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AMBULATORY STUDY COMPARING *AD LIBITUM* USE OF USUAL BRAND CIGARETTES TO DUAL USE OF CAMEL SNUS WITH REDUCED SMOKING

OBJECTIVE

The objective of this study was to evaluate changes in product use patterns, biomarkers of tobacco exposure, and subjective responses of smokers as they changed their daily tobacco usage to include Camel Snus and reduce smoking.

SUMMARY

Thirty-two smokers completed a three-week transition from only smoking usual brand (UB) cigarettes to dual use of UB cigarettes and Camel Snus. Participants were instructed to decrease smoking by 25% per week to attain a targeted reduction of 75% of their initial *ad libitum* use by the end of the study. Although participants did not generally meet this goal, they reported a significant average smoking reduction of 59%. They started the study smoking an average of 22 cigarettes per day (CPD) and completed the study smoking an average of 9 CPD and using an average of 3.5 snus pouches per day.

Consistent with other reports, decreases in the biomarkers of smoke exposure measured in this study were not of the same magnitude as CPD smoking reductions. Expired carbon monoxide (ECO) and carboxyhemoglobin saturation (%COHb) significantly decreased an average of 28% and 21% respectively. In addition, biomarkers of the vapor phase compounds acrolein, acrylonitrile, benzene, crotonaldehyde, ethylene oxide, hydrogen cyanide, and 1, 3 butadiene were examined in 24-hour urine samples. Levels of all vapor phase biomarkers measured showed significant median decreases of 21-39%. Reductions in these biomarkers suggest participants did reduce smoke exposure. Yield-in-use analysis of cigarette filters showed a nominally significant mean 8.7% decrease in nicotine per cigarette ($p=0.0697$) and no difference in 'tar' per cigarette. These results suggest participants did not change puffing behavior as they reduced cigarette-per-day consumption.

Biomarker levels of toxicants found in the particulate phase of cigarette smoke and in snus were also examined. Biomarkers of naphthalene, fluorene, acrylamide, NAB, and four aromatic amines showed significant median decreases of 21-27%. Nicotine equivalents nominally significantly decreased a median of 17%. Total NNAL and total TSNA's nominally significantly decreased a median of 9%. No biomarkers examined significantly increased during dual use.

Subjective responses were also assessed. Initially, participants rated Camel Snus as "Quite Good" on the thermometer scale and, by the end of the study, rated it closer to "Very Good," with a significant upward

trend in rating over time. Participants rated the sweetness, tobacco taste, and texture of Camel Snus to be “Just Right” throughout the study. They rated it as having slightly too much flavor at Visit 1, but flavor ratings trended toward “Just Right” in Visits 2, 3, and 4.

As participants decreased smoking, a significant downward trend was observed in the overall opinion of their UB cigarettes. The average rating of UB cigarettes at the beginning of the study was 84 on a 100-point scale, with an average rating of 77 by study conclusion. Significant downward trends were also observed in satisfaction, smoothness, strength of taste, and tobacco taste. Significant upward trends were observed in harshness and aftertaste of their UB cigarettes. As the study progressed, participants also reported experiencing increased impact in the nose and chest while smoking.

Although participants reported a decrease in CPD, and urinary nicotine equivalents decreased during the study, responses to the Minnesota Nicotine Withdrawal Scale did not indicate a significant increase in tobacco abstinence symptoms. Of the nine validated symptoms of nicotine withdrawal, only weight gain/appetite showed a small, marginally significant increase as the study progressed. In contrast, small but significant reductions were seen in anxiety, insomnia, restlessness, and coughing.

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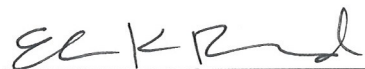
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KEYWORDS

1, 3 butadiene; 2-aminonaphthalene; 2-CEMA; 2-HEMA; 3-aminobiphenyl; 3-HMPMA; 3-HPMA; 3-SPMA; 4-aminobiphenyl; AAMA; acceptance; acrolein; acrylamide; acrylonitrile; benzene; biomarkers; Camel Snus; carbon monoxide; carboxyhemoglobin; COHb; crotonaldehyde; ethylene oxide; expired CO; fluorene; hydrogen cyanide; GAMA; MHBMA; migration study; modern smoke-free tobacco; MSFT; naphthalene; nicotine equivalents; NAB; NAT; NNAL; NNK; NNN; o-toluidine; phenanthrene; preferences; pyrene; serum cotinine; serum nicotine; snus; thiocyanate; tobacco-specific nitrosamines; urinary biomarkers; urine; UB; usual brand; yield in use; YIU

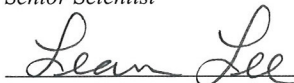
AMBULATORY STUDY COMPARING *AD LIBITUM* USE OF USUAL BRAND CIGARETTES TO DUAL USE OF CAMEL SNUS WITH REDUCED SMOKING

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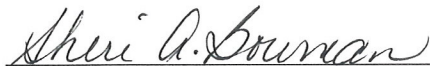
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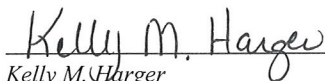
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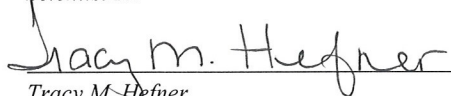
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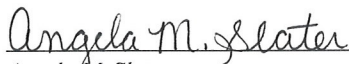
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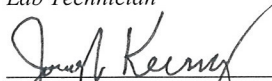
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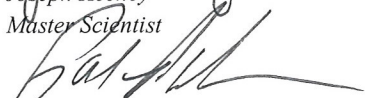
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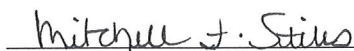
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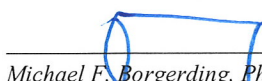
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INTRODUCTION

R.J. Reynolds Tobacco Company (RJRT) has developed several modern smoke-free tobacco (MSFT) products. The company introduced the first of this product category, Camel Snus, nationwide in January 2009. Camel Snus is a pouched, pasteurized moist snuff product designed to be used without the need to spit. Although snus is a relatively new product to the United States, it has been commercially available to Swedish tobacco users for over 100 years. Epidemiologic data of snus users in Sweden associated reduced health risks with snus use compared to smoking cigarettes (Levy *et al.* 2004). Studies performed in Norway associated snus use with smoking cessation (Lund *et al.* 2010).

In this study, we examined the use of Camel Snus by smokers throughout a three-week product transition from exclusive, *ad libitum* use of usual brand cigarettes to dual use with snus. During dual use, snus was used *ad libitum* together with an instructed reduction in cigarette usage. We evaluated the changes in a number of vapor phase and particulate phase biomarkers of tobacco exposure in 24-hour urine collections. We measured carbon monoxide (CO) levels in expired breath samples and carboxyhemoglobin (COHb) levels in whole blood as additional measures of cigarette smoke inhalation. Serum nicotine and cotinine levels were measured following product use in the lab as an initial effort to understand the nicotine absorption resulting from the use of Camel Snus. Used cigarette filters were collected by participants the day before each study visit and analyzed to estimate mouth-level exposure to mainstream smoke ‘tar’ and nicotine. Used snus pouches were collected each day for the last two weeks of the study for determination of tobacco constituent extraction. In addition, we assessed participants’ overall opinions of usual brand cigarettes and Camel Snus, sensory and nicotine withdrawal experiences, and individual patterns of usual brand cigarette and snus usage.

The design of this study was based on previous RJRT studies (Round *et al.* 2009 RDM, Round *et al.* 2010 RDR, Bowman *et al.* 2010 RDR). In those studies, smokers were instructed to switch their tobacco use from cigarettes only to dual use with one MSFT product (Tobacco Orbs, Strips, or Sticks) over a two- or three-week period. Learnings from the previous studies influenced modifications to product-use guidelines, study duration, sample collection, and biomarker choice.

METHODS

Participants. Bellomy Research Inc. in Winston-Salem recruited eligible smokers from the Winston-Salem community. Recruits were asked to attend an orientation session that provided an overview of all study requirements and gave those interested the option to sample Camel Snus before consenting to participate. Smokers who agreed to follow the study protocol provided written informed consent for study participation before beginning any study procedures.

To be eligible for inclusion, smokers were required to be 21–55 years of age, be in generally good health with no active oral lesions, and have no history of major health conditions. In addition, participants reported smoking at least 7 cigarettes per day of a usual brand (UB) cigarette with Cambridge Filter Method (CFM) ‘tar’ levels of 8.0–14.0 mg/cigarette when machine smoked according to the following regimen: 35 mL puffs, two seconds in duration; one puff per minute¹. If smokers reported they were in the process of quitting, they were not included in the study.

¹ At the time this study was executed, this ‘tar’ range of cigarette was considered to be Full Flavor Low Tar (FFLT) and referred to as such during the study. Use of the term “low” and similar descriptors has since been banned by the Family Smoking Prevention and Tobacco Control Act. The Cambridge Filter Method (CFM) has been previously referred to as the

The R&D Human Research Review Committee (HRRC) approved this study after a review of the experimental protocol (HRRC proposal #0905 and #0905A). Participant activities were conducted 3/30/09 through 6/5/09.

Procedure. Following the orientation session but before the start of study visits, participants were required to have an oral exam performed by a physician or nurse practitioner. Participants judged to have active oral lesions during that exam were to be excluded from the study. No participants in this study were excluded based on results from the screening oral exam.

Study procedures were conducted over four weeks, with each seven-day period ending in a study visit. Participants reported to the offsite testing facility for one study visit per week, the same day and time each week. Study sessions lasted 45-120 minutes and were held Tuesday through Friday afternoons at 12:00, 2:00, and 4:00. Participants were asked to refrain from tobacco use for 30 minutes prior to each test session. The time and type of tobacco product last used were recorded at each visit.

Participants smoked UB cigarettes without restriction for the first week of the study and recorded the number of cigarettes smoked each day on a log sheet. Based on self-reported Week 1 smoking levels, participants were given individual weekly goals for cigarette-per-day reductions. During the second week of the study, participants were instructed to reduce their smoking by 25% of the average daily cigarette consumption calculated from their Week 1 reports. During Week 3, participants were instructed to reduce smoking by 50% of their daily Week 1 average, and during Week 4, they were instructed to reduce smoking by 75% of their daily Week 1 average. Participants were told at Orientation that if they did not meet their weekly goal for reducing cigarette consumption, they would not be dismissed from the study if they honestly and accurately recorded their actual cigarette use. Participants were given Camel Snus to use during Weeks 2, 3, and 4 of the study as they reduced cigarette consumption. At the conclusion of Visit 1, participants were given snus to take home for the first time and were asked to incorporate it into their daily tobacco-use routines. At Visits 2 and 3, participants were told that they might find that increasing their use of snus might provide tobacco satisfaction while reducing smoking. Participants were permitted to take home one variety of Camel Snus at each visit (Frost or Mellow, 600 mg pouches), but could switch varieties at successive visits if desired. The variety provided to the participant during test session use was the same variety the participant took home to use the previous week.

At Visit 1, participants were asked to smoke one UB cigarette. Study procedures at Visit 1 included product log sheet collection, return of 24-hour urine samples, timed blood sample collection for nicotine and cotinine measurements, expired breath CO and blood COHb measurements, collection of spot urine samples, and questionnaire completion. Fifteen blood samples for nicotine and cotinine measurements were collected over approximately 92 minutes.

At Visits 2 and 3, participants returned product log sheets, collected spot urine samples, provided expired breath and one blood sample for the measurement of expired CO (ECO) and COHb, respectively, and completed questionnaires. Participants used one snus pouch, but did not have timed blood samples collected for nicotine and cotinine analysis.

FTC method ("FTC Rescinds Guidance from 1966 on Statements Concerning Tar and Nicotine Yields," FTC, <http://www.ftc.gov/opa/2008/11/cigarettetesting.shtm>, accessed 2/26/09). Prior to its rescission in 2008 (*ibid.*), the method was prescribed by the FTC as the standardized method for reporting cigarette "tar" and nicotine values (Fed. Reg. 32 (147): 11178 (1967)).

