Original Investigation

Assessment of Mouth-Level Exposure to Tobacco Constituents in U.S. Snus Consumers

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Abstract

Introduction: When assessing the health risks associated with different tobacco product categories (e.g., combustible and noncombustible), it is important to understand exposure. Snus, a smokeless tobacco product with lower levels of most tobacco-related toxicants than cigarette smoke, has been recently introduced in the United States. The objective of this study was to evaluate the mouth-level exposure (MLE) to selected tobacco constituents from snus by adult consumers of Camel SNUS (CSNUS).

Methods: For 7 days, 53 adult CSNUS consumers used their usual brand styles *ad libitum*, collecting their snus pouches after use. The collected pouches and unused product were analyzed for nicotine, *N*′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*′-nitrosoanabasine (NAB), *N*′-nitrosoanatabine (NATh), benzo[a]pyrene (B[a]P), arsenic, cadmium, chromium, lead, and nickel. MLE was estimated using the difference between the constituent amounts in the used and unused snus products.

Results: CSNUS consumption averaged 3.3 pouches/day or approximately 1.98 g/day. Mean nicotine MLE was 2.8 mg/pouch or 9.4 mg/day. Mean MLE to total tobacco-specific nitrosamines (TSNAs: NNK, NNN, NAB, and NATh) was 171 ng/pouch or 527.7 ng/day. B[a]P MLE averaged 0.2 ng/pouch or 0.68 ng/day.

Conclusions: This study is the first to report snus MLE under normal conditions of use in a group of adult, U.S. snus consumers. On average, approximately 60%–90% of the amounts of nicotine, TSNAs, and B[a]P initially present in a snus pouch remained in the pouch after use by snus consumers in this study. The results from this study provide a preliminary assessment of exposure to constituents present in snus, which is potentially useful in risk assessment.

Introduction

Significant health risks are associated with the use of tobacco products. The risks of tobacco product use vary depending on the type of product used, the duration of product use, and the frequency and manner of use. Risk is greatest for combustible tobacco products, considering cigarette smoking increases the risk for developing cardiovascular and respiratory disease and cancers (U.S. Department of Health and Human Services, 2004). Smokers may reduce the risk for serious diseases by quitting smoking. Recognizing that many cigarette smokers are unwilling to stop using tobacco, or are unsuccessful at quitting tobacco use with or without nicotine replacement or other smoking-cessation therapies, recent debate among members of the public health community has centered around tobacco harm-reduction strategies. The tobacco harm-reduction concept is based on the premise that tobacco-related mortality and morbidity may be decreased without completely eliminating the use of tobacco products (Stratton, Shetty, Wallace, & Bondrant, 2001). One approach toward achieving tobacco harm reduction for smokers who choose to continue using tobacco products is to migrate from cigarette smoking to consuming tobacco products that are less harmful.

For consumers who choose to continue using tobacco, the health risks associated with consumption of smoke-free tobacco products are generally regarded as lower than those associated with smoking cigarettes (Levy et al., 2004; Royal College of Physicians, 2007; Zeller et al., 2009). Snus is an oral, smoke-free product made from finely ground, cured tobacco. Snus has been available in Sweden for many years as a loose form of tobacco and as tobacco portions contained in pouches. Snus is typically placed in the mouth under the upper lip and used without chewing or spitting. During manufacturing, snus tobacco is subjected to a heat treatment process. Because snus tobacco is not subjected to fermentation processes that are reported to be largely responsible for the formation of nitrosamines, snus generally has a lower concentration of carcinogenic nitrosamines in comparison to U.S. moist snuff (Foulds, Ramstrom, Burke, & Fagerström, 2003). Previous studies in Swedish populations suggest that the risks for lung disease, cardiovascular disease, and cancer associated with Swedish snus consumption are much less than the risks associated with smoking (Foulds et al., 2003; Roth, Roth, & Liu, 2005; Schildt, Eriksson, Hardell, & Magnuson, 1998).

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Key elements critical to assessing the effects and health risks associated with tobacco product consumption include studies to determine exposure and biological effects. Individual exposure to a tobacco product can be assessed by external exposure measures that are not invasive. For example, mouth-level exposure (MLE) to a tobacco constituent in snus can be estimated by determining the difference between the constituent levels in used and unused products (Andersson, Bjornberg, & Curvall, 1994). Because expectoration is not typical when using snus, MLE is expected to provide an estimate off maximum potential exposure at the mouth level.

In 2006, R. J. Reynolds Thobacco Company introduced Camel SNUS (CSNUS) into lead markets in the United States (Biener & Bogen, 2009) and subsequently expanded it to national distribution. As a result off the relatively limited time these products have been in market, little infformation exists on the extent off MLE to tobacco constituents ffrom these products among U.S. consumers. The aim off this study was to assess the MLE to selected tobacco constituents due to snus use among U.S. consumers who regularly use CSNUS.

Methods

The study was approved by the R. J. Reynolds Thobacco Company Research and Development Human Research Review Committee prior to study initiation. Written infformed consent was obtained ffrom all subjects beffore enrollment in the study.

Participants

Eligible snus consumers were recruited during September 2008 in the local metropolitan areas off Dallas, ThX, Kansas City, MO, Orlando, FL, and Raleigh, NC. Participants were ffree to withdraw ffrom the study at any time ffor any reason. Tho be eligible ffor enrollment, snus consumers were required to be 21–55 years off age with selff-reported, weekly consumption off at least 15 pouches off a single variety off CSNUS (Original, Spice, or Frost) ffor at least the previous three months. Anyone interested in quitting the consumption off tobacco products was excluded ffrom the study. Women who were pregnant, lactating, or intending to get pregnant were also excluded.

During the study, participants were allowed to consume other tobacco products and nicotine-containing products, but participants were not permitted to consume any snus brands other than CSNUS.

Study Products

The snus product varieties used in this study were Camel SNUS Original, Camel SNUS Spice, and Camel SNUS Frost. Participants purchased their own usual variety off CSNUS at retail ffor their consumption during the study. One sample off each variety was purchased at a store near each central location test (CLTh) ffacility when the study was conducted. A total off 18 samples were obtained: nine samples ffrom Dallas, three samples ffrom Kansas City, three samples ffrom Orlando, and three samples ffrom Raleigh. The products purchased near the CLTh ffacilities were used to determine tobacco constituent baseline levels in unused CSNUS. The CSNUS products were available at retail in 0.6-g pouch fformat and sold in packaging tins containing 15 pouches each.

Study Conduct

Recruited CSNUS consumers visited CLTh ffacilities in Kansas City, MO, Orlando, FL or Raleigh, NC, and three sites in Dallas, ThX, to receive infformation regarding the study. Interested, eligible recruits provided infformed consent and were enrolled in the study. The enrolled participants completed questionnaires to record their usual CSNUS variety, consumption off other tobacco- or nicotine-containing products, and product consumption behavior. Participants received a collection kit ffor used snus pouches, which included an amber glass jar ffor each study day and a product collection diary ffor recording the number off CSNUS pouches consumed but not collected.

Participants were asked to consume their usual CSNUS variety *ad libitum* in their normal liffe settings and to collect their used snus pouches each day ffor 7 days. The product consumption and used-pouch collection period began upon departure ffrom the CLTh and ended at the return visit. Participants were scheduled ffor their return visit 7 days later at approximately the same time off day as their initial visit. Each day, participants collected their used snus pouches in a jar speciffed ffor that day and were asked to place the jar in a ffreezer affer collecting their last pouch consumed that evening. Iff a participant consumed any CSNUS but did not collect the pouches, the participant was asked to record the number off uncollected pouches daily in their diary. At the return visit, participants turned in their used snus collection jars and their diaries. Participants also completed questionnaires to assess their tobacco- and nicotine-containing product consumption behavior during the study. Participants were compensated ffor their time and travel upon completion off the study.

Analysis of Tobacco Constituents of Snus Products

All snus samples were analyzed at Labstat International, ULC (Kitchener, ON, Canada) using the methods described below. Samples were stored at approximately −20 °C and were equilibrated to room temperature prior to analysis. A summary off the methods, lower limits off quantitation (LLOQ), and lower limits off detection (LOD) is given in Thable 1. Analyte content was reported on a mass-per-pouch "as received" basis.

Nicotine. Nicotine content in snus pouches was determined according to Health Canada Offcial Thest Method Th-301 (Health Canada, 1999a). One snus pouch was extracted per replicate test sample. The pouch was ffrst lyophilized ffor 48 hr. The pouch was cut in halff to ffacilitate extraction, and both the pouch contents and ffeece material were placed into a 50-ml Pyrex extraction tube with 24 ml off extraction solution. The sample was extracted in an ultrasonic bath ffor 3 hr, ffollowed by 0.5 hr off shaking on a wrist-action shaker. The sample was centriffuged ffor 5 min at low speed to separate the solid material ffrom the solution. The supernatant was then analyzed per the standard test method.

