



Consumption patterns and biomarkers of exposure in cigarette smokers switched to Snus, various dissolvable tobacco products, Dual use, or tobacco abstinence



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ABSTRACT

The objectives of this clinical study were to evaluate changes in tobacco product use behavior and levels of selected biomarkers of exposure (BOEs) for smokers who switched to one of six conditions during clinical confinement: exclusive use of; Camel Snus, Sticks, Strips or Orbs, controlled Dual use of cigarettes and Camel Snus, or tobacco abstinence. The controlled Dual use (DU) condition mandated a 60% reduction in cigarettes smoked per day (CPD). 167 healthy U.S. male and female smokers were randomized to the six groups ($n = 25\text{--}30/\text{group}$). Subjects smoked their usual brand of cigarette for 1 day prior to switching to their designated intervention condition. Levels of thirty-two BOEs in plasma, whole blood, urine and feces were determined before and after switching. Questionnaires that scored nicotine dependence and withdrawal discomfort were also administered. After 5 days, exclusive Snus, Sticks, Strips, or Orbs use averaged 6.1, 5.9, 13.5, and 8.5 units/day, respectively. DU subjects smoked 7.6 CPD and used 3.2 Snus pouches/day, on average. After 5 days, substantial reductions of most biomarkers, including nicotine, were observed in all groups. Toxicant exposures were similar to being tobacco abstinent after switching exclusively to Camel Snus, Sticks, Strips or Orbs. DU reductions were more modest.

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1. Introduction

In recent years, the tobacco industry, including R.J. Reynolds Tobacco Company (RJRT), has introduced smokeless tobacco products containing reduced levels of harmful and potentially harmful constituents (HPHCs) present in cigarette smoke. Camel Snus and a relatively new tobacco product category, referred to as dissolvable tobacco products (DTPs), that includes Camel Sticks, Strips, and Orbs are examples of such products. Snus comes in a portioned pouch. It is placed between the cheek and gum for about 20–30 min and does not require spitting. Camel DTPs are intended to be placed in the mouth until completely dissolved or dispersed and entirely consumed, and also do not require spitting. While no tobacco product has been shown to be safe and without risks, the increased risk of harm from tobacco use is generally recognized to proceed along a pronounced continuum; significantly influenced by the type of tobacco product, its associated toxicant profile, and the manner and frequency of use (Zeller et al., 2009; Zeller, 2013). Products that burn tobacco during use produce tobacco

combustion-related toxicants. As such, the risks for serious disease are greatest with combustible tobacco products.

The objectives of this clinical trial were to characterize tobacco product use patterns and subjective responses, estimate daily mouth-level exposure (MLE) to ‘tar’ and/or nicotine, and to quantify levels of select biomarkers of tobacco or tobacco smoke exposure in biological specimens after smokers are switched for a 5-day period to one of six groups: exclusive use of Snus, Sticks, Strips, or Orbs, Dual use of cigarettes and Snus (Dual use), or smoking/tobacco abstinence (Abstinent). The Abstinent group was included to determine the maximum declines possible for biomarker responses under the trial conditions utilized. The biomarkers of exposure selected for analysis represent toxicants which have been identified as hazardous or potentially hazardous, and shown in other clinical intervention trials to change when cigarette smokers significantly change their smoking behavior, switch to the use of tobacco products with lower toxicant levels or abstain from tobacco use (Sarkar et al., 2009; Breland et al., 2006; Hatsukami et al., 2004; Mendoza-Baumgart et al., 2007).

2. Materials and methods

This trial was a randomized, controlled, open-label, parallel group study design, conducted by Covance Clinical Research Unit

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Inc. (Madison, WI) at three clinical sites; Daytona Beach, FL, Evansville, IN, and Madison, WI, with the clinical phase performed between September, 2010 and February, 2011. The trial was approved by Independent Institutional Review Board, Inc. (currently Shulman Associates IRB, Inc., Fort Lauderdale, Florida USA) and was conducted in accordance with Good Clinical Practice, Financial Disclosure by Clinical Investigators and Institutional Review Boards and consistent with the Declaration of Helsinki. All participants signed informed consent before any trial procedures were performed and were paid for their participation.

2.1. Participants

Generally healthy adult male and female smokers between the ages of 21 and 65 were recruited by local radio, TV and printed advertisement. Potential enrollees were prescreened by phone and those eligible asked to make two screening visits within a 28-day period. Primary inclusion criteria required: a self-report of smoking ≥ 10 cigarettes/day for ≥ 12 months (menthol or non-menthol; high or medium machine measured 'tar' yield), a Fagerström test for nicotine dependence (FTND) score ≥ 3 (minimal dependence or higher) (Heatherton et al., 1991). Primary exclusionary criteria were: use of any nicotine-containing products other than cigarettes within 30 days prior to trial, evidence of drug abuse, a serious medical condition, current oral lesions, or being or becoming pregnant or breast feeding. Participants with chronic diseases whose symptoms were controlled by oral medication(s) (e.g., diabetes or hypertension) were enrolled if deemed acceptable by the principle investigator.

During the first screening visit enrollees signed informed consent, had their urine cotinine level measured (pass ≥ 200 ng/mL) and were asked to 'taste-test' one of each of the four test products for palatability. If acceptable, enrollees had a physical and oral exam and provided demographic and medical history information. Urine and blood specimens were collected for standard screenings (clinical chemical series-20, hematology, urinalysis, pregnancy, FSH and estradiol, hepatitis, HIV, urinary illicit drugs and alcohol breathalyzer) and the FTND was administered and scored. During the second visit, vital signs and expired carbon-monoxide (ECO) measurements were made, clinical laboratory results reviewed and eligibility determined. Participants were randomized into one of six groups (but were blinded to their assigned group until first day of new condition) and scheduled for clinic check-in. Participants were informed they could leave the trial at any time for any reason without penalty, and if they wanted to quit smoking, they were encouraged to discuss treatment options with their private physician.

2.2. Test products

Participant usual brand (UB) cigarettes were provided by the clinical sites and purchased locally at retail outlets. Both menthol and non-menthol brands were allowed.

Camel Snus (Snus) is consistent in form and general composition to other commercial products referred to as "Swedish snus", primarily available in Scandinavian countries. It is widely accepted that Swedish snus, while addictive, is less risky than cigarettes and may lower risks of tobacco-related diseases (e.g., WHO, 2008; European Commission, 2008; Le Houezec et al., 2011). The Snus tested in this trial was made as individual 600-mg pouches, with a moisture content of $\sim 30\%$, and a total alkaloid content of ~ 6.9 mg/pouch. Nicotine is the primary tobacco alkaloid, comprising about 95% of the total alkaloid content (Benowitz et al., 2009). On a dry-weight basis (dwb), Snus is mainly comprised of tobacco, with the pouch material and food-grade minor ingredients accounting for the remainder: sodium carbonate, sodium bicarbonate, sucralose, propylene glycol, salt, and natural and/or

artificial flavors. The relatively low salt content in Snus minimizes salivation and the need to spit. In this trial, two Snus variants were available for participant selection; Frost (mint) or Mellow (non-mint). Both variants were comprised of the same tobacco blend and substantially similar formulations, differing primarily in the proprietary flavor mixture used. Because flavor mixtures account for relatively small proportions of the final products, both variants are considered equivalent in nature and composition for the purpose of this study.

The Sticks, Strips, and Orbs tested are generally similar in composition and consist of finely-milled tobacco and other food-grade ingredients that include: binders, fillers, acid-base modifiers, processing aids, humectants, colorants, artificial sweeteners, and natural and/or artificial flavors. Having some differences in non-tobacco ingredients, Sticks, Strips, and Orbs in this study differ primarily by their physical attributes (unit weight, shape, size, and dissolution time) and organoleptic characteristics (texture and taste). Sticks, Strips, and Orbs are low moisture products having a unit weight of approximately 486, 207, and 227 mg, and a total alkaloid content of approximately 3.2, 1.0, and 1.1 mg, respectively.

Given tobacco blend selection, other food-grade ingredients and manufacturing conditions, all products tested in this trial had relatively low concentration levels of select toxicants (i.e., total tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), aromatic amines (AAs), and metals).

2.3. Trial design and conduct

Trial conduct confined participants on an on-going rotational basis until total enrollment required per group ($n = 25$) was achieved. Five to 14 participants were confined/rotation/site; check-in occurred the day before baseline (D -2), followed by baseline day (Day -1), followed by Days 1–5 of intervention. The following morning after Day 5 (Day 6) participants completed procedures and were discharged after medical approval. Six rotations were required to complete enrollment at each of the three clinical sites, with 73, 30, and 64 completed participants recruited at the Daytona, Evansville, and Madison sites, respectively. During confinement, participants were offered meals and snacks that excluded: grilled, charbroiled or barbequed foods (potential sources of PAHs and AAs) and full-bodied beer, almonds and kale (potential sources of cyanogens). Participants could elect to drink up to two alcoholic beverages (12-oz ultralight beer or 6-oz of white or rose wine per serving) during evening meals. Tobacco product usage was unrestricted (except as noted) although site staff dispensed all products (one cigarette or Sticks, or one or two Snus, Strips or Orbs) between the hours of 07:00 and 23:00 whenever requested. Each site had a sheltered outdoor smoking area and participants wore color-coded wrist-bands to facilitate staff surveillance of proper product use.

During baseline day, all participants smoked as many UB cigarettes as desired until 23:00. Their daily consumption, i.e., cigarettes per day (CPD) was determined based upon the number of cigarettes smoked during baseline day. As a precautionary measure to prevent the potential for significantly increased nicotine exposure, a product maximum-use-level (MUL) was calculated for each participant that limited their total daily tobacco usage to 130% of their baseline cigarette consumption. For this purpose, one cigarette was considered a single unit of exposure (i.e., 1.0–1.25 mg nicotine exposure assumed). Based on the nicotine content of the 4 products tested and assuming bioavailability of 50% for Snus and 100% for the DTPs: SNUS and Sticks = 2 units each and Orbs and Strips = 1 unit each. All participants were encouraged during informed consent to use as much of their assigned product as they may wish, but not to exceed their MUL. The morning after baseline day, participants started their assigned intervention as a member of one of six groups:

1. *Dual use group (Dual use)*: Participants were allowed to continue to smoke their UB cigarettes at any time, but restricted to a maximum of 40% of their baseline CPD. These participants were also allowed to consume as many Snus pouches as they wanted at any time, but not while smoking.
2. *Exclusive Snus group (Snus)*: Participants discontinued smoking entirely and switched to consuming as many Snus pouches as they wanted.
3. *Exclusive Sticks group (Sticks)*: Participants discontinued smoking entirely and switched to consuming as many Sticks as they wanted.
4. *Exclusive Strips group (Strips)*: Participants discontinued smoking entirely and switched to consuming as many Strips as they wanted.
5. *Exclusive Orbs group (Orbs)*: Participants discontinued smoking entirely and switched to consuming as many Orbs as they wanted.
6. *Tobacco Abstinent group (Abstinent)*: Participants discontinued smoking/tobacco use entirely.

All participants had unrestricted access to standard and sugarless hard candies and gum. Throughout the confinement period all tobacco product use was voluntary.