At Visit 4, participants were asked to use one snus pouch. Participants were not required to use snus for a minimum amount of time but were asked to remove the pouch after 30 minutes if it was still in use. All other procedures performed at Visit 4 were identical to those performed at Visit 1.

At the completion of Visit 4, participants were asked to return for a final oral exam performed by a physician or nurse practitioner to determine whether any oral conditions had developed over the course of the study.

Participants were compensated for their time and travel at the end of Orientation, each oral exam, and each study visit.

Study Products. Participants provided their own UB cigarettes throughout the study (see Table 1 for complete list). Mainstream smoke yield ranges when machine smoked using the Cambridge Filter Method under 35/2/60 conditions for the UB cigarettes of participants in this study are shown in Table 2.

Table 1. Usual brand cigarette styles for study completers.

Brand Style	# of Participants
Marlboro 85 HP FFLT	6
Camel 85 HP FFLT	3
Winston 85 HP FFLT	3
Doral 85 HP FFLT	2
Doral 100 HP FFLT	2
Marlboro 100 HP FFLT	2
Salem (Menthol) 85 HP FFLT	2
Camel (Menthol) 85 HP FFLT	1
Camel Wides 85 HP FFLT	1
Doral (Menthol) 85 HP FFLT	1
Kool Milds (Menthol) 85 HP FFLT	1
Monarch (Menthol) 85 SP FFLT	1
Newport (Menthol) 85 HP FFLT	1
Pall Mall 85 HP FFLT	1
Pall Mall 100 HP FFLT	1
Tahoe 85 SP FFLT	1
Tahoe 100 SP FFLT	1
USA Gold 100 FFLT	1
Winston 85 SP FFLT	1
Total	32

Table 2. ‘Tar,’ nicotine, and carbon monoxide yield ranges for UB cigarettes for study completers.

	‘Tar’ mg/cig	Nicotine mg/cig	Carbon Monoxide mg/cig
Usual Brands	8.7 - 11.3	0.63 - 0.95	9.3 - 12.4

‘Tar,’ nicotine, and CO yield information was obtained from TITL #51 (TITL=Tobacco Institute Testing Laboratory). Information was not available for Tahoe 85 SP FFLT, Tahoe 100 SP FFLT, or USA Gold 100 FFLT.

Pouches of Frost and Mellow Camel Snus (600 mg) were distributed to participants for use in this study. Camel Snus was provided for in-lab use during Visits 2, 3, and 4 and was provided for home use at the end of Visits 1, 2, and 3. Participants were permitted to take home one variety of snus following each study visit, but could switch varieties to take home at subsequent visits if desired. For in-lab testing, participants were provided with the same variety they took for home use the week prior. Pouches from one lot each of Frost (C9TJ080) and Mellow (D9FJ038) were distributed to participants during this study. Combined analytical data for tobacco constituents in both lots are reported in Table 3.

Table 3. Analytical data of tobacco constituents in Camel Snus distributed for study use.^a

Constituent	Units	N	Mean	SD
Nicotine	(mg/pouch)	6	7.33	0.25
Normicotine	(µg/pouch)	6	107.1	7.24
Anatabine	(µg/pouch)	6	60.53	3.11
B[a]P	(ng/pouch)	6	0.47	0.06
Cd	(ng/pouch)	6	183	37
Cr	(ng/pouch)	6	232	64
Ni	(ng/pouch)	6	361	64
Pb	(ng/pouch)	6	91.1	23.5
As	(ng/pouch)	6	53.6	13.9
Se	(ng/pouch)	6	55.4	3.5
NNN	(ng/pouch)	6	421	17
NAT	(ng/pouch)	6	203	7.4
NAB	(ng/pouch)	6	24.4	1.1
NNK	(ng/pouch)	6	137	7.1

^a Summary of both varieties, Frost Lot C9TJ080 n=3, Mellow Lot D9FJ038 n=3.

Product Use Logs. Participants recorded daily cigarette consumption and snus use throughout the study. Blank log sheets containing spaces to record product usage for eight days were distributed at Orientation and at Visits 1–3. Completed logs were collected at Visits 1–4.

Yield-In-Use Analysis. Participants were asked to collect filters from all cigarettes smoked the day before each study visit. Butt collection materials were provided at Orientation and at Visits 1, 2, and 3. Participants collected cigarette butts in individual vials and returned them at their next study visit. Each week, participants were provided fewer vials to reinforce weekly smoking reduction guidelines. Butts were stored at -20°C until processing. An approximately 10-mm piece was cut from the mouth end of each butt and pieces were batched by participant and frozen at -70°C or below. Samples were shipped frozen to Arista Laboratories (Richmond, VA). For analysis of ‘tar’ and nicotine levels, samples were batched according to participant in groups of 4-6 tips.

Snus-After-Use Analysis. Participants were asked to collect all used snus pouches each day during the third and fourth weeks of the study. Pouches were sent to Labstat International (Kitchener, ON, Canada) for extraction and measurement of the remaining nicotine, TSNAs, trace metals, and B[a]P in the pouches after use. Extraction of nicotine required one pouch per measurement, and extraction of TSNAs, trace metals, and B[a]P required four pouches per measurement. The total number of pouches returned by each participant each week determined the number and type of extractions performed. The number of used pouches returned per participant per week ranged from a minimum of 8 to a maximum of 80. Extractions were performed with the following priority: up to 4 alkaloid measurements, TSNAs, trace metals, and B[a]P. The type and number of extractions are listed according to the number of pouches returned per participant per week in Table 4.

Table 4. Constituent extractions performed according to the number of used pouches collected per participant per week.

Number of used pouches returned	# of alkaloid measurements	# of TSNA measurements	# of Trace Metals measurements	# B[a]P measurements
8	4	1	0	0
9	1	1	1	0
10	2	1	1	0
11	3	1	1	0
12	4	1	1	0
13	1	1	1	1
14	2	1	1	1
15	3	1	1	1
16	4	1	1	1
17	1	2	1	1
18	2	2	1	1
19	3	2	1	1
20	4	2	1	1
21	1	2	2	1
22	2	2	2	1
...
80	4	7	6	6

Expired Carbon Monoxide Measurements. Participants provided breath samples for determination of expired carbon monoxide concentrations just prior to and 25 minutes after the start of product use at Visits 1, 2, 3, and 4. For proper sample measurement, participants were asked to inhale deeply, hold their breath for 15 seconds, then exhale slowly and completely through a disposable cardboard mouth tube attached to a Bedfont Micro 4 Smokerlyzer unit. This instrument utilized an electrochemical sensor to measure CO levels (to the nearest ppm) detected in the breath expired through the unit.

Carboxyhemoglobin Measurements. Whole blood samples (~3 ml each) were collected 2 minutes before and 25 minutes following the start of product use at Visits 1 and 4, and within 10 minutes prior to product use at Visits 2 and 3. Samples were collected in tubes containing EDTA and were measured for carboxyhemoglobin saturation (%COHb), defined as the percentage of total hemoglobin to which CO is bound. Measurements were generally performed within 15 minutes of collection. Carboxyhemoglobin saturation was measured using Instrumentation Laboratories IL-682 CO-oximeters.

Urine Samples. Participants were asked to collect all urine voided for two 24-hour periods during the study, once at the end of the *ad libitum* smoking phase (Week 1) and once at the end of the final smoking reduction phase (Week 4). Participants started each 24-hour urine collection with the second void of the morning the day before their study visit and collected all urine voided up to and including the first-morning void the day of their study visit. Samples were kept cold for up to 36 hours from the start of collection using ice packs and storage coolers provided at Orientation. Samples were stirred for 10 minutes, aliquoted, and frozen at approximately -70°C or below. Aliquots of all 24-hour urine samples were sent to ABF Laboratories (Munich, Germany) for biomarker analyses.

Single-void urine samples ("spot" urine samples) were collected from participants during each study visit. Samples were chilled in coolers with ice packs for 4-7 hours, then aliquoted and frozen at approximately -70°C or below. Spot urine samples were retained for possible future analyses.

Blood Sample Collection and Processing. Venous access was started and maintained at Visits 1 and 4 by the insertion of an indwelling catheter into the antecubital region of the arm. The catheter remained in place for up to 120 minutes. Heparin solution (0.1 cc) was injected into the access port between blood draws to prevent clot formation. Prior to each blood collection, approximately 1.5 ml of blood was drawn and discarded to flush the heparin from the catheter port. Blood (~3 ml) was drawn into individual gold-topped serum separator tubes to obtain serum for nicotine and cotinine analyses at -2, 0, 3, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 minutes with respect to the start of product use. Samples were allowed to clot at room temperature for at least 30 minutes. Tubes containing clotted blood were spun in a refrigerated centrifuge (8°C) at 3000 rpm for 20 minutes. Serum was aliquoted into cryovials (~750 µl each) and stored at approximately -70°C or below. Samples were shipped frozen to ABF Laboratories (Munich, Germany) for nicotine and cotinine measurements.

At Visits 1 and 4, additional whole blood samples were drawn at -2 and 25 minutes with respect to the start of product use for determination of %COHb. At Visits 2 and 3, one whole blood sample was drawn for this purpose just prior to the start of product use. For these samples, whole blood (~3 ml) was drawn into tubes containing EDTA to prevent clotting (see Carboxyhemoglobin Measurements above).

Serum Nicotine Concentration Corrections. (b) (4)

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Questionnaires. At each visit, participants completed questionnaires to assess their sensory impressions of UB cigarettes and Camel Snus. Cigarette questionnaires included: 1) an overall rating (the thermometer score), 2) various cigarette attribute ratings, and 3) scales assessing the perceived level of physical impact at four upper body locations (Attachments 1-3, respectively). Similar questionnaires assessed subjective measures related to snus use including: 1) an overall snus rating (the “thermometer” score), 2) various snus attribute ratings, and 3) scales assessing the level of perceived physical impact at five upper body locations (Attachments 4-6, respectively). All questionnaires were administered at each visit and, when applicable, were presented following product use.

In addition, at Orientation following informed consent and at Visit 4, participants completed the Fagerström Test for Nicotine Dependence (FTND) (Attachment 7). This is a validated instrument that provides a short, reliable, self-reported measure of nicotine dependence related to cigarette smoking (reviewed in de Meneses-Gaya *et al.* 2009). Participants were not given scoring information for this questionnaire. At Visit 4, participants completed a version of the FTND for smokeless tobacco products (Attachment 8).

To evaluate tobacco abstinence symptoms over the course of the study, participants also completed the Minnesota Nicotine Withdrawal Scale (MNWS) at Visits 2-4 (see Attachment 9). The first 9 of 15 questions have been validated as an accurate measure of nicotine withdrawal symptoms (reviewed in Hughes 2007). These items were summed to create an overall nicotine withdrawal discomfort score.

At the end of Visit 4, participants completed an exit questionnaire. Topics for inquiry included final opinions about snus, opinions about the study and study staff, and questions intended to assess protocol compliance.

Adverse Events. Adverse events (AEs) are defined as any untoward medical events occurring during the use of study product, whether or not related to its use. AEs were recorded by study staff and were assessed for relationship to study product by a contracted physician who served as the medical advisor for the study. AEs were judged by the medical advisor to have one of the following relationships to the use of study product: definitely related, probably related, possibly related or not related.

Data Analysis. Descriptive statistical analyses were performed for all study endpoints. Arithmetic means were reported for variables with data points that were normally distributed, which included CPD, snus per day, ECO, %COHb and questionnaire responses. Medians were reported for urinary biomarkers because data points showed evidence of skewed distribution.

A mixed model with repeated measures (MMRM) was used to assess changes across time in product use, expired CO, %COHb, and FTND. A Wilcoxon Sign-Rank test was performed to assess within-subject changes of urinary biomarkers. Changes in questionnaire responses (thermometer, attributes, impact, and

MNWS) were analyzed using Kendall's τ test for trend. Questionnaire endpoints were normalized to account for participant scale-usage differences prior to testing.

Statistical significance was specified as $p \leq 0.05$, and all references to significance are made with regard to this criterion. Nominal significance was specified as $0.05 < p \leq 0.10$. Final analyses were based on data from the 32 participants who completed the study.

RESULTS

Participants. A total of 43 participants provided informed consent to participate in the study following an orientation session. Thirty-six participants met all of the inclusion and none of the exclusion criteria and were enrolled for study procedures. Thirty-two participants (18 female, 14 male) completed the study. Of the four participants who did not complete the study, one withdrew due to a family emergency, one withdrew due to health concerns unrelated to product use, one was dismissed due to protocol noncompliance, and one was dismissed due to illness.

Product Use. Over the course of the study, average reported CPD significantly decreased and average reported snus per day significantly increased (Tables 5 and 6). Average smoking reductions calculated from participant reports were 24%, 41%, and 59% from Visit 1 to Visit 2, Visit 3, and Visit 4, respectively. Participants began using snus in Week 2 and, based on their reports, increased snus use an average of 20% and 39% from Visit 2 to Visit 3 and from Visit 2 to Visit 4, respectively. Snus use overall and by variety is reported in Table 6.

CPD did not statistically significantly differ between genders at any point during the study. However at Visits 1 and 2, females reported smoking fewer CPD with nominal statistical significance. Results are reported in Table 7.

In contrast, overall snus-per-day reports, Week 3 reports and Week 4 reports were statistically significantly higher for males than females. During participants' first week of use (Week 2), male snus-per-day reports were higher than female reports with nominal statistical significance. Results are reported in Table 8.

Table 5. Cigarette use patterns among completers. N=32

Visit	Cigarettes / day		Reduction in CPD Compared to Visit 1	
	Mean* (SD)	Min, Max	Mean	
1	22.3 (8.5)	6.7, 39.6	.	
2	17.2 (7.5)	4.2, 34.7	24%	
3	13.2 (5.9)	3.7, 25.7	41%	
4	9.3 (5.2)	1.5, 22.3	59%	

*All pairwise comparisons are statistically significant ($p < 0.0001$).

Table 6. Snus use patterns among completers.

											Increase in Snus per day compared to Visit 2		
Snus / day													
Visit	All Varieties				Frost				Mellow			All Varieties	
	N	Mean*	(SD)	Min, Max	N	Mean	(SD)	Min, Max	N	Mean	(SD)	Min, Max	Mean
2	32	2.7	(1.2)	0.7, 5.5	22	2.6	(1.1)	1.0, 5.5	10	2.9	(1.4)	0.7, 5.2	..
3	32	3.0	(1.5)	1.3, 7.3	19	3.0	(1.6)	1.3, 2.7	13	3.0	(1.2)	1.5, 5.7	20%
4	32	3.5	(2.3)	1.2, 11.2	21	3.3	(2.5)	1.2, 2.3	11	3.9	(1.8)	1.3, 7.7	39%

*Pouches per day reported at Visit 4 > Visit 2 ($p = 0.0276$). Pouches per day reported at Visit 4 > Visit 3 ($p = 0.0088$)
No differences were observed with regard to variety.

Table 7. Cigarette use according to gender

CPD								
Visit	Females				Males			p-value
	N	Mean (SD)	Min, Max		N	Mean (SD)	Min, Max	
1	18	19.9 (7.7)	6.7, 36.3		14	25.3 (8.9)	11.1, 39.6	0.0747
2	18	15.1 (6.6)	4.2, 29.5		14	19.9 (7.9)	8.2, 34.7	0.0730
3	18	11.9 (5.8)	3.7, 23.7		14	14.8 (5.8)	5.3, 25.7	0.1770
4	18	8.6 (4.9)	2.8, 19.3		14	10.2 (5.6)	1.5, 22.3	0.3788

Table 8. Snus use according to gender.

Pouches / day								
Visit	Females				Males			p-value
	N	Mean (SD)	Min, Max		N	Mean (SD)	Min, Max	
2	18	2.3 (0.9)	0.7, 4.7		14	3.1 (1.3)	1.0, 5.5	0.0517
3	18	2.4 (0.9)	1.3, 4.7		14	3.8 (1.7)	1.5, 7.3	0.0039
4	18	2.6 (1.2)	1.2, 6.0		14	4.7 (2.9)	1.3, 11.2	0.0104
all	54	2.4 (1.0)	0.7, 6.0		42	3.9 (2.1)	1.0, 11.2	0.0116

Expired Carbon Monoxide and Percent Carboxyhemoglobin Measurements. Two methods were used to measure carbon monoxide (CO) exposure at each study visit. CO levels were measured in expired breath samples, and carboxyhemoglobin levels were measured as a percentage of total hemoglobin in whole blood (%COHb). Expired CO (ECO) and %COHb were measured just prior to product use and 25 minutes after the start of product use during study visits. Results observed at both time points are reported in Tables 9 and 10.

Table 9. Expired CO and %COHb measurements just prior to product use for participants who completed all visits.

0 minute time point								
Visit	Expired CO (ppm)		% Reduction in CO Relative to Visit 1		%COHb of Total Hb		% Reduction in %COHb Relative to Visit 1	
	N	Mean* (SD)	N	Mean	N	Mean# (SD)	N	Mean
1	31	31.4 (16.8)		.	32	6.7 (2.5)		.
2	32	28.7 (14.3)	31	5.5	32	6.3 (2.5)	32	4.8
3	29	25.1 (15.2)	28	17.4	31	5.6 (2.2)	31	16.8
4	31	27.7 (22.5)	30	11.2	32	6.2 (2.3)	32	8.8

*Statistically significant and nominally significant differences were observed for ECO as follows:
Visit 1 > Visit 3, p=0.0316, Visit 2 > Visit 3, p=0.0962

#Statistically significant differences were observed for %COHb as follows:
Visit 1 > Visit 3, p=0.0085, Visit 2 > Visit 3, p=0.0327, Visit 3 < Visit 4, p=0.0385

Table 10. Expired CO and %COHb measurements 25 minutes after the start of product use for participants who completed all visits.

Visit	25 minute time point							
	Expired CO (ppm)		% Reduction in CO Relative to Visit 1		%COHb of Total Hb		% Reduction in %COHb Relative to Visit 1	
	N	Mean* (SD)	N	Mean	N	Mean# (SD)	N	Mean
1	32	34.7 (16.1)		.	32	7.4 (2.8)		.
2	31	26.9 (12.8)	31	23.3		.		.
3	32	22.4 (13.6)	32	37.3		.		.
4	32	26.0 (19.6)	32	27.8	32	6.0 (3.2)	32	21.1

*Statistically significant differences were observed for ECO as follows:

Visit 1 > Visit 2 and Visit 1 > Visit 3, $p < 0.0001$, Visit 1 > Visit 4, $p = 0.0038$,

Visit 2 > Visit 3, $p = 0.0283$

Visit 4 > Visit 3, $p = 0.0554$ (nominal significance)

#A nominally significant difference was observed for %COHb:

Visit 1 > Visit 4, $p = 0.0693$

Biomarker Measurements in 24-Hour Urine Samples. Participants collected 24-hour urine samples at baseline (Week 1) and after 3 weeks of dual use (Week 4) for comparison of the levels of biomarkers of tobacco exposure before and after their tobacco-use transition. The baseline sample was collected the day before the first study visit, and the second sample was collected the day before the final visit, which generally corresponded to participants' largest cigarette reductions and greatest use of snus. Several biomarkers of tobacco exposure of both particulate and vapor phases were measured in the samples. Results are reported in Table 11.

Table 11. Biomarkers of tobacco exposure measured in 24-hour urine samples at baseline and after three weeks of dual use. N=32

			Visit 1	Visit 4	Median Percent Change	p-value	
Category	Biomarker	Metabolite of:	Median	Median			
Particulate Phase Constituents	Nicotine	Nicotine Equivalents (mg/24h)	nicotine	20.5	16.8	-17.3	#0.0525
	Tobacco-Specific Nitrosamines	NNN-T (ng/24h)	NNN	27.0	19.9	-1.8	0.5704
		NAT-T (ng/24h)	NAT	395.3	399.6	-19.1	0.1761
		NAB-T (ng/24h)	NAB	78.8	66.2	-20.9	*0.0007
		NNAL-T (ng/24h)	NNK	879.3	932.4	-9.3	#0.0719
			NNN, NAT, NAB,				
		TSNA-T (ng/24h)	NNK	1540	1474	-8.5	#0.0689
	Aromatic Amines	3-Aminobiphenyl (ng/24h)	-	8.8	7.9	-28.3	*0.0009
		4-Aminobiphenyl (ng/24h)	-	24.2	18.9	-13.7	*0.0306
		2-Aminonaphthalene (ng/24h)	-	27.4	24.7	-26.1	*<0.0001
		o-Toluidine (ng/24h)	-	181.6	175.9	-16.3	*0.0133
	PAHs	1-OH-Naphthalene (µg/24h)	naphthalene	13.4	9.5	-27.4	*<0.0001
		2-OH-Naphthalene (µg/24h)	naphthalene	16.0	12.9	-21.6	*0.0002
		2-OH-Fluorene (ng/24h)	fluorene	2436	2195	-25.6	*0.0045
		1-OH-Phenanthrene (ng/24h)	phenanthrene	219.3	194.5	-10.9	0.1755
		2-OH-Phenanthrene (ng/24h)	phenanthrene	175.1	123.8	-20.2	0.1034
		3-OH-Phenanthrene (ng/24h)	phenanthrene	268.1	265.4	+22.0	0.8483
4-OH-Phenanthrene (ng/24h)		phenanthrene	39.8	45.4	-4.1	0.2038	
9-OH-Phenanthrene (ng/24h)		phenanthrene	182.7	173.7	-13.1	0.1805	
1-OH-Pyrene (ng/24h)		pyrene	267.5	234.9	-5.8	0.7018	
	AAMA (µg/24h)	acrylamide	296.3	239.0	-22.0	*0.0055	
	GAMA (µg/24h)	acrylamide	40.4	39.1	-21.7	*<0.0001	
Vapor Phase Constituents							
		HPMA (µg/24h)	acrolein	2250	1782	-23.4	*0.0036
		SPMA (µg/24h)	benzene	6.06	5.51	-35.5	*0.0006
		HMPMA (µg/24h)	crotonaldehyde	8545	6679	-23.4	*0.0031
		MHBMA (µg/24h)	1, 3 butadiene	5.08	5.03	-30.8	*0.0022
		CEMA (µg/24h)	acrylonitrile	235.8	204.4	-21.4	*0.0223
		HEMA ((µg/24h)	ethylene oxide	14.0	13.4	-25.2	*0.0003
	Thiocyanate (ng/24h)	hydrogen cyanide	3456	2197	-39.3	*0.0002	

*Indicates statistical significance.

#Indicates nominal statistical significance.

Yield-In-Use (YIU) Measurements. Participants collected the filters of all cigarettes smoked the day before each study visit and returned them to study staff for analysis. The amounts of nicotine and ‘tar’ deposited in the last 10 mm of the filter were measured to estimate the maximum amount of nicotine and ‘tar’ available to smokers at the mouth level.

A nominally significant 8.7% reduction in nicotine per cigarette was seen from Visit 1 to Visit 4 ($p=0.0697$). ‘Tar’ per cigarette did not change significantly from Visit 1 to Visit 4 ($p=0.2367$). Interestingly, significant reductions in nicotine and ‘tar’ per cigarette were seen from Visit 1 to Visit 2, $p=0.0151$ and $p=0.0029$, respectively. Statistically significant reductions in nicotine and ‘tar’ per day were seen over the study, primarily as a result of the reduction in CPD. (For all pair-wise comparisons, $p\leq 0.0002$.) Results are reported in Table 12.

Table 12. Yield-in-use cigarette data for participants who completed all visits.