Tobacco-Specific Nitrosamines. The determination off tobacco-speciffc nitrosamines (ThSNAs: *N*-nitrosonornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], *N′*-nitrosoanabasine [NAB], *N′*-nitrosoanatabine [NATh]) in snus pouches was perfformed according to Health Canada

Table 1. Tobacco Constituents and Analytic Test Methods

Tobacco constituent	Method	Lower limit ofi quantitation	Lower limit ofi detection
Nicotine	GC–TSD	169 μg/g	51 μg/g
NNN	GC–TEA	180 ng/g	5.4 ng/g
NNK	GC–TEA	272 ng/g	82 ng/g
NAT	GC–TEA	213 ng/g	67 ng/g
NAB	GC–TEA	103 ng/g	31 ng/g
B[a]P	HPLC–UV	0.141 ng/g	0.042 ng/g
Arsenic	AAS	40.6 ng/g	17.1 ng/g
Cadmium	AAS	97.5 ng/g	29.3 ng/g
Chromium	AAS	26.9 ng/g	8.06 ng/g
Lead	AAS	85.3 ng/g	25.7 ng/g
Nickel	AAS	88.7 ng/g	26.6 ng/g

Note. AAS = atomic absorption spectroscopy; B[a]P = benzo[a] pyrene; GC = gas chromatography; HPLC = high perfiormance liquid chromatography; NAB = *N′*-nitrosoanabasine; NAT = *N′*-nitrosoanatabine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N′*-nitrosonornicotine; TEA = thermal energy analyzer; TSD = thermionic specific detector; UV = ultraviolet detector.

Offcial Thest Method Th-309 (Health Canada, 1999b). Four snus pouches were extracted per replicate test sample. Each pouch was cut in halff, and both the pouch contents and ffeece material were added into the extraction ffask. The test sample was extracted as per the standard test method. A ffnal sample volume off 2ml was used instead off the 5-ml volume speciffed in the standard test method. The ffnal sample was then analyzed per the standard test method.

Benzo[a]pyrene. Benzo[a]pyrene (B[a]P) content in snus pouches was determined according to Health Canada Offcial Thest Method Th-307 (Health Canada, 1999c). Four snus pouches were extracted per replicate test sample, instead off 2g off tobacco as per the standard method. Each pouch was cut in halff, and both the contents and ffeece material were added to the extraction ffask. The remainder off the analysis was perfformed according to the standard test method.

Trace Metals. The determination off arsenic, cadmium, chromium, lead, and nickel in snus pouches was perfformed according to Health Canada Offcial Thest Method Th-306 (Health Canada, 1999d). Four snus pouches were digested per replicate test sample. The pouches were independently lyophilized ffor 48 hr. Each pouch was cut in halff, and both the pouch contents and ffeece material were placed into the microwave digestion vessel. The remainder off the analysis was perfformed as per the standard test method.

Mouth-Level Exposure

The used snus pouches collected by each participant were analyzed to determine levels off nicotine, ThSNAs, trace metals, and B[a]P in the pouches affer consumption. Replicate test samples (one to ffour replicates) were analyzed ffor a subject depending on the quantity off collected used snus pouches. For each subject, the observed value was taken as the average off replicate measurements ffrom the collected pouches. The total number off snus pouches consumed by a subject during the collection period was estimated by adding the total number off used pouches collected and the total number off uncollected used pouches recorded by the subject. The number off snus pouches consumed per day ffor each subject was calculated by dividing the total number off consumed pouches by 7, corresponding to the number off days. Samples off each variety off CSNUS were purchased at retail near each CLTh site and were used to determine the baseline level off each constituent in unused snus. Three replicate test samples were measured ffor each variety ffrom each site. The average off replicate measurements ffor a variety ffrom a site was used as the observed value ffor that site sample. The baseline level off constituent in a variety off unused snus was taken as the average off the observed values ffrom all sites ffor that variety. Estimated constituent MLE per pouch (i.e., the amount extracted per pouch) was calculated ffor each subject as the baseline constituent level in the variety used by the subject minus the observed constituent level in the used product collected by the subject. Estimated constituent MLE per day (i.e., the amount extracted per day) was obtained ffor each subject by multiplying the appropriate per-pouch value by the number off snus pouches consumed per day by the subject. The proporfion off constituent removed during CSNUS consumption was calculated ffor each subject by dividing their per-pouch MLE to the constituent by the matched variety baseline constituent level and multiplying by 100%.

Data Analysis

Descriptive statistics were calculated ffor subject age, snus pouches consumed per day, and each MLE endpoint. Descriptive statistics were calculated ffor each constituent baseline level in unused snus by variety. A one-way analysis off variance (ANOVA) test was applied to test ffor differences in (a) the measured constituents among unused CSNUS varieties and (b) MLE endpoints among subjects grouped by usual CSNUS variety and among subjects grouped by selff-reported typical product-usage time. Thukey-Kramer honestly signiffcant difference test ($\alpha = 0.05$) was used to determine which means were signiffcantly different ffrom each other. Four (4%) off the NAB determinations were below the LLOQ. Three (3%) off the NNK determinations were below the LLOQ, and one (1%) off the NNK determinations was below the LOD. Thotal ThSNAs was calculated as the sum off the NNN, NNK, NATh, and NAB observed values. When a value was reported as below the LOD, one-halff off the LOD was imputed ffor the calculations. The average off the LLOQ and LOD was used when a value below the LLOQ was reported.