2.4. Filter processing and mouth-level exposure (MLE) estimates

Smoked cigarette filter processing: The cigarette butt length is defined as the length of unburnt cigarette remaining at the moment when the cigarette is extinguished or stops smoldering. As such, the final cigarette butt length results from the cigarette burning down to a shorter length during active smoking and, in some cases if the cigarette is not extinguished at the end of active smoking, from additional reduction in length due to smoldering. All individual cigarette butts collected during the trial had their butt lengths measured (mm) with a standard ruler. Then ~10-mm filter segments were cut and composited into daily samples for analysis of the amounts of ‘tar’ and nicotine that passed through the filter. Filter segments generated by each participant were composited in amber glass jars as daily samples and stored frozen prior to analysis (shipped at ambient temperature). Butts or filter segments were not analyzed if they were from an incorrect brand or were damaged, i.e., burnt, excessively crushed or extinguished in liquid.

Yield-in-use (YIU): YIU provides a non-invasive means to estimate the amount of smoke that passes through the filter of a cigarette and into the mouth of a smoker, i.e., mouth-level exposure (MLE) (Nelson et al., 2011). To measure YIU, smoked cigarette butt filter segments were extracted in methanol and the extracts analyzed by standardized methods (Labstat International ULC, Kitchener, ON) to determine the amount of ‘tar’ (colorimetric) and nicotine (GC) trapped by the filter. The ‘tar’ and nicotine quantified are correlated to the amount of smoke passing through the filter, as determined on a cigarette brand-specific basis by calibration curves derived from machine-smoked cigarettes using five smoking regimes of differing intensity, and results in an estimate of maximum MLE. Comparisons between daily cigarette usage, butt length and MLE were used to determine smoking behavior between groups at baseline and whether changes were observed during intervention in the Dual use group.

2.5. Whole blood, plasma, urine and feces specimen collections

It is noted that while certain samples and analytes noted below were collected and analyzed more frequently than reported herein, for brevity, results are limited to D -1 and D5, and 22:00 h

sampling periods, representing the greatest analyte concentration changes observed.

Whole blood: Whole blood (EDTA) samples were collected on days D -1, D 1, D 3, and D 5 at approximately 22:00 each day to quantify carboxyhemoglobin concentrations spectroscopically with a CO oximeter at Covance Central Clinical Laboratory (Indianapolis, IN). Samples were kept chilled prior to analysis.

Plasma: Plasma (sodium heparin) samples were collected on days D -1, D 1, D 3, and D 5 at approximately 07:00, 12:00, and 22:00 each day to quantify nicotine and cotinine concentrations, and at 22:00 to quantify thiocyanate concentrations. Plasma samples were stored frozen at -20 or -70 °C prior to analysis.

Urine: 24-Hour urine samples were collected on days D -1, D 1, D 3, and D5 following the collection procedure where the morning's first void was discarded and all subsequent voids collected to include the first void from the following morning. Samples were stored refrigerated during collection, total daily (24-h) urine volumes recorded, and bioanalytical aliquots stored frozen at -20 or -70 °C prior to analysis. Feces: 24-Hour feces samples were collected from up to six participants per group on days D -1 and D5 using a stool collection hat where daily composited samples were collected throughout the collection day. Daily samples were weighed and combined with deionized water (2:1, w:v), homogenized using a probe-type homogenizer, and subsampled (~20 g/subsample) adding 1 g sodium azide/subsample as preservative. Feces homogenate samples were stored frozen at -20 or -70 °C prior to analysis.

2.6. Urine mutagenicity

24-Hour urine mutagenicity (total revertants/day) was determined using a microsusension-modified Ames test using *Salmonella typhimurium* strain YG1024 with 5% Aroclor induced S9 (MolTox™; Molecular Toxicology Incorporated, USA) from male Sprague Dawley rats. Strain YG1024 is an O-acetyltransferase-over producing derivative of TA98 that is known to be more sensitive in detecting mutagenicity in human urine caused by cigarette smoking than TA98 (Kuenemann-Migeot et al., 1997). A 250-mL aliquot of filtered and pH neutralized 24-h urine sample was passed through a Varian Megabond Elute C18 column (2 g C18 sorbent) pre-conditioned by 2 × 5 mL each of methanol and then water. The loaded column was washed with 2 × 5 mL water, and urinary hydrophobic components eluted with 2 × 5 mL methanol/acetonitrile (1:1, v/v). Eluate was evaporated to dryness at 46 °C under nitrogen and resuspended in 500 µL DMSO. Resuspended solutions were stored frozen at -80 °C until assayed, and diluted with DMSO to appropriate dose levels.

2.7. Biomarker analysis

Urinary and fecal biomarkers: All biomarker analyses were performed by the bioanalytical laboratory (ABF GmbH, Munich, Germany). Total daily excretion values presented for the urinary and fecal analytes were calculated by converting analytically determined values to units of original concentration (i.e., biomarker mass/mL urine or g feces), and multiplying the observed concentration by total daily output (total mL urine or g feces/24-h) to yield total excreted biomarker mass/day.

Total nicotine equivalents (T-NicEq) concentrations (comprised of nicotine plus nine metabolites) were determined in urine and feces homogenates by the same analytical methods; liquid chromatography–tandem mass spectroscopy (LC–MS/MS) according to Meger et al. (2002), with major modifications. The analysis was performed as two separate methods: (i) Nic + 5 (nicotine, cotinine, trans-3'-hydroxycotinine, nicotine-glucuronide, cotinine-N-glucuronide, trans-3'-hydroxycotinine-N,O-glucuronide); and (ii)

nor-compounds and N-oxides (nornicotine, norcotinine, nicotine-N-oxide, cotinine-N-oxide). Total daily T-NicEq excreted was calculated by first transforming the observed daily analyte concentrations (total mass/day) for each of the ten analytes to their respective molar nicotine equivalent (NE) values (mass NE/day), and then summing all NE values to yield total mass NE/day (T-NicEq). The lower limits of quantitation (LLOQ) for all ten analytes ranged between 1.5 and 25 ng/mL in urine, and between 4 and 10 ng/g in feces.

Total tobacco-specific nitrosamine (TSNA) concentrations were determined by liquid chromatography–tandem electrospray ionization (ESI) mass spectroscopy (LC–MS/MS) according to Kavvadias et al. (2009). Samples were enzymatically hydrolyzed with β -glucuronidase (type IX-A from *Escherichia coli*) prior to analysis of four analytes: 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanol (total NNAL) [a metabolite of 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone (NNK)], N-nitrosornicotine (total NNN), N-nitrosoanatabine (total NAT), and N-nitrosoanabasine (total NAB). The LLOQ for all four analytes was 2 pg/mL.

Aromatic amine (AA) concentrations were determined by gas chromatography/mass spectroscopy (GC/MS) in negative ion chemical ionization (NICI) mode according to Riedel et al. (2006). Samples were derivatized prior to analysis of four analytes: 3-aminobiphenyl (3-ABP); 4-aminobiphenyl (4-ABP); 2-amino naphthalene (2-AN); and o-toluidine (o-T). The LLOQ for all four analytes ranged between 1.0 and 2.5 pg/mL.

Hydroxylated polyaromatic hydrocarbon (PAH) concentrations were determined by gas chromatography–mass spectroscopy, selected reaction monitoring (SRM) mode (GC–MS/MS) with modified methods of Chetianukornkul et al. (2006). Samples were enzymatically hydrolyzed with β -glucuronidase/arylsulfatase (from *Helix pomatia*) and derivatized prior to analysis of nine analytes: 1-OH-pyrene, 1- and 2-OH-naphthalene, 2 OH-flourene, 1-, 2-, 3-, 4-, and 9-OH-phenanthrene. The LLOQ for all nine analytes ranged between 0.01 and 1.00 ng/mL.

3-Hydroxypropylmercapturic acid (HPMA), a metabolite of acrolein had concentrations determined by liquid chromatography–tandem electrospray ionization (ESI, negative) mass spectroscopy (LC–MS/MS) according to Mascher et al. (2001). N-acetyl-S-(2-hydroxy-2-carbamoyl)thyl)cysteine (GAMA) and N-acetyl-S-(2-carbamoyl)thyl)cysteine (AAMA), metabolites of acrylamide, had concentrations determined by liquid chromatography–tandem electrospray ionization (ESI, negative) mass spectroscopy (LC–MS/MS) according to Urban et al. (2006). The LLOQ for HPMA, GAMA and AAMA were 20, 1.0 and 4.1 ng/mL, respectively. S-phenylmercapturic acid (SPMA), a metabolite of benzene, had concentrations determined by liquid chromatography–tandem atmospheric pressure chemical ionization (negative mode) (APCI, negative) mass spectroscopy (LC–MS/MS) by a modified method of Paci et al. (2007) that includes hydrochloric acid hydrolysis of urine to convert pre-SPMA to SPMA (Sterz et al., 2010). The LLOQ for SPMA was 0.05 ng/mL. Monohydroxybutenyl-mercapturic acids (MHBMA) and dihydroxy-butylmercapturic acid (DHBMA), metabolites of 1,3-butadiene had concentrations determined by liquid chromatography–tandem electrospray ionization (ESI, negative) mass spectroscopy (LC–MS/MS) according to Urban et al. (2003). The LLOQ for MHBMA and DHBMA were 0.5 and 4.4 ng/mL, respectively. 2-Hydroxyethylmercapturic acid (HEMA), a metabolite of ethylene oxide, and 2-cyanoethylmercapturic acid (CEMA), a metabolite of acrylonitrile had concentrations determined by liquid chromatography–tandem electrospray ionization (ESI, positive) mass spectroscopy (LC–MS/MS) according to Scherer et al. (2010). Thiocyanate urinary concentrations were determined by a GC–MS method according to Riedel et al. (2013), with a LLOQ of 0.7 μ mol/L.

Plasma biomarkers: Nicotine and cotinine plasma concentrations were determined by liquid chromatography–tandem mass

spectroscopy (LC–MS/MS) after protein precipitation according to a validated method by the analytical laboratory (ABF GmbH, Munich, Germany). The LLOQ for both analytes was 1.0 ng/mL. Thiocyanate plasma concentrations were determined according to Degiampietro et al. (2009). Absorption was measured with a SLT Spectra multi-channel-reader (Tecan GmbH, Crail, Germany) at 492 nm, using a 620 nm reference filter. The LLOQ for thiocyanate was 0.7 μ mol/L.

2.8. Questionnaire administration

MNWS-R: The self-reported Minnesota Nicotine Withdrawal Scales-Revised (MNWS-R) questionnaire can be scored with 9-Items or 15-Items, where the first nine items are validated (angry/irritable/frustrated, anxious/nervous, depressed mood/sad, desire or craving to smoke, difficulty concentrating, increased appetite/hungry/weight gain, insomnia/sleep problems/awakening at night, restless, and impatient) and the remaining 6 items investigational, but seemingly relevant for evaluating DTPs (i.e., constipation, dizziness, coughing, dreaming/nightmares, nausea, and sore throat) (Hughes and Hatsukami, 2007). Participants rated each item on a five point scale from 0 (none) to 4 (severe) in the evenings on baseline day and Days 1, 3, and 5.