Visit	N	Nicotine mg/cig	‘Tar’ mg/cig	‘Tar’:Nicotine mg/mg	Nicotine mg/day	‘Tar’ mg/day
		Mean* (SD)	Mean# (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1	32	1.38 (0.5)	13.6 (4.7)	9.9 (1.1)	27.8 (12.3)	274 (122)
2	32	1.29 (0.4)	12.5 (3.7)	9.7 (1.1)	20.0 (9.9)	194 (93)
3	32	1.31 (0.4)	13.1 (4.3)	10.0 (1.2)	15.5 (7.7)	156 (78)
4	31	1.26 (0.5)	13.0 (4.9)	10.8 (5.0)	10.8 (6.6)	109 (66)

*Nicotine per cigarette, significant or nominally significant pair-wise comparisons:

Visit 1 > Visit 2, $p=0.0151$, Visit 1 > Visit 3, $p=0.0668$, Visit 1 > Visit 4, $p=0.0697$

#‘Tar’ per cigarette, significant or nominally significant pair-wise comparisons:

Visit 1 > Visit 2, $p=0.0029$, Visit 2 < Visit 3, $p=0.0550$

Snus After Use Measurements. The extraction of tobacco constituents from snus was calculated by subtracting the amount of constituent remaining in the pouch after use from the amount of constituent present in the paired unused snus lot. Descriptive statistics are reported in Table 13. Negative extractions are an artifact of the variability of the analytical method used to detect trace metals. Results were also analyzed by variety and gender. No statistically significant differences were seen in extraction levels between genders for any constituent (data not shown). Of the constituents shown to be extracted (*i.e.*, all but trace metals), extraction amounts of nicotine, B[a]P, and all TSNAAs did not differ between varieties (data not shown). An average of 9.7 μg more normicotine ($p=0.0424$) and an average of 3.8 μg more anatabine ($p=0.0978$) were extracted from the Mellow variety.

Table 13. Amount of tobacco constituents extracted per pouch from snus collected during *ad libitum* use.^a

Constituent	Units	N	Amount extracted		Percent extracted	
			Mean	SD	Mean	SD
Nicotine	(mg/pouch)	32	1.6	1.1	22.2%	14.7%
Nornicotine	(µg/pouch)	32	20.0	14.2	18.7%	13.1%
Anatabine	(µg/pouch)	31	10.3	6.1	17.0%	10.1%
B[a]P	(ng/pouch)	27	0.0	0.1	-6.6%	13.8%
Cd	(ng/pouch)	32	4.0	37.0	-1.2%	21.3%
Cr	(ng/pouch)	32	-24.6	65.8	-16.3%	31.8%
Ni	(ng/pouch)	32	-48.8	85.6	-15.6%	25.7%
Pb	(ng/pouch)	32	-17.6	26.4	-24.5%	33.9%
As	(ng/pouch)	32	-17.1	13.6	-35.1%	31.1%
Se	(ng/pouch)	32	-4.9	5.6	-8.8%	10.1%
NNN	(ng/pouch)	27	78.4	50.2	18.6%	11.9%
NAT	(ng/pouch)	32	10.7	38.8	5.2%	19.5%
NAB	(ng/pouch)	32	-3.6	7.5	-14.7%	30.5%
NNK	(ng/pouch)	32	18.1	19.1	13.4%	13.9%

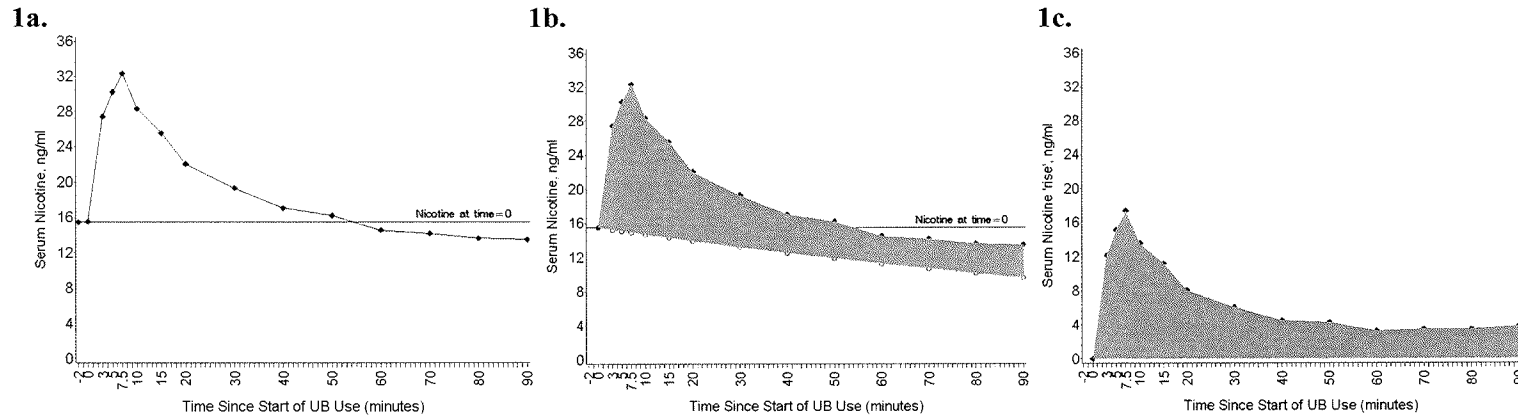
^a Summaries were calculated using one average extraction per participant if multiple extractions of a single constituent were performed on pouches returned by a single participant.

Serum Nicotine and Cotinine Analysis. Serum nicotine profiles following UB and snus use in the lab are shown in Figures 1 and 2 respectively. In contrast to results from the Strips and Sticks local studies, which followed similar protocols (Round *et al.*, 2010 RDR; Bowman *et al.*, 2010 RDR), a serum nicotine rise was observed following snus use; nevertheless, a similar correction technique applied in those studies were also applied to these data. Corrections were applied to better estimate the nicotine uptake following UB and snus use after a minimal, 30-minute tobacco abstinence. Average serum nicotine AUC and peak nicotine concentrations following smoking of one UB cigarette were significantly higher than concentrations following use of one Camel Snus pouch. This was true for observed and corrected values. Averages of observed and corrected results are reported in Table 14. Other work is in progress to address confounding background nicotine levels.

Average starting serum cotinine levels did not change significantly from Visit 1 to Visit 4: participants who completed the study averaged 308.9 ± 199.8 ng/ml at the start of Visit 1 and 286.8 ± 155.0 ng/ml at the start of Visit 4 ($p=0.42$).

Table 14. Observed and “corrected” average serum nicotine results.

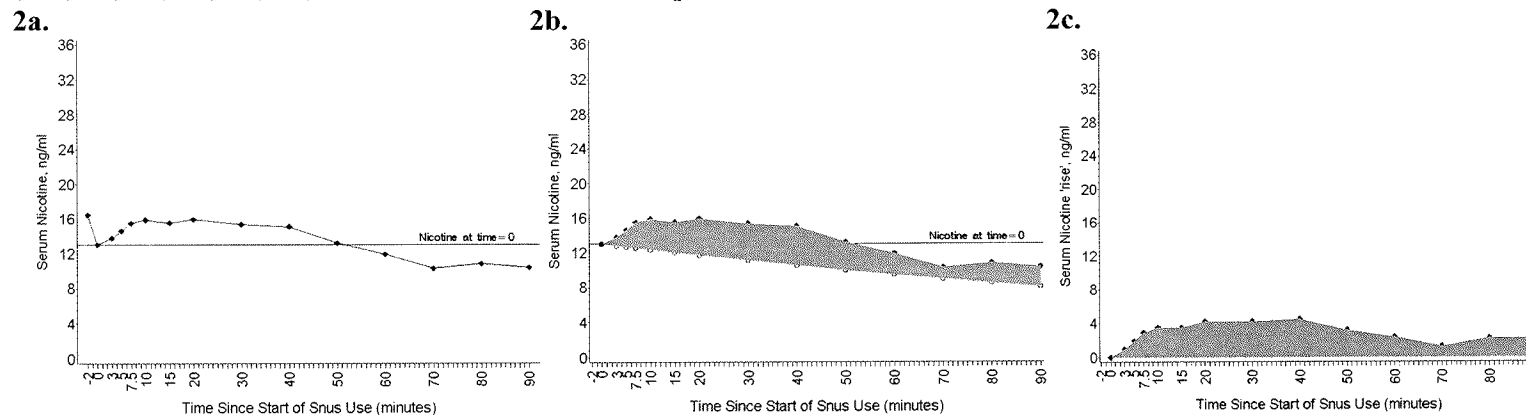
Product-Visit	Observed values				Values after baseline nicotine clearance correction			
	AUC		Peak concentration (ng/ml)		AUC		Peak concentration (ng/ml)	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
UB-Visit 1	32	1655.7 (724.8)	32	37.8 (18.0)	32	546.6 (354.3)	32	23.2 (14.2)
Snus-Visit 4	29	1189.1 (694.4)	31	20.1 (11.9)	29	299.3 (269.1)	31	9.2 (6.3)
p-value		0.0161		<0.0001		0.005		<0.0001

Figure 1. Average serum nicotine concentration vs. time curves following initiation of UB cigarette use. N=32 for all time points.

1a. Average serum nicotine concentrations observed for the duration of testing.

1b. Top curve: observed serum nicotine concentrations. Bottom curve: estimated clearance of starting nicotine. Shaded area represents estimated nicotine uptake from one UB cigarette.

1c. Cleared nicotine estimations have been subtracted from observed values. Shaded area represents estimated nicotine uptake from one UB cigarette.

Figure 2. Average serum nicotine concentration vs. time curves following initiation of snus use. N=32 for -2 minute time point. N=31 for 0, 3, 10, 15, 20, 30, 50, 60, 70, 80, and 90 minute time points. N=30 for 5, 7.5, and 40 minute time points.

2a. Average serum nicotine concentrations observed for the duration of testing.

2b. Top curve: observed serum nicotine concentrations. Bottom curve: estimated clearance of starting nicotine. Shaded area represents estimated nicotine uptake from one snus pouch.

2c. Cleared nicotine estimations have been subtracted from observed values. Shaded area represents estimated nicotine uptake from one snus pouch.

Questionnaires. Questionnaires captured data relating to participants' acceptance of Camel Snus, their experiences of using snus and cigarettes, sensory perceptions of snus and cigarettes, nicotine withdrawal symptoms, and nicotine dependence over the course of the study. The Cigarette and Snus Thermometers captured overall product ratings each week (Attachments 1, 4). The Cigarette and Snus Evaluation Questionnaires captured participants' sensory opinions about product attributes (Attachments 2, 5). The Cigarette and Snus Impact Questionnaires captured perceived physical impact in different regions of the body during product use (Attachments 3, 6). The Minnesota Nicotine Withdrawal Scale (Attachment 9) captured withdrawal symptoms reported by participants, and the Fagerström Test for Nicotine Dependence was given for cigarettes (Attachment 7) at the beginning and end of the study, and for smokeless tobacco (Attachment 8) at the end of the study only.

Initially, participants rated Camel Snus as "Quite Good" on the thermometer scale and, by the end of the study, rated it closer to "Very Good," with a significant upward trend in rating over time. Participants rated the sweetness, tobacco taste, and texture of snus to be "Just Right" throughout the study. They rated snus as having slightly too much flavor at Visit 1, but flavor ratings trended toward "Just Right" in Visits 2, 3, and 4. Results are reported in Table 15 and Table 16.

Participants also reported changes in their UB cigarette perceptions over the course of the study (see Table 17). Differences included a significant downward trend in thermometer rating, satisfaction, smoothness, strength of taste, and tobacco taste for their UB cigarettes. Participants reported significant increases in harshness and aftertaste of their UB cigarettes. They also reported experiencing increased impact in the nose and chest while smoking.

Table 15. Mean Snus questionnaire responses. (N=32)

Question	Visit			trend p-value
	2	3	4	
Thermometer	67	70	75	*<0.0001
Sweetness	4.2	4.0	4.0	#0.062
Flavor	4.6	4.2	4.3	#0.079
Tobacco taste	3.7	4.1	3.9	0.443
Texture	3.8	3.6	3.7	0.571
Bitterness	2.1	2.3	2.7	*0.001
Mouth burn	3.9	3.0	3.0	*0.011
Throat burn	2.6	2.6	2.9	0.543
Side Effects	2.1	2.3	2.0	0.283
Aftertaste	4.4	4.8	4.9	0.102
Overall taste	4.9	5.2	5.0	0.750
Overall likeability	4.4	4.9	4.7	0.181
Nose impact	0.6	0.5	0.6	0.573
Mouth impact	4.7	4.0	3.6	*0.002
Throat impact	2.8	2.7	3.2	0.327
Chest impact	0.4	0.6	0.8	0.132
GI Tract impact	1.5	1.7	1.7	0.554

*Indicates statistical significance.

