The Journal of Clinