed by ultra performance liquid chromatography coupled to Waters XEVO TQ tandem mass spectrometry (UPLC-MS-MS) in MRM mode with an "electrospray" source in positive mode and MassLynx software. For total NNAL, the limit of detection and limit of quantification were 1.3 pg/mL and 4.3 pg/mL, respectively, and reproducibility was 3.0%. Measures of urine creatinine were also available from the MEC visits. Creatinine was measured using the colorimetric end-point Jaffé method on a Hitachi chemistry autoanalyzer. The limit of detection for creatinine was 0.31 mmol/L.

Statistical Analyses

Tobacco users were identified on the basis of a urinary free cotinine concentration of 50 ng/mL or higher (SRNT Subcommittee on Biochemical Verification, 2002) and self-reported consumption of a minimum of one cigarette per day. Geometric mean concentrations of urinary total NNAL and creatinine-corrected urinary total NNAL were calculated for tobacco users. Prior to conducting multivariate analyses, measures of urinary free cotinine and urinary total NNAL were log-transformed in order to reduce the skewness in their distributions. A linear regression model was fitted, with the log of urinary NNAL as the dependent variable, and age, gender, ethnicity, daily cigarette consumption, use of alternative tobacco products, log of urinary free cotinine, and urinary creatinine included as independent variables. The level of significance was set at $\alpha = 0.05$. All analyses were conducted using SPSS Version 21 (IBM, Illinois).

RESULTS

The tobacco sub-sample consisted of 2,481 participants. Of these, 2,352 participants had a valid cotinine measure. Using the urinary free cotinine cut-point of 50 ng/mL, we identified 1,728 non-tobacco users and 624 tobacco users. Fifty-five self-reported tobacco users were excluded as a result of the urinary free cotinine cut-point. Among those identified as tobacco users using the cut-point, a further 117 who denied smoking at least 1 cigarette per day were excluded. Thus, 507 participants were identified as tobacco users, on the basis of their urinary free cotinine level and self-reported daily consumption of cigarettes, and were included in the analyses. Although the majority of these were cigarette smokers, 50 reported use of alternative tobacco products. Due to the fact that all forms of tobacco are sources of TSNAs, all 507 tobacco users were included in the analyses. Weighted sample characteristics are presented in Table 1. The identified tobacco users had a geometric mean concentration (95% confidence interval) of urinary free cotinine of 1,283.81 (1,184.40, 1,391.55) ng/mL and consumed an average of 14 ± 9 cigarettes per day.

Geometric means of total urinary NNAL (uncorrected, in pg/mL) and creatinine-corrected total urinary NNAL (creatinine corrected, in pg/mg creatinine) are presented in Table 2. The mean population level of total urinary NNAL (95% confidence interval) was 71.2 (63.1, 80.4) pg/mL. Following adjustment for creatinine, the mean population level of creatinine-corrected total urinary NNAL was 82.0 (74.4, 90.4) pg/mg creatinine.

A linear regression model examining urinary total NNAL was conducted, adjusting for age, sex, ethnicity, daily cigarette consumption, use of alternative tobacco products, urinary free cotinine, and urinary creatinine, the results of which are presented in Table 3. NNAL levels were higher among female respondents ($p = 0.04$), among respondents with greater daily cigarette consumption ($p < 0.0001$), and among respondents with greater levels of urinary free cotinine (p

< 0.0001) and urinary creatinine ($p < 0.0001$). NNAL levels were also associated with age ($p = 0.02$): respondents aged 20-44 years had significantly lower NNAL levels than those aged 45-64 years ($p = 0.03$), and those aged 65-79 years ($p = 0.01$). There were no significant differences for ethnicity and use of alternative tobacco products.

DISCUSSION

The findings provide the first nationally representative characterization of TSNA exposure among Canadian tobacco users. Factors that predicted urinary NNAL concentrations included age, sex, daily cigarette consumption, urinary free cotinine, and urinary creatinine. Significant relationships between NNAL concentrations and female gender, daily cigarette consumption, urinary free cotinine and urinary creatinine are consistent with findings among American tobacco users (Xia et al, 2011). The current findings also revealed significant differences in urinary NNAL across age, with higher NNAL levels among older smokers. Given that the analysis also adjusted for daily cigarette consumption, it is unclear whether the differences in age reflect different smoking patterns or brand preferences. In contrast to the findings of Xia et al (2011), no significant differences were found across ethnicity after adjusting for covariates. Importantly, these data establish a basis for future surveillance and evaluation of trends in exposure over time within and across populations.

