The Journal of Clinical Pharmacology
2022, 62(11) 1445–1458

DOI: 10.1002/jcph.2098

A Randomized, Controlled Study to Assess Biomarkers of Exposure in Adult Smokers Switching to Oral Nicotine Products

Jeffery Edmiston, PhD, Jianmin Liu, MD, Jingzhu Wang, MS, and Mohamadi Sarkar, MPharm,PhD,FCP

Abstract

This open-label, randomized, controlled, in-clinic, 6-parallel-group study evaluated changes in biomarkers of exposure (BoEs) to select harmful and potentially harmful constituents in adult smokers (N = 213) not planning to quit smoking. Adult smokers were randomized to continue smoking (CS), reduce smoking by 50% and dual use oral tobacco-derived nicotine (OTDN) products (VERVE chews/discs), stop smoking and exclusively use discs or chews, or stop using all tobacco products (NT). The primary objective compared 24-hour urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; a biomarker for the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) in dual and exclusive use of discs and chews to continue smoking and NT on day 7. NNAL levels on day 7 were significantly lower ($P < .05$) among dual and exclusive users of discs/chews compared to continue smoking; median percent reductions were ≈30% and ≈73%, respectively. NNAL levels were not significantly different between those who used discs/chews and the NT group. Many of the additional secondary biomarkers of exposure were significantly lower in dual users (10/19) and exclusive users of discs/chews (17/19) compared to the continue smoking group. Overall, reductions in secondary biomarkers of exposure were greater in exclusive users than dual users. The 24-hour urinary nicotine equivalents were significantly lower ($P < .05$) among exclusive users of discs/chews compared to continue smoking. The discs/chews appeared to be well tolerated. These results demonstrate that while switching completely to discs/chews substantially reduces exposure to select harmful and potentially harmful constituents, dual use with 50% reduction in cigarette consumption also reduces exposure. oral tobacco-derived nicotine products like discs/chews may present a harm reduction opportunity for adult smokers, particularly those not intending to quit smoking.

Keywords

adult smokers, biomarkers of exposure, nicotine, NNAL, oral nicotine, oral tobacco-derived nicotine products

There is overwhelming scientific evidence regarding a risk continuum in the range of tobacco products currently available on the market. Cigarette smoking is the leading preventable cause of death in the United States, primarily due to lung cancer, respiratory disease, and cardiovascular disease.[1] The US Food and Drug Administration (FDA) and many in public health acknowledge that noncombustible tobacco products (eg, e-vapor, smokeless tobacco [ST], and heat-not-burn) present relatively lower risks.[2-4] There is a growing category of oral tobacco consumer products like VERVE chews and discs (referred to as the test products in this study; Altria Group, Inc., Richmond, Virginia) that are tobacco leaf free and contain tobacco-derived nicotine, flavors, and excipients. The test products do not contain cut, ground, powdered, or leaf tobacco—a point of differentiation compared to most oral tobacco products in the United States. The test products are tobacco products intended as switching products for adult smokers not planning to quit smoking. While being similar in format to nicotine replacement therapies, the test products should not be considered cessation products and are not intended to intercept adult smokers who intend to quit smoking. On the contrary, the test products complement and may motivate adult smokers who are interested in continuing to use tobacco products to switch away from cigarettes to a less harmful alternative, although not risk free (because they contain nicotine). The evidence indicates this might be possible because, in a previous pharmacokinetic study, the chews test products demonstrated significantly higher nicotine delivery

Altria Client Services LLC, Richmond, Virginia, USA

Submitted for publication 13 March 2022; accepted 30 May 2022.

Corresponding Author:
Mohamadi Sarkar, MPharm, PhD, FCP, Altria Client Services LLC, Center for Research and Technology, 601 East Jackson Street, Richmond, VA 23219
Email: mohamadi.a.sarkar@altria.com

Table 1. Participant Demographics (Safety Population)

Parameter	Group 1 continue smoking (n = 40)	Dual Use of own-brand cigarette and Test Products: Group 2 DUD (n = 42)	Dual Use of own-brand cigarette and Test Products: Group 3 DUC (n = 41)	Exclusive Use of Test Products: Group 4 EUD (n = 30)	Exclusive Use of Test Products: Group 5 EUC (n = 30)	Group 6 NT (n = 30)	Overall (N = 213)
Age, y	36.6 ± 11.1	39.8 ± 11.6	43.7 ± 12.3	38.3 ± 12.6	38.2 ± 13.1	41.5 ± 8.1	39.8 ± 11.7
Sex, n (%)							
Male	27 (67.5)	23 (54.8)	26 (63.4)	15 (50.0)	18 (60.0)	18 (60.0)	86 (40.4)
Female	13 (32.5)	19 (45.2)	15 (36.6)	15 (50.0)	12 (40.0)	12 (40.0)	127 (59.6)
Race, n (%)							
Black	13 (32.5)	14 (33.3)	14 (34.1)	8 (26.7)	12 (40.0)	14 (46.7)	75 (35.2)
White	27 (67.5)	26 (61.9)	22 (53.7)	22 (73.3)	18 (60.0)	16 (53.3)	131 (61.5)
Other	0 (0.0)	2 (4.8)	5 (12.2)	0 (0.0)	0 (0.0)	0 (0.0)	7 (3.3)
BMI, kg/m^2	28.1 ± 4.6	28.7 ± 4.7	29.1 ± 5.5	28.8 ± 5.4	26.7 ± 5.1	29.7 ± 4.8	28.5 ± 5.0
Number of cigarettes smoked per day	16.8 ± 4.6	17.8 ± 6.0	18.4 ± 6.0	15.9 ± 3.5	16.7 ± 5.4	17.8 ± 5.9	17.3 ± 5.4
Number of years of smoking	17.6 ± 11.3	18.9 ± 12.2	23.6 ± 13.7	21.7 ± 13.4	21.7 ± 12.9	22.2 ± 9.4	20.8 ± 12.3
FTCD score	5.9 ± 1.8	5.4 ± 1.6	5.2 ± 1.9	5.4 ± 1.4	5.6 ± 1.3	5.6 ± 1.6	5.5 ± 1.6

BMI, body mass index; CS, continued smoking; DUC, dual use with chews; DUD, dual use with discs; EUC, exclusive use of chews; EUD, exclusive use of discs; FTCD, Fagerström Test for Cigarette Dependence; NT, no tobacco product use; OBC, own-brand cigarette; SD, standard deviation.
Data are presented as mean ±SD unless otherwise noted.

than the 2-mg Nicorette gum (GlaxoSmithKline, London, England), with similar scores of satisfaction and the willingness to use the product again.[5] Completely switching to oral products like the test products presents a unique harm reduction opportunity for those adult smokers who are unable or unwilling to quit using tobacco products.[6-8] Adult smokeless tobacco users, who are accustomed to using oral tobacco products—particularly dual users of smokeless tobacco and combustible cigarettes—may similarly experience reductions in harm by switching completely to the test products. Many of the chemicals identified by the FDA[9] as harmful and potentially harmful constituents (HPHCs) in cigarette smoke (eg, carbon monoxide) are related to the combustion of tobacco, and these HPHCs would not be expected to be present in noncombustible products. Reduction in exposure to select combustible cigarette HPHCs is a key step in assessing the potential impact on risks of smoking-related diseases.

The biomarkers of exposure (BoEs) were selected for evaluation based on previous publications, including reports by the Institute of Medicine: "Clearing the Smoke" and "Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease."[2,10-13] Furthermore, while there are close to 7000 chemicals in cigarette smoke, 70 of which are carcinogens, we selected biomarkers for cigarette smoke constituents or metabolites of cigarette smoke constituents that are representative of the particulate and gas/vapor phase of cigarette smoke. These smoke constituents were selected primarily based on the toxicologic relevance and their usefulness as surrogates for chemical classes of smoke constituents, as well as availability of validated analytical methods. This approach is well established and generally considered reasonable by many researchers,[2,14-16] including the FDA.[17] The biomarkers of exposure characterize exposure to select HPHCs as shown in Table 1 and are classified by the FDA as carcinogens, respiratory toxicants, cardiovascular toxicants, reproductive or developmental toxicants, or as addictive constituents (Table S1) for combustible cigarettes by the FDA.[9]

In this study we investigated changes in biomarkers of exposure among complete switchers and dual users for 2 different formats of test products (discs and chews), each in 2 flavor variants: blue mint and green mint. We characterized dual users for 2 reasons: First, dual use is a transition state for adult smokers, since many adult smokers may experience difficulty in abruptly and immediately switching completely and may start by gradually reducing cigarette consumption as they start using the test products. Second, some reports indicate that dual users of e-vapor products and cigarettes have *higher* exposure to select HPHCs,[18] suggesting that adult smokers may be altering their smoking behavior as they transition from cigarettes. Therefore, we investigated this phenomenon by measuring BoE levels among dual users who reduced cigarette consumption by at least 50% and used the test products. In this randomized, controlled study, we compared the BoE levels in switchers and dual users to adult smokers who either continued smoking cigarettes or discontinued all tobacco product use for 7 days.