2.9. Statistical analysis

This trial was a “complete-case” trial, i.e., only data from participants who successfully completed the trial were included in the statistical analysis. The sample size of ‘completers’ was determined based on a previous trial when participants switched from smoking their UB to the use of a similar dissolvable tobacco product, to provide at least 80% statistical power to detect a 20% reduction in NicEq-T and NNAL in 24-h urine at a 5% significance level and two-sided testing. All participants receiving test products were included in the reporting of demographics and adverse events. All statistical analyses were done using SAS and statistical significance was considered at $p \leq 0.05$.

Categorical and continuous variables were summarized by randomization group and, where relevant, by sampling time. Summaries of categorical variables included counts and percentages per applicable category. Descriptive statistics for continuous variables included the number of non-missing values, mean, geometric mean, standard deviation (SD), median, minimum, and maximum. Summaries of biomarker data also included percent change of mean from baseline, which was defined as the values from Day -1. One-way Analysis of variance (ANOVA) model was used to perform baseline comparisons among appropriate groups for endpoints related to ‘product use’ including UB cigarette dispensation, butt length, YIU (‘tar’ and nicotine MLE), and Snus-After-Use (nicotine MLE). The p -values for pairwise comparison between any two groups were derived. Within each of five product groups, t -test was used to assess the significance of the changes from baseline (defined as Day 1) to Day 5 and regression analysis was performed to assess the product usage trend over time, where day was treated as a continuous variable.

For biomarker data, analytical values above the limit of detection (LOD) will be reported unchanged. Values below the LOD were reported as $\frac{1}{2}$ the LOD. For blood level %COHb measurements, if %saturation is below the level of quantification, the value was replaced by 0.15%. Missing data were not imputed or replaced by any value. ANOVA models were used to compare baseline biomarker level across groups as well as to compare product effect on the absolute changes from baseline to Day 5 for biomarkers in all categories. Multiple comparisons of all pairwise groups were performed. For endpoints which were collected on more than 2 days, variable “day” and the interaction between product group

and day were included in the ANOVA model and pairwise comparison of groups was performed on each day. Paired *t*-test was used to assess the significance of the changes from baseline to Day 5 for each group.

For all questionnaire measures, when data were available, descriptive statistics were calculated by group and time points. Statistical analysis was performed for the FTND and MNWS questionnaires by scoring according to published algorithms. Observed as well as adjusted mean change in scale scores from baseline were reported for the MNWS by randomized group and assessment period (Day 1, 3, and 5). The adjustment was carried out by modeling observed change from Baseline scale scores as a function of randomization group, subject, and assessment period, adjusting for Baseline scale scores using analysis of covariance (ANCOVA) model. The 95% confidence interval of the least square mean of change scores was obtained. Differences in adjusted scale change scores were examined for all paired randomization groups and the *p*-values were obtained. For FTND data, one-way ANOVA model was built for pairwise comparisons between randomization groups.

3. Results

A total of 181 participants were enrolled, randomized and at a minimum started their intervention. Of these, 167 participants completed the trial: two withdrew due to an adverse event (AE), both considered not product related; 11 withdrew consent; and one withdrew for personal reasons. Numbers of participants with chronic health conditions controlled by daily use of prescription or OTC medications were generally evenly distributed between groups. Of the overall number of participants (167), 29 were treated with a single medication, whereas 19 had multiple medications. Medications were taken to treat: hypertension (15); chronic pain (12); depression/anxiety/ADD (9); hormones for birth control or transgender (7); cardiac prophylaxis (6); hypercholesterolemia (5); chronic headache (5); insomnia (4); allergies (3);

and type 2 diabetes (1). Besides medications, it is noted 16 participants used health supplements daily, usually a multivitamin. Demographic data at time of screening are presented in Table 1, with attributes appearing relatively balanced although no formal comparisons were made except for FTND. Group mean FTND scores ranged between 5.3 and 6.3 with some statistical differences noted between groups, attributed to instrument (questionnaire) variability. Of the 288 reported AEs, 282 were considered mild and 6 moderate. 152 AEs were considered related to study products, most commonly headache, nausea and flatulence.

Daily smoking behavior measures of CPD, 'tar' and nicotine MLEs, butt lengths, and daily test product usage rates are presented in Table 2. On baseline day, group mean smoking rates were only significantly different between the lowest Snus and highest Strips groups. Not surprisingly, given the mandatory 60% baseline CPD reduction, the Dual use group had a significantly reduced CPD at Day 5, where participants smoked the mean maximum allowable rate (7.6 CPD) during all 5 days. No statistical differences were observed between groups on baseline day for mean MLE to 'tar' (393–467 mg/day) or nicotine (32–39 mg/day). For the Dual use group during the intervention period, daily MLE to 'tar' and nicotine were both reduced to ~50% of baseline levels. It is notable that the daily MLE reductions observed occurred notwithstanding the fact that when MLE is computed on a per cigarette basis, MLE to 'tar' and nicotine increased by ~27% and 28%, respectively. Some slight but significant differences were also noted between group mean butt lengths on baseline day, which ranged between 35.0 and 37.3 mm. For the Dual use group during the intervention period, butt lengths were slightly but significantly shorter (~2.5 mm) on Day 5 compared to baseline. Overall, while minor baseline differences in group comparisons for CPD and butt length were observed, given no differences in daily MLE to 'tar' and nicotine between groups, the differences noted were not considered meaningful. For the Dual use group during the intervention period, increases in per cigarette 'tar' and nicotine MLEs and a decrease in

Table 1
Participant demographic characteristics at time of screening.

Demographic variable	Dual use N = 29	SNUS N = 30	Sticks N = 29	Strips N = 25	Orbs N = 29	Abstinence N = 25	Total N = 167
Age (years)							
Mean ± SD	42.45 ± 12.28	37.60 ± 11.42	40.72 ± 12.22	40.64 ± 11.76	39.34 ± 12.71	43.32 ± 11.20	40.60 ± 11.93
(Min–max)	23–63	21–59	24–64	22–63	21–63	21–58	21–64
Weight (kg)							
Mean ± SD	81.66 ± 22.49	79.82 ± 15.50	79.51 ± 14.84	77.09 ± 19.33	76.95 ± 17.22	79.02 ± 22.74	79.06 ± 18.60
(Min–max)	51.9–146.0	55.4–118.2	49.2–114.5	44.7–119.0	48.3–107.6	54.2–151.9	44.7–151.9
Height (cm)							
Mean ± SD	168.20 ± 7.63	172.02 ± 7.82	172.44 ± 8.46	172.48 ± 8.26	169.24 ± 7.93	173.17 ± 8.99	171.19 ± 8.26
(Min–max)	156.0–182.8	158.5–184.2	156.7–189.5	152.1–183.8	154.7–186.1	149.1–189.5	149.1–189.5
BMI (kg/m ²)							
Mean ± SD	28.76 ± 7.12	27.14 ± 5.99	26.77 ± 4.97	25.87 ± 6.10	26.83 ± 5.83	26.18 ± 6.35	26.97 ± 6.07
(Min–max)	19.2–46.0	19.9–43.6	18.3–44.2	17.6–40.9	18.4–40.2	18.6–48.3	17.6–48.3
Gender (n [%])							
Male	14 (48.3)	18 (60.0)	17 (58.6)	14 (56.0)	13 (44.8)	14 (56.0)	90 (53.9)
Female	15 (51.7)	12 (40.0)	12 (41.4)	11 (44.0)	16 (55.2)	11 (44.0)	77 (46.1)
Ethnicity (n [%])							
Hispanic or Latino	3 (10.3)	–	1 (3.4)	4 (16.0)	–	1 (4.0)	9 (5.4)
Not Hispanic or Latino	26 (89.7)	30 (100)	28 (96.6)	21 (84.0)	29 (100)	24 (96.0)	158 (94.6)
Race (n [%])							
White	25 (86.2)	23 (76.7)	21 (72.4)	22 (88.0)	19 (65.5)	21 (84.0)	131 (78.4)
Black or African American	4 (13.8)	6 (20.0)	6 (20.7)	1 (4.0)	6 (20.7)	3 (12.0)	26 (15.6)
Asian	–	–	–	–	1 (3.4)	–	1 (0.6)
American Indian/Alaskan native	–	–	1 (3.4)	–	1 (3.4)	–	2 (1.2)
Other	–	1 (3.3)	1 (3.4)	2 (8.0)	2 (6.9)	1 (4.0)	7 (4.2)
Fagerström test score							
Mean ± SD	5.52 ± 1.79	5.13 ± 1.46 ^a	5.41 ± 1.40 ^b	6.08 ± 1.38	5.28 ± 1.62 ^b	6.32 ± 2.04	na

^a Significantly (*p* ≤ 0.05) lower compared to Strips and Abstinent groups.

^b Significantly (*p* ≤ 0.05) lower compared to Abstinent group.

Table 2

Daily UB cigarette use rates and 'tar' and nicotine MLE, and smokeless product use rates.

Measure/intervention day	Dual use	Snus	Sticks	Strips	Orbs	Abstinent
<i>Daily "n", M ± SD CPD* smoked</i>						
Baseline	29 19.24 ± 7.40	30 16.33 ± 4.30 ^b	29 18.48 ± 5.65	25 20.88 ± 5.82 ^b	29 19.03 ± 4.32	25 19.44 ± 7.11
Day 1	29 7.62 ± 2.99	na	na	na	na	na
Day 2	29 7.62 ± 2.86	na	na	na	na	na
Day 3	29 7.55 ± 2.81	na	na	na	na	na
Day 4	29 7.55 ± 3.02	na	na	na	na	na
Day 5	29 7.62 ± 2.80 ^a	na	na	na	na	na
<i>Daily "n", M ± SD 'tar' MLE† (mg/day)</i>						
Baseline	29 415.3 ± 209.1	30 392.8 ± 178.1	29 406.8 ± 137.1	25 466.5 ± 194.4	29 400.4 ± 119.9	25 442.2 ± 187.9
Day 1	28 214.7 ± 209.1	na	na	na	na	na
Day 2	28 205.6 ± 105.1	na	na	na	na	na
Day 3	29 204.9 ± 100.3	na	na	na	na	na
Day 4	29 206.9 ± 106.0	na	na	na	na	na
Day 5	28 207.4 ± 107.1 ^a	na	na	na	na	na
<i>Daily "n", M ± SD nicotine MLE† (mg/day)</i>						
Baseline	29 34.26 ± 16.06	30 32.11 ± 17.98	29 33.00 ± 11.36	25 38.82 ± 17.51	29 32.13 ± 9.41	25 36.25 ± 15.42
Day 1	28 17.44 ± 8.63	na	na	na	na	na
Day 2	28 16.87 ± 8.10	na	na	na	na	na
Day 3	29 16.80 ± 7.23	na	na	na	na	na
Day 4	29 16.82 ± 7.95	na	na	na	na	na
Day 5	28 17.21 ± 7.89 ^a	na	na	na	na	na
<i>Daily "n", M ± SD butt length (mm/butt)</i>						
Baseline	29 37.29 ± 2.52 ^c	30 34.99 ± 4.99 ^{c,d}	29 35.79 ± 4.08	25 36.22 ± 3.72	29 35.44 ± 4.38	25 37.18 ± 3.66 ^{c,d}
Day 1	28 35.06 ± 3.14	na	na	na	na	na
Day 2	28 34.75 ± 3.47	na	na	na	na	na
Day 3	29 34.35 ± 4.44	na	na	na	na	na
Day 4	29 35.11 ± 3.66	na	na	na	na	na
Day 5	28 34.75 ± 3.53 ^a	na	na	na	na	na
<i>Daily "n", M ± SD # units consumed</i>						
Baseline	na	na	na	na	na	na
Day 1	29 3.62 ± 1.32	30 6.10 ± 2.02	29 5.33 ± 2.69	25 12.04 ± 7.75	29 7.52 ± 24.11	na
Day 2	27 2.81 ± 1.36	28 5.71 ± 2.00	28 6.01 ± 4.20	24 13.67 ± 8.10	29 7.72 ± 5.02	na
Day 3	27 3.44 ± 1.58	30 6.27 ± 2.29	29 6.41 ± 3.99	25 15.16 ± 8.86	29 9.55 ± 6.37	na
Day 4	23 3.00 ± 1.76	28 5.82 ± 2.87	26 6.09 ± 4.02	22 14.18 ± 8.48	26 9.46 ± 6.59	na
Day 5	27 3.26 ± 1.97	30 6.43 ± 2.96	29 6.39 ± 4.44	25 13.48 ± 9.03	29 8.93 ± 5.57	na

* CPD = cigarettes per day.