The findings suggest marked differences in TSNA exposure between Canadian and US tobacco users. Overall, the mean level of urinary total NNAL among Canadian tobacco users was approximately one-fourth that of their US counterparts (Xia et al, 2011). This marked difference is consistent with the findings of Ashley et al (2010), and demonstrates the feasibilty of reducing TSNA levels in cigarettes. The need for effective tobacco product regulation has been recognized by the World Health Organization (WHO) Framework Convention on Tobacco

Control, Articles 9 and 10 (WHO, 2003). Recommendations put forth by the WHO Study Group on Tobacco Product Regulation (TobReg) call for mandated lowering of toxicants in cigarette smoke, including levels of TSNAs (WHO, 2008). As of 2009, the US Food and Drug Administration (FDA) has been granted authority to regulate the manufacture, distribution, and marketing of tobacco products through the Family Smoking Prevention and Tobacco Control Act (FDA, 2013). Specifically, the FDA has authority to establish standards for tobacco products as appropriate to protect public health, which could potentially include regulating TSNA levels.

Although it may seem intuitive to set limits for TSNA levels in cigarette tobacco, it remains unclear whether the differences between US and Canadian cigarettes translate into meaningful differences in risk for these populations. Although some epidemiological studies have shown a direct association of NNK exposure with risk of lung cancer in smokers (Church et al, 2009; Yuan et al, 2009), there is a lack of epidemiological data indicating differential lung cancer rates among smokers across markets with different tobacco blends and TSNA levels. In addition, because tobacco and tobacco smoke are comprised of many harmful constituents, changes made to levels of TSNAs must be considered in the context of exposure to other toxins and their impact on human health. For instance, although Canadian cigarette emissions consist of lower TSNA levels, other toxic constituents are notably higher, including polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (Hammond & O'Connor, 2008). Indeed, a "negative" relationship between TSNA and PAH emissions has been noted, wherein selective reduction of PAHs resulted in unintended increases in TSNA emissions (King et al, 2007). Thus, the threat of "risk swapping" due to counteracting causes of emissions of harmful agents in cigarette smoke must be carefully considered in any regulatory strategy.

Even if it were possible to selectively reduce TSNAs without increasing exposure to other toxicants, the benefit may be negligible given the levels of exposure associated with cigarette smoking; as some commentators have noted, the risk differential may be akin to jumping from a 19-storey rather than a 20-storey building, with the same outcome in either case. In the absence of a public health benefit from toxicant reductions, regulatory limits have the potential to be counter-productive. For example, TSNA limits may provide consumers with false reassurances about the the risk of tobacco products, much in the same way as "lower tar" cigarettes provided false reassurance to health concerned smokers (USDHHS, 2001). Overall, any regulatory strategy that seeks to make cigarette smoke less harmful to inhale must proceed very carefully.

A key strength of the current study is the use of biomarkers of exposure, which provide more reliable measures of human exposure compared to product measures alone. In addition, the study utilized nationally representative data, thereby providing robust measures of exposure for Canadian tobacco users. However, there were limitations relating to the nature of secondary data analysis. Firstly, although the study inclusion criteria aimed to identify tobacco users on the basis of urinary cotinine measures and self-reported daily consumption of cigarettes, we were unable to exclude individuals who use nicotine replacement therapy. Similarly, although we were able to identify dual users of cigarettes and alternative tobacco products, we were unable to distinguish users of specific types of alternative tobacco products and examine their exposure to NNK. In addition, we suggest using caution in interpreting findings for ethnicity groups due to small sample sizes. Finally, we were limited in the discussion of our findings in relation to the literature, given that the wide variety of methods and measures used to assess TSNA exposure makes comparisons across studies difficult.

CONCLUSIONS

The study findings provide the first nationally representative characterization of TSNA exposure among Canadian tobacco users. Overall, the mean level of urinary total NNAL among Canadian tobacco users was approximately one-fourth that of their US counterparts. These findings are consistent with other studies suggesting marked differences in TSNA exposure between Canadian and American populations of tobacco users, despite similar patterns of tobacco use. In the absence of epidemiological data, it is not known whether these differences in exposure translate into differences in risk. Importantly, these data establish a basis for future surveillance and evaluation of trends in exposure over time within and across populations.