Results

Participants

A total off 56 CSNUS consumers were enrolled, and 54 participants completed the study. Thwo participants chose to discontinue the study ffor personal reasons. One off the participants that completed the study deviated ffrom instructions by using both Original and Spice CSNUS varieties during the collection period. This participant was excluded ffrom the subject group, leaving a total off 53 subjects (46 males/7 ffemales). Among these,

25 (47.2%) off the subjects used the Frost variety, 16 (30.2%) used Spice, and 12 (22.6%) used Original. The mean age off the subjects was 31.9 ± 7.6 years, and the ages ranged ffrom 21–47 years.

Consumption and Behavior

Forty (75.5%) off the subjects reported consuming CSNUS regularly ffor the previous 3–12 months, and 13 (24.5%) off the subjects reported consuming the product ffor longer than twelve months. Subjects were allowed to use other tobacco products during the study, and most (86.8%) off the subjects reported using other tobacco products concurrently with snus. Seven (13.2%) off the subjects consumed only snus, whereas 26 (49.1%) off the subjects were dual users off both snus and cigarettes. Thwo (3.8%) off the subjects were dual users off both snus and moist snuff and two (3.8%) were dual users off both snus and a tobacco product other than cigarettes or moist snuff. Moreover, 16 (30.2%) off the subjects were users off snus, cigarettes, and at least one other tobacco product.

Most (88.7%) off the subjects reported using one pouch at a time when consuming snus. Six (11.3%) off the subjects reported using two or more pouches simultaneously. Off the six subjects using more than one pouch simultaneously, ffour subjects used two pouches, and two subjects used three pouches simultaneously. Subjects were asked how long they typically kept their snus pouch in the mouth during use, and their responses were limited to a group off categories: 14 (26.4%) off the subjects reported that they typically used the product ffor less than ten min; 25 (47.2%) off the subjects reported that they usually kept their snus in their mouths ffor 10–30min, and 14 subjects (26.4%) reported they used the products ffor more than thirty min. The subjects were also asked whether they moved the pouch around or repositioned the pouch in the mouth while using the product. A similar number off subjects moved the pouch around during use (50.9%) as those that maintained the pouch in the same location while using the product (49.1%).

Overall, subjects consumed on average 3.3 ± 1.9 snus pouches/day. Subjects who consumed only snus used 5.4 ± 3.7 pouches/day, and dual users off snus and cigarettes consumed 2.8 ± 1.2 pouches/day.

CSNUS Baseline Results

CSNUS products purchased at retail locations near each participating CLTh ffacility were analyzed to determine baseline levels off nicotine, ThSNAs, B[a]P, and trace metals in unused snus pouches. Thable 2 summarizes the baseline results ffor unused snus by variety. The tobacco constituent levels measured in these products do not exceed the maximum levels deffned by the GothiaThek® standard (Rutqvist, Curvall, Hassler, Ringberger, & Wahlberg, 2011), based on a 0.6-g pouch with 32.3% moisture content. There were no statistically signiffcant (p > .05) differences in the mean baseline levels off nicotine, NNN, NATh, NAB, arsenic, cadmium, and chromium between the Frost, Original, and Spice varieties. Mean baseline NNK level ffor the Spice variety was 59.0–64.0ng/pouch lower than that off the Frost and Original varieties. Mean baseline B[a]P levels ffor the Frost and Original varieties were 0.26–0.32ng/pouch lower than the same in the Spice variety. Mean baseline level off lead ffor the Spice variety was 5.0ng/pouch lower than that ffor the Frost variety, and the mean baseline level off nickel ffor the Spice variety was 43.0 ng/pouch lower than the same in the Frost variety. Regional variations in tobacco constituent levels in CSNUS have been reported previously (Stepanov et al., 2012). Statistical comparisons among products ffrom different cities were not perfformed ffor the current study because product was not sampled ffor assessment off geographic differences. Samples were obtained ffrom just one store in each off the cities off Kansas City, Orlando, and Raleigh.

Constituent Exposure

The MLEs (per pouch and per day) to tobacco constituents and the proportion off constituents removed during CSNUS consumption were calculated and are presented in Thable 3. MLE to trace metals and B[a]P could not be estimated ffor some participants because these subjects collected an inadequate amount off used pouches to complete all constituent determinations. Overall, mean nicotine MLE was 2.8 mg/pouch and 9.4 mg/day, corresponding to 39% off the original amount off nicotine in the product. MLE to NNN averaged 97.1 ng/pouch and 302.4 ng/day (23%), NNK averaged 37.5 ng/pouch and 124.4 ng/day (30%), NATh LE

Table 2. Baseline Constituent Levels in Unused CSNUS Varieties (Mean ± *SD*)

Constituent	Frost (n = 6)	Original (n = 6)	Spice (n = 6)	ANOVA p value
Nicotine (mg/pouch)	7.3 ± 0.7[a]	6.9 ± 0.7[a]	6.5 ± 0.3[a]	.1196
NNN (ng/pouch)	427.5 ± 47.5[a]	425.4 ± 39.1[a]	392.2 ± 31.0[a]	.2584
NNK (ng/pouch)	142.7 ± 15.0[a]	137.7 ± 6.9[a]	78.7 ± 7.6[b]	<.0001
NAT (ng/pouch)	220.5 ± 34.1[a]	217.8 ± 15.8[a]	216.3 ± 19.3[a]	.9544
NAB (ng/pouch)	29.3 ± 3.5[a]	29.0 ± 1.4[a]	28.0 ± 3.9[a]	.7683
Total TSNAs (ng/pouch)	820.0 ± 96.5[a]	809.9 ± 48.7[a]	715.2 ± 56.9[a]	.0396
B[a]P (ng/pouch)	0.59 ± 0.03[a]	0.53 ± 0.07[a]	0.85 ± 0.10[b]	<.0001
Arsenic (ng/pouch)	49.7 ± 4.4[a]	49.8 ± 3.5[a]	45.8 ± 6.1[a]	.2934
Cadmium (ng/pouch)	198.8 ± 15.8[a]	196.5 ± 4.4[a]	195.5 ± 7.8[a]	.9745
Chromium (ng/pouch)	261.8 ± 11.3[a]	257.8 ± 17.9[a]	251.9 ± 33.7[a]	.7604
Lead (ng/pouch)	72.3 ± 2.4[a]	71.4 ± 3.9[a,b]	67.3 ± 2.9[b]	.0331
Nickel (ng/pouch)	385.9 ± 28.1[a]	367.2 ± 25.8[a,b]	342.9 ± 20.1[b]	.0293

Note. ANOVA = analysis ofi variance; NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine; TSNAs = tobacco-specific nitrosamines; B[a]P = benzo[a]pyrene. For a constituent, mean variety baseline levels that share the same letter ([a] or [b]) were not fiound to be significantly difierent using the Tukey–Kramer honestly significant difierence test (α = 0.05).

Table 3. Constituent Mouth-Level Exposure From Snus Pouches Used by Subjects (Mean ± *SD*)

Mouth-level exposure endpoint	All	Frost	Original	Spice	ANOVA *p* value
Nicotine					
MLE per pouch (mg/pouch)	2.8 ± 1.7	3.4 ± 1.7[a]	2.7 ± 1.5[a,b]	1.8 ± 1.2[b]	.0092
MLE per day (mg/day)	9.4 ± 7.8	12.2 ± 7.9[a]	7.7 ± 5.6[a,b]	6.3 ± 7.9[b]	.0429
Proportion removed (%)	39.2 ± 23.0	46.6 ± 23.6[a]	39.2 ± 21.9[a,b]	27.8 ± 18.8[b]	.0346
N	53	25	12	16	
NNN					
MLE per pouch (ng)	97.1 ± 92.7	123.7 ± 95.3[a]	83.3 ± 86.7[a]	65.8 ± 85.8[a]	.1253
MLE per day (ng)	302.4 ± 341.4	412.9 ± 401.1[a]	254.1 ± 304.3[a]	166.1 ± 191.7[a]	.0641
Proportion removed (%)	23.1 ± 22.1	28.9 ± 22.2[a]	19.6 ± 20.4[a]	16.8 ± 21.9[a]	.1876
N	53	25	12	16	
NNK					
MLE per pouch (ng)	37.5 ± 28.2	50.0 ± 28.3[a]	35.6 ± 28.5[a,b]	19.5 ± 16.6[b]	.0020
MLE per day (ng)	124.4 ± 119.2	173.0 ± 127.4[a]	114.8 ± 123.3[a,b]	55.6 ± 55.1[b]	.0062
Proportion removed (%)	29.9 ± 20.6	35.1 ± 19.8[a]	25.9 ± 20.7[a]	24.8 ± 21.1[a]	.2275
N	53	25	12	16	
NAT					
MLE per pouch (ng)	34.1 ± 57.4	43.0 ± 61.3[a]	17.8 ± 69.5[a]	32.4 ± 39.2[a]	.4627
MLE per day (ng)	94.1 ± 215.2	123.6 ± 265.9[a]	53.9 ± 226.6[a]	78.2 ± 82.2[a]	.6234
Proportion removed (%)	15.6 ± 26.2	19.5 ± 27.8[a]	8.2 ± 31.9[a]	15.0 ± 18.1[a]	.4753
N	53	25	12	16	
NAB					
MLE per pouch (ng)	2.8 ± 11.7	4.1 ± 12.0[a]	1.3 ± 15.5[a]	1.7 ± 8.0[a]	.7245
MLE per day (ng)	6.7 ± 44.6	10.8 ± 54.1[a]	3.9 ± 49.2[a]	2.6 ± 19.5[a]	.8263
Proportion removed (%)	9.5 ± 40.4	14.1 ± 40.8[a]	4.3 ± 53.6[a]	6.2 ± 28.4[a]	.7367
N	53	25	12	16	
Total TSNAs					
MLE per pouch (ng)	171.5 ± 171.3	220.9 ± 181.6[a]	137.9 ± 170.6[a]	119.5 ± 140.7[a]	.1346
MLE per day (ng)	527.7 ± 642.9	720.3 ± 765.3[a]	426.7 ± 613.5[a]	302.5 ± 309.6[a]	.1039
Proportion removed (%)	21.6 ± 21.4	26.9 ± 22.1[a]	17.0 ± 21.1[a]	16.7 ± 19.7[a]	.2338
N	53	25	12	16	
B[a]P					
MLE per pouch (ng)	0.20 ± 0.12	0.15 ± 0.07[a]	0.13 ± 0.08[a]	0.33 ± 0.11[b]	<.0001
MLE per day (ng)	0.68 ± 0.55	0.57 ± 0.35[a]	0.35 ± 0.19[a]	1.08 ± 0.73[b]	.0014
Proportion removed (%)	29.0 ± 13.8	24.9 ± 11.2[a]	24.4 ± 14.8[a]	38.5 ± 12.7[b]	.0058
N	45	21	10	14	
Arsenic					
MLE per pouch (ng)	−10.1 ± 13.4	−5.5 ± 10.7[a]	−14.8 ± 16.8[a]	−13.7 ± 13.1[a]	.0655
MLE per day (ng)	−29.5 ± 41.3	−13.1 ± 32.4[a]	−39.0 ± 37.4[a,b]	−47.4 ± 48.0[b]	.0220
Proportion removed (%)	−21.0 ± 27.8	−11.0 ± 21.6[a]	−29.8 ± 33.6[a]	−29.8 ± 28.5[a]	.0522
N	51	24	11	16	
Cadmium					
MLE per pouch (ng)	21.2 ± 29.2	16.7 ± 24.9[a]	23.9 ± 31.3[a]	26.2 ± 34.4[a]	.5819
MLE per day (ng)	56.2 ± 86.6	55.5 ± 98.8[a]	57.0 ± 77.2[a]	56.7 ± 77.8[a]	.9986
Proportion removed (%)	10.8 ± 14.9	8.5 ± 12.7[a]	12.2 ± 15.9[a]	13.4 ± 17.6[a]	.5739
N	51	24	11	16	
Chromium					
MLE per pouch (ng)	−15.8 ± 44.3	−20.5 ± 39.9[a]	−9.4 ± 32.1[a]	−13.2 ± 57.8[a]	.7660
MLE per day (ng)	−66.2 ± 153.8	−85.3 ± 167.3[a]	−32.8 ± 100.3[a]	−60.3 ± 166.6[a]	.6423
Proportion removed (%)	−6.1 ± 17.3	−7.8 ± 15.2[a]	−3.7 ± 12.5[a]	−5.2 ± 23.0[a]	.7859
N	51	24	11	16	
Lead					
MLE per pouch (ng)	−11.4 ± 19.2	−11.8 ± 21.0[a]	−17.7 ± 20.9[a]	−6.3 ± 14.3[a]	.3242
MLE per day (ng)	−39.1 ± 57.5	−43.1 ± 67.8[a]	−50.3 ± 46.8[a]	−25.3 ± 46.9[a]	.4906
Proportion removed (%)	−16.0 ± 26.9	−16.4 ± 29.0[a]	−24.7 ± 29.3[a]	−9.4 ± 21.3[a]	.3542
N	51	24	11	16	
Nickel					
MLE per pouch (ng)	32.1 ± 72.9	45.3 ± 57.3[a]	43.1 ± 53.7[a]	4.7 ± 97.8[a]	.1946
MLE per day (ng)	97.6 ± 226.9	167.3 ± 236.9[a]	105.2 ± 132.6[a,b]	−12.4 ± 230.6[b]	.0456
Proportion removed (%)	8.5 ± 20.3	11.7 ± 14.9[a]	11.7 ± 14.6[a]	1.4 ± 28.5[a]	.2432
N	51	24	11	16	

Note. ANOVA = analysis ofi variance; MLE = mouth-level exposure; NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine; TSNAs = tobacco-specific nitrosamines; B[a]P = benzo[a]pyrene.
For an MLE endpoint, CSNUS variety mean levels that share the same letter ([a] or [b]) were not fiound to be significantly difierent using the Tukey–Kramer honestly significant difierence test (α = 0.05). Proportion removed = the percentage ofi the unused snus baseline constituent level corresponding to the estimated amount ofi constituent removed during product consumption.

averaged 34.1 ng/pouch and 94.1 ng/day (16%), NAB averaged 2.8 ng/pouch and 6.7 ng/day (9.5%), and total ThSNAs averaged 171.5 ng/pouch and 527.7 ng/day (22%). B[a]P MLE averaged 0.20 ng/pouch and 0.68 ng/day (29%), whereas cadmium MLE averaged 21.2 ng/pouch and 56.2 ng/day (11%) and nickel MLE averaged 32.1 ng/pouch and 97.6 ng/day (8.5%).

Nicotine MLE (mean ± *SD*) per pouch was 2.6 ± 1.7 mg/pouch (37% off the original amount off nicotine in the product) ffor subjects who consumed only snus and was 2.5± 1.6 mg/pouch (35%) ffor dual users off snus and cigarettes. For subjects consuming only snus, total ThSNAs MLE was 94.2± 122.8 ng/pouch (12%). Thotal ThSNAs MLE was 176±8156.9 ng/pouch (23%) ffor dual users off snus and cigarettes.

The usual CSNUS variety off each subject was identiffed during the study. The mean values ffor the nicotine MLE endpoints, NNK MLE per pouch, and NNK MLE per day were signiffcantly ($p < .05$) lower among the subjects who used the Spice variety than among those who used Frost (Thable 3). There was no statistically signiffcant difference in the mean proportion off NNK removed ffrom the product between the Frost, Original, and Spice subjects during use ($p > .05$). The mean B[a]P MLE endpoints were signiffcantly ($p < .05$) higher among the Spice subjects than among the Frost and Original subjects.

During the study, subjects selff-reported how long they typically kept their CSNUS in their mouths, and their responses were limited to a group off time categories. The ANOVA ffor usage-time groups showed that there were no signiffcant differences (*p* value range: 0.11–0.94) in any off the mean MLE endpoints among subjects who reported keeping their snus in their mouths ffor less than ten, 10–30, and greater than 30 min (data not shown).

Discussion

This is the ffrst reported study where MLE to tobacco constituents due to snus use was determined among U.S. snus consumers under normal liffe conditions. On average, 61% off the original amount off nicotine and 78% off the original amount off total ThSNAs remained in the snus pouch affer use by snus consumers in the current study. Previous studies off MLE among snus users in Sweden have also shown that substantial proportions off the original amounts off nicotine and ThSNAs remain in the snus pouch affer use. In one such study off Swedish men who used portion-bag (pouch) snus products *ad libitum* over a 24-hr period, 63% off the original amount off nicotine and 44% off the original amount off total ThSNAs remained in the pouch affer use (Andersson et al., 1994). In a study off Swedish snus consumers using ffour different brand styles off pouched Swedish snus under standardized conditions, 56%–78% off the original amount off nicotine remained in the pouch affer use (Lunell & Lunell, 2005). A study off Swedish smokers who were asked to use 1-g snus products under standardized conditions showed that, on average, 75%–79% off the original amount off nicotine remained in the pouch affer use (Lunell & Curvall, 2011).

The mean nicotine MLE per day, mean total ThSNA MLE per day, and mean proportion off total ThSNA removed ffrom the pouch affer snus consumption in this study were lower than the results reported by Andersson et al. (1994) ffor *ad libitum* use off pouch snus products among Swedish snus consumers. It appears that the differences between MLE levels per day among pouch snus consumers in the current study and that by Andersson et al. (1994) are primarily a result off the difference in amount off snus consumed per day as well as product differences (e.g., composition). The average daily consumption among the portion-bag snus users in the study by Andersson et al. (1994) was 14.4 g snus/day, whereas the average daily consumption was ~2.1 g snus/day in the current study. It has been suggested that MLE ffrom snus may be inffuenced by the level off snus consumption experience, ffor example, exclusive snus consumers versus smokers that were never users off smokeless tobacco (Lunell & Curvall, 2011). The differences between MLE among the subjects in the current study and that by Andersson et al. (1994) may also have been affected by the consumption behavior among the subjects: Subjects in the Andersson et al. (1994) study were exclusive snus consumers, whereas the subject group in the current study included dual- and multiple-tobacco-product users. The diffference between the mean proportion off total ThSNA removed during product use in the current study and that by Andersson et al. (1994) may be explained by the higher concentration off ThSNA in the pouch snus products used by subjects in the study by Andersson et al. . The mean baseline level off total ThSNA in CSNUS in the current study was 1.3 μg/g and was lower than the concentration off total ThSNA (~5.2 μg/g) in the pouch snus products used by subjects in the Andersson et al. (1994) study.

For each off the tobacco constituents evaluated in the current study, MLE ffrom snus was not related to duration off use, that is, selff-report off either less than 10, 10–30, or greater than 30 min in the mouth. However, interpretations off this result should be approached with caution. Subject responses regarding their usage time were limited to a small group off time categories. A previous study suggested that limiting product-usage responses to time categories may not provide a robust dataset (Digard, Errington, Richter & McAdam, 2009). Subject responses were also based on their typical consumption behavior, which may not accurately represent their product consumption behavior during their participation in this study.