† MLE = mouth level exposure from cigarette smoking.

^a Statistically ($p \leq .05$) lower at Day 5 compared to baseline.^b Statistically ($p \leq .05$) different between Snus and Strips groups.^c Statistically ($p \leq .05$) different between Snus and Dual use groups.^d Statistically ($p \leq .05$) different between Snus and Abstinent groups.

butt length suggested the cigarettes were smoked with somewhat greater intensity compared to baseline.

Daily use rates of all the smokeless test products were uniform throughout the intervention period, with no statistical differences (Table 2). Of note was the use of ~6 pouches/day in the exclusive Snus group compared to about half that amount (~3 pouches/day) in the Dual use group.

Biomarker of exposure concentrations and relative percent changes on Day 5 compared to baseline observed in urine, blood, and plasma samples are provided in Table 3. Group biomarker percent changes relative to the Abstinent group, whose biomarker changes from baseline to Day 5 are considered to be –100% of the maximum potential change possible, are depicted in Fig. 1 for T-NicEq, TSNA and other select biomarkers, in Fig. 2 for AAs and PAHs, and in Fig. 3 for select vapor phase biomarkers. For urinary biomarkers, T-NicEq levels excreted at baseline were not statistically different (NSD) between groups, while on Day 5 significant reductions were seen in all groups compared to baseline, with Dual use having the least and the Abstinent group the greatest decline, with intermediate declines in the remaining groups. Day 5 group comparisons showed the Abstinent group had a statistically greater reduction compared to all the other groups, and the Strips, Orbs, and Abstinent groups had statistically

greater reductions compared to Dual use. Relative to the Abstinent group, reductions in the other groups ranged between –33% and –75% (Fig. 1).

Total TSNA levels excreted at baseline were NSD between groups, while on Day 5 significant reductions were seen in all groups compared to baseline, with Dual use having the least and the Abstinent group the greatest decline. Day 5 group comparisons showed the Abstinent group had a statistically greater reduction compared to all the other groups. Relative to the Abstinent group, reductions in the other groups ranged between –28% and –47% (Fig. 1). While levels of the four individual TSNA excreted were generally similar to the total TSNA findings, some exceptions were noted. Total NNAL levels on Day 5 were significantly reduced from baseline only in the Dual use and Abstinent groups, whereas reductions were nominal in the Sticks (–1%) and Snus (–17%) groups, with nominal increases seen in the Strips (9%) and Orbs (3%) groups, all NSD from baseline. These findings may be related to observations that NNK is converted to total NNAL to a greater extent (~3- to 4-fold) in consumers of smokeless tobacco compared to cigarette smokers (Hecht et al., 2008; Stepanov et al., 2008). Lastly, while most group comparisons at baseline for the individual and total TSNA were NSD, there was one exception; the total NNN level was significantly greater in the Snus group

Table 3

Biomarker of exposure concentrations in 24-h urine, whole blood, or plasma, and percent changes on Day 5 compared to baseline.

Group	Total nicotine equivalents (mg/24-h Urine)			Total TSNA (ng/24-h Urine)			Total NNAL (ng/24-h Urine)		
	Baseline	Day 5	% Change	Baseline	Day 5	% Change	Baseline	Day 5	% Change
Dual use	22.59 ± 9.70	15.40 ± 7.17	−31.8% ^{a,b}	1222.20 ± 540.79	907.90 ± 477.59	−25.7% ^{a,b}	717.73 ± 326.22	572.90 ± 304.62	−20.2% ^{a,b}
Snus	21.46 ± 7.49	11.25 ± 9.83	−47.6% ^{a,b}	1115.05 ± 503.76	712.32 ± 588.06	−36.1% ^{a,b}	596.37 ± 282.61	496.67 ± 330.54	−16.7% ^a
Sticks	21.21 ± 7.42	11.08 ± 8.61	−47.8% ^{a,b}	1148.44 ± 568.84	780.02 ± 517.13	−32.1% ^{a,b}	598.44 ± 300.04	594.30 ± 360.60	−0.7% ^a
Strips	22.48 ± 6.57	9.45 ± 5.98	−58.0% ^{a,b,d}	1208.17 ± 562.17	946.40 ± 568.66	−21.7% ^{a,b}	663.77 ± 336.83	725.59 ± 408.01	9.3% ^{a,d}
Orbs	21.18 ± 6.58	5.71 ± 4.55	−73.0% ^{a,b,d}	1250.65 ± 585.14	814.47 ± 547.28	−34.9% ^{a,b}	677.69 ± 303.52	700.11 ± 454.35	3.3% ^{a,d}
Abstinent	22.89 ± 9.53	0.56 ± 0.47	−97.6% ^{b,d}	1217.17 ± 548.40	272.86 ± 148.44	−77.6% ^{b,d}	672.00 ± 299.84	267.89 ± 146.77	−60.1% ^{b,d}
Total NNN (ng/24-h Urine)				Total NAB (ng/24-h Urine)			Total NAT (ng/24-h Urine)		
Dual use	24.08 ± 21.93 [*]	28.43 ± 41.40	18.1% ^a	68.30 ± 35.44	42.57 ± 26.88	−37.7% ^{a,b}	412.92 ± 238.52	264.00 ± 184.62	−36.1% ^{a,b}
Snus	42.70 ± 69.46 [*]	22.62 ± 50.55	−47.0% ^{b,d}	68.26 ± 37.74	29.73 ± 41.51	−56.4% ^{a,b}	407.72 ± 250.76	163.30 ± 252.32	−59.9% ^{a,b}
Sticks	27.71 ± 19.94	7.98 ± 7.44	−71.2% ^b	74.48 ± 45.07	14.42 ± 13.11	−80.6% ^{b,d}	447.81 ± 299.20	163.32 ± 180.74	−63.5% ^{a,b,d}
Strips	26.90 ± 26.10	10.91 ± 16.55	−59.5% ^b	71.77 ± 37.08	15.59 ± 13.88	−78.3% ^{b,d}	445.73 ± 261.64	194.31 ± 190.48	−56.4% ^{a,b}
Orbs	29.66 ± 17.88	9.34 ± 17.37	−68.5% ^{b,d}	76.90 ± 39.14	9.24 ± 8.54	−88.0% ^{b,d}	467.42 ± 264.52	95.77 ± 95.17	−79.5% ^{a,b,d}
Abstinent	28.29 ± 19.77	1.79 ± 2.06	−93.7% ^{b,d}	74.85 ± 43.16	1.25 ± 0.87	−98.3% ^{b,d}	442.03 ± 236.39	1.93 ± 2.29	−99.6% ^{b,d}
COHb (% Saturation in Whole Blood)				Thiocyanate (μmol/24-h Urine)			Thiocyanate (μmol/L Plasma)		
Dual use	5.58 ± 2.27	2.90 ± 0.90	−48.1% ^{a,b}	201.49 ± 100.07	157.65 ± 79.35	−21.8% ^b	126.76 ± 45.11	99.78 ± 32.21	−21.3% ^{a,b}
Snus	5.24 ± 1.62	0.98 ± 0.27	−81.3% ^{b,d}	224.24 ± 122.60	119.29 ± 46.74	−46.8% ^{b,d}	121.12 ± 39.48	80.55 ± 26.74	−33.5% ^{b,d}
Sticks	5.23 ± 1.89	0.98 ± 0.46	−81.3% ^{b,d}	200.22 ± 124.40	108.49 ± 57.15	−45.8% ^{b,d}	109.34 ± 36.43 [*]	69.66 ± 21.66	−36.3% ^{b,d}
Strips	6.16 ± 2.22	1.07 ± 0.31	−82.7% ^{b,d}	246.58 ± 130.32	138.12 ± 68.36	−44.0% ^{b,d}	138.17 ± 52.49 [*]	84.50 ± 24.94	−38.8% ^{b,d}
Orbs	5.82 ± 1.54	1.02 ± 0.30	−82.5% ^{b,d}	203.56 ± 101.39	120.86 ± 58.45	−40.6% ^b	120.11 ± 40.16	80.75 ± 26.56	−32.8% ^{b,d}
Abstinent	6.19 ± 2.55	1.08 ± 0.22	−82.5% ^{b,d}	222.38 ± 144.84	135.91 ± 92.48	−38.9% ^b	122.36 ± 51.37	79.50 ± 28.04	−35.0% ^{b,d}
YG1024 Mutagenicity (Revertants/10 ³ /24-h Urine)				3-Aminobiphenyl (ng/24-h Urine)			4-Aminobiphenyl (ng/24-h Urine)		
Dual use	172.62 ± 118.10	100.44 ± 61.22	−41.8% ^{a,b}	11.75 ± 6.34	6.46 ± 3.88	−45.0% ^{a,b}	26.42 ± 11.39	14.64 ± 6.24	−44.6% ^{a,b}
Snus	164.51 ± 85.17	49.74 ± 81.45	−69.8% ^b	12.22 ± 5.49	1.41 ± 0.75	−88.5% ^{b,d}	26.09 ± 11.36	3.74 ± 1.41	−85.6% ^{b,d}
Sticks	191.86 ± 155.48	37.46 ± 41.53	−80.5% ^{b,d}	11.42 ± 5.05 [*]	1.02 ± 0.73	−91.0% ^{b,d}	26.14 ± 9.35	3.64 ± 1.25	−86.1% ^{b,d}
Strips	184.74 ± 110.68	27.86 ± 38.36	−84.9% ^{b,d}	14.85 ± 7.73 [*]	1.47 ± 0.97	−90.1% ^{b,d}	27.03 ± 9.53	4.10 ± 1.74	−84.8% ^{b,d}
Orbs	199.66 ± 105.00	24.67 ± 21.72	−87.6% ^{b,d}	12.67 ± 5.57	1.14 ± 0.68	−91.0% ^{b,d}	29.49 ± 12.33	3.86 ± 1.43	−86.9% ^{b,d}
Abstinent	187.78 ± 107.49	34.36 ± 43.41	−81.7% ^{b,d}	14.10 ± 6.15	1.33 ± 0.87	−90.6% ^{b,d}	27.44 ± 10.79	4.31 ± 1.67	−84.3% ^{b,d}
2-Aminonaphthalene (ng/24-h Urine)				o-Toluidine (ng/24-h Urine)			1-OH-Pyrene (ng/24-h Urine)		
Dual use	37.92 ± 17.13	20.35 ± 9.27	−46.3% ^{a,b}	228.39 ± 86.02 [*]	159.04 ± 62.47	−30.4% ^{a,b}	392.62 ± 173.07	404.56 ± 386.46	3.0% ^{nsd}
Snus	37.65 ± 14.77	3.28 ± 1.43	−91.3% ^{b,d}	223.18 ± 75.07 [*]	89.82 ± 36.41	−59.8% ^{b,d}	410.85 ± 172.96	377.07 ± 520.67	−8.2% ^{nsd}
Sticks	36.28 ± 13.09	3.07 ± 1.28	−91.5% ^{b,d}	241.05 ± 88.97	88.47 ± 27.95	−63.3% ^{b,d}	350.76 ± 149.21	372.23 ± 527.22	6.1% ^{nsd}
Strips	40.38 ± 16.66	3.64 ± 1.22	−91.0% ^{b,d}	275.86 ± 99.88 [*]	117.41 ± 46.99	−57.4% ^{b,d}	428.60 ± 293.91	359.90 ± 485.29	−16.0% ^{nsd}
Orbs	45.24 ± 35.02	3.39 ± 1.36	−92.5% ^{b,d}	233.36 ± 58.47	101.04 ± 43.33	−56.7% ^{b,d}	347.68 ± 103.30	355.26 ± 511.36	2.2% ^{nsd}
Abstinent	39.34 ± 16.97	3.53 ± 1.35	−91.0% ^{b,d}	255.76 ± 84.22	102.82 ± 43.38	−59.8% ^{b,d}	408.13 ± 218.78	241.13 ± 259.45	−40.9% ^{nsd}
1-OH-Naphthalene (μg/24-h Urine)				2-OH-Naphthalene (μg/24-h Urine)			2-OH-Flourene (ng/24-h Urine)		
Dual use	12.08 ± 5.72	7.60 ± 3.68	−37.1% ^{a,b}	17.75 ± 7.24	11.33 ± 5.27	−36.2% ^{a,b}	2048.77 ± 813.21	1406.72 ± 565.19	−31.3% ^{a,b}
Snus	12.80 ± 5.95	2.59 ± 4.03	−79.7% ^{b,d}	18.76 ± 6.63	4.08 ± 3.08	−78.2% ^{b,d}	2166.66 ± 780.94	574.39 ± 217.58	−73.5% ^{b,d}
Sticks	11.72 ± 4.46	2.95 ± 4.42	−74.8% ^{b,d}	17.91 ± 5.52	3.62 ± 2.73	−79.8% ^{b,d}	2039.09 ± 612.29	561.52 ± 196.04	−72.5% ^{b,d}
Strips	13.73 ± 5.39	2.42 ± 3.24	−82.4% ^{b,d}	18.93 ± 5.90	3.48 ± 1.36	−81.6% ^{b,d}	2261.26 ± 804.78	610.34 ± 236.88	−73.0% ^{b,d}
Orbs	12.38 ± 4.51	1.69 ± 1.63	−86.4% ^{b,d}	17.77 ± 5.03	4.65 ± 4.04	−73.8% ^{b,d}	2094.01 ± 607.56	613.78 ± 362.33	−70.7% ^{b,d}
Abstinent	12.60 ± 6.29	2.60 ± 4.59	−79.4% ^{b,d}	18.01 ± 6.22	3.30 ± 1.69	−81.7% ^{b,d}	2141.86 ± 860.33	597.85 ± 260.32	−72.1% ^{b,d}
1-OH-Phenanthrene (ng/24-h Urine)				2-OH-Phenanthrene (ng/24-h Urine)			3-OH-Phenanthrene (ng/24-h Urine)		
Dual use	263.02 ± 88.87	204.90 ± 63.79	−22.1% ^{a,b}	149.49 ± 48.21	120.28 ± 38.69	−19.5% ^{a,b}	316.06 ± 102.81	242.37 ± 85.47	−23.3% ^{a,b}
Snus	284.52 ± 91.67	139.17 ± 62.90	−51.1% ^{b,d}	153.17 ± 59.28	80.57 ± 54.85	−47.4% ^{b,d}	332.80 ± 94.68	129.17 ± 73.03	−61.2% ^{b,d}
Sticks	261.98 ± 72.10	142.95 ± 79.11	−45.4% ^{b,d}	158.64 ± 92.57	94.37 ± 57.32	−40.5% ^{b,d}	305.62 ± 114.22	141.38 ± 90.96	−53.7% ^{b,d}
Strips	286.42 ± 93.60	152.58 ± 70.78	−46.7% ^{b,d}	165.00 ± 61.05	85.52 ± 51.18	−48.2% ^{b,d}	351.35 ± 124.71	148.06 ± 94.20	−57.9% ^{b,d}
Orbs	283.99 ± 74.59	151.91 ± 64.04	−46.5% ^{b,d}	152.50 ± 51.45	93.21 ± 53.12	−38.9% ^b	340.48 ± 98.67	145.65 ± 84.79	−57.2% ^{b,d}
Abstinent	284.93 ± 111.25	131.74 ± 57.74	−53.8% ^{b,d}	153.75 ± 62.31	74.60 ± 51.04	−51.5% ^{b,d}	336.08 ± 130.20	118.55 ± 55.34	−64.7% ^{b,d}