#Indicates nominal statistical significance.

Table 16. Percent of participants reporting snus to be acceptable. (N=32)

Visit	Is this product acceptable?
	% responding "Yes"
2	91
3	97
4	97

Table 17. Mean cigarette questionnaire responses. (N=32)

Question	Visit				trend p-value
	1	2	3	4	
Thermometer	84	84	82	77	*<0.0001
Strong tasting	4.2	3.8	3.7	3.8	*0.028
Smoke through filter	3.0	3.0	2.8	2.7	0.594
Harsh	2.3	2.8	2.9	3.3	*<0.0001
Smooth	5.5	4.7	4.8	4.5	*<0.0001
Satisfying	6.1	5.5	5.3	5.0	*<0.0001
Tobacco taste	4.7	4.3	4.2	4.2	*0.013
Strong aftertaste	3.3	3.2	3.3	3.8	*0.005
Nose impact	1.9	2.4	2.3	2.8	*0.001
Mouth impact	3.5	3.3	3.2	3.7	0.576
Throat impact	3.3	2.8	3.4	3.4	0.463
Chest impact	3.4	3.0	3.4	4.0	*0.024

*Indicates statistical significance.

Changes in tobacco product usage over the course of the study resulted in a minimal change in nicotine withdrawal symptoms. Of the nine symptoms validated as accurate measures of nicotine withdrawal, only weight gain/appetite showed a small, nominally significant increase in rating as the study progressed (reviewed in Hughes 2007). In contrast, small but significant reductions were seen in anxiety, desire to smoke, insomnia, restlessness, and coughing. Results are reported in Table 18.

Table 18. Average withdrawal symptom ratings and overall withdrawal symptom scores measured using the Minnesota Nicotine Withdrawal Scale. (N=32)

Symptom description	Visit			trend p-value
	2	3	4	
Angry, irritable, frustrated	1.0	1.0	0.7	0.182
Anxious, nervous	1.0	0.7	0.6	*0.014
Depressed mood, sad	0.7	0.4	0.4	0.281
Desire or craving to smoke	2.4	2.0	2.1	*0.045
Difficulty concentrating	0.8	0.7	0.5	#0.092
Increased appetite, hungry, weight gain	0.7	0.9	1.0	#0.076
Insomnia, sleep problems, awakening at night	0.9	0.8	0.5	*0.014
Restless	0.9	0.8	0.5	*0.018
Impatient	0.9	0.8	0.7	0.468
Constipation	0.3	0.3	0.3	0.335
Dizziness	0.2	0.3	0.2	0.997
Coughing	0.9	0.8	0.5	*0.004
Dreaming or nightmares	0.7	0.7	0.7	0.321
Nausea	0.5	0.7	0.3	0.351
Sore throat	0.5	0.6	0.3	0.189
Nicotine Withdrawal Discomfort Score	9.3	8.1	7.0	0.117

*Indicates statistical significance.

#Indicates nominal statistical significance.

FTND for cigarettes was administered at the start and end of the study, and the FTND for smokeless tobacco (modified for snus) was administered at the end of the study. These instruments measure nicotine dependence from a single source; however, participants in this study received nicotine from two sources, cigarettes and snus, the last three weeks of the study. Due to this caveat, the validity of score comparisons is unknown; nevertheless, results are reported in Table 19.

Table 19. Fagerström Tests for Nicotine Dependence results: response means by question and overall scores for participants who completed all visits. (N=32)

Question	Visit		p-value
	1	4	
How soon after you wake up do you smoke your first cigarette?	2.3	1.9	*0.016
Is it difficult to refrain from smoking in places where it is forbidden?	0.4	0.2	*0.035
Which cigarette would you most hate to give up?	0.8	0.7	0.572
How many cigarettes per day do you smoke?	1.4	0.6	*<0.0001
Do you smoke more frequently after waking than during the rest of the day?	0.5	0.4	0.625
Do you smoke if you are so ill that you are in bed most of the day?	0.5	0.3	0.211
FTND Score: level of nicotine dependence, cigarettes	5.8	4.1	*<0.0001
FTND Score: level of nicotine dependence, smokeless tobacco	.	3.4	.

*Indicates statistical significance..

Adverse Events. Adverse events (AEs) that were determined by the medical advisor to be possibly, probably or definitely related to the use of snus included: nausea, throat irritation/burn, mouth burn, indigestion/heartburn/stomach discomfort, hiccups, headache, and worsening of acid reflux. Participants generally reported resolution of these events within 20 minutes of onset. The numbers of participants reporting these events at each visit are reported in Table 20. Participants reported the most AEs at Visit 2, the visit following their first full week of snus use. AEs decreased as the study progressed. No serious adverse events (SAEs) were reported during this study.

Table 20. Number of participants reporting adverse events at each visit and overall.

Adverse Event	Total # participants reporting	# Participants reporting at Visit 1	# Participants reporting at Visit 2	# Participants reporting at Visit 3	# Participants reporting at Visit 4
Nausea	7	0	3	5	1
Throat Irritation/ Burn	7	0	7	4	4
Mouth Burn	4	0	3	2	2
Indigestion/Heartburn/Stomach Discomfort	3	0	2	1	1
Hiccups	3	0	1	1	2
Headache	2	0	2	0	0
Worsening of Acid Reflux	1	0	0	0	1

DISCUSSION

The design of this study was similar to previous studies in which smokers were instructed to reduce smoking and use Tobacco Strips or Tobacco Sticks (Round *et al.* 2010 RDR, Bowman *et al.* 2010 RDR). Modifications to the study design were minor. Changes included measurement of an expanded number of urinary biomarkers of tobacco exposure, extension of the timed blood collections from 60 minutes to 90 minutes following the start of in-lab product use, and collection of used snus pouches for measurement of constituent extraction.

Product Use

Overall, participants successfully reduced cigarette consumption and incorporated Camel Snus use as the study progressed. Although participants did not reach the final targeted cigarette reduction of 75%, they did report an average reduction of 59%. Participants also increased snus use to an average of 3.5 pouches per day by the end of the study. Snus use among males was higher than females. As the study progressed, males increased snus use from an average of 3.1 pouches per day in Week 2 to 4.7 pouches per day in Week 4. Snus use among females remained constant, averaging 2.3 pouches per day in Week 2 and 2.6 pouches per day in Week 4.

Urinary Biomarker Analyses

To evaluate the reported smoking reductions, biomarkers of several vapor phase tobacco constituents were examined in 24-hour urine samples. These biomarkers are all mercapturic acids, which have elimination half-lives of 5 and 9 hours (van Welie *et al.*, 1992; van Sittert *et al.*, 1993). Analysis of biomarkers in 24-hour samples (rather than single void samples) with half-lives of this range reduce confounding effects of smoking fluctuations throughout the day and provide an estimate of smoke exposure over one to several days. All biomarkers of the vapor phase tobacco compounds measured in this study showed statistically significant median decreases of 21.4% to 39.3% from baseline. These results suggest participants significantly decreased smoke exposure.

Biomarkers of tobacco constituents found in the particulate phase of smoke and in non-combustible forms of tobacco such as snus were also evaluated. Of the 20 particulate phase biomarkers examined, ten showed significant median decreases of 13.7%-28.3%. Nicotine equivalents nominally significantly decreased a median of 17.3%, suggesting participants did not increase their nicotine intake during dual use. Total NNAL and total TSNA's nominally decreased 9.3% and 8.5%, respectively. No biomarkers examined significantly increased from baseline.

Biomarker results from this study can be compared to those observed in the RJRT Quality of Life (QOL) study (Ogden *et al.*, 2009) and the Marlboro Snus study [hereafter, MS (Sarkar *et al.*, 2009)]. In the QOL study, one cohort of smokers reduced CPD and used Camel Snus for 24 weeks. Twenty-four hour urine samples were collected under confinement conditions at baseline prior to dual use, and at 12 weeks and 24 weeks after switching to dual use with snus. Results from participants whose Camel Snus use consisted of $\geq 50\%$ of the total number of tobacco units consumed (1 unit = 1 snus pouch or 1 cigarette) were reported.

In contrast to the longer study duration of QOL, the MS study evaluated smokers who were randomized to one of four cohorts while confined for ten days. One cohort was required to reduce cigarette consumption $\geq 50\%$ and use Marlboro Snus for eight days ($n = 60$). Other cohorts included a continue-smoking group ($n = 30$), a snus-only group ($n = 15$), and a no-tobacco group ($n = 15$). Twenty-four hour urine samples were collected at baseline before randomization and on days 7 and 8 of dual use. Selected biomarkers were measured in the 24-hour urine samples of both studies.

A comparison of urinary biomarker results and CPD reductions for the cigarette/snus dual use cohorts of the three studies is shown in Table 21. While the biomarker reductions seen in all studies were not of the same magnitude as CPD reductions, they do suggest participants decreased overall tobacco exposure from baseline.

Table 21. Comparison of changes in biomarkers of tobacco exposure across studies with cigarette/snus dual-use cohorts.

Category	Biomarker	Metabolite of:	QOL Study- Week 24	MS Study - Days 7, 8	This Study - Visit 4
			Mean % Change	Mean % Change	Median % Change ^a
Nicotine Eq	Nicotine Equivalents (mg/24h)	nicotine	-9.0%	-34.3%	-17.3%
Tobacco-Specific					
Nitrosamines	NNAL-T (ng/24h)	NNK	-34.5%	-30.4%	-9.3%
Aromatic Amines	3-Aminobiphenyl (ng/24h)	-	-50.6%	ND	-28.3%
	4-Aminobiphenyl (ng/24h)	-	-44.8%	-44.9%	-13.7%
	2-Aminonaphthalene (ng/24h)	-	-55.1%	-48.1%	-26.1%
	o-Toluidine (ng/24h)	-	-42.1%	-21.4%	-16.3%
PAHs	1-OH-Naphthalene ($\mu\text{g}/24\text{h}$)	naphthalene	+12.7%	ND	-27.4%
	2-OH-Naphthalene ($\mu\text{g}/24\text{h}$)	naphthalene	-32.0%	ND	-21.6%
	2-OH-Fluorene (ng/24h)	fluorene	-34.3%	ND	-25.6%
	1-OH-Pyrene (ng/24h)	pyrene	-35.1%	ND	-5.8%
Vapor Phase Constituents	AAMA ($\mu\text{g}/24\text{h}$)	acrylamide	-39.3%	ND	-22.0%
	GAMA ($\mu\text{g}/24\text{h}$)	acrylamide	-21.5%	ND	-21.7%
	HPMA ($\mu\text{g}/24\text{h}$)	acrolein	-41.5%	ND	-23.4%
	SPMA ($\mu\text{g}/24\text{h}$)	benzene	-50.0%	-37.1%	-35.5%
	HMPMA ($\mu\text{g}/24\text{h}$)	crotonaldehyde	-48.0%	ND	-23.4%
	MHBMA ($\mu\text{g}/24\text{h}$)	1, 3 butadiene	-55.5%	ND	-30.8%
CPD ^b			-74% (4.8)	-52% (8.4)	-59% (9.3)
Snus per day ^c			10.2	2.2	3.5

^a Median percent changes are reported due to the presence of outlier data. CPD reduction is calculated as a mean % change.

^b Mean percent changes of CPD are reported with absolute CPD means in parentheses.

^c Mean values of snus pouches used per day are reported

ND indicates analyses were not done.

Biomarker comparisons between the current study and the QOL study reveal several differences. Biomarker reductions generally show similar trends, but are consistently greater in the QOL study. Differing study conditions make direct comparison difficult. CPD reductions were greater in the QOL study and were sustained over 24 weeks, allowing toxicant metabolism to reach equilibrium.

Direct comparison of biomarker results from the current study may be more appropriate with the MS study due to the shorter duration of product switching and the similar CPD reductions observed. The MS study measured one vapor phase biomarker, SPMA, a metabolite of the combustion product benzene. Results from that study showed a similar percent reduction to the results observed in our study, 37% and 36%, respectively, reflecting similar reductions in smoke exposure. Reductions in the aromatic amine o-toluidine were also similar, 16% and 21%, respectively.

