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Research Article

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Carcinogen Exposure among Canadian Tobacco Users: Changes in NNK Exposure from 2007–2009 through 2012–2013

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Abstract

Background: Tobacco-specific nitrosamines (TSNAs) are a class of carcinogens found in tobacco products, whose levels can vary considerably depending on tobacco blends and manufacturing processes. The current study examined whether recent increases in levels of the TSNA NNK [4-(methylnitrosamino-1-(3-pyridyl)-1-butanone] in Canadian cigarettes translated into differences in exposure among Canadian tobacco users.

Methods: Nationally representative data from the Canadian Health Measures Survey (CHMS) were used to measure levels of total urinary NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol], a metabolite of the TSNA NNK, among tobacco users. Data from CHMS Cycle 3 (2012–13) were used to examine NNAL, and linear regression was used to examine predictors. Data from CHMS Cycle 1 (2007–09) and Cycle 3 (2012–13) were used to examine changes in NNAL over time.

Results: From 2007–2009 through 2012–2013, levels of creatinine-corrected NNAL increased by 64% ($P < 0.0001$). Levels of NNAL in 2012–2013 were higher among older respondents ($P = 0.04$), among females ($P = 0.03$), among respondents identifying as "white" and "Aboriginal" ($P < 0.0001$), and among those with greater daily cigarette consumption ($P < 0.001$), as well as greater levels of urinary free cotinine ($P < 0.0001$) and urinary creatinine ($P < 0.0001$).

Conclusions: The findings indicate that exposure to the TSNA NNK among Canadian tobacco users has increased considerably from 2007–2009 through 2012–2013, in parallel to changes in TSNA levels in Canadian cigarettes. In the absence of epidemiologic data, it is unclear whether this change translates into increased risk.

Impact: The study findings have potential implications for tobacco manufacturers, who bear a responsibility to reduce levels of tobacco carcinogens to the full extent possible. *Cancer Epidemiol Biomarkers Prev; 27(3); 1–6. ©2018 AACR.*

Introduction

Despite substantial declines in smoking prevalence over several decades, tobacco use remains the leading preventable cause of death in Canada (1). As of 2015, 13.0% of Canadian adults reported smoking (2).

Tobacco use exposes smokers to more than 7,000 chemicals, including 70 known carcinogens (3, 4). Many of these harmful tobacco smoke constituents have been associated with increased risk for the development of cardiovascular disease, respiratory diseases, and various cancers (4). Tobacco-specific nitrosamines (TSNAs) are a family of potent carcinogens that have been implicated in the development of human cancers. TSNAs include NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], NNN [N′-nitrosonornicotine], N-nitrosoanabasine, and N-nitrosoanatabine. These constituents are predominantly formed during the curing and processing of tobacco, and through combustion that occurs when cigarettes are smoked (3, 4).

Levels of TSNAs in cigarettes can vary considerably across markets. Historically, the Canadian market has been characterized by lower levels of TSNAs compared with other markets for two primary reasons. First, cigarettes made with Virginia flue-cured tobacco, which has notably lower levels of NNK and other TSNAs compared with U.S.-blended cigarettes, dominate the market (3). Second, government-led changes in tobacco manufacturing and processing in 2000 introduced heat exchangers for tobacco curing, which lowered levels of TSNAs in Canadian cigarettes (5–7). Differences in the TSNA levels measured in tobacco have been associated with differences in biomarkers of exposure among smokers. Evidence from nationally representative population-level data of tobacco users in the United States and in Canada collected from 2007 through 2009 indicates that mean levels of urinary total NNAL, a metabolite of NNK, among Canadian tobacco users was approximately one-fourth that of their U.S. counterparts (8, 9). Specifically, mean urinary total NNAL among U.S. tobacco users was found to be 299 pg/mL (95% CI, 253–353), compared with 71 pg/mL (95% CI, 63–80) among Canadian tobacco users (8, 9).