Nearly halff (49%) off the subjects reported dual use off snus and cigarettes. Mean nicotine MLE per pouch was similar between subjects who consumed only snus and dual users off snus and cigarettes. Mean total ThSNA MLE per pouch was higher ffor the dual users off snus and cigarettes compared to subjects who consumed only snus, but the dual users used ffewer pouches per day. A statistical comparison off these two subsets off subjects was not perfformed because a small number off subjects consumed only snus. In addition, this comparison was outside the scope off the study design.

Few data speciffc to snus consumption in the United States are available, but smokeless tobacco use (snuff and chewing tobacco inclusive) in the United States has been reported to be more prevalent among adult males than ffemales (male to ffemale ratio: ~ 6:1; Substance Abuse and Mental Health Services Administration, 2008). Although the sample off subjects in the current study was limited, the gender makeup off the subject group was primarily male (male to ffemale ratio: ~7:1) and similar to data previously reported ffor a survey off consumption patterns and behaviors off Swedish snus consumers (Digard et al., 2009).

A number off limitations off this study should be noted. Nicotine and ThSNAs have been detected in the saliva off

tobacco consumers (Feyerabend, Higenbottam & Russell, 1982; Osterdahl & Slorach, 1988). Constituents that may be present in saliva from previous tobacco use could transfer to the study product. Therefore, the estimate of MLE may not explicitly represent the amount of constituents directly extracted from snus during use. However, substantial TSNA transfer from saliva to the study product is unlikely, considering that, in previous studies, TSNAs were either not detected or detected at trace levels in the saliva of smokers and moist snuff users after the product was removed from the mouth (Jarczyk, Maier, Born, Scherer & Adlkofer, 1991;Nair et al., 1985; Osterdahl & Slorach, 1988; Wenke, Brunnemann, Hoffmann & Bhide, 1984). Nevertheless, the approach used in previous studies (Andersson et al., 1994; Lunell & Curvall, 2011; Lunell & Lunell, 2005) and in the current study estimates the net amounts of tobacco constituents introduced at the mouth level because constituents analyzed in the used product are no longer in the mouth of the consumer.

Another potential limitation of this study is the possibility that TSNA formation may occur when saliva is introduced into smokeless products. A previous study reported that the concentration of NNN in a sample of chewing tobacco increased after stirring the sample with human saliva for 3hr at 37 °C (Hecht, Ornaff, & Hoffmann, 1975), but, in a separate study, TSNA levels did not increase in smokeless tobacco samples stirred with enzyme-containing artificial human saliva for two hours at 37 °C (Fowler, Bombick, Hill-Hart, Arimilli, & Prasad, 2012). The results of these studies suggest that, in the current study, formation of additional TSNA may be nominal while the pouch is in the mouth of the subject, considering 75% of the subjects in the current study indicated that they typically kept their snus in their mouths for only 30min or less. As a result of the ambulatory collection of used snus in this study, subjects stored used pouches at ambient temperatures during the day. Under these conditions, there is the potential for the formation of additional TSNA in the used pouches, and additional studies would be required to assess the extent of any TSNA formation.

Constituent levels in a tobacco product can vary as a result of the inherent variability of tobacco and variability in product manufacturing (Ogden, 2011;Tso, 1990). Tobacco is a natural product, and its constituent levels are influenced by a number of factors, including genetic and varietal differences, agricultural practices, and climatic conditions. The differences in baseline constituent levels observed between the CSNUS varieties and the negative MLE estimates observed for arsenic, chromium, and lead are probably a result of this variability. Production-lot variability could have some effect on MLE estimates because the products used to determine constituent baseline levels and the products consumed by subjects were not from matched production lots. Sampling product for baseline level determinations from the same packaging tins used by subjects would reduce the effect of production-lot variability on MLE estimates.

Most subjects reported using other types of tobacco products in addition to snus during the study. Additional research efforts are necessary to effectively compare consumers who use snus exclusively and those who also use other tobacco products. For example, a future study could focus on recruiting a larger number of adult U.S. consumers who use only snus and who use both snus and cigarettes in order to explore tobacco constituent exposure and product consumption behavior among these populations.

In summary, this study is the first to report the MLE to tobacco constituents from snus consumption among a group of adult, U.S. snus consumers under normal life conditions. On average, approximately 60%–90% of the original amounts of nicotine, TSNA, and B[a]P remained in the snus pouch after use by U.S. snus consumers in this study. The mean MLE to some tobacco constituents was substantially lower among snus consumers in the current study than that previously reported among exclusive snus consumers in Sweden. At the time of this study, CSNUS had been available in selected U.S. markets for 28 months. The results of this study represent an initial cross-sectional investigation of CSNUS consumers and may not represent future marketplace dynamics nor future consumer behavior. Further studies can provide the opportunity to confirm these results as generally applicable after the product has been established in the market for a longer period of time.

Declaration of Interests

None declared.

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