Although biomarker reductions occurred in both studies, reductions in particulate phase biomarkers, including nicotine, were greater in the MS study. Several factors could contribute to these differences. First, there is some indication that smoking behavior may have changed for participants in the MS study. In the continue-smoking cohort, CPD did not change but nicotine equivalents decreased 14%, suggesting the confinement condition and/or smoking restrictions enforced by the study altered smoking behavior. Similar reductions were seen in the continue-smoking cohort for seven of the eight additional urinary biomarkers examined.

Second, participants' opinions of the different snus products may have affected biomarker reductions indirectly. Participants in our study rated Camel Snus as "Quite Good" to "Very Good" and 97% judged the product to be acceptable. Average product use at the end of the study was 3.5 pouches per day and 9.3 CPD. Product acceptability ratings were not measured in the MS study; however, the authors report CPD and snus per day use on Days 1 and 8. In that study, average snus per day decreased for both the dual-use (Day 1: 3.2 ± 2.3 , Day 8: 2.2 ± 2.6) and snus-only cohorts (Day 1: 4.5 ± 2.5 , Day 8: 3.5 ± 2.3). In addition, the number of participants in those cohorts who used snus also decreased from Day 1 to Day 8. (Because snus use was not required, participants could choose to reduce smoking without using Marlboro Snus.) The number of participants in the dual-use cohort who used Marlboro Snus was 54/60 on Day 1 and 38/59 on Day 8. Thus, 10% and 36% of participants in the dual-use cohort on Days 1 and 8, respectively, were not true dual users, but smokers who reduced smoking at least 50%. The number of participants in the snus-only cohort who used Marlboro Snus was 13/15 on Day 1 and 10/15 on Day 8. Thus, 13% and 33% of participants in the snus-only cohort on Days 1 and 8, respectively, were behaving similar to the no-tobacco cohort. These numbers suggest participants in the MS study may not have judged Marlboro Snus to be acceptable. Lack of participant acceptability also may have decreased usage time of Marlboro Snus and therefore the constituent amounts extracted from each pouch. Extraction measurements were not performed on the used Marlboro Snus, but constituent levels were reported for unused Marlboro Snus. A comparison of the constituent levels of the unused snus dispensed in both studies is shown in Table 22.

Table 22. Comparison of snus pouch constituent amounts reported in the MS study and this study.

Constituent	Units	Marlboro Snus (300 mg pouch)	Camel Snus (600 mg pouch)	
		Range	Mean	SD
Nicotine	(mg/pouch)	4.6 - 8.7	7.33	0.25
NNN	(ng/pouch)	204.6 - 335.1	421	17
NNK	(ng/pouch)	22.8 - 67.2	137	7.1
B[a]P	(ng/pouch)	0.11 - 0.20	0.47	0.06
pH		6.8 - 7.2	7.78	.

Overall, expected results for all three studies are unclear because a systematic study of incremental CPD reductions with corresponding urinary biomarker measurements has not been completed. In addition, studies to assess urinary biomarkers of tobacco exposure in natural adopters of snus are in progress; therefore, expected biomarker levels resulting from snus use alone are currently unknown.

Extraction of Tobacco Constituents from Snus

This study is one of several RJRT studies to evaluate tobacco constituent extraction resulting from use of Camel Snus. In the QOL study, constituent extraction analysis was performed on pouches used by participants in the snus cohort (Caraway and Lee, 2010 RDM). The snus product used in the QOL study contained 400 mg of tobacco in contrast to the 600 mg pouches used in the current study. Nevertheless, the amount of tobacco constituents extracted by participants was similar in both studies. Results reported from the QOL study included extraction levels of nicotine, NNN, NAT, and NNK. Although nicotine levels in the unused 600 mg pouches were greater than levels in the 400 mg pouches, participants extracted approximately the same amount of nicotine from both products, 1.8 and 1.6 mg per pouch, respectively. NAT extraction per pouch was also similar in the two studies. In contrast, NNN and NNK extraction per pouch was larger in the current study compared to QOL by 1.9 and 3.6 fold, respectively.

Another RJRT study that evaluated constituent extraction from Camel Snus was the U.S. Market Adopters study [hereafter, USMA (Caraway and Chen, 2009)]. Natural adopters of Camel Snus were recruited to collect their used snus pouches over seven days. Participants reported using at least 15 pouches of their usual brand of Camel Snus per week for at least three months immediately prior to study participation. Exclusive use of Camel Snus was not required for participation. Participants purchased Camel Snus at retail, which was sold in 600 mg pouches at the time of the study. Average extraction amounts and percent extraction in the USMA study were consistently higher than the results seen in the current study, although pouch size was the same. A comparison of results among the three studies is shown in Table 23.

Several caveats apply when comparing these results. The Camel Snus used by participants in the QOL and USMA studies was not lot controlled; therefore, the levels of constituents in unused pouches may have varied. Additionally, natural adopters may have used Camel Snus differently than smoking participants who were randomly assigned to use the product, regardless of their preferences. Also, the length of time participant used Camel Snus differed among the three studies. Participants in the current study used Camel Snus for a total of three weeks, whereas participants in the QOL study used Camel Snus for 24 weeks, and participants in the USMA study used Camel Snus for at least three months prior to sampling.

Table 23. Comparison of snus extraction levels between the current study, the Quality of Life study and the U.S. Market Adopters study.

Constituent	Current Study ^a								Quality of Life ^b						U.S. Market Adopters ^c			
	Unused Pouches (<i>n</i> =6)			Used Pouches ^d					Unused Pouches (<i>n</i> =45)			Used Pouches ^d - Week 24 (<i>n</i> =30)			Used Pouches ^c			
	Amount		Amount extracted		Percent extracted		Amount		Amount extracted		Percent extracted		Amount extracted		Percent extracted			
	Mean	SD	<i>n</i>	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Nicotine (mg/pouch)	7.33	0.25	32	1.6	1.1	22.2%	14.7%	5.55	0.73	1.8	1.1	34.4%	20.7%	2.8	NR	39%	23%	
NNN (ng/pouch)	421	17	27	78.4	50.2	18.6%	11.9%	242	5.2	41	52	17.2%	21.5%	97.1	NR	23%	22%	
NAT (ng/pouch)	203	7.4	32	10.7	38.8	5.2%	19.5%	170	34	4	47	2.1%	27.9%	34.1	NR	16%	26%	
NNK (ng/pouch)	137	7.1	32	18.1	19.1	13.4%	13.9%	57.6	27	5	21	7.1%	38.3%	37.5	NR	30%	21%	

^a Products distributed in the current study included Frost and Mellow Camel Snus in 600 mg pouches.

^b Products distributed in QOL included Frost, Spice, and Original Camel Snus in 400 mg pouches.

^c Products purchased by participants in the USMA study included Frost, Spice, and Original Camel Snus in 600 mg pouches.

^d Summaries were calculated to weigh each participant's average extraction amounts equally, regardless of the number of pouches used per participant.

^e Summaries were calculated to weigh each analysis performed per constituent equally.

NR indicates the values were not reported.

Table 24. Comparison of endpoints among ambulatory Strips, Sticks, and Camel Snus studies.

Endpoint	Units	Strips				Sticks				Snus			
		W1	W4	p-value	% Change	W1	W4	p-value	% Change	W1	W4	p-value	% Change
CPD*		20.5	8.0	<0.0001	-60%	22.4	9.2	<0.0001	-60%	22.3	9.3	<0.0001	-59%
MSFT per day*		4.7	8.6	<0.0001	+92%	2.0	4.6	<0.0001	+138%	2.7	3.5	0.0280	+39%
ECO - 25 min*	ppm	32.3	23.7	<0.0001	-29%	36.5	24.4	0.0007	-30%	34.7	26.0	0.0038	-28%
COHb - 25 min*	%	6.4	5.1	<0.0001	-21%	7.1	5.2	<0.0001	-25%	7.4	6.0	0.0693	-21%
YIU nicotine*	mg/cig	1.28	1.34	>0.05	+4%	1.39	1.56	0.002	+12%	1.38	1.26	0.0697	-9%
YIU 'tar'*	mg/cig	16.0	16.5	>0.05	+2%	18.4	20.8	0.002	+13%	13.6	13.0	0.2367	-4%
NicEq-T [#]	mg/24 hr	17	15.8	0.0403	-6%	15.3	14.3	0.3996	-6%	20.5	16.8	0.0525	-17%
NNAL-T [#]	ng/24 hr	754	671	0.0018	-10%	621	690	0.9118	-1%	879	932	0.0719	-9%
HPMA [#]	µg/24 hr	2242	1971	0.0639	-14%	2103	1458	0.0227	-23%	2250	1782	0.0036	-23%

*Mean Week 1 and Week 4 values and mean % changes are reported for these endpoints.

[#]Median Week 1 and Week 4 values and median % changes are reported for these endpoints.

Metabolism of NNK

NNK is a tobacco-specific nitrosamine formed in tobacco during the curing process. Due to the rapid metabolism of NNK in humans, the compound in its unaltered form is not found in the urine of smokers. One major metabolic pathway of NNK is its conversion to NNAL, which is subsequently *O*-glucuronidated to form NNAL-Gluc. Both NNAL and NNAL-Gluc are detected in the urine of smokers and are a reliable measure of NNK exposure (Hecht, 1998).

The percentage of NNK metabolized to NNAL and NNAL-Gluc (hereafter, total NNAL or NNAL-T) has been shown previously to differ according to whether the source of NNK is cigarette smoke or smokeless tobacco. Stepanov *et al.* (2008) showed that ~5% of NNK absorbed from smoking, presumably via the lung, is metabolized to NNAL. In contrast, Hecht *et al.* (2008) showed that ~14% of NNK absorbed from smokeless tobacco use, presumably via the mouth, is metabolized to NNAL. For the purposes of this discussion, it is assumed that the percent NNK metabolized to NNAL-T is completely dependent on route of absorption.

The metabolic differences described above could result in a 2.8 fold overestimate of NNK uptake from smokeless tobacco use if the route of absorption is not taken into account. Likewise, participants in studies that switch smokers to dual use with smokeless tobacco will have higher 24-hour urinary NNAL levels than expected if these metabolic differences are not incorporated when determining expected values. When these metabolic differences are applied to reported product use in the current study and QOL, expected urinary NNAL-T output is better estimated. Nevertheless, expected reductions of urinary NNAL-T are of greater magnitude than the reductions observed for both studies. Participants in the QOL study showed a 33% decrease in average NNAL-T; however, a 66% reduction in average NNAL-T would be expected based on reported product use. Participants' NNAL-T levels in the current study showed a median nominally significant decrease of 9.3%, but a median 49% reduction would be expected based on reported product use. These calculations assume a consistent amount of NNK is absorbed per cigarette for all brand styles and all participants throughout each study. Calculations to determine expected NNAL-T reductions for participants in the QOL study used average NNK extraction, average CPD at Week 0 and Week 24, and average pouches per day for all participants. For the current study, data for each participant were used to calculate participant-specific expected reductions.