Methods

All pertinent study documents were reviewed by an independent institutional review board, Advarra (Columbia, Maryland), before study initiation. All participants in this study reviewed, signed, and dated the informed consent form before study initiation. This multicenter study was conducted at QPS Bio-Kinetic (Springfield, Michigan) and Inflamax/Hill Top Research (Neptune, New Jersey).

Ethics Approval

The investigator and all research staff conducted the study in accordance with the ethical standards in the Declaration of Helsinki, applicable sections of the US Code of Federal Regulations, and International Conference on Harmonization E6 Guideline for Good Clinical Practice.

Study Participants

This study enrolled healthy adult male and female self-affirmed combustible cigarette smokers, 21 to 65 years of age, who were willing to abstain from smoking and use all 4 test products. All participants had an average consumption of at least 10 but no more than 30 combustible cigarettes per day for at least 12 months before screening and positive urine cotinine ($\geq$500 ng/mL) test. As part of the informed consent, participants were made aware that the test product contains nicotine, which is addictive. They were informed that nicotine can harm an unborn baby or infant in people who are pregnant or nursing; increase heart rate and blood pressure; aggravate diabetes; and cause dizziness, nausea, and stomach pain. Health evaluations included physical exams, measurements of vital signs, and an electrocardiogram. Primary exclusion criteria included any clinically significant medical condition that would jeopardize the safety of the participant or impact the validity of the study results, including women who were pregnant or lactating, dentition that prevented using the test products, and allergies or intolerance to mint flavoring agents or phenylalanine. Participants were also excluded if they had attempted to quit smoking in the 30 days before the screening visit or used any tobacco- or nicotine-containing products other than combustible cigarettes within 1 week of check-in. Moreover, we provided the participants access to quit-smoking resources at screening and at the end of the study.

Study Products

The 4 test products used in this study have been marketed under the brand name VERVE chews and VERVE discs, both in 2 flavors: blue mint and green mint. The test products consist of $\approx$1.5 mg of US Pharmacopeia–grade tobacco-derived nicotine and food or biocompatible medical grade nontobacco ingredients, including flavors. The VERVE discs have a firm, flexible texture, and VERVE chews have a soft, flexible texture. Both product types are placed in the mouth and, once chewing is completed, removed and discarded. The reference product used in this study was the participants' own-brand cigarette (OBC).

Study Design

This was an open-label, randomized, controlled, 10-day, in-clinic, 6-parallel-group study. Eligible participants checked into the clinic on day –3 and completed a product trial (using each of the 4 test products ad libitum for 10 minutes, separated by $\approx$30 minutes). Participants continued to smoke their own-brand cigarette through 11:00 pm on day –3 and from 7:00 am to 11:00 pm on days –2 and –1. Participants were randomized into 6 groups (groups 1-6) using an interactive web response system on study day 1 after completion of 24-hour urine collection and before product use. Groups were stratified by men or women (no more than 60% of either in any group) and cigarettes per day. Participants were randomized into following groups:

Group 1 (n = 35): Continue smoking (CS) continued to use their own-brand cigarette for the duration of the study (days 1-7).

Group 2 (n = 35): Dual users of discs and own-brand cigarette. Adult smokers were instructed to reduce their cigarette consumption by at least 50% of their baseline use (the average number of cigarettes per day on days –2 and–1) and use the discs ad libitum (except for 3 specific discs use opportunities at 11:00 am, 3:00 pm, and 7:00 pm each study day, where they were asked to use the discs for at least 10 minutes to ensure use of the discs).

Group 3 (n = 35): Dual users of chews and own-brand cigarette. Adult smokers were instructed to reduce their cigarette consumption by at least 50% of their baseline use and use the chews ad libitum (except for 3 specific chew use opportunities at 11:00 am, 3:00 pm, and 7:00 pm each study day, where they were asked to use the chews for at least 10 minutes to ensure use of the chew).

Group 4 (n = 25): Exclusive users of discs. Adult smokers were instructed to stop smoking own-brand cigarette and use discs ad libitum (except for 3 specific disc use opportunities at 11:00 am, 3:00 pm, and 7:00 pm each study day, where they were asked to use the disc for at least 10 minutes to ensure use of the disc).

Group 5 (n = 25): Exclusive users of chews. Adult smokers were instructed to stop smoking own-brand cigarette and use chews ad libitum (except for 3 specific chew use opportunities at 11:00 am, 3:00 pm, and 7:00 pm each study day, where they were asked to

use the chews for at least 10 minutes to ensure use of the chews).

Group 6 (n = 25): Stop using all tobacco products. Adult smokers were instructed to stop smoking own-brand cigarette and not allowed access to any other tobacco products, including the discs/chews.

Biomarkers of Exposure

The primary objective was to compare 24-hour urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in adult smokers who reduced cigarette consumption by at least 50% from their baseline use with supplementary (dual) usage of the test products to those who continued to smoke cigarettes for 7 days. Secondary objectives included comparison of additional biomarkers of exposure: total N-nitrosonornicotine (NNN), nicotine equivalents (NEs), 2-aminonaphthalene (2-AN), 4-aminobiphenyl (4-ABP), 2-hydroxyethyl mercapturic acid (HEMA), 2-cyanoethylmercapturic acid (CEMA), S-phenyl mercapturic acid (S-PMA), 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA), 3-hydroxypropylmercapturic acid (3-HPMA), 2-hydroxypropyl-mercapturic acid (2-HPMA), N-acetyl-S-(2-carbamoylethyl)-l-cysteine (AAMA), N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine (GAMA), 2-hydroxybutenyl-mercapturic acid, 2-OH-fluorene, 2-naphthol, 1-OH-phenanthrene, urine mutagenicity, 1-hydroxypyrene (1-OHP), and carboxyhemoglobin (COHb; listed in Table S1) in adult smokers who reduced cigarette consumption by at least 50% from their baseline use with dual usage of the test products to those who continued to smoke cigarettes for 7 days, as well as those who ceased all tobacco use.

Twenty-four-hour urine collections for biomarkers of exposure and blood sampling for COHb were performed on days –1, 5, and 7. Each 24-hour urine collection was from ≈07:00 am on the scheduled day to ≈07:00 am the following day. The 24-hour urine collection began on each scheduled day after the first morning void (and any void before 07:00 am) and finished the following morning with the last void collected at ≈7:00 am (including first morning void). Participants were specifically instructed to collect all urine voided, and any missed collection during the 24-hour interval was documented as a protocol deviation.

Subjective Measures

Subjective effects were assessed using the Questionnaire of Smoking Urges–Brief (QSU-Brief),[19] the Modified Cigarette Evaluation Questionnaire (mCEQ),[20,21] and the Use the Product Again Questionnaire.[22] The QSU-Brief (all groups) and appropriate mCEQ (groups 1-5) were completed on days –1, 1, 5, and 7 ≈07:00 am before product use (QSU-Brief) and at ≈9:30 pm (QSU-Brief and mCEQ); group 6 completed the mCEQ on day –1 only. The mCEQ was further modified to reflect the test products. The Use the Product Again questionnaire for own-brand cigarette (groups 1-3) and the test products (groups 2-5) was completed on day 7 at ≈9:30 pm. The full list of items contained in the subjective questionnaires is included in Table S2.

Product Use Behavior

Product use behavior was characterized by the clinic staff documenting the number of each product used per day during each day in a product use period. The average product use duration of each test product was documented by measuring the time the product was placed in and removed from the mouth per use each day in a product use period. The total product use duration of each test product used was the sum of product use durations during each day in a product use period.

Clinical Safety End Points

Clinical safety end points (adverse events [AEs], electrocardiograms, physical examinations, vital signs, clinical chemistry, urinalysis, and hematology) were also characterized.