However, evidence indicates that TSNA levels in tobacco have changed in recent years. TSNA levels in whole ("unburned") tobacco and smoke emissions of cigarettes sold in Canada increased from 2007 through 2011–12, following initial reductions over the previous two years (10). For example, levels of NNK in whole tobacco decreased from 49 ng/cigarette in 2005 to 3 ng/cigarette in 2007, and then increased to 44 ng/cigarette in 2012. In addition, NNK smoke emissions were generally constant

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between 2005 and 2009 (mean level: 3 ng/cigarette), although significant increases were recorded in 2010 (13 ng/cigarette) and 2011 (14 ng/cigarette; ref. 10). Previously published data collected through Cycle 1 (2007–09) of the Canadian Health Measures Survey (CHMS) provided the first nationally representative characterization of TSNA exposure among Canadian tobacco users (8), and correspond to the early portion of the time period for which TSNA levels in cigarettes sold in Canada were examined. The current study sought to assess TSNA exposure in Canadian tobacco users in the latter portion of this time period (2012–13), and examine whether differences in the TSNA levels of cigarettes sold in Canada translated into differences in human exposure.

Materials and 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 ages 3–79 years. Persons living on reserves, Aboriginal settlements or Crown lands, residents of institutions, full-time members of the Canadian Forces, persons living in Canada's three territories, and residents of certain remote regions were excluded from the survey. Overall, a total of 5,604 and 5,071 participants were examined in Cycle 1 (2007–09) and Cycle 3 (2012–13), respectively, representing approximately 96% of the Canadian population. Given that methodologic details pertaining to CHMS Cycle 1 have been described previously (8, 11), the focus here will be on CHMS Cycle 3.

The survey used a multi-stage sampling strategy to provide national-level estimates for 11 age–sex groups (ages 3–5, both sexes combined; ages 6–11, males and females; ages 12–19, males and females; ages 20–39, males and females; ages 40–59, males and females; and ages 60–79, males and females). The first sampling frame consisted of geographic units created using census geography and grouped with respect to provincial boundaries, metropolitan-area boundaries, health regions, and population density criteria. This sampling frame was used to construct a total of 16 data collection sites, allocated by geographical region (Atlantic, Quebec, Ontario, Prairies, and British Columbia). Within each region, 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. The second sampling frame consisted of a list of dwellings drawn from the 2011 Census. Dwellings were stratified according to 6 age groups, using the date of birth of household members present at the time of the Census. Participants were selected at random from within each household. Survey weights were assigned to each survey respondent, corresponding to the number of people represented by the respondent in the population as a whole. A full description of the survey methodology and details of the survey weights are available online (11).

Protocol. Cycle 3 data were collected from January 2012 through December 2013. 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 first-catch 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, 74.1% agreed to participate. In each responding household, one or two members were selected; 88.4% of selected household members completed the household interview, and 78.8% of the responding household members participated in the subsequent MEC component. The overall response rate, after adjusting for the sampling strategy, was 51.7%. To ensure that internationally recognized ethical standards for human research were met and maintained, review and approval of the CHMS was obtained from the Health Canada and Public Health Agency of Canada Research Ethics Board (REB# 2005-0025). Participation in the CHMS was voluntary and respondents were asked to provide their written informed consent prior to participation in the study.

Tobacco subsamples. Similar to Cycle 1 of the CHMS (8), a subsample of clinic participants ages 12–79 years were randomly selected for the analysis of urinary nicotine, nicotine metabolites, and NNAL. In Cycle 3, the subsample was allocated by CHMS age group, sex, and smoking status derived from the respondents' answers to the household questionnaire. Adjustment factors were applied to respondents in this subsample to account for those who did not provide urine, did not consent, or on whom nicotine analysis was not performed. The overall response rate was 50.3%.

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); sex (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 1.1 ng/mL and 1.4 ng/mL, respectively, and reproducibility was 7.3%.

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-S tandem mass

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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 1.4 pg/mL and 4.8 pg/mL, respectively, and reproducibility was 1.7%. Measures of urine creatinine were also available from the MEC visits. Creatinine was measured using the colorimetric endpoint 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 (12) 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 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. Changes in levels of urinary total NNAL from 2007–2009 and 2012–2013 were examined using independent samples *t* tests. Analyses were conducted using bootstrap weights to account for design-based variance estimation. The level of significance was set at $\alpha = 0.05$. Analyses were conducted using SPSS Version 22 (IBM, Illinois) and SAS Version 9.4.