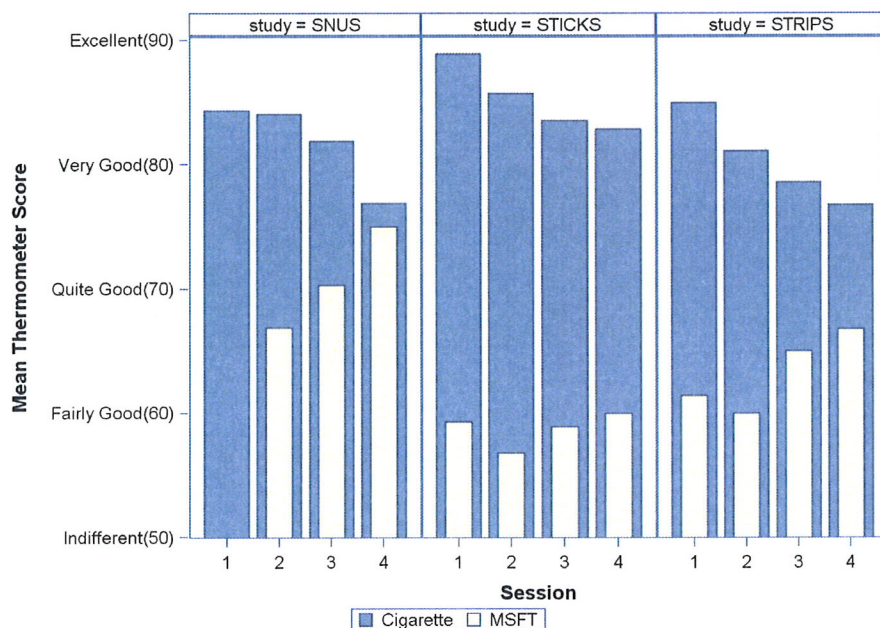
In contrast to the shorter elimination half-lives of the other urinary biomarkers analyzed in this study, NNAL-T has a longer, 40-45 day elimination half-life (Hecht *et al.*, 1999, Hecht *et al.*, 2002). This provides an additional challenge for determining expected urinary NNAL-T reductions in studies that employ shorter tobacco-product-switching periods. The longer half-life indicates that NNAL is stored in human tissue and is slowly released and eliminated from the body. These conditions could contribute to the higher NNAL-T levels observed in the final 24-hour urine samples of participants in the current study. Unlike the QOL and MS studies in which stable smoking reductions were required at enrollment, participants in the current study were required to work toward targeted CPD reductions each week of dual use. As a result, participants reported smoking an average of 13.2 CPD one week before the second urine collection, only a 41% reduction from baseline compared to the 59% reduction (9.3 CPD) achieved the day of the second 24-hour urine collection. This difference together with the longer half-life may be, in part, responsible for the larger-than-expected amounts of NNAL-T observed in the final 24-hour urine collections. A different study design, one that requires participants to reduce smoking by 60% starting

after the first urine collection and extending 21 days until the second urine collection, may result in reductions closer to expectation.

Comparison to Results from Previous Ambulatory MSFT Studies

Several results observed in this study were consistent with results observed in previous studies in which smokers switched to dual use with Tobacco Strips or Sticks (Round *et al.*, 2010 RDR; Bowman *et al.*, 2010 RDR). Participants in the Strips and Sticks studies reduced CPD 60% by the end of the study. In comparison, participants in this study reduced CPD by 59%. Reductions of CO and %COHb measured at equivalent time points were also consistent across studies. Subjective responses and nicotine withdrawal symptoms were similarly consistent; however, of the MSFT products, participants in this study scored Camel Snus better overall than Strips or Sticks and a greater percentage of participants rated Camel Snus to be an acceptable product than Strips or Sticks. Comparisons of major endpoints among ambulatory MSFT dual-use studies are shown in Table 24 and Figure 3.

Figure 3. Comparison of overall “Thermometer” ratings among ambulatory Strips, Sticks, and Camel Snus dual-use studies.



In contrast to the Strips and Sticks studies, the number of urinary biomarkers of tobacco exposure analyzed was increased in this study. Urinary biomarkers of exposure measured in the Strips and Sticks studies were limited to NicEq-T, NNAL-T and HPMa. NicEq-T decreased to the greatest extent in the current study, 17%, but with nominal statistical significance. Among the three studies, NNAL-T statistically significantly decreased only in the Strips study, but showed a similar nominally statistically significant decrease in the current study, 10% and 9%, respectively. HMPA statistically significantly decreased 23% in the current and Sticks studies and showed a nominally statistically significant decrease of 14% in the Strips study.

The results of the three biomarkers in the Strips study may indicate a small reduction in smoke exposure with minimal contribution of nicotine and NNK from Strips use. In contrast, the results of the Sticks and Camel Snus studies indicate a greater reduction in smoke exposure. Participants in the Sticks study showed a non-statistically significant decrease in urinary NicEq-T of 6%, while participants in the Camel Snus study showed a nominally statistically significant decrease of 17%. This difference is expected because Sticks study participants used more units per day and were exposed to more nicotine from each unit than participants in the Snus study. Participants used an average of 4.6 and 3.5 units per day, respectively, with an average nicotine exposure of 3.2 mg and 1.6 mg per unit respectively. Based on individual participant values for CPD, snus per day, YIU nicotine, and nicotine extraction from snus, a 32% decrease in urinary NicEq-T is expected in Week 4 of the current study. In contrast, according to similar information for Sticks study participants, substituting Sticks per day and the known amount of nicotine per Stick, a 1% decrease in NicEq-T is expected.

Urinary NNAL-T also decreased to a greater extent in Camel Snus study participants. This may be expected when related to the number of MSFT units used and amount of NNK exposure per unit. Analytical data showed one Stick to have less than 95 ng of NNK. Because subjects consumed the entire Stick, it can be assumed that subjects were exposed to all NNK present. In contrast, participants in the Camel Snus study extracted an average of 18 ng of NNK per pouch used. Because participants in both studies decreased CPD a similar amount and participants in the Camel Snus study consumed fewer MSFT units per day than participants in the Sticks study, it can be assumed that the overall percent reduction in NNK exposure would be greater for participants in the current study. Urinary NNAL-T decreased 9% with nominal statistical significance in the current study; however, a reduction of 49% was expected. A similar discrepancy was observed for urinary NNAL-T reductions in the Sticks study: urinary NNAL-T levels did not change in Week 4, but a 35% reduction was expected. See the previous section, Round *et al.* (2010 RDR), and Bowman *et al.* (2010 RDR) for further discussion.

Blood sample collection for the measurement of serum nicotine and cotinine differed among the studies. In contrast to the 60-minute blood collections in the Strips and Sticks studies, this study collected blood samples for nicotine and cotinine measurement for 90 minutes following in-lab product use. Nicotine levels following use of a Strip or Stick in the lab declined overall, probably due to the clearance of nicotine present from prior product use the day of testing. In contrast, serum nicotine levels increased following in-lab snus use, suggesting the amount and/or rate of nicotine absorbed during and following snus use is higher than Strip or Stick use. The same correction technique was applied to the serum nicotine results in all three studies; however, a different method was used in this study to calculate the average serum nicotine half-life. The average half-life calculated for participants in this study was 131 minutes, different than the 30 and 32-minute half-lives calculated for the participants in the Strips and Sticks studies, respectively, and more consistent with published reports (reviewed in Hukkanen *et al.*, 2005). A study designed to enforce a longer tobacco abstinence period would reduce the amount of starting nicotine and provide a more direct measurement of nicotine uptake from use of a single MSFT product. A study to evaluate nicotine uptake under those conditions is currently in progress and will be reported separately.

Expired Carbon Monoxide and Carboxyhemoglobin Measurements

Carbon monoxide measurement in expired breath is the least invasive method for estimating smoke exposure. However, CO is not well suited for use as an indicator of daily smoke exposure because of its quick association with hemoglobin and its short 1-4 hour half-life (reviewed in Scherer, 2006). Protocol requirements that restrict and/or require smoking at different times can significantly affect CO levels and confound the interpretation of study results.

Previous MSFT product studies measured expired CO and %COHb 25 minutes after product use in the lab during two different study visits. The measurement during the first visit was taken after smoking a cigarette; the measurement during the second visit was taken after using a MSFT product. Comparison of these measures was confounded by the requirement to smoke on the first occasion and not to smoke on the second. To eliminate this bias, expired CO and %COHb were measured just prior to product use in the lab during the same two study visits.

ECO and %COHb values were clearly affected by the different tobacco products used in the lab. Average reductions in ECO and %COHb from Visit 1 to Visit 4 after product use were 27.8% and 21.1%, respectively. In contrast, average reductions in ECO and %COHb when the values were measured just prior to product use were 11.2% and 8.8%, respectively.

To better reduce confounding factors when estimating smoke exposure, biomarkers of compounds with longer elimination half-lives than CO, yet still relatively specific to the vapor phase of tobacco, were examined. The compounds examined included 1, 3 butadiene, acrolein, benzene, and crotonaldehyde. The longer half-lives reduced the effect of smoking fluctuations within a given day. The relative specificity to tobacco smoke ensured the majority of the participants' exposure to the compound was from cigarette smoking. Changes in these compounds were measured in 24-hour urine samples and were discussed earlier in this report.

Conclusions

This study evaluated smokers who incorporated Camel Snus into their tobacco use routines while reducing smoking over three weeks. Participants reduced smoking 59% and used an average of 3.5 snus pouches per day by the end of the study. Biomarkers of tobacco exposure were measured before and after dual use. Urinary biomarkers of vapor phase tobacco constituents decreased significantly 21-39% from baseline indicating participants reduced short-term smoke exposure. Of the statistically significant changes in biomarkers of particulate phase constituents, all decreased, indicating a reduction of overall tobacco exposure during the dual-use phase of the study.

Yield-in-use analysis of spent cigarette filters indicated participants did not alter puffing behavior in a way that significantly changed their maximum mouth-level exposure to 'tar' and nicotine from cigarettes. Tobacco constituent extraction from snus was similar to results seen in the QOL study, although pouch size differed.

Over the course of the study, participants' overall ratings of their UB cigarettes decreased significantly. Significant downward trends were also observed in satisfaction, smoothness, strength of taste, and tobacco taste for UB cigarettes. Significant upward trends were observed in harshness and aftertaste.

Participants also reported experiencing increased impact in the nose and chest while smoking. In contrast to UB ratings, participants' ratings of Camel Snus significantly increased during the study.

The in-clinic phase of a study to evaluate the nicotine uptake from use of one MSFT product following a 12-hour tobacco abstinence has recently been completed. Results from that study will provide information for nicotine uptake without the confounding effects of prior smoke exposure on the test day.

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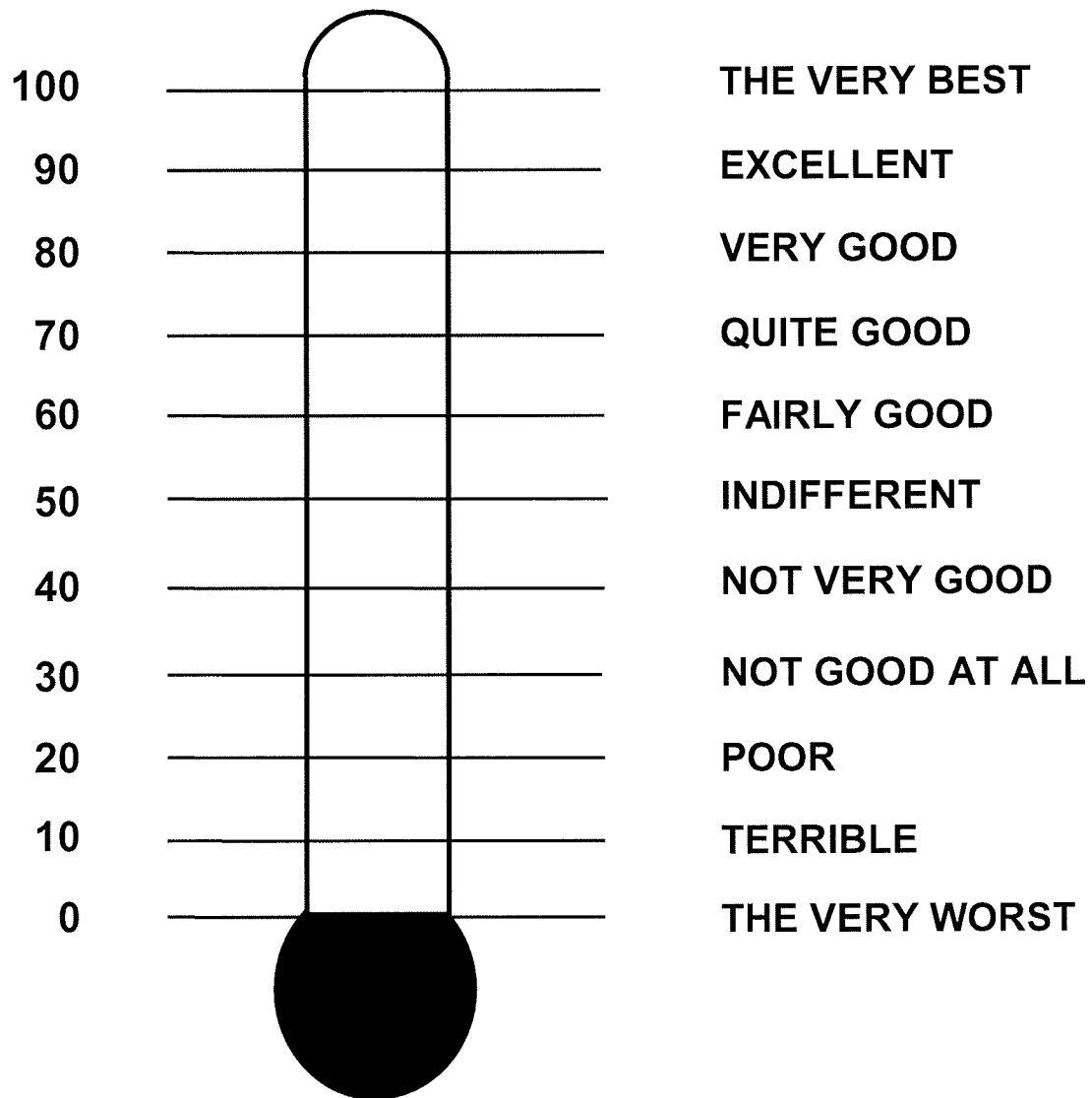
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Attachment 1

Cigarette Thermometer

Please circle the number that best describes your opinion of the cigarette you are smoking this week. Circle ONE NUMBER ONLY.