4-OH-Phenanthrene (ng/24-h Urine)				9-OH-Phenanthrene (ng/24-h Urine)				CEMA (μg/24-h Urine)			
Dual use	65.91 ± 25.71	49.71 ± 18.43	–24.6% ^{a,b}	270.68 ± 140.19	194.75 ± 85.07	–28.1% ^{a,b}		274.56 ± 137.25	150.79 ± 74.27	–45.1% ^{a,b}	
Snus	69.69 ± 24.21	25.48 ± 17.12	–63.4% ^{b,d}	289.94 ± 116.74	71.71 ± 64.85	–75.3% ^{b,d}		278.63 ± 122.74	37.69 ± 17.42	–86.5% ^{b,d}	
Sticks	68.45 ± 28.02	27.23 ± 17.65	–60.2% ^{b,d}	279.72 ± 115.51	80.51 ± 68.16	–71.2% ^{b,d}		262.50 ± 92.15	40.83 ± 18.78	–84.4% ^{b,d}	
Strips	75.27 ± 27.23	30.33 ± 21.51	–59.7% ^{b,d}	306.75 ± 126.81	83.83 ± 50.28	–72.7% ^{b,d}		322.98 ± 147.2	45.99 ± 20.62	–85.8% ^{b,d}	
Orbs	76.92 ± 27.10	30.43 ± 22.62	–60.4% ^{b,d}	311.35 ± 121.88	87.38 ± 87.79	–71.9% ^{b,d}		262.73 ± 85.55	33.03 ± 13.12	–87.4% ^{b,d}	
Abstinent	75.48 ± 35.44	23.08 ± 16.83	–69.4% ^{b,d}	317.14 ± 155.69	68.33 ± 37.21	78.5% ^{b,d}		287.24 ± 117.63	40.99 ± 22.94	–85.7% ^{b,d}	
HEMA (μg/24-h Urine)				HPMA (μg/24-h Urine)				AAMA (μg/24-h Urine)			
Dual use	12.33 ± 6.91	8.73 ± 5.50	–29.2% ^{a,b}	2820.28 ± 1352.86	1415.78 ± 558.73	–49.8% ^{a,b}		359.58 ± 115.27 [*]	265.04 ± 95.57	–26.3% ^{a,b}	
Snus	16.07 ± 8.40 [*]	6.73 ± 2.99	–58.1% ^{b,d}	2678.81 ± 978.80	445.39 ± 288.51	–83.4% ^{b,d}		428.89 ± 140.62 [*]	152.92 ± 73.80	–64.3% ^{b,d}	
Sticks	11.64 ± 4.87 [*]	7.19 ± 4.03	–38.2% ^{b,d}	2607.31 ± 1012.32	470.06 ± 174.55	–82.0% ^{b,d}		395.63 ± 149.36	146.73 ± 54.85	–62.9% ^{b,d}	
Strips	12.40 ± 6.78	6.48 ± 3.87	–47.7% ^{b,d}	3113.38 ± 1268.96	466.45 ± 180.00	–85.0% ^{b,d}		407.21 ± 140.53	135.55 ± 28.14	–66.7% ^{b,d}	
Orbs	13.06 ± 7.07	7.62 ± 3.89	–41.7% ^{b,d}	2696.71 ± 866.21	452.57 ± 217.75	–83.2% ^{b,d}		374.78 ± 110.44	146.92 ± 55.08	–60.8% ^{b,d}	
Abstinent	15.64 ± 12.86	7.27 ± 4.49	–53.5% ^{b,d}	2865.21 ± 1210.80	473.70 ± 212.59	–83.5% ^{b,d}		405.57 ± 124.00	146.11 ± 37.85	–64.0% ^{b,d}	
GAMA (μg/24-h Urine)				HMPMA (μg/24-h Urine)				MHBMA (ng/24-h Urine)			
Dual use	49.79 ± 20.00	39.18 ± 15.33	–21.3% ^{a,b}	642.88 ± 287.21	363.11 ± 164.18	–43.5% ^{a,b}		7688.81 ± 4716.43	3833.51 ± 2285.38	–50.1% ^{a,b}	
Snus	57.31 ± 22.00	27.64 ± 10.53	–51.8% ^{b,d}	617.87 ± 242.53	125.78 ± 57.97	–79.6% ^{b,d}		8911.75 ± 5896.10	707.07 ± 247.61	–92.1% ^{b,d}	
Sticks	52.00 ± 18.74	27.41 ± 11.25	–47.3% ^{b,d}	599.68 ± 239.58	139.07 ± 77.14	–76.8% ^{b,d}		6186.13 ± 4700.37 [*]	753.70 ± 315.36	–87.8% ^{a,b}	
Strips	45.47 ± 16.79	22.44 ± 5.72	–50.6% ^{b,d}	687.95 ± 304.11	130.63 ± 63.32	–81.0% ^{b,d}		6688.63 ± 4592.95 [*]	664.74 ± 325.73	–90.1% ^{a,b}	
Orbs	47.32 ± 18.66	25.62 ± 9.17	–45.9% ^{b,d}	600.96 ± 233.99	117.34 ± 56.34	–80.5% ^{b,d}		9092.82 ± 5563.85	670.29 ± 286.10	–92.6% ^{b,d}	
Abstinent	49.06 ± 20.82	24.81 ± 7.72	–49.4% ^{b,d}	614.94 ± 231.47	125.49 ± 62.93	–79.6% ^{b,d}		10702.67 ± 8487.79 [*]	738.32 ± 328.26	–93.1% ^{b,d}	
SPMA (ng/24-h Urine)				Nicotine (ng/mL Plasma @ 22:00)				Cotinine (ng/mL Plasma @ 22:00)			
Dual use	6349.75 ± 4247.47	3411.91 ± 2350.75	–46.3% ^{a,b}	28.43 ± 14.34	15.34 ± 6.84	–46.0% ^{a,b}		300.63 ± 135.61	191.91 ± 103.83	–36.2% ^{a,b}	
Snus	6302.54 ± 3168.36	523.27 ± 301.82	–91.7% ^{b,d}	27.25 ± 9.21	10.37 ± 9.18	–61.9% ^{a,b}		296.65 ± 108.06	143.09 ± 126.69	–51.8% ^{a,b}	
Sticks	4784.78 ± 3046.34 [*]	493.12 ± 263.16	–89.7% ^{b,d}	25.9 ± 10.10	7.49 ± 5.27	–71.1% ^{a,b}		280.60 ± 80.61	139.52 ± 104.09	–50.3% ^{a,b}	
Strips	5555.02 ± 3783.57	531.81 ± 260.01	–90.4% ^{b,d}	31.48 ± 9.32	7.21 ± 4.68	–77.1% ^{b,d}		321.59 ± 98.22	115.42 ± 67.90	–64.1% ^{a,b,d}	
Orbs	7059.41 ± 4057.39 [*]	543.05 ± 226.15	–92.3% ^{b,d}	26.49 ± 7.39	3.98 ± 2.17	–85.0% ^{a,b,d}		306.53 ± 111.80	59.89 ± 37.76	–80.5% ^{a,b,d}	
Abstinent	7288.75 ± 4947.08 [*]	612.93 ± 408.37	–91.6% ^{b,d}	29.90 ± 14.91	0.78 ± 0.90	–97.4% ^{b,d}		317.39 ± 149.40	3.85 ± 5.88	–98.8% ^{b,d}	