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Table 1: Sample characteristics of Canadian tobacco users[a,b]

Characteristic		%	n
All			507
Age (years)	12-19	5.1	26
	20-44	49.1	249
	45-64	38.4	194
	65-79	7.4	37
Sex	Male	52.7	267
	Female	47.3	240
Ethnicity	White	88.2	447
	Aboriginal	6.2	32
	Other	5.5	28
Cigarettes per day	< 5	10.8	55
	5-10	28.8	146
	11-20	42.1	213
	> 20	18.3	93
Use of alternative tobacco products	No	90.2	457
	Yes	9.8	50

[a] Analyses were conducted using weighted data

[b] Tobacco users defined as having urinary free cotinine concentrations ≥ 50 ng/mL and self-reported daily cigarette consumption ≥ 1

Table 2: Geometric means of total urinary NNAL and creatinine-corrected total urinary NNAL among Canadian tobacco users[a,b]

Characteristic		NNAL (95% CI) [pg/mL]	CC-NNAL (95% CI) [pg/mg creatinine]
All		71.2 (63.1, 80.4)	82.0 (74.4, 90.4)
Age (years)	12-19	94.8 (58.9, 152.4)	62.6 (41.7, 93.9)
	20-44	61.5 (51.1, 73.9)	64.7 (56.3, 74.3)
	45-64	83.2 (69.1, 100.2)	109.4 (94.1, 127.3)
	65-79	68.8 (45.5, 103.9)	107.0 (73.7, 155.3)
Sex	Male	93.7 (80.5, 109.0)	76.0 (66.9, 86.2)
	Female	52.5 (43.6, 63.2)	89.3 (76.7, 103.8)
Ethnicity	White	76.2 (67.6, 86.0)	87.5 (79.2, 96.7)
	Aboriginal	53.6 (29.1, 99.0)	67.5 (47.0, 96.9)
	Other	33.1 (14.7, 74.9)	36.2 (19.9, 65.6)
Cigarettes per day	< 5	19.4 (12.3, 30.6)	25.7 (18.7, 35.3)
	5-10	56.3 (46.3, 68.3)	60.5 (52.3, 69.9)
	11-20	83.0 (70.2, 98.2)	99.7 (86.9, 114.3)
	> 20	156.6 (122.3, 200.5)	168.0 (137.8, 204.9)
Use of alternative tobacco products	No	68.8 (60.4, 78.5)	83.0 (74.7, 92.3)
	Yes	97.0 (74.3, 126.6)	73.1 (57.7, 92.5)

[a] Analyses were conducted using weighted data

[b] Tobacco users defined as having urinary free cotinine concentrations ≥ 50 ng/mL and self-reported daily cigarette consumption ≥ 1 (n = 507)

Table 3: Linear regression analysis examining factors associated with total urinary NNAL among Canadian tobacco users[a,b]

Variables		Beta (SE)	Significance
Intercept		- 0.05 (0.25)	$p = 0.83$
Age (years)		$\chi^2 = 10.25$	$p = 0.02$
	20-44 vs 12-19	- 0.12 (0.10)	$p = 0.23$
	45-64 vs 12-19	- 0.03 (0.10)	$p = 0.78$
	65-79 vs 12-19	0.09 (0.12)	$p = 0.47$
	45-64 vs 20-44	0.09 (0.04)	$p = 0.03$
	65-79 vs 20-44	0.12 (0.08)	$p = 0.01$
	65-79 vs 45-64	0.12 (0.08)	$p = 0.12$
Sex		$\chi^2 = 4.27$	$p = 0.04$
	Female vs Male	0.09 (0.04)	$p = 0.04$
Ethnicity		$\chi^2 = 4.46$	$p = 0.11$
	Aboriginal vs White	- 0.10 (0.08)	$p = 0.22$
	Other vs White	- 0.16 (0.09)	$p = 0.07$
	Other vs Aboriginal	- 0.07 (0.11)	$p = 0.55$
Cigarettes per day		$\chi^2 = 39.51$	$p < 0.0001$
	5-10 vs < 5	0.15 (0.07)	$p = 0.04$
	11-20 vs <5	0.23 (0.08)	$p < 0.01$
	>20 vs <5	0.47 (0.08)	$p < 0.0001$
	11-20 vs 5-10	0.08 (0.05)	$p = 0.08$
	>20 vs 5-10	0.32 (0.06)	$p < 0.0001$
	>20 vs 11-20	0.23 (0.05)	$p < 0.0001$
Use of alternative tobacco products		$\chi^2 = 0.14$	$p = 0.71$
	Yes vs No	- 0.03 (0.07)	$p = 0.71$
Log urinary cotinine		0.57 (0.06)	$p < 0.0001$
Urinary creatinine		0.04 (0.01)	$p < 0.0001$

[a] Analyses were conducted using weighted data

[b] Tobacco users defined as having urinary free cotinine concentrations ≥ 50 ng/mL and self-reported daily cigarette consumption ≥ 1 (n = 507)