Statistical Analysis

A linear mixed model for repeated measures analysis was used for comparing each test group (groups 2-5) to the control groups (groups 1 and 6) for the primary end point (24-hour urinary total NNAL [ng/24 h] excreted on day 7) and secondary end points. In the model, study group, study day, study group by study day interaction, and sex were the fixed-effect factors. A sensitivity analysis was performed for the primary end point, as applicable. The baseline value of the biomarkers was included as a covariate in the model. A restricted maximum likelihood estimation method was applied, and 5 candidate covariance structures were considered: compound symmetry, first-order autoregressive, first-order autoregressive with a random subject effect, unstructured, and Toeplitz. The most appropriate covariance structure was determined on the basis of the Akaike information criterion (the covariance structure with the smallest Akaike information criterion was chosen). The pairwise comparisons of each of the test groups vs each control group were performed using a Dunnett's test at a 2-sided significance level of 0.05 to adjust for multiplicity, and analysis was conducted on both the modified intent-to-treat and per-protocol data sets. No multiplicity adjustment was done for multiple biomarkers. For the test groups compared to the reference groups for each biomarker, Dunnett's method was used for the adjustment of multiple comparisons. A standard residual analysis using a PROC MIXED procedure was used to examine the validity of normality assumptions for the primary end point. The data were found to be log-normally distributed; therefore, the statistical

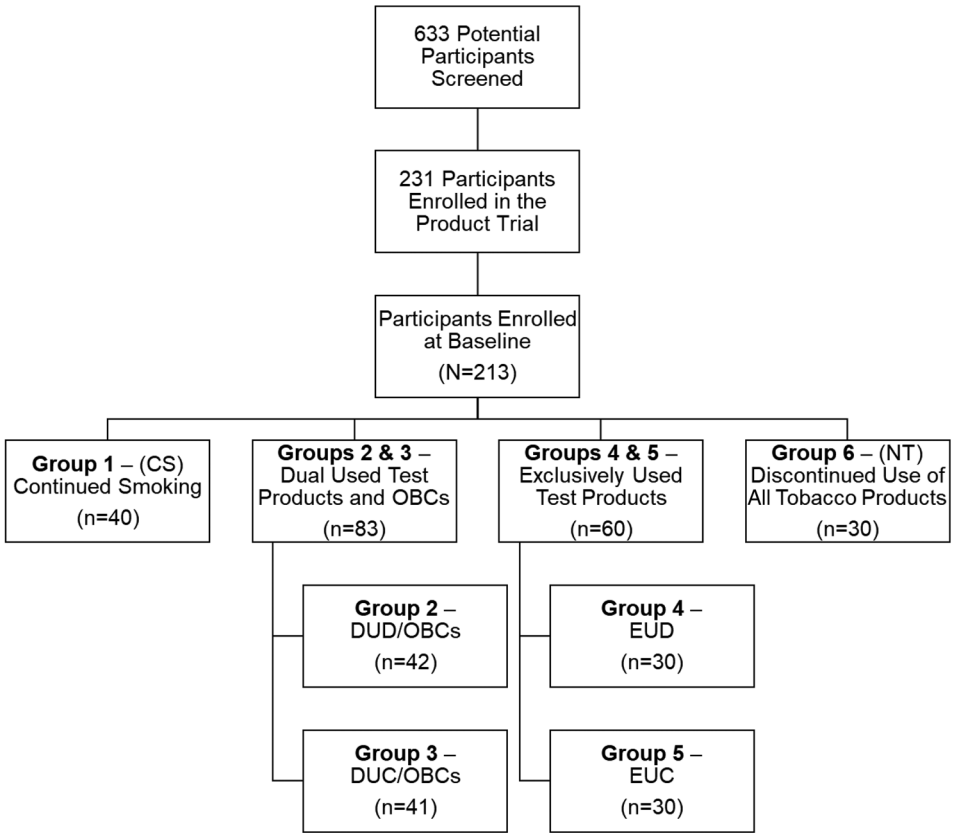

Figure 1. Participant disposition. CS, continued smoking; DUC, dual use with chews; DUD, dual use with discs; EUC, exclusive use of chews; EUD, exclusive use of discs; NT, no tobacco; OBC, own-brand cigarette.

analyses were performed on log-transformed data for all urine HPHC biomarkers and blood COHb. Square root transformation was used for urine mutagenicity statistical analysis, as mutagenicity data typically have a Poisson distribution. Data outliers were examined for the primary end point through a PROC MIXED model residual diagnosis (±4 studentized residuals). Sensitivity analysis by excluding outliers was performed for the primary analysis variable if any outliers were found.

Statistical analysis of subjective effects response scores was performed on QSU-Brief change from preuse score and mCEQ original scores to compare each of the test groups with each control group. The questions from QSU-Brief (Table S2) were used to derive factor scores for "anticipation of pleasure from smoking" (factor 1) and "relief of nicotine withdrawal" (factor 2).[19] This analysis was conducted on the modified intent-to-treat data sets. No multiple adjustment was performed for participant effect scores.

Sample Size Estimation

We assumed an effect size between the dual-use groups (groups 2 and 3) and continue smoking (group 1) to be similar to a previously reported study with an oral tobacco product using a similar design.[14] We estimated a sample size of 35 to complete, per group, would be needed for a 2-sided t-test, 85% power, and an $\alpha = 0.025$ type I error rate to account for the multiplicity adjustment for the 2 comparisons. We expected that the effect size would be much larger in the exclusive users (groups 4 and 5) and group 6; therefore, 25 participants to complete were estimated for these groups.

Results

Participant disposition is described in Figure 1, and participant demographics, cigarette consumption, and Fagerström Test for Cigarette Dependence scores are presented in Table 1, with no notable differences among groups. The age of the participants ranged from an average of 37 to 42 years, and the sex distribution was generally even (about 50%-60% men and 40%-50% women) and included ≈30% to 40% Black smokers. The study participants had smoked an average of 16 to 18 cigarettes per day for 18 to 24 years and the mean ± standard deviation Fagerström Test for Cigarette Dependence score was 5.5 ± 1.6.

Product Use Behavior

The number of test products and cigarettes used are shown in Figure S1. The mean total number of test products used per day in the test product/own-brand cigarette dual-use groups (groups 2 and 3) ranged from a total of 5.2 to 6.5 units of the 2 test products across study days, with both flavor variants used at similar amounts (≈3 units of each flavor/day) and most of the study participants used both flavors every day. The participants in groups 2 and 3 reduced their cigarette consumption by ≈52% from ≈17 to 18 cigarettes per day at baseline to ≈8 to 9 cigarettes per day on day 7. For the test product exclusive-use groups (groups 4 and 5), the total number of test products used per day ranged from a total of 8.5 to 11.6 pieces. The mean duration of product use in the test product/own-brand cigarette dual-use groups ranged from 16.3 to 20.2 minutes; for the test product exclusive-use groups, the mean duration of use ranged from 20.7 to 29.3 minutes.

Biomarkers of Exposure

The descriptive statistics of biomarkers of exposure measured on days –1 and 7 and median percent change are presented in Table 2 and Figure 2. Urine total NNAL was significantly reduced ($P < .05$) in group 2 (dual users–discs/own-brand cigarette) and 3 (dual users–chews/own-brand cigarette) compared to group 1 continue smoking. Many (10 of 19) of the secondary biomarkers of exposure (urine 2-AN, 4-ABP, CEMA, S-PMA, 3-HMPMA, 3-HPMA, 2-HPMA, 2-Naphthol, mutagenicity, and whole blood COHb) were also significantly reduced ($P < .05$) in groups 2 and 3 compared to group 1. Not surprisingly, 17 of the 19 biomarkers of exposure (urine total NNAL, NNN, NEs, 2-AN, 4-ABP, HEMA, CEMA, S-PMA, 3-HMPMA, 3-HPMA, 2-HPMA, AAMA, GAMA, 2-hydroxybutenyl-mercapturic acid, 2-Naphthol, urine mutagenicity, and whole blood COHb) were significantly higher ($P < .05$) among the dual users in groups 2 and 3 compared to those who stopped using all tobacco products in group 6. All biomarkers of exposure, except 3-HMPMA and 1-OHP were significantly reduced ($P < .05$) in group 4 (exclusive users of discs) and group 5 exclusive users of chews) compared to group 1 continue smoking.

The mean urine total NNAL amount excreted dropped on day 5, and the decline was less pronounced between days 5 and 7 (Figure 3); this trend was more pronounced for the exclusive users (groups 4 and 5) than the dual users (groups 2 and 3). The 50% reduction in cigarettes per day in the dual users was accompanied by ≈30% reduction in NNAL levels across days 5 and 7. The mean urine total NNAL amount excreted was decreased by 61% to 67% on day 5 and ≈70% on day 7 among exclusive users of the test products in groups 4 and 5, which was similar to the reductions observed in group 6. A relatively small change (–9.4%) was observed in urine total NNAL amount excreted in the continue smoking group at the end of the study. The average urinary NE levels on day 7 among the dual users (12.8 mg/24 h in group 2 and 15.4 mg/24 h in group 3), while lower compared to group 1 (16.8 mg/24 h), were not significantly different. The average NE levels on day 7 among exclusive users in group 4 (5.5 mg/24 h) and group 5 (7.4 mg/24 h) were significantly lower compared to group 1 and significantly higher compared to group 6 (0.5 mg/24 h).