^{*} Statistically ($p \leq 0.05$) different for between group pair-wise comparisons at baseline.

^{nsd} Not statistically different in either between group pair-wise, or within group baseline comparisons.

^a Statistically ($p \leq 0.05$) different compared to Abstinent group.

^b Statistically ($p \leq 0.05$) different compared to within group baseline comparison.

^d Statistically ($p \leq 0.05$) different compared to the Dual use group.

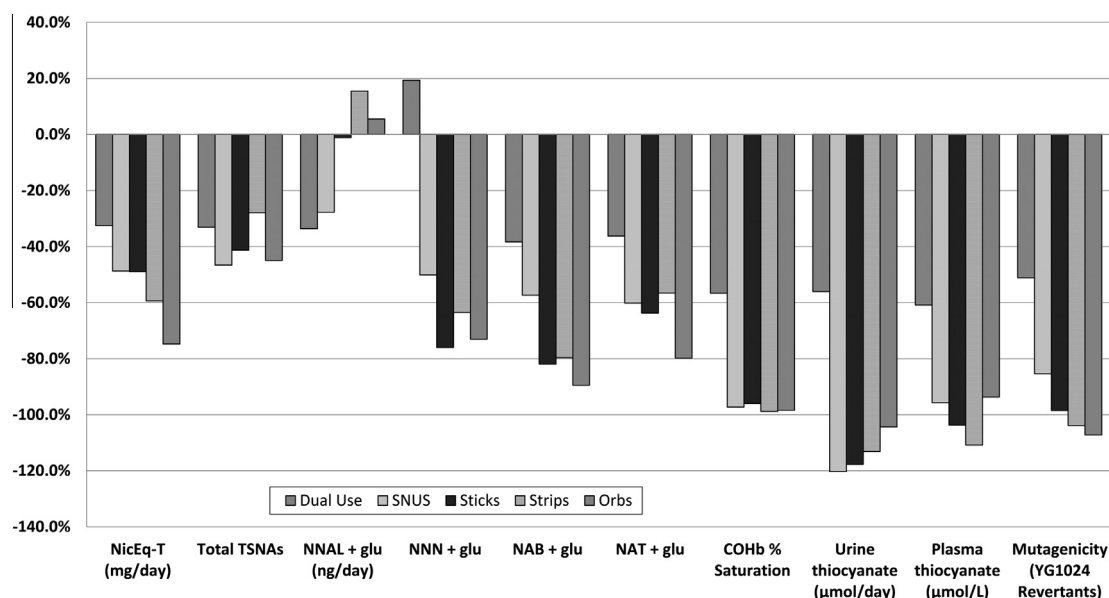


Fig. 1. Select urinary, whole blood or plasma biomarker level percent changes relative to the Abstinent group at Day 5 compared to baseline.

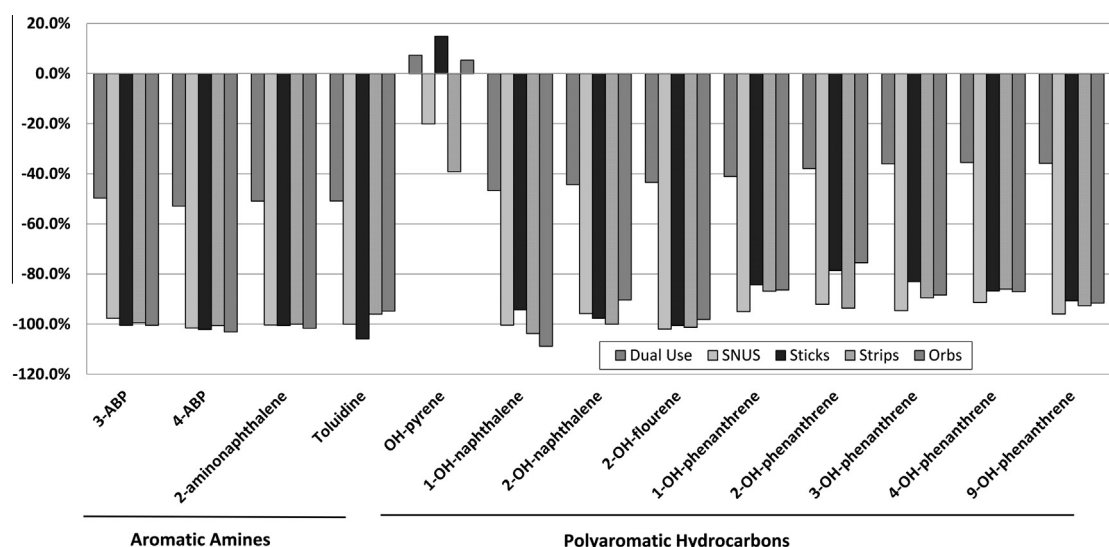


Fig. 2. Aromatic amine and polyaromatic hydrocarbon urinary biomarker level percent changes relative to the Abstinent group at Day 5 compared to baseline.

compared to the Dual use group, a finding attributed to the unusually high mean \pm SD variability observed in the Snus group.

Aromatic amine (AA) levels excreted at baseline were NSD between all groups for 4-aminobiphenyl and 2-aminonaphthalene. Significant differences were observed for 3-aminobiphenyl between the lowest Sticks and highest Strips groups, and for o-toluidine between the two lowest Dual use and Snus groups and highest Strips group. Group reductions for all four individual AAs on Day 5 were substantial for the non-smoking Snus, Sticks, Strips, Orbs, and Abstinent groups, and all groups had significant reductions compared to baseline. Notably, the magnitude of Day 5 reductions for the four individual AAs in the non-smoking (Snus, Sticks, Strips, and Orbs) groups were comparable to the Abstinent group (ranging between -95% and -102% relative to Abstinent), whereas the Dual use group reductions were consistently approximately one-half (ranging between -50% and -53% relative to

Abstinent; Fig. 2) of those seen in the non-smoking groups, with all Day 5 Dual use AA comparisons between the non-smoking groups being significantly different.

Polyaromatic hydrocarbon (PAH) levels excreted at baseline were NSD between all groups for all nine of the PAH biomarkers. Similar to the AA findings, group reductions for eight of the individual PAHs on Day 5 were substantial for the non-smoking groups (ranging between -76% and -109% relative to Abstinent; Fig. 2), and all groups had significant reductions compared to baseline. Dual use group reductions were approximately one-third to one-half of those seen in the non-smoking groups (ranging between -35% and -47% relative to Abstinent), with all Dual use group PAH comparisons between the non-smoking groups significantly different, except the 2-OH-phenanthrene Orbs group that was NSD. Of exception to these findings was 1-OH-pyrene, which showed only nominal changes on Day 5, ranging between -39%

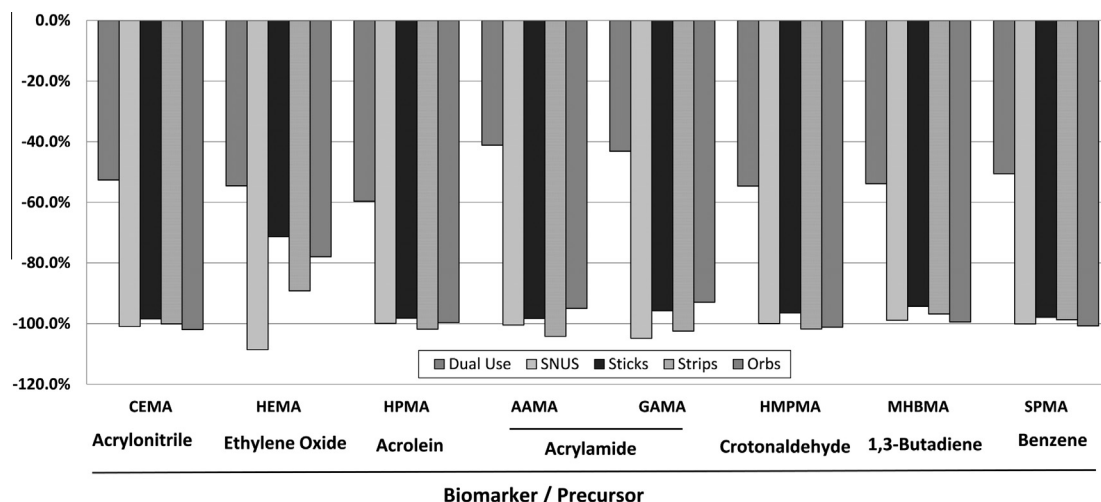


Fig. 3. Select vapor phase urinary biomarker level percent changes relative to the Abstinent group at Day 5 compared to baseline.

and 15% relative to Abstinent, and were NSD in both baseline and Day 5 group comparisons.

For the vapor phase biomarkers, levels excreted at baseline were NSD in group comparisons for CEMA, HPMA, GAMA, and HMPMA. Significant differences were observed for HEMA between the lowest Sticks and highest Snus groups, for AAMA between the lowest Dual use and highest Snus groups, for MHBMA between the two lowest Sticks and Strips groups and highest Abstinent group, and for SPMA between the lowest Sticks and two highest Orbs and Abstinent groups. Similar to the previous findings, group reductions for all vapor phase biomarkers on Day 5 were substantial for the non-smoking groups (ranging between –71% and –109% relative to Abstinent; Fig. 3), and all groups had significant reductions compared to baseline. Day 5 Dual use group reduction levels were consistently approximately one-half of those seen in the non-smoking groups, with all Dual use group comparisons between the non-smoking groups being significantly different.