Results

The CHMS Cycle 3 tobacco subsample consisted of 2,220 participants, 2,215 of whom had a valid cotinine measure. Using the urinary free cotinine cutoff point of 50 ng/mL, we identified 1,532 nontobacco users and 683 tobacco users. Among self-reported tobacco users, 59 were excluded following application of the cutoff point. Among those identified as tobacco users using the cutoff point, a further 122 were excluded due to the fact that they did not report smoking at least 1 cigarette per day. Thus, 561 participants were identified as tobacco users and included in the analyses. Among tobacco users, the majority reported smoking cigarettes, while 61 reported also using alternative tobacco products. Weighted sample characteristics are presented in Table 1. The identified tobacco users had a geometric mean concentration of urinary free cotinine of 922.4 ng/mL (95% CI, 866.8–981.8) and consumed an average of 15.5 (SD = 10) cigarettes per day.

Geometric means of total urinary NNAL (uncorrected, in pg/mL; and creatinine corrected, in pg/mg creatinine) are presented in Table 2. The mean population levels of total urinary NNAL and creatine-corrected total urinary NNAL were 146.9 pg/mL (95% CI, 133.7–161.4), and 134.4 pg/mg creatinine (95% CI, 122.6–146.3), respectively.

Results of the linear regression analysis examining urinary total NNAL in 2012–2013 are presented in Table 3. NNAL levels were higher among female respondents ($P = 0.03$), among respondents with greater daily cigarette consumption ($P < 0.001$), 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 ($P = 0.04$): respondents ages 20–44 years had significantly lower NNAL levels than those ages 45–64 years ($P = 0.02$), and those ages 65–79 years ($P = 0.04$); and with ethnicity ($P < 0.0001$): white and Aboriginal respondents had significantly greater NNAL levels than respondents identifying as "other" ($P < 0.0001$ for both), and Aboriginal respondents had significantly greater NNAL levels compared with white respondents ($P = 0.01$).

Changes in total urinary NNAL from 2007–2009 through 2012–2013 are presented in Tables 4 and 5. Overall, mean levels of creatinine-corrected total urinary NNAL increased by 64% ($P < 0.0001$). Over this time period, NNAL levels increased

Table 1. Sample characteristics of Canadian tobacco users[a,b], 2012–13 ($n = 561$)

Characteristics	*n* (%)
All	561
Age (years)	
12–19	27 (4.9)
20–44	219 (39.1)
45–64	202 (35.9)
65–79	113 (20.1)
Sex	
Male	349 (62.2)
Female	212 (37.8)
Ethnicity	
White	474 (84.4)
Aboriginal	23 (4.1)
Other	64 (11.5)
Cigarettes per day	
<5	75 (13.3)
5–10	128 (22.8)
11–20	231 (41.2)
>20	127 (22.6)
Use of alternative tobacco products	
No	500 (89.1)
Yes	61 (10.9)

[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], 2012–13 ($n = 561$)

Characteristics	NNAL (95% CI) [pg/mL]	CC-NNAL (95% CI) [pg/mg creatinine]
All	146.9 (118.6–181.9)	134.4 (110.1–164.0)
Age (years)		
12–19	126.2 (63.9–249.1)	81.0 (46.1–142.2)
20–44	122.2 (100.8–148.3)	94.5 (71.4–125.1)
45–64	174.4 (125.8–241.7)	188.9 (149.1–239.3)
65–79	160.3 (225.8–223.9)	163.7 (122.0–219.6)
Sex		
Male	154.2 (119.4–199.1)	119.2 (90.2–157.6)
Female	135.6 (94.8–194.0)	163.5 (123.5–216.5)
Ethnicity		
White	166.9 (130.1–214.2)	157.8 (128.2–194.1)
Aboriginal	192.9 (107.2–347.1)	160.6 (99.9–258.3)
Other	52.1 (30.2–89.9)	38.7 (17.9–83.7)
Cigarettes per day		
<5	36.1 (23.0–56.9)	26.9 (13.3–54.5)
5–10	111.4 (74.8–165.9)	115.9 (82.3–163.3)
11–20	201.4 (155.0–261.6)	160.3 (133.0–193.3)
>20	249.1 (182.9–339.2)	290.7 (247.2–341.8)
Use of alternative tobacco products		
No	149.6 (121.8–183.6)	140.2 (117.5–167.2)
Yes	126.9 (77.4–208.0)	95.1 (56.8–159.4)

Abbreviations: CC, creatinine-corrected; CI, confidence interval.
[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.