Subjective Measures

QSU-Brief Questionnaire. Overall, changes in the QSU-Brief factor scores were small, with few significant differences between study groups and without any consistent trends. The change in factor score related to "anticipation of pleasure from smoking" (factor 1) and "relief of nicotine withdrawal" (factor 2) in the evening compared to morning was –0.70 and +0.50 on average, respectively, on day 7 for group 1. The magnitude of change in score from pre–product use in the morning vs post–product use in the evening across the various other groups were relatively small (ranging from –0.31 to –0.01 for factor 1 and –0.36 to +0.28 for factor 2).

Modified Cigarette Evaluation Questionnaire. In general, scores for "smoking satisfaction," "psychological reward," "enjoyment of sensation," and "craving reduction" were generally lower for the test products compared to cigarettes, with slightly higher "aversion" scores for the test products compared to cigarettes. The test products were rated as "a little" to "moderately" satisfying and cigarettes were rated as "moderately" to "a lot." Scores on the mCEQ in the areas of "smoking satisfaction," "psychological reward," and "craving reduction" were significantly lower for the discs test product, but not the chews, dual-use groups compared to group 1 continue smoking on day 7.

Use the Product Again Questionnaire. Overall, slightly more participants indicated they were more likely to use the chews test product again (64%) than the discs test products (44%) in the exclusive-user groups (Figure 4). The majority of participants (81%-85% in groups 2 and 3 and 95% in group 1) indicated that they would use own-brand cigarette again.

Adverse Events

In general, the use of the test products under the study conditions appeared to be well tolerated by the healthy adult smokers in this study, and there were no serious AEs reported. After randomization, 60 participants

Table 2. Biomarker of Exposure Amount Excreted on Day –1 and Day 7 and Percent Change

	Group 1		Dual Use of own-brand cigarette and Test Products				Exclusive Use of Test Products				Group 6	
	Continue Smoking		Group 2 - DUD		Group 3 - DUC		Group 4 - EUD		Group 5 - EUC		NT	
Biomarker	Day –1	Day 7	Day –1	Day 7	Day –1	Day 7	Day –1	Day 7	Day –1	Day 7	Day –1	Day 7
Total NNAL (ng/24 h)	511.9 ± 273.1	476.1 ± 296.6	581.7 ± 317.9	389.1 ± 226,9	548.8 ± 260.0	394.3 ± 240.8	638.8 ± 309.2	167.3 ± 100.2	599.2 ± 387.3	176.9 ± 135.2	527.3 ± 208.7	146.4 ± 60.4
% Change		–9.4		–31.3[a,b]		–29.5[a,b]		–73.0[a]		–73.5[a]		–72.0
Total NNN (ng/24 h)	17.2 ± 14.6	16.2 ± 11.8	25.7 ± 32.2	34.9 ± 142.4	20.3 ± 12.8	26.5 ± 94.8	37.6 ± 93.0	0.9 ± 1.3	21.1 ± 17.5	1.9 ± 3.9	20.3 ± 15.2	1.2 ± 1.6
% Change		–3.6		–45.5[b]		–46.8[b]		–97.3[a]		–96.6[a]		–96.6
NE (mg/24 h)	16.9 ± 5.5	16.8 ± 5.9	17.5 ± 6.0	12.8 ± 4.2	17.9 ± 7.2	15.4 ± 6.1	16.0 ± 5.9	5.5 ± 7.4	15.8 ± 5.0	7.4 ± 5.2	16.3 ± 5.0	0.5 ± 0.3
% Change		–3.8		–27.4[b]		–12.8[b]		–84.6[a,b]		–64.3[a,b]		–97.3
2-AN (ng/24 h)	35.1 ± 14.7	35.8 ± 16.7	38.4 ± 16.6	20.0 ± 8.1	39.1 ± 20.6	20.7 ± 10.4	36.9 ± 18.4	2.8 ± 2.6	35.4 ± 13.9	1.8 ± 0.8	39.8 ± 18.0	2.8 ± 2.2
% Change		–4.7		–45.1[a,b]		–36.9[a,b]		–93.7[a]		–95.1[a]		–93.7
4-ABP (ng/24 h)	23.5 ± 7.5	21.7 ± 8.6	27.5 ± 11.1	14.3 ± 4.9	26.5 ± 9.3	15.6 ± 6.2	25.8 ± 11.3	3.5 ± 1.8	24.8 ± 8.8	3.4 ± 1.2	26.2 ± 7.4	3.6 ± 1.2
% Change		–10.2		–46.0[a,b]		–39.7[a,b]		–86.1[a]		–86.5[a]		–87.3
HEMA (μg/24 h)	13.6 ± 6.7	11.3 ± 5.6	17.8 ± 12.7	12.5 ± 8.9	15.7 ± 12.9	10.6 ± 6.4	17.7 ± 15.1	8.4 ± 6.4	20.0 ± 12.5	10.3 ± 5.6	17.6 ± 12.9	9.3 ± 7.1
% Change		–11.0		–28.1[b]		–25.1[b]		–50.3[a]		–45.5[a]		–44.3
CEMA (μg/24 h)	252.7 ± 95.8	242.6 ± 100.3	262.5 ± 105.6	162.3 ± 75.7	283.7 ± 126.9	170 ± 77.6	265.6 ± 136.8	34.4 ± 24.6	255.5 ± 90.0	33.7 ± 14.6	255.4 ± 88.6	35.1 ± 16.9
% Change		–6.8		–41.5[a,b]		–37.2[a,b]		–87.8[a]		–86.1[a]		–85.0
S-PMA (μg/24 h)	7.0 ± 3.8	6.8 ± 3.6	8.1 ± 5.6	4.8 ± 3.1	9.1 ± 5.9	5.4 ± 3.8	8.5 ± 5.1	0.5 ± 0.5	6.5 ± 3.7	0.3 ± 0.1	6.8 ± 5.1	0.5 ± 0.3
% Change		–0.4		–40.6[a,b]		–39.5[a,b]		–94.3[a]		–95.0[a]		–92.3
3-HMPMA (μg/24 h)	550.3 ± 205.3	521.1 ± 217.2	566.2 ± 212.3	368.7 ± 160.3	620.3 ± 282.8	383.7 ± 158.7	606.4 ± 388.1	150.4 ± 63.8	569.7 ± 236.6	157.6 ± 79.9	563.3 ± 237.7	155.1 ± 75.4
% Change		–8.0		–37.4[a,b]		–35.5[a,b]		–74.5[a]		–74.1[a]		–72.4
3-HPMA (μg/24 h)	1743.2 ± 668.1	1658.8 ± 610.0	1766.8 ± 615.7	1047.9 ± 369.6	1986.4 ± 853.9	1152.7 ± 512.4	1865.7 ± 901.9	384.7 ± 186.5	1811.9 ± 878.3	414.8 ± 193.3	1922.8 ± 697.3	465.6 ± 181.1
% Change		–3.2		–38.8[a,b]		–38.0[a,b]		–81.5[a]		–76.6[a]		–74.9
2-HPMA (μg/24 h)	105.3 ± 54.6	100.7 ± 51.5	110.4 ± 62.5	72.4 ± 39.9	113.3 ± 64.2	70.6 ± 31.1	116.0 ± 64.4	22.0 ± 9.4	108.4 ± 63.0	33.0 ± 18.5	115.2 ± 54.4	26.8 ± 9.4
% Change		–2.3		–34.0[a,b]		–32.5[a,b]		–81.4[a]		–71.0[a]		–78.2
AAMA (μg/24 h)	205.8 ± 70.0	196.3 ± 79.0	213.8 ± 84.3	162.1 ± 78.0	234.4 ± 111.0	186.0 ± 85.9	179.4 ± 60.7	85.6 ± 53.8	203.2 ± 69.9	84.9 ± 36.1	212.3 ± 72.7	112.1 ± 48.3
% Change		–3.3		–27.3[a,b]		–16.2[b]		–59.1[a]		–62.6[a]		–48.7
GAMA (μg/24 h)	28.2 ± 7.9	27.3 ± 8.9	30.3 ± 9.1	24.8 ± 9.1	30.4 ± 12.3	25.7 ± 9.2	25.4 ± 8.0	14.2 ± 6.1	26.3 ± 9.0	14.3 ± 5.1	30.7 ± 10.1	18.9 ± 6.6
% Change		–3.4		–20.1[b]		–11.6[b]		–46.5[a]		–50.1[a]		–37.8
2-MHBMA (μg/24 h)	5.2 ± 4.3	5.3 ± 4.5	6.2 ± 6.0	3.4 ± 3.1	7.1 ± 6.4	4.1 ± 3.4	5.9 ± 4.1	0.2 ± 0.2	4.1 ± 3.9	0.2 ± 0.1	5.1 ± 4.5	0.2 ± 0.2
% Change		1		–45.9[a,b]		–33.2[b]		–97.5[a]		–95.0[a]		–95.5
2-OHFLe (μg/24 h)	6.0 ± 5.5	5.5 ± 5.0	6.5 ± 4.2	4.0 ± 4.2	4.9 ± 4.3	3.2 ± 3.7	5.4 ± 4.1	2.2 ± 2.6	8.1 ± 17.0	2.5 ± 3.5	4.9 ± 2.4	2.0 ± 2.2
% Change		3.3		–42.3		–38.2[b]		–70.6[a]		–78.4[a]		–72.0
2-Naphthol (μg/24 h)	26.4 ± 13.5	23.2 ± 8.1	28.2 ± 9.1	16.2 ± 8.3	28.2 ± 12.7	15.5 ± 7.2	27.7 ± 12.7	6.7 ± 4.5	26.3 ± 8.7	5.4 ± 4.4	24.8 ± 9.8	8.2 ± 9.0
% Change		–4.3		–42.0[a,b]		–40.8[a,b]		–80.2[a]		–78.1[a]		–77.3