Mutagenicity levels excreted at baseline ranged between 164,510 and 191,860 revertants/24-h urine, which were NSD between all groups. Group reductions on Day 5 were substantial for the non-smoking groups (ranging between –85% and –107% relative to Abstinent; Fig. 1), and all groups had significant reductions compared to baseline. Similar to the discrete chemical biomarkers, the magnitude of Day 5 mutagenicity reductions were comparable between the Abstinent and non-smoking groups, whereas the Dual use group reduction was approximately one-half (–51% relative to Abstinent) of those seen in the non-smoking groups, with all Day 5 Dual use group comparisons between the non-smoking groups being significantly different, except the Snus group.

Evening (~22:00) plasma nicotine and cotinine levels at baseline were both NSD between all groups. Similar to the urinary T-NicEq results, Day 5 reductions in both plasma nicotine and cotinine were observed in all groups, where the least was in the Dual use and greatest in the Abstinent groups, while the magnitude of reductions varied by test product, such that Orbs > Strips > Sticks = Snus. Day 5 Dual use reductions for nicotine and cotinine were approximately one-half and one-third of those seen in the Abstinent group, respectively. Evening blood COHb percent saturation levels at baseline were all $\geq 5\%$, indicative of being an active smoker, and were NSD between all groups. Expectedly, group COHb reductions on Day 5 for the non-smoking groups were substantial (ranging between –96% and –99% relative to Abstinent; Fig. 1), and all groups had significant reductions compared to

baseline. The magnitude of Day 5 reductions were comparable between the Abstinent and the non-smoking groups, whereas the Dual use group reduction level was slightly greater than one-half (–57% relative to Abstinent) of those seen in the non-smoking groups, with all Day 5 Dual use group comparisons between the non-smoking groups being significantly different.

Evening plasma thiocyanate levels at baseline were NSD in all group comparisons, except for the lowest Sticks and highest Strips groups. Also expectedly, group reductions on Day 5 for the non-smoking groups were robust (ranging between –94% and –111% relative to Abstinent), and all groups had significant reductions compared to baseline. The magnitude of Day 5 reductions were comparable between the Abstinent and non-smoking groups, whereas the Dual use group reduction level was slightly greater than one-half (–61% relative to Abstinent) of those seen in the non-smoking groups, with all Day 5 Dual use group comparisons between the non-smoking groups being significantly different. Thiocyanate levels were also quantified as $\mu\text{mol}/24\text{-h}$ urine, and findings were generally comparable with the plasma results (Table 3).

Fecal T-NicEq levels excreted at baseline and Day 5 were quantified in a small subset (3–5) of participants in each group (data not shown). For all groups at both time points, fecal excretion was negligible relative to urinary excretion, with mean levels ranging between 8 and 118 $\mu\text{g}/24\text{-h}$, and unlike urine, no conjugated metabolites were detected. Notably, analytical intra- and inter-group variability appeared excessive, attributed to urine cross-contamination of the feces samples collected. Nevertheless, based on T-NicEq masses recovered in both urine and feces, fecal excretion accounted for only $\leq 2\%$ of the total, a value similar to the 1% fecal excretion level reported for smokers in a 14C-nicotine mass balance study (Armitage et al., 1975).

In the self-reported 15-Item-MNWS-R questionnaire, higher scores connote a greater level of withdrawal discomfort. At baseline, group mean \pm SD scores ranged between 5.5 ± 6.0 and 9.1 ± 7.5 , and were NSD between all groups, except for the lowest Orbs and highest Strips groups, which were significantly ($p \leq 0.05$) different. By Day 5 scores were lowest (6.5 ± 7.5) for Dual use and highest (12.1 ± 11.7) for the Abstinent groups. All Day 5 to baseline comparisons were NSD, with the exception of the Abstinent group whose score increased by 74%. Day 5 group comparisons showed the Abstinent's group score was significantly greater than the Dual use and Snus groups, but NSD between the Sticks, Strips and Orbs groups. It is noted that the group comparison results from

the self-reported 9-Item-MNWS-R questionnaire were similar to the 15-Item results, and are not reported.

4. Discussion

Methods used in this trial were adapted from a previous similar trial (Krautter and Borgerding, 2014) that studied an earlier version of Orbs for use patterns, toxicant exposure and subjective effects. While benefitting from good compliance inherent in a confinement trial, participants were allowed limited alcoholic beverages during evening meals, a feature 77% of participants choose to use. Another feature was use of a controlled diet, low in potentially confounding biomarkers (i.e., PAHs, AAs, and cyanogens). Recognizing that some smokers will Dual use cigarettes with various smokeless tobacco products (STPs), a Dual use cohort was included that restricted their CPD by 60% to standardize reduced smoking rates. Randomization of participants into the 6 groups was well balanced, given that no statistical differences were observed at baseline between groups for the primary clinical parameters of cigarette smoke exposure, namely: blood COHb%, urinary T-NicEq and plasma nicotine and cotinine concentrations.

Results demonstrate that when active smokers switch to exclusive use of Snus, Sticks, Strips, or Orbs, after 5 days their biomarkers of exposure to toxicants in cigarette smoke were substantially and significantly reduced, usually comparable in magnitude to being tobacco abstinent. Similarly, when smoking rates are reduced by 60% in Dual users, significant but less robust toxicant reductions are still evident. In a similar biomarker study of Dual users of cigarettes and Snus, the authors suggest that Dual use does not result in compensatory changes in the way each cigarette was smoked, but do acknowledge the lack of data where a systematic assessment of cigarette smoke exposure during Dual use is available (Sarkar et al., 2009). This trial did include such an assessment and results indicate that MLE did increase on a per cigarette basis, but when participants reduced their CPD by 60%, their daily 'tar' and nicotine MLE was reduced by ~50%, indicating a net benefit in reduced toxicant exposures under these product use conditions.

Of the 32 biomarkers of exposure studied, there are two notable biomarkers that yielded apparent anomalous results; 1-OH-pyrene and NNAL. 1-OH-pyrene has long been used as a surrogate biomarker for occupational, environmental and dietary exposures to PAHs. While pyrene is known to be present in cigarette smoke, it has no known toxicological consequences. Perhaps a better biomarker of exposure for PAHs from cigarette smoke or smokeless tobacco is benzo(a)pyrene (BaP), by quantifying one of its urinary metabolites, 3-OH-BaP. During planning of this trial, methods for urinary BaP were not widely available and we elected to use a PAH panel of nine metabolites, including 1-OH-pyrene. Eight PAH metabolites showed consistent and substantial PAH reductions on Day 5 for all the non-smoking groups, whereas changes in 1-OH-pyrene were NSD on Day 5 between all groups and baseline comparisons. Variability in 1-OH-pyrene urinary excretion and its poor correlation with nicotine smoke exposure have been noted by others (Hecht et al., 2004; St. Helen et al., 2012; USDHHS, 2010). The lack of 1-OH-pyrene reductions noted in this trial again suggests it is not a reliable biomarker in tobacco studies.

Similarly, NNAL has long been used in tobacco studies as a biomarker to estimate relative NNK exposure, a potent TSNA carcinogen which is present in both tobacco and tobacco smoke (IARC, 2007). Our results did not show reductions of NNAL in all groups, whereas significant reductions for the other three TSNA were observed in all groups. This apparent anomaly, as previously mentioned, adds to the evidence that STP users convert a greater proportion of NNK to NNAL, confounding direct comparisons between NNK exposure from combustible and non-combustible tobacco products. Given that NNK conversion to NNAL is a step

in NNK's detoxification metabolic pathway, up-regulation of this conversion by using STPs is not necessarily a negative aspect, but rather highlights the limitations of NNAL as a predictive and quantitative biomarker in such product switching studies.

The self-reported 15-Item-MNWS-R questionnaire is a measure of subjective effects of nicotine withdrawal. Day 5 group score comparisons were all NSD, except the Abstinent group which had a significantly higher score compared to the Dual use and Snus groups, indicating heightened withdrawal discomfort in the Abstinent group.

Several limitations are noted in this trial that are inherent to the trial design. Most notably, the mandated 60% reduction in CPD (from baseline) in the Dual use group during intervention may or may not accurately reflect the way consumers' actually Dual use. Longer-term ambulatory studies would be required to make this determination. A second potential limitation may appear to be the limited group size and duration of the intervention period. However, with the exceptions of NNAL and 1-OH-pyrene, virtually all other analytes showed significant and substantial reductions, seemingly validating our statistical plan to detect a 20% change, 80% of the time. While NNAL is known to have a relatively long terminal half-life (17.6 day) (Goniewicz et al., 2009), levels in the Abstinent group was reduced by ~60% after 5 days intervention, less than most other biomarkers measured, but still suggesting group size (>25) and intervention duration (5 day) was adequate to detect the biomarker level changes studied. As previously discussed, the two exceptions that lacked observable reductions may be caused by other factors. Lastly, some investigators may consider not having a 'positive' control group, that is, participants who enter the clinic and continue smoking their UB cigarettes throughout the trial period, as a limitation. However, in a previous trial of very similar design, that did include a UB group, no material changes in product usage and biomarker levels were noted during the 5 day intervention period (Krautter and Borgerding, 2014).

In summary, we report results from a clinical confinement trial where cigarette smokers were switched to exclusive or Dual use of two relatively new types of STPs; Camel-branded Snus and dissolvable tobacco Sticks, Strips, and Orbs. Changes in exposure for 32 biomarkers, representing toxicants commonly associated with tobacco-related morbidity and mortality, were measured after 5 days. As expected, because many of the biomarkers studied result directly from the combustion of tobacco, the majority of Day 5 biomarker levels were substantially reduced in all exclusive STP users, reductions generally similar in magnitude to being tobacco abstinent, with more modest but generally significant reductions seen in Dual users. Of exception was 1-OH-pyrene which does not appear to be a reliable surrogate biomarker for PAHs in tobacco studies. Moderate to substantial reductions of biomarkers endogenous to tobacco (i.e., TSNA) were also seen, again less so in Dual users, with the exception of NNAL, a biomarker of exposure that appears to have limited utility based upon confounding factors in trials that switch smokers from combustible to non-combustible tobacco products. Lastly, given the unique nature of DTPs, which are entirely consumed, we investigated whether such oral ingestion causes a shift in urinary to fecal nicotine excretion. Results suggest no shift occurs and the major excretory pathway remains urinary excretion, regardless of the route of nicotine exposure. It is noted however, levels of T-NicEq in his trial analyzed for nicotine + 9 metabolites (nic + 9) in urine and feces, known to account for ≥90% of systemic exposure in urine when an additional metabolite, 4-OH-4-(3-puridyl)-butanoic acid is included (Benowitz et al., 2009). Recent advances in urinary analysis has verified these earlier estimates, and when 4-OH-4-(3-puridyl)-butanoic acid is included, total accountability is 95% of the absorbed exposure (Piller et al., 2014), with 4-OH-4-(3-puridyl)-butanoic acid accounting for approximately 5% of the T-NicEq. Based on this trial,

the presence of this minor metabolite in feces cannot be discounted.