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Table 3. Linear regression analysis examining factors associated with total urinary NNAL among Canadian tobacco users[a,b], 2012–2013 ($n = 561$)

Variables	Beta (SE)	Significance
Intercept	0.51 (0.17)	$P = 0.003$
Age (years)	$\chi^2 = 8.52$	$P = 0.04$
20–44 vs. 12–19	0.05 (0.08)	$P = 0.52$
45–64 vs. 12–19	0.14 (0.08)	$P = 0.09$
65–79 vs. 12–19	0.14 (0.08)	$P = 0.10$
45–64 vs. 20–44	0.09 (0.04)	$P = 0.02$
65–79 vs. 20–44	0.09 (0.04)	$P = 0.04$
65–79 vs. 45–64	0.00 (0.04)	$P = 0.95$
Sex	$\chi^2 = 4.52$	$P = 0.03$
Female vs. male	0.07 (0.03)	$P = 0.03$
Ethnicity	$\chi^2 = 28.08$	$P < 0.0001$
Aboriginal vs. white	0.21 (0.08)	$P = 0.01$
Other vs. white	−0.22 (0.05)	$P < 0.0001$
Other vs. Aboriginal	−0.43 (0.09)	$P < 0.0001$
Cigarettes per day	$\chi^2 = 140.56$	$P < 0.0001$
5–10 vs. < 5	0.40 (0.05)	$P < 0.0001$
11–20 vs. <5	0.48 (0.06)	$P < 0.0001$
>20 vs. <5	0.67 (0.06)	$P < 0.0001$
11–20 vs. 5–10	0.08 (0.04)	$P = 0.04$
>20 vs. 5–10	0.30 (0.05)	$P < 0.0001$
>20 vs. 11–20	0.22 (0.04)	$P < 0.0001$
Use of alternative tobacco products	$\chi^2 = 0.27$	$P = 0.60$
Yes vs. no	0.03 (0.05)	$P = 0.60$
Log urinary cotinine	0.50 (0.06)	$P < 0.0001$
Urinary creatinine	0.03 (0.01)	$P < 0.0001$

Abbreviation: SE = standard error.
[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.

significantly for adult respondents [including those ages: 20–44 years (46%, $P < 0.0001$), 45–64 years (73%, $P < 0.0001$), and 65–79 years (53%, $P = 0.034$)]; for both male (57%, $P < 0.0001$) and female respondents (83%, $P < 0.0001$); for respondents identifying as "white" (80%, $P < 0.0001$), and as Aboriginal (138%, $P = 0.001$); for respondents consuming 5–10 cigarettes per day (92%, $P < 0.0001$), 10–20 cigarettes per day (61%, $P < 0.0001$), and greater than 20 cigarettes per day (73%, $P < 0.0001$); and for respondents who reported not using alternative tobacco products (69%, $P < 0.0001$).

Discussion

The study findings indicate that exposure to NNK among Canadian tobacco users increased significantly between 2007–2009 and 2012–2013. This change parallels significant increases in levels of NNK in whole tobacco and smoke emissions of cigarettes sold in Canada from 2007 through 2011–2012 (10), indicating that increased levels of TSNAs in cigarettes have translated into increased exposure at the population level.

Levels of urinary NNAL in 2012–2013 were associated with age, sex, daily cigarette consumption, urinary free cotinine, and urinary creatinine. These findings are consistent with research examining tobacco users in Canada and the United States (8, 9). In addition, the current findings revealed a significant relationship between NNAL concentrations and ethnicity, after adjusting for covariates. Although this relationship was not detected in previously published analyses conducted among Canadian tobacco users (8), it is consistent with U.S.-based research by Xia and colleagues (9). However, given that the analysis adjusted for daily cigarette consumption, it is unclear whether differences in NNK exposure by age and ethnicity reflect different smoking patterns or other factors.

Although levels of exposure among Canadian tobacco users in 2012–2013 remain lower than those of U.S. tobacco users in 2007–2008 (9), further comparative evaluations of these markets

Table 4. Geometric means of total urinary NNAL among Canadian tobacco users[a,b], Canadian Health Measures Survey (CHMS) Cycle 1 (2007–09) ($n = 507$) and Cycle 3 (2012–13) ($n = 561$)

Characteristic	CHMS Cycle 1 (2007–09) NNAL (95% CI) [pg/mL]	CHMS Cycle 3 (2012–13) NNAL (95% CI) [pg/mL]	% change[c]	p-value[d]
All	71.2 (52.7–96.1)	146.9 (118.6–181.9)	106	$P < 0.0001$
Age				
12–19	94.8 (57.2–157.0)	126.2 (63.9–249.1)	33	$P = 0.410$
20–44	61.5 (43.8–91.2)	122.2 (100.8–148.3)	99	$P < 0.0001$
45–64	83.2 (61.4–112.7)	174.4 (125.8–241.7)	110	$P < 0.0001$
65–79	68.8 (46.2–102.4)	160.3 (225.8–223.9)	133	$P < 0.0001$
Sex				
Male	93.7 (67.4–130.1)	154.2 (119.4–199.1)	65	$P < 0.0001$
Female	52.5 (37.3–73.8)	135.6 (94.8–194.0)	158	$P < 0.0001$
Ethnicity				
White	76.2 (58.6–99.1)	166.9 (130.1–214.2)	119	$P < 0.0001$
Aboriginal	53.6 (13.1–219.7)	192.9 (107.2–347.1)	260	$P = 0.001$
Other	33.1 (7.3–149.6)	52.1 (30.2–89.9)	57	$P = 0.287$
Cigarettes per day				
<5	19.4 (9.0–41.7)	36.1 (23.0–56.9)	86	$P = 0.019$
5–10	56.3 (43.3–73.2)	111.4 (74.8–165.9)	98	$P < 0.0001$
10–20	83.0 (61.0–113.0)	201.4 (155.0–261.6)	143	$P < 0.0001$
>20	156.6 (93.5–262.1)	249.1 (182.9–339.2)	59	$P = 0.002$
Use of alternative tobacco products				
No	68.8 (49.9–94.9)	149.6 (121.8–183.6)	117	$P < 0.0001$
Yes	97.0 (67.4–139.8)	126.9 (77.4–208.0)	31	$P = 0.156$

Abbreviations: CC, creatinine-corrected; CI, confidence interval.
[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.
[c]Percent change calculated as value of NNAL in Cycle 3 minus value of NNAL in Cycle 1, divided by value of NNAL in Cycle 1, multiplied by 100%.
[d]*P* value calculated using independent samples *t* tests.

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Table 5. Geometric means of creatinine-corrected total urinary NNAL among Canadian tobacco users[a,b], Canadian Health Measures Survey (CHMS) Cycle 1 (2007–2009; $n = 507$) and Cycle 3 (2012–2013; $n = 561$)

Characteristics	CHMS Cycle 1 (2007–09) CC-NNAL (95% CI) [pg/mg creatinine]	CHMS Cycle 3 (2012–13) CC-NNAL (95% CI) [pg/mg creatinine]	% change[c]	p-value[d]
All	82.0 (64.4–104.4)	134.4 (110.1–164.0)	64	$P < 0.0001$
Age				
12–19	62.6 (40.0–97.9)	81.0 (46.1–142.2)	29	$P = 0.366$
20–44	64.7 (50.7–82.5)	94.5 (71.4–125.1)	46	$P < 0.0001$
45–64	109.4 (86.6–138.3)	188.9 (149.1–239.3)	73	$P < 0.0001$
65–79	107.0 (68.2–167.8)	163.7 (122.0–219.6)	53	$P = 0.034$
Sex				
Male	76.0 (59.8–96.4)	119.2 (90.2–157.6)	57	$P < 0.0001$
Female	89.3 (68.3–116.7)	163.5 (123.5–216.5)	83	$P < 0.0001$
Ethnicity				
White	87.5 (68.7–111.5)	157.8 (128.2–194.1)	80	$P < 0.0001$
Aboriginal	67.5 (35.8–127.1)	160.6 (99.9–258.3)	138	$P = 0.001$
Other	36.2 (13.5–96.8)	38.7 (17.9–83.7)	7	$P = 0.836$
Cigarettes per day				
<5	25.7 (16.9–39.2)	26.9 (13.3–54.5)	5	$P = 0.826$
5–10	60.5 (47.1–77.7)	115.9 (82.3–163.3)	92	$P < 0.0001$
10–20	99.7 (76.9–129.2)	160.3 (133.0–193.3)	61	$P < 0.0001$
>20	168.0 (122.9–229.8)	290.7 (247.2–341.8)	73	$P < 0.0001$
Use of alternative tobacco products				
No	83.0 (64.5–106.8)	140.2 (117.5–167.2)	69	$P < 0.0001$
Yes	73.1 (56.4–94.6)	95.1 (56.8–159.4)	30	$P = 0.156$

Abbreviations: CC, creatinine-corrected; CI, confidence interval.
[a]Analyses were conducted using weighted data.
[b]Tobacco users defined as having urinary free cotinine concentrations $\geq$ 50 ng/mL and self-reported daily cigarette consumption $\geq$ 1.
[c]Percent change calculated as: value of NNAL in Cycle 3 minus value of NNAL in Cycle 1, divided by value of NNAL in Cycle 1, multiplied by 100%.
[d]*P* value calculated using independent samples t-tests.

cannot be made without more data from the United States. The results presented here highlight the importance of national monitoring and surveillance of tobacco products and smoking behaviour. The assessment of biomarkers of exposure in national health surveys can provide useful toxicologic data regarding tobacco users, and may prove valuable in monitoring exposure among this subpopulation as novel tobacco and nicotine products, such as electronic cigarettes and heat-not-burn products, enter the market.

Although the study findings indicate that differences in the TSNA levels of cigarettes have translated into differences in human-level exposure within the Canadian context, it is not clear whether these differences will in turn translate into meaningful differences in risk for tobacco-related disease. Despite some epidemiologic evidence showing a direct association between NNK exposure and risk of lung cancer among smokers (13, 14), there is no epidemiologic data indicating differential lung cancer rates among smokers across markets or over time with different TSNA levels. In addition, TSNAs are just one family of constituents found in the complex mixture of tobacco smoke, meaning changes in TSNA levels must be considered in the context of exposure to other toxins and their health impacts (4). These issues make the consideration of regulatory strategies targeting specific product constituents, such as those recommended by the World Health Organization Study Group on Tobacco Product Regulation (15), very challenging. Nevertheless, despite the lack of evidence regarding a meaningful threshold for risk, tobacco manufacturers bear a responsibility to reduce the levels of known carcinogens and other toxicants to the full extent possible. This is particularly relevant for TSNAs, given the feasibility of reducing TSNA levels in cigarettes (3), as well as evidence indicating that cigarette manufacturers possess the necessary knowledge and tools to do so (16). Failure to act upon this potential would seriously call into question the industry's stated commitment to minimizing the harm from using a product sold to millions of consumers.

The current study had several strengths. The use of nationally representative data across two time points allowed for a robust analysis of exposure among Canadian tobacco users over time. In addition, the use of biomarkers of exposure provided reliable measures of human exposure, in contrast to product- and machine-based measures. However, the study also faced several limitations. Although the 2012–2013 sample of Canadian tobacco users had a greater proportion of males as compared to that of 2007–2009, the data were weighted to account for sample differences across the years on gender and age (8). Future research should consider alternative methods to control for the confounding effect of creatinine (17), particularly given the substantial differences by sex between the creatinine-adjusted and unadjusted analyses. In addition, although average daily consumption of cigarettes increased slightly from 2007–2009 through 2012–2013, exposure to cotinine decreased over this time period (8), suggesting that consumption of more cigarettes or more intense smoking of cigarettes cannot account for the increased exposure to NNK. Furthermore, significant increases in exposure were observed among almost all examined groups of tobacco users, suggesting a widespread effect. Finally, due to the constraints of secondary data analysis, we were unable to exclude individuals who used nicotine replacement therapy or distinguish between users of specific types of alternative tobacco products.

Conclusions

The study findings indicate that exposure to the tobacco-specific nitrosamine NNK increased considerably from 2007–2009 through 2012–2013. Parallel changes in NNK levels in cigarettes sold in Canada may account for the observed changes

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in human exposure. However, in the absence of epidemiologic data, it is unclear whether this change translates into increased risk for tobacco-related disease. Future research should continue to monitor tobacco use and exposure among the Canadian population, particularly as new tobacco/nicotine products enter the market.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.D. Czoli, D. Hammond
Development of methodology: C.D. Czoli
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.D. Czoli, D. Hammond
Writing, review, and/or revision of the manuscript: C.D. Czoli, D. Hammond
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.D. Czoli

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