(*Continued*)

Table 2. Continued

	Group 1	Dual Use of own-brand cigarette and Test Products				Exclusive Use of Test Products				Group 6	
	Continue Smoking	Group 2 - DUD		Group 3 - DUC		Group 4 - EUD		Group 5 - EUC		NT	
Biomarker	Day −1	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7
1-OHPhe (μg/24 h)	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
% Change		−11.5	−42.7[a]		−38.9[a]		−63.2[a]		−61.9[a]		−63.0
1-OHP (μg/24 h)	0.5 ± 0.5	0.4 ± 0.3	0.4 ± 0.3	0.6 ± 0.5	0.5 ± 0.4	0.7 ± 0.7	0.5 ± 0.4	0.5 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.4
% Change		−2.3	−27.5		−33.1		−15.3		−27.9		−10.6
Urine mutagenicity (revertants/24 h)	42695.9 ± 31628.4	40008.3 ± 32211.8	21884.6 ± 13846.2	31725 ± 22029.9	23575.3 ± 16787.6	38042 ± 25874.2	5231 ± 6316.6	39185.5 ± 23427.2	2962.7 ± 4112.2	45475.7 ± 24556.0	4642.7 ± 6147.9
% Change		3.4	−44.6[a,b]		−23.1[a,b]		−88.9[a]		−93.0[a]		−90.5
Blood COHb (% saturation)	6.7 ± 1.8	6.8 ± 1.5	6.8 ± 1.7	4.6 ± 1.2	7.1 ± 1.9	5.1 ± 1.3	7.2 ± 2.3	1.9 ± 0.3	7.0 ± 1.7	1.8 ± 0.4	6.9 ± 2.0	1.8 ± 0.3
% Change		0	−31.0[a,b]		−27.6[a,b]		−73.1[a]		−73.6[a]		−72.9	

1-OHP, 1-hydroxypyrene; 1-OHPhe, 1-OH-phenanthrene; 2-AN, 2-aminonaphthalene; 2-HPMA, 2-hydroxypropyl-mercapturic acid; 2-MHBMA, 2-hydroxybutenyl-mercapturic acid; 2-OHFLe, 2-OH-fluorene; 3-HPMA, 3-hydroxypropylmercapturic acid; 3-HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 4-ABP, 4-aminobiphenyl; AAMA, N-acetyl-S-(2-carbamoylethyl)-l-cysteine; CEMA, 2-cyanoethylmercapturic acid; COHb, carboxyhemoglobin; CS, continued smoking; DUC, dual use with chews; DUD, dual use with discs; EUC, exclusive use of chews; EUD, exclusive use of discs; GAMA, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine; HEMA, 2-hydroxyethyl mercapturic acid; mITT, modified intent-to-treat; NE, nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, N-nitrosonornicotine; NT, no tobacco product use; OBC, own-brand cigarette; S-PMA, S-phenyl mercapturic acid.

Biomarkers of exposuredata are presented as mean ± SD, and percent change data are presented as median.

[a]Indicates that the least-squares mean compared on day 7 is statistically significantly different ($P < .05$) from group 1 continue smoking.

[b]Indicates that the least-squares mean compared on day 7 is statistically significantly different ($P < .05$) from group 6 (NT).

(28%) experienced a total of 85 AEs. Headache was the most frequently reported event, experienced by a total of 17 participants (8%); all remaining AEs were experienced by ≤12 fewer participants (≤6%) each. The majority of AEs (81) were mild in severity, and 4 were moderate (back pain, headache, eyelid stye, and allergic rhinitis). The principal investigator considered 1 event (hiccups) to be definitely related to the study products, 4 events (nausea, salivary gland enlargement, and 2 events of throat irritation) to be likely related, 15 events to be possibly related, and the remaining 65 events unlikely/not related to any product. No discernable patterns for the AEs were reported between the discs and chews test products.

Discussion

In this open-label, randomized, parallel-group, 10-day, in-clinic study, significant reductions in urinary NNAL were observed among dual users of test products who reduced cigarette consumption by 50% compared to the continue smoking group on day 7. These reductions were even more striking among exclusive users of the test products, reaching similar levels as those participants who stopped using all tobacco products. Similar reductions were observed for many of the other biomarkers of exposure. Urinary NE levels, while lower among dual users, were not statistically significantly different compared to the continue smoking group. On the other hand, NE levels among exclusive users were significantly lower compared to the continue smoking group. A modest proportion of the participants indicated that they would use the test products again, suggesting that switching to these products may present a harm reduction option for adult smokers.

We note that some of the biomarkers of exposure representing combustion-related HPHCs were not reduced by 100% in group 6, despite stopping all tobacco use, for example: COHb (72.9%), 3-HPMA (74.9%), 3-HMPMA (72.4%), HEMA (44.3%), AAMA (48.7%), GAMA (37.8%), and others. These biomarkers represent volatile organic constituents, which typically have relatively short half-lives. Other researchers have reported measurable levels of many of these biomarkers of exposure among smokers who have stopped smoking and measurements within in-clinic studies[14,23] as well as in ambulatory studies among nonsmokers.[15,24,25] Therefore, the observations from our study are comparable to other literature reports and suggest that some of the combustion-related constituents are present endogenously (eg, carbon monoxide and acrolein). Indeed, other sources of exposure cannot be completely eliminated for some of the HPHCs, and we had taken precautions to minimize