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References

- Armitage, A.K., Dollery, C.T., George, C.F., Houseman, T.H., Lewis, P.J., Turner, D.M., 1975. Absorption and metabolism of nicotine from cigarettes. *Br. Med. J.* 4 (5992), 313–316. <http://dx.doi.org/10.1136/bmj.4.5992.313>.
- Benowitz, N.L., Hukkanen, J., Jacob III, P., 2009. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb. Exp. Pharmacol.* 192, 29–60. http://dx.doi.org/10.1007/978-3-540-69248-5_2.
- Breland, A.B., Kleykamp, B.A., Eissenberg, T., 2006. Clinical laboratory evaluation of potential reduced exposure products for smokers. *Nicotine Tob. Res.* 8 (6), 727–738. <http://dx.doi.org/10.1080/14622200600789585>.
- Chetiyanukornkul, T., Toriba, A., Kameda, T., Tang, N., Hayakawa, K., 2006. Simultaneous determination of urinary hydroxylated metabolites of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene as multiple biomarkers of exposure to polycyclic aromatic hydrocarbons. *Anal. Bioanal. Chem.* 386 (3), 712–718. <http://dx.doi.org/10.1007/s00216-006-0628-6>.
- Degiampietro, P., Peheim, E., Drew, D., Graf, H., Colombo, J.P., 2009. Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *Clin. Chem. Lab. Med.* 25 (10), 711–718. <http://dx.doi.org/10.1515/ccm.1987.25.10.711>.
- European Commission, 2008. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Health Effects of Smokeless Tobacco Products.
- Goniewicz, M.L., Havel, C.M., Peng, M.W., Jacob 3rd, P., Dempsey, D., Yu, L., Zielinska-Danch, W., Koszowski, B., Zogala, J., Sobczak, A., Benowitz, N.L., 2009. Elimination kinetics of tobacco specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNAL). *Cancer Epidemiol. Biomark. Prev.* 18, 3421–3425. <http://dx.doi.org/10.1158/1055-9965.EPI-09-0874>.
- Hatsukami, D.K., Lemmonds, C., Zhang, Y., Murphy, S.E., Le, C., Carmella, S.G., Hecht, S.S., 2004. Evaluation of carcinogen exposure in people who used “reduced exposure” tobacco products. *J. Natl. Cancer Inst.* 96 (11), 844–852.
- Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Fagerström, K.O., 1991. The Fagerström test for nicotine dependence: a revision of the Fagerström tolerance questionnaire. *Br. J. Addiction* 86, 1119–1127.
- Hecht, S.S., Carmella, S.G., Stepanov, I., Jensen, J., Anderson, A., Hatsukami, D.K., 2008. Metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to its biomarker total NNAL in smokeless tobacco users. *Cancer Epidemiol. Biomark. Prev.* 17 (3), 732–735. <http://dx.doi.org/10.1158/1055-9965.EPI-07-2843>.
- Hecht, S.S., Carmella, S.G., Le, K.A., Murphy, S.E., Li, Y.S., Le, C., Jensen, J., Hatsukami, D.K., 2004. Effects of reduced cigarette smoking on levels of 1-hydroxypyrene in urine. *Cancer Epidemiol. Biomark. Prev.* 13 (5), 834–842.
- Hughes, J.R., Hatsukami, D.K., 2007. Background on the Minnesota withdrawal scale-revised (MNWS-R). Accessed at www.uvm.edu/~hbpl; dated September, 2007. Scales originally reported in: Hughes, J. R., & Hatsukami, D. K. (1986). Signs and symptoms of tobacco withdrawal. *Arch. Gen. Psychiatry* 43, 289–294.
- IARC – International Agency for Research on Cancer; World Health Organization. (2007). IARC monographs on the evaluation of carcinogenic risks to humans; smokeless tobacco and some tobacco-specific N-nitrosamines. Lyon, France.
- Kavvadias, D., Scherer, G., Cheung, F., Errington, G., Shepperd, J., McEwan, M., 2009. Determination of tobacco-specific N-nitrosamines in urine of smokers and non-smokers. *Biomarkers* 14 (8), 547–553. <http://dx.doi.org/10.3109/13547500903242883>.
- Krautter, G.R., Borgerding, M.F., 2014. Comparison of consumption patterns, biomarkers of exposure and subjective effects in cigarette smokers switched to dissolvable tobacco (Camel Orbs), dual use, or tobacco abstinence. *Nicotine Tob. Res.* 16 (10), 1336–1347. <http://dx.doi.org/10.1093/ntr/ntu082>.
- Kuenemann-Migeot, C., Callais, F., Momas, I., Festy, B., 1997. Use of *Salmonella typhimurium* TA 98, YG 1024 and YG 1021 and deconjugating enzymes for evaluating the mutagenicity from smokers' urine. *Mut. Res./Genetic Toxicol. Environ. Mutagen.* 390 (3), 283–291. [http://dx.doi.org/10.1016/S1383-5718\(97\)00029-6](http://dx.doi.org/10.1016/S1383-5718(97)00029-6).
- Houezec, Le., Jacques, McNeill, Ann, Britton, John., 2011. Tobacco, nicotine and harm reduction. *Drug Alcohol Rev.* 30 (2). <http://dx.doi.org/10.1111/j.1465-3362.2010.00264.x>.
- Mascher, D.G., Mascher, H.J., Scherer, G., Schmid, E.R., 2001. High-performance liquid chromatographic-tandem mass spectrometric determination of 3-hydroxypropylmercapturic acid in human urine. *J. Chromatogr. B Biomed. Sci. Appl.* 750, 163–169. Retrieved from [http://dx.doi.org/10.1016/S0378-4347\(00\)00385-6](http://dx.doi.org/10.1016/S0378-4347(00)00385-6).
- Meger, M., Meger-Kossein, I., Schuler-Metz, A., Janket, D., Scherer, G., 2002. Simultaneous determination of nicotine and 8 nicotine metabolites in urine of smokers using liquid chromatography-tandem mass spectrometry. *J. Chromatogr.* 778, 251–261. Retrieved from [http://dx.doi.org/10.1016/S0378-4347\(01\)00451-0](http://dx.doi.org/10.1016/S0378-4347(01)00451-0).
- Mendoza-Baumgart, M.I., Tulunay, O.E., Hecht, S.S., Zhang, Y., Murphy, S., Le, C., Jensen, J., Hatsukami, D.K., 2007. Pilot study on lower nitrosamine smokeless tobacco products compared with medicinal nicotine. *Nicotine Tob. Res.* 9 (12), 1309–1323.
- Nelson, P.R., Chen, P., Dixon, M., Steichen, T., 2011. A survey of mouth level exposure to cigarette smoke in the United States. *Regul. Toxicol. Pharmacol.* 61 (Suppl. 3), S25–S38.
- Paci, E., Pigini, D., Cialdella, A.M., Faranda, P., Tranfo, G., 2007. Determination of free and total S-phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure. *Biomarkers* 12 (2), 111–122. <http://dx.doi.org/10.1080/13547500601007943>.
- Piller, M., Gilch, G., Scherer, G., Scherer, M., 2014. Simple, fast and sensitive LC-MS/MS analysis for the simultaneous quantification of nicotine and 10 of its major metabolites. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 1 (951–952), 7–15. <http://dx.doi.org/10.1016/j.jchromb.2014.01.025>.
- Riedel, K., Scherer, G., Engl, J., Hagedorn, H.-W., Tricker, A., 2006. Determination of three carcinogenic aromatic amines in urine of smokers and never-smokers. *J. Anal. Toxicol.* 30, 1–8. <http://dx.doi.org/10.1093/jat/30.3.187>.
- Riedel, K., Hagedorn, H.W., Scherer, G., 2013. Thiocyanate in plasma and saliva. *Biomonitoring Methods* 13. <http://dx.doi.org/10.1002/3527600418.bi5712sale0013>.
- Sarkar, M., Liu, J., Koval, T., Wang, J., Feng, S., Serafin, R., Jin, Y., Xie, Y., Newland, K., Roethig, H.J., 2009. Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro Snus. *Nicotine Tob. Res.* 12 (2), 105–116. <http://dx.doi.org/10.1093/ntr/ntp183>.
- Scherer, G., Urban, M., Hagedorn, H.W., Serafin, R., Feng, S., Kapur, S., Muhammad, R., Jin, Y., Sarkar, M., Roethig, H.J., 2010. Determination of methyl-, 2-hydroxyethyl- and 2-cyanoethylmercapturic acids as biomarkers of exposure to alkylating agents in cigarette smoke. *J. Chromatogr. B.* 878 (12), 2520–2528.
- Stepanov, I., Upadhyaya, P., Carmella, S.G., Feuer, R., Jensen, J., Hatsukami, D.K., Hecht, S.S., 2008. Extensive metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers. *Cancer Epidemiol. Biomarkers Prev.* 17 (7), 1764–1773. <http://dx.doi.org/10.1158/1055-9965.EPI-07-2844>.
- Sterz, K., Kohler, D., Schettgen, T., Scherer, G., 2010. Enrichment and properties of urinary pre-S-phenylmercapturic acid (pre-SPMA). *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* 878, 2502–2505. Retrieved from <http://dx.doi.org/10.1016/j.jchromb.2009.08.043>.
- St. Helen, G., Goniewicz, M.L., Dempsey, D., Wilson, M., Jacob III, P., Benowitz, N.L., 2012. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. *Chem. Res. Toxicol.* 25 (4), 952–964. <http://dx.doi.org/10.1021/tx300043k>.
- Urban, M., Gilch, G., Schepers, G., van Miert, E., Scherer, G., 2003. Determination of the major mercapturic acids of 1,3-butadiene in human and rat urine using liquid chromatography with tandem mass spectrometry. *J. Chromatogr. B.* 796, 131–140. <http://dx.doi.org/10.1016/j.jchromb.2003.08.009>.
- Urban, M., Kavvadias, D., Riedel, K., Scherer, G., Tricker, A., 2006. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and never-smokers. *Inhal. Toxicol.* 18, 831–839. Retrieved from <http://dx.doi.org/10.1080/08958370600748430>.
- USDHHS – U.S. Department of Health and Human Services. (2010). How Tobacco smoke causes disease – the biology and behavioral basis for tobacco-attributable disease: A Report of the Surgeon General. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.
- WHO, 2008. The scientific basis of tobacco product regulation: second report of a WHO study group. (WHO technical report series; No. 951).
- Zeller, M., Hatsukami, D. and the Strategic Dialogue on Tobacco Harm Reduction Group, 2009. The strategic dialogue on tobacco harm reduction: a vision and blueprint for action in the US. *Tob. Control* 18 (4), 324–332.
- Zeller, M., 2013. Reflections on the ‘endgame’ for tobacco control. *Tob. Control* 22, i40–i41. <http://dx.doi.org/10.1136/tobaccocontrol-2012-050789>.