Attachment 2

Cigarette Attributes Questionnaire

Please circle the number you feel best describes the cigarette you are smoking this week.
Please circle only one number for each of the following phrases.

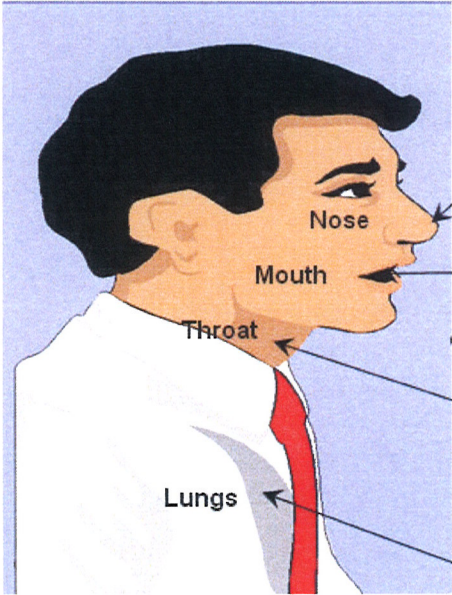
THE CIGARETTE WAS:

Not strong tasting at all	1	2	3	4	5	6	7	Extremely strong tasting
Extremely easy to get smoke through filter	1	2	3	4	5	6	7	Extremely hard to get smoke through filter
Not harsh at all	1	2	3	4	5	6	7	Extremely harsh
Not smooth at all	1	2	3	4	5	6	7	Extremely smooth
Not satisfying at all	1	2	3	4	5	6	7	Extremely satisfying
Cigarette had: No tobacco taste	1	2	3	4	5	6	7	Extremely strong tobacco taste
Cigarette left: No strong aftertaste	1	2	3	4	5	6	7	Extremely strong aftertaste

Attachment 3

Cigarette Impact Questionnaire

Subject # _____
Appearance _____
Tobacco Product _____



The diagram shows a profile of a man's head and neck. Four arrows point from specific areas to corresponding impact scales:

- Nose:** An arrow points from the bridge of the nose to the first scale.
- Mouth:** An arrow points from the mouth to the second scale.
- Throat:** An arrow points from the throat area to the third scale.
- Lungs:** An arrow points from the chest area to the fourth scale.

Each scale is a horizontal line with vertical tick marks at intervals of 1, from 0 to 8. The word "None" is above the 0 mark and "Extreme" is above the 8 mark.

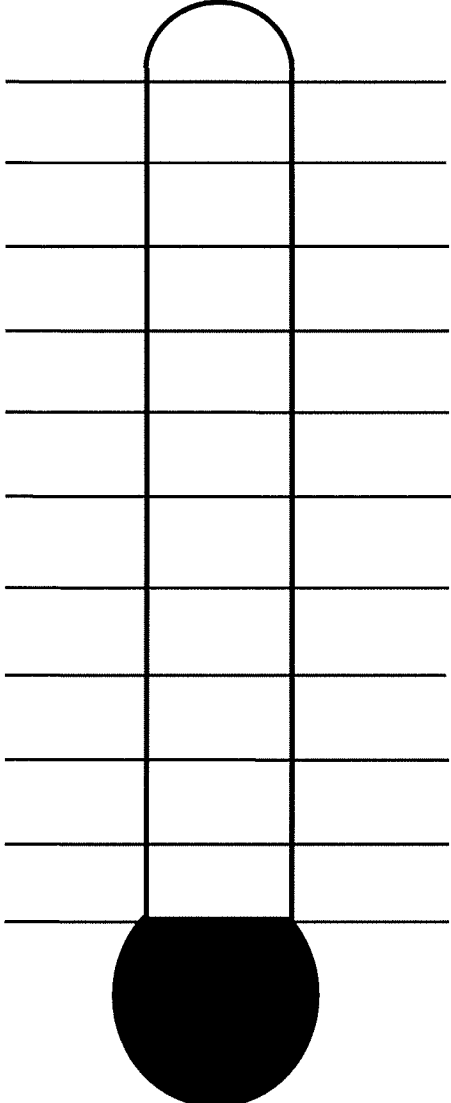
Area	None	1	2	3	4	5	6	7	Extreme
Nose									
Mouth									
Throat									
Lungs									

With the cigarette you are smoking this week, rate the level of IMPACT you feel (from None to Extreme) in EACH of the areas indicated BY CIRCLING THE NUMBER.

Attachment 4

Snus Thermometer

Please circle the number that best describes your opinion of the oral tobacco product you are using this week. Circle ONE NUMBER ONLY.

100		PERFECT
90		EXCELLENT
80		VERY GOOD
70		QUITE GOOD
60		FAIRLY GOOD
50		INDIFFERENT
40		NOT VERY GOOD
30		NOT GOOD AT ALL
20		POOR
10		TERRIBLE
0		CAN'T USE AT ALL

Attachment 5

Snus Evaluation Form

Participant # _____ Visit # _____ Date _____

Please circle the appropriate rating for each attribute.

Attribute	Rating Scale							Comments
Sweetness	1	2	3	4	5	6	7	
	Too Little		Just Right			Too Much		
Flavor	1	2	3	4	5	6	7	
	Too Little		Just Right			Too Much		
Tobacco Taste	1	2	3	4	5	6	7	
	Too Little		Just Right			Too Much		
Texture	1	2	3	4	5	6	7	
	Too Slimy		Just Right			Too Coarse		
Bitterness	1	2	3	4	5	6	7	
	None					Extreme		
Mouth Burn	1	2	3	4	5	6	7	
	None					Extreme		
Throat Burn	1	2	3	4	5	6	7	
	None					Extreme		
Side Effects (hiccups, nausea, etc.)	1	2	3	4	5	6	7	
	None					Extreme		
Aftertaste	1	2	3	4	5	6	7	
	Unpleasant					Pleasant		
Overall Taste	1	2	3	4	5	6	7	
	Unpleasant					Pleasant		
Overall Likeability	1	2	3	4	5	6	7	
	Hate it					Love it		

In your mouth, where
did you use?

Do you consider this product Acceptable?

(Circle One)

Yes

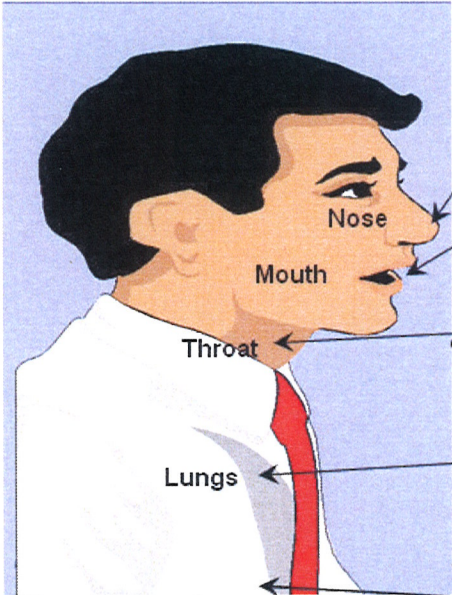
No

Attachment 6

Snus Impact Questionnaire

Subject # _____
 Appearance _____
 Tobacco Product _____

	None	0	1	2	3	4	5	6	7	8	Extreme
Nose											
Mouth											
Throat											
Lungs											
Stomach, Esophagus, GI Tract											



With the oral tobacco product you are using this week, rate the level of IMPACT you feel (from None to Extreme) in EACH of the areas indicated BY CIRCLING THE NUMBER.

Attachment 7

The Fagerström Test – Cigarettes

1. How soon after you wake up do you smoke your first cigarette?

Within 5 minutes _____ (3)
 6 - 30 minutes _____ (2)
 31- 60 minutes _____ (1)
 After 60 minutes _____ (0)

2. Do you find it difficult to refrain from smoking in places where it is forbidden e.g. in church, at the library, in cinema, etc.?

Yes _____ (1)
 No _____ (0)

3. Which cigarette would you hate most to give up?

The first one in the morning _____ (1)
 Any other.. _____ (0)

4. How many cigarettes per day do you smoke?

≤ 10. _____ (0)
 11-20 _____ (1)
 21-30 _____ (2)
 ≥ 31. _____ (3)

5. Do you smoke more frequently during the first hours after waking than during the rest of the day?

Yes _____ (1)
 No _____ (0)

6. Do you smoke if you are so ill that you are in bed most of the day?

Yes _____ (1)
 No _____ (0)

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Source: Heatherton, T. F., L. T. Kozlowski, *et al.* (1991). "The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire." *Br J Addict* **86**(9): 1119-27

Attachment 8

The Fagerström Test – Smokeless Tobacco

1. How soon after you wake up do you use your first snus pouch?
 Within 5 minutes.....☐₃
 6 – 30 minutes.....☐₂
 31 – 60 minutes.....☐₁
 After 60 minutes.....☐₀
2. How often do you intentionally swallow tobacco juice?
 Always.....☐₂
 Sometimes.....☐₁
 Never.....☐₀
3. Which snus would you hate to give up most?
 The first one in the morning.....☐₁
 Any other.....☐₀
4. How many tins per week do you use?
 More than 3.....☐₂
 2 – 3.....☐₁
 1.....☐₀
5. Do you use snus more frequently during the first hours after awakening than during the rest of the day?
 Yes.....☐₁
 No.....☐₀
6. Do you use snus if you are so ill that you are in bed most of the day?
 Yes.....☐₁
 No.....☐₀

Modified from the original and reproduced with permission.

Source: Ebbert JO *et al.* (2006). "The Fagerström Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST)." *Addictive Behaviors* **31**:1716-1721

Attachment 9

Minnesota Nicotine Withdrawal Scale**Please rate yourself for the last week:****0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe**

1. Angry, irritable, frustrated	0	1	2	3	4
2. Anxious, nervous	0	1	2	3	4
3. Depressed mood, sad	0	1	2	3	4
4. Desire or craving to smoke	0	1	2	3	4
5. Difficulty concentrating	0	1	2	3	4
6. Increased appetite, hungry, weight gain	0	1	2	3	4
7. Insomnia, sleep problems, awakening at night	0	1	2	3	4
8. Restless	0	1	2	3	4
9. Impatient	0	1	2	3	4
10. Constipation	0	1	2	3	4
11. Dizziness	0	1	2	3	4
12. Coughing	0	1	2	3	4
13. Dreaming or nightmares	0	1	2	3	4
14. Nausea	0	1	2	3	4
15. Sore throat	0	1	2	3	4

Original reference (with additions made by the University of Vermont, Department of Human Behavioral Pharmacology):

Hughes, JR and Hatsukami, D (1986). Signs and Symptoms of Tobacco Withdrawal. Arch Gen Psychiatry **43**: 289-94. Available through public domain.