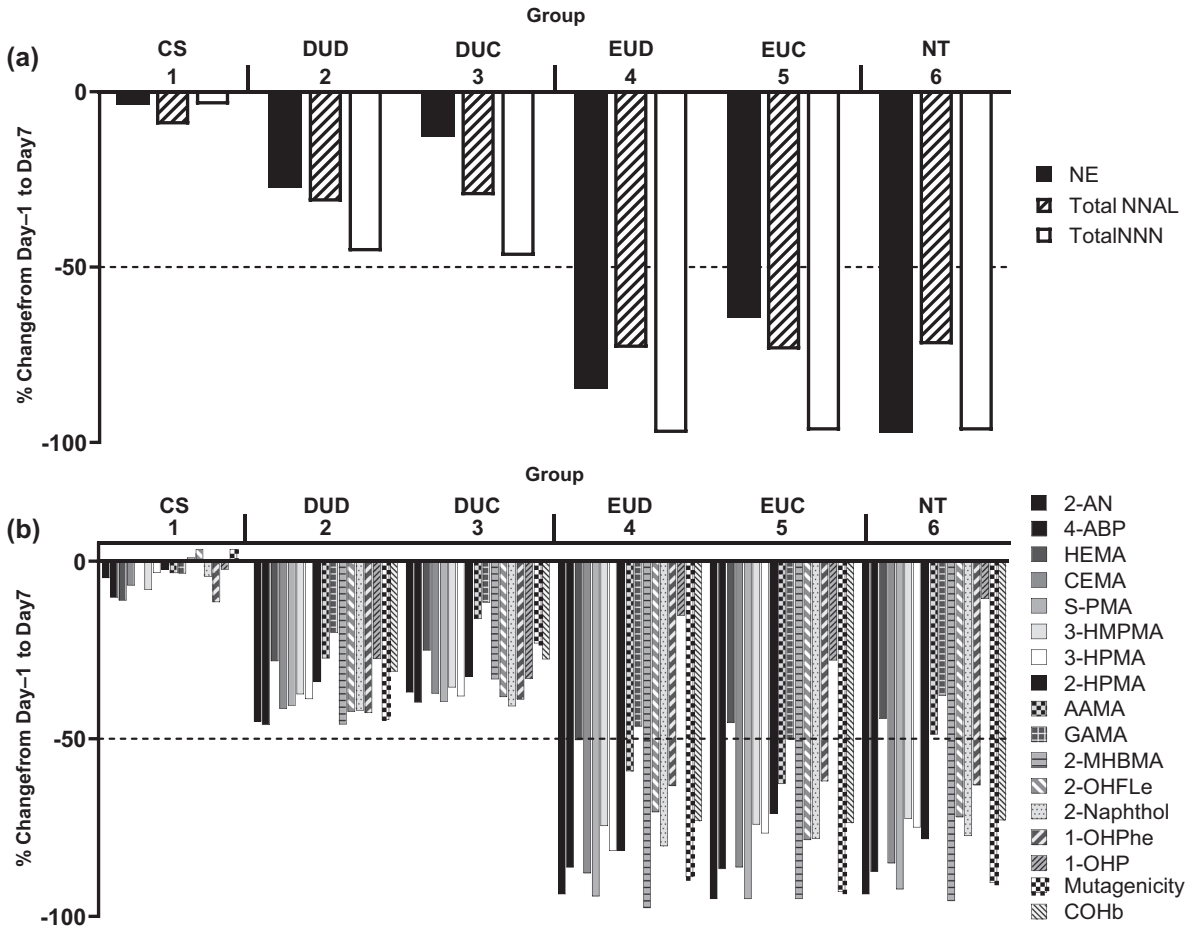

Figure 2. Median percent changes of tobacco-specific (a) and non–tobacco-specific (b) biomarkers of exposure from day -1 to day 7 . 2-AN, 2-aminonaphthalene; 4-ABP, 4-aminobiphenyl; COHb, carboxyhemoglobin; CS, continued smoking; DUC, dual use with chews; DUD, dual use with discs; EUC, exclusive use of chews; EUD, exclusive use of discs; NE, nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, N-nitrosonornicotine; NT, no tobacco product use; S-PMA, S-phenyl mercapturic acid.

such exposure. For example, the participants in the complete switching and tobacco cessation groups were completely separated to minimize likely secondhand smoke exposure. Furthermore, despite control of dietary exposure (eg, no charbroiled meat, a significant source[26] of polycyclic aromatic hydrocarbons [PAHs]), we observed that 1 of the biomarkers of exposure to PAHs, 1-OHP, although reduced by ≈30% in the dual-user groups, was reduced by only ≈10% in group 6 (stopped using all tobacco) and by ≈15% and ≈28% in groups 4 (exclusive use of discs) and 5 (exclusive use of chews), respectively. We were unable to resolve this discrepancy and could not associate this observation to analytical issues. Nonetheless, levels of another biomarkers of exposure to PAHs, 2-naphthol,1-OH-phenanthrene, was indeed reduced substantially in group 6 (63%) and indicated a dose-response relationship with ≈40% reduction in dual-user groups (groups 2 and 3) and ≈60% reduction in complete switcher groups (groups 4 and 5). These observations confirm that the lack of measurable levels of PAHs in the test products[27] does indeed manifest into reduction in exposure to PAHs among both dual users and complete switchers.

Some of the reductions in biomarkers of exposure observed in the dual-user groups are not proportionate to the 50% reduction in cigarettes from baseline usage. These observations are unlikely to be due to compensatory smoking behavior, because median levels of NNN (a tobacco-specific biomarker unique to cigarettes) were reduced by ≈46% among dual users. The reduction in urinary NNAL levels by ≈30% among the dual-user groups is likely due to the relatively long half-life of the constituents. For example, the elimination half-life of urinary NNAL has been reported to range from 10 to 18 days.[28] Given that the test products do not contain tobacco leaf and have nondetectable levels of NNK,[27] the median percent reduction in urinary NNAL levels (≈73%) from exclusive use of the test products (groups 4 and 5) as well as among those who stopped all tobacco use (72%, group 6) reflect the

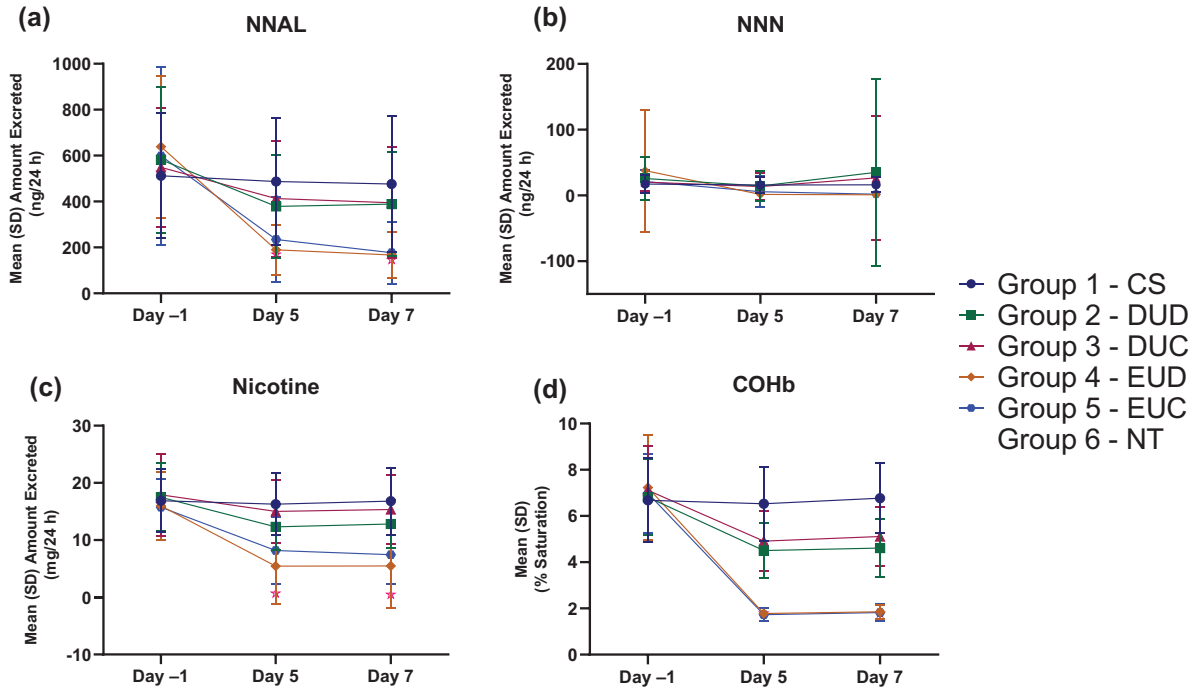

Figure 3. Amount excreted (a) NNAL, (b) NNN, (c) NE, and (d) mean (SD) % saturation COHb over time. COHb, carboxyhemoglobin; CS, continued smoking; DUC, dual use with chews; DUD, dual use with discs; EUC, exclusive use of chews; EUD, exclusive use of discs; NE, nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, N-nitrosonornicotine; NT, no tobacco product use; SD, standard deviation.

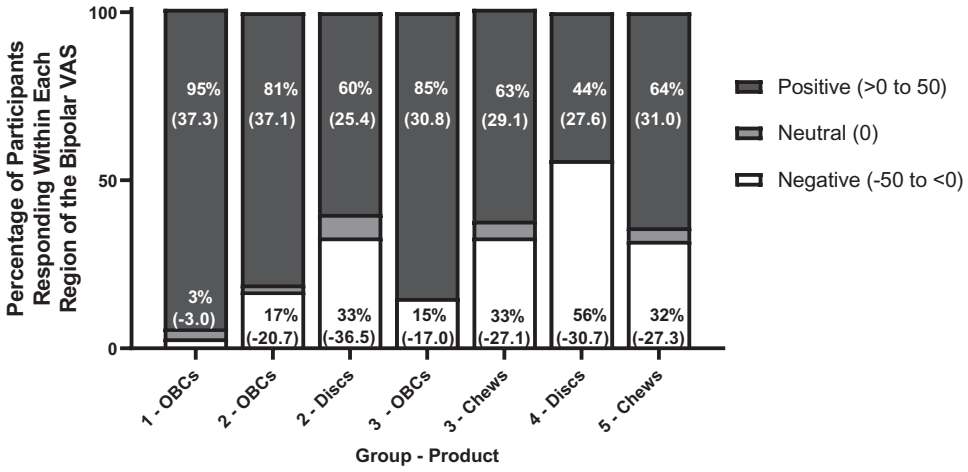

Figure 4. Percentage of participants in each response region of the bipolar VAS to want to use the product again. Each bar represents the percentage of participants, rounded to the nearest whole number, who responded within the negative, neutral, or positive response regions of the bipolar VAS by product on the Use the Product Again Questionnaire (described in Table S2). Average scores within the positive and negative response regions are depicted parenthetically. OBC, own-brand cigarette; VAS, visual analog scale.

residual carryover from baseline smoking due to the long half-life.

Additionally, urine mutagenicity was substantially reduced among dual users (≈45% in group 2 and ≈23% in group 3) and exclusive users of test products (≈89% in group 4 and ≈93% in group 5). The lower magnitude of reduction in group 3 was likely due to lower baseline values (31 725 revertants/24 h) compared to the other groups (range, 38 042-45 475 revertants/24 h). The urine mutagenicity provides insights regarding the mutagenic compounds excreted in the urine; cigarette smoke contains various mutagenic and carcinogenic compounds, including nitroso-compounds, PAHs, and heterocyclic amines.[29-31] Most of these carcinogens and mutagens are metabolized to more active forms in the human body and can form adducts to DNA, inducing chromosomal alterations, and are finally excreted in urine.[32,33] Therefore, significant reductions in urine

mutagenic revertants in both dual users and exclusive users provides mechanistic insights regarding possible favorable biological outcomes from the reductions in exposure that can be expected after switching from cigarettes to the test products.

Overall, our results suggest that dual use of test products with cigarettes does not lead to compensatory changes in smoking behavior, and substantial reduction in exposure to HPHCs can be expected with ≥50% reduction in cigarette consumption. Furthermore, dual use of the test products with substantially reduced cigarette consumption should also result in a reduction of smoking-related disease risks. In a recent systematic review and meta-analysis, Chang et al[34] reported that, while not proportionate, large reductions in cigarette consumption (≥50%) will reduce lung cancer risks. Others have reported similar reductions in disease risk[35-37] with smoking reduction. Therefore, dual use of the test products with large reduction in cigarette consumption among adult smokers unwilling or unable to quit may be a step in the right direction, especially if it eventually leads to switching to exclusive use of the test products without the use of combusted tobacco products.

We note that the NE levels on day 7 among dual users of the chews test product were higher (average, 15.4 ± 6.1 mg/24 h) relative to the dual users of the disc test products (12.8 ± 4.2 mg/24 h). Similar observations were noted among the exclusive users of the test products. These differences were primarily observed for nicotine exposure but not for the other HPHCs. The biomarkers of exposure related to other HPHCs were comparable for both the test product groups for the dual-user as well as exclusive-user groups. These findings are not surprising since the test products primarily contain nicotine and all the other HPHCs are either nondetectable or present at substantially lower levels compared to cigarettes. We hypothesize that the differences in nicotine exposure observed between the 2 test products may be attributed to the greater efficiency of extraction of nicotine by the users of chews vs the disc test products. The firmer polymer matrix of the disc test product may lead to slower release of nicotine relative to the softer, gum-based matrix of the chews. We note that these differences in nicotine exposure between the test products were not discernable in the subjective measures. Nonetheless, the nicotine levels reflect the tobacco product use behavior with cigarettes being primarily accountable for nicotine exposure. NE levels among the exclusive users of the test products (5.5 ± 7.4 mg/24 h for exclusive disc test product users and 7.4 ± 5.2 mg/24 h for the chews test product users) were substantially lower than the exclusive users of cigarettes (16.9 ± 5.5 mg/24 h). These observations, along with the modest proportions (44%-64%) of participants with positive responses to using the test products again, suggest that some smokers may find the test products to be reasonable switching products. The switching potential of the test products was also demonstrated in a 6-week ambulatory study,[38] where ≈23% of adult smokers not intending to quit smoking switched completely to the test products by the end of the study, and a sizeable proportion (≈60%) reduced their cigarette consumption by ≥50%. These changes were observed in the absence of any instructions to change smoking behavior. Additionally, both the blue mint and green mint flavor variant test products were used by all the participants in the dual-use and complete-switching groups. While dual users appeared to equally prefer both flavor variants, the average consumption of the blue mint test product trended to be higher among the exclusive users of the disc test products, likely driven by a few heavy users (eg, the maximum number of test products used was 39 units a day by 1 participant.) Moreover, although the usage level peaked on day 5 and declined thereafter such that the use behavior was similar for the 2 test products on day 7. We have previously observed, in the 6-week ambulatory study mentioned earlier, that the availability of different flavors plays an important role in facilitating switching from cigarettes to the test products.[38] Given the similarity of test products across the flavor variants, the nominal differences in product use behavior will not offset the reductions in exposure to the toxicologically relevant HPHCs.

To date there are no studies investigating changes in biomarkers of exposure among adult smokers who have switched or are dual using tobacco leaf–free oral tobacco-derived nicotine products. The results from our study are similar to the 2 reports that have evaluated BoE measurements among adult smokers either exclusively using or dual using novel oral products that contain ground tobacco (eg, Orbs, Strips, or Sticks[39,40]). For example, comparable levels of reductions in biomarkers of exposure (≈30%-90%) were observed in exclusive users of Orbs for 5 days, which were similar to those abstaining from tobacco use.[39] However, unlike our study, dual use of Orbs with cigarettes did not result in significant reductions in biomarkers of exposure, probably due to the relatively small reductions in cigarette consumption.[39] These observations indicate the importance of substantial reductions in cigarette use among dual users to manifest meaningful reductions in biomarkers of exposure. As observed by Krautter et al,[40] substantial reduction in biomarkers of exposure was observed among dual users of Snus, Orbs, Sticks, or Strips (all tobacco leaf–containing products), with cigarette consumption reduced by 60%. Because

these products contained ground tobacco leaves, some of the biomarkers of exposure were not reduced to the same extent as observed with the test products in our study, since they do not contain any tobacco leaf. For example, the authors report of urinary NNN levels in dual users essentially remained unchanged (+18%) and reduced by –27% when switching to Snus or by –60% when switching to Strips, whereas in our study the reductions observed among dual use was –45% and among exclusive users of test products was –97%.

The results of this study must be interpreted within the context of its limitations, for example, the study was conducted under controlled conditions within the clinic with free access to the test products, which may not reflect product use behavior under real-world ambulatory settings. However, the in-clinic environment allows precise determination of product use and exposure that reflects how the products are actually used by the study participants. Additionally, the in-clinic environment allows for accurate characterization of BoE levels in 24-hour urine samples that are difficult to assess in an ambulatory setting. Another potential limitation may be that the duration of 7 days may not be long enough to stabilize the product use behavior. We believe that the study duration is reasonable enough based on the relatively consistent use behavior of the test products (Figure S1). Furthermore, we have observed that most of the product use transitions happen within the first week, based on the previously mentioned 6-week ambulatory study among adult smokers, with gradual and nominal changes in use behavior occurring over the 6-week period.[38] In order to manifest the greatest harm reduction potential of the test products, adult smokers must switch completely and sustain their exposure reductions over a long time period. Given that measurable changes in disease outcomes can take years, such assessments are best obtained from real-world evidence gathered from postmarket or epidemiological studies. However, any potential benefits from switching to the test products can be offset if adult smokers return to smoking.

Conclusions

The harmful effects of cigarettes primarily arise from exposure to HPHCs present in smoke. However, smokers who are unable or unwilling to quit could potentially reduce harm by switching to oral tobacco-derived nicotine products like the test products. We have previously reported that the test products have demonstrably none or very low levels of HPHCs.[27] In this study, we report that adult smokers switching to the test products will indeed experience a substantial reduction in exposure to many of the HPHCs found in cigarette smoke, and these reductions are comparable to complete abstinence from tobacco products. Some smokers may be unable to make the switch immediately and may gradually transition with some period of dual use of cigarettes and test products. We also demonstrate that adult smokers who use the test products accompanied by a substantial reduction (by at least 50%) in cigarette consumption will also experience significant reduction in exposure. Sustained and prolonged reduction in exposure to the HPHCs, including the carcinogens like NNK, is reasonably likely to reduce the chances of morbidity and mortality in adult smokers switching to test products.

The select biomarkers of exposure investigated in this study are a reasonable representation of HPHCs from several important chemical classes (eg, carbonyls, aromatic amines, PAHs, and nitrosamines) associated with smoking-related disease outcomes (eg, carcinogenic, cardiovascular, respiratory, and reproductive). The FDA has acknowledged the relevance of clinical studies demonstrating a reduction in biomarkers of exposure in its decision to grant a modified exposure authorization to a heated tobacco product.[17] Based on the reductions in biomarkers of exposure, along with other evidence, the FDA in its decision summary stated that a measurable and substantial reduction in morbidity or mortality among individual tobacco users is reasonably likely when switching to the heated tobacco product.[17] The data presented here are consistent with that conclusion and therefore indicate that oral tobacco-derived nicotine products like these test products present a harm reduction opportunity for adult smokers.

Acknowledgment

Scientific writing support was provided by Meghan L. Thompson, PharmD, PhD, of Altria Client Services LLC.

Conflicts of Interest

JE, JL, JW, and MS are employees of Altria Client Services LLC.

Funding

This study was funded by Altria Client Services LLC.

Data Sharing

The underlying data related to this article will be shared on reasonable request to the corresponding author.

References

1. National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. *The Health Consequences of Smoking–50 Years of Progress: A Report of the Surgeon General*. Atlanta (GA): Centers for Disease Control and Prevention (US); 2014.

2. Hatsukami DK, Joseph AM, Lesage M, et al. Developing the science base for reducing tobacco harm. *Nicotine Tob Res*. 2007;9(4):S537-S553.
3. Cappelleri JC, Bushmakin AG, Baker CL, Merikle E, Olufade AO, Gilbert DG. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. *Addict Behav*. 2007;32(5):912-923.
4. Gottlieb S, Zeller M. A nicotine-focused framework for public health. *N Engl J Med*. 2017;377(12):1111-1114.
5. Liu J, Wang J, Vansickel A, Edmiston J, Graff D, Sarkar M. Characterization of the abuse potential in adult smokers of a novel oral tobacco product relative to combustible cigarettes and nicotine polacrilex gum. *Clin Pharmacol Drug Dev*. 2021;10(3):241-250.
6. Robichaud MO, Seidenberg AB, Byron MJ. Tobacco companies introduce 'tobacco-free' nicotine pouches. *Tob Control*. 2020;29(e1):e145-e146.
7. Blank MD, Eissenberg T. Evaluating oral noncombustible potential-reduced exposure products for smokers. *Nicotine Tob Res*. 2010;12(4):336-343.
8. Blank MD, Sams C, Weaver MF, Eissenberg T. Nicotine delivery, cardiovascular profile, and subjective effects of an oral tobacco product for smokers. *Nicotine Tob Res*. 2008;10(3):417-421.
9. Food and Drug Administration. Guidance for Industry Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act, Draft Guidance, March, 2012. https://www.fda.gov/media/83375/download. Accessed July 7, 2022.
10. Hatsukami DK, Benowitz NL, Rennard SI, Oncken C, Hecht SS. Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine Tob Res*. 2006;8(4):600-622.
11. Hatsukami DK, Hecht SS, Hennrikus DJ, Joseph AM, Pentel PR. Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. 25-26 October 2001, Minneapolis, Minnesota. *Nicotine Tob Res*. 2003;5(3):387-396.
12. Institute of Medicine (US) Committee on Qualification of Biomarkers and Surrogate Endpoints in Chronic Disease. In: Micheel CM, Ball JR, eds. *Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease*. Washington (DC): National Academies Press (US); 2010.
13. Stratton K, Shetty P, Wallace R, Bondurant S. Clearing the smoke: the science base for tobacco harm reduction–executive summary. *Tob Control*. 2001;10(2):189-195.
14. Sarkar M, Liu J, Koval T, et al. Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro snus. *Nicotine Tob Res*. 2010;12(2):105-116.
15. Roethig HJ, Munjal S, Feng S, et al. Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers. *Nicotine Tob Res*. 2009;11(10):1216-1225.
16. Gale N, McEwan M, Camacho OM, Hardie G, Murphy J, Proctor CJ. Changes in biomarkers of exposure on switching from a conventional cigarette to the glo tobacco heating product: a randomized, controlled ambulatory study. *Nicotine Tob Res*. 2021;23(3):584-591.
17. Food and Drug Administration. Scientific review of IQOS modified risk application-technical project lead summary. https://www.fda.gov/media/156829/download. Published 2020. Accessed July 7, 2022.
18. Rostron BL, Corey CG, Chang JT, van Bemmel DM, Miller ME, Chang CM. Associations of cigarettes smoked per day with biomarkers of exposure among u.s. adult cigarette smokers in the population assessment of tobacco and health (PATH) study wave 1 (2013-2014). *Cancer Epidemiol Biomarkers Prev*. 2019;28(9):1443-1453.
19. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine Tob Res*. 2001;3(1):7-16.
20. Rose JE, Salley A, Behm FM, Bates JE, Westman EC. Reinforcing effects of nicotine and non-nicotine components of cigarette smoke. *Psychopharmacology (Berl)*. 2010;210(1):1-12.
21. St Helen G, Havel C, Dempsey DA, Jacob P, 3rd, Benowitz NL. Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes. *Addiction*. 2016;111(3):535-544.
22. Griffiths RR, Bigelow GE, Ator NA. Principles of initial experimental drug abuse liability assessment in humans. *Drug Alcohol Depend*. 2003;70(3):S41-S54.
23. Theophilus EH, Coggins CR, Chen P, Schmidt E, Borgerding MF. Magnitudes of biomarker reductions in response to controlled reductions in cigarettes smoked per day: a one-week clinical confinement study. *Regul Toxicol Pharmacol*. 2015;71(2):225-234.
24. Chang CM, Edwards SH, Arab A, Del Valle-Pinero AY, Yang L, Hatsukami DK. Biomarkers of tobacco exposure: summary of an FDA-sponsored public workshop. *Cancer Epidemiol Biomarkers Prev*. 2017;26(3):291-302.
25. De Jesus VR, Bhandari D, Zhang L, et al. Urinary biomarkers of exposure to volatile organic compounds from the population assessment of tobacco and health study wave 1 (2013-2014). *Int J Environ Res Public Health*. 2020;17(15).
26. Strickland P, Kang D, Sithisarankul P. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. *Environ Health Perspect*. 1996;104(5):927-932.
27. Danielson TL, McFarlane CB, Brown AP, et al. Evaluation of novel, oral tobacco-derived nicotine products for HPHCs. CORESTA Congress 2018; October 22-26, 2018; Kunming, China. https://sciences.altria.com/library/-/media/Project/Altria/Sciences/library/conferences/2018%20CORESTA%20Danielson-Verve_HPHC.pdf. Accessed July 7, 2022.
28. Goniewicz ML, Havel CM, Peng MW, et al. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Cancer Epidemiol Biomarkers Prev*. 2009;18(12):3421-3425.
29. Hoffmann D, Patrianakos C, Brunnemann KD, Gori GB. Chromatographic determination of vinyl chloride in tobacco smoke. *Anal Chem*. 1976;48(1):47-50.
30. Yamashita M, Wakabayashi K, Nagao M, et al. Detection of 2-amino-3-methylimidazo[4,5-f]quinoline in cigarette smoke condensate. *Jpn J Cancer Res*. 1986;77(5):419-422.
31. International Agency for Research on Cancer. In: O'Neill IK, Chen J, Bartsch H. eds. *Relevance to human cancer of N-nitroso compounds, tobacco and mycotoxins*. IARC Sci Publ.; 1991;105:1-594.
32. Sarkar M, Unyime N, Bao-Zhen Z. Effect of pH on mutagenicity of urine from smokers and nonsmokers. *Environ Toxicol Pharmacol*. 2003;13(1):21-27.
33. Peluso M, Castegnaro M, Malaveille C, et al. 32P-postlabelling analysis of DNA adducted with urinary mutagens from smokers of black tobacco. *Carcinogenesis*. 1990;11(8):1307-1311.
34. Chang JT, Anic GM, Rostron BL, Tanwar M, Chang CM. Cigarette smoking reduction and health risks: a systematic review and meta-analysis. *Nicotine Tob Res*. 2021;23(4):635-642.
35. Godtfredsen NS, Holst C, Prescott E, Vestbo J, Osler M. Smoking reduction, smoking cessation, and mortality: a 16-year follow-up of 19,732 men and women from the Copenhagen centre for prospective population studies. *Am J Epidemiol*. 2002;156(11):994-1001.

36. Godtfredsen NS, Osler M, Vestbo J, Andersen I, Prescott E. Smoking reduction, smoking cessation, and incidence of fatal and non-fatal myocardial infarction in Denmark 1976-1998: a pooled cohort study. *J Epidemiol Community Health*. 2003;57(6):412-416.
37. Godtfredsen NS, Prescott E, Osler M. Effect of smoking reduction on lung cancer risk. *JAMA*. 2005;294(12):1505-1510.
38. Vansickel A, Apkarian L, Schendel J, Largo E. Cigarette smoking behavior among adult cigarette smokers using VERVE® discs or chews during 6-weeks of at-home use. 25th Annual Meeting of the Society for Research on Nicotine and Tobacco; February 20-23, 2019; San Francisco, CA. https://sciences.altria.com/library/-/media/Project/Altria/Sciences/library/conferences/2019%20SRNT_Vansickel%20VerveBehavior.pdf. Accessed July 7, 2022.
39. Krautter GR, Borgerding MF. Comparison of consumption patterns, biomarkers of exposure, and subjective effects in cigarette smokers who switched to dissolvable tobacco (Camel Orbs), dual use, or tobacco abstinence. *Nicotine Tob Res*. 2014;16(10):1336-1347.
40. Krautter GR, Chen PX, Borgerding MF. Consumption patterns and biomarkers of exposure in cigarette smokers switched to Snus, various dissolvable tobacco products, dual use, or tobacco abstinence. *Regul Toxicol Pharmacol*. 2015;71(2):186-197.

Supplemental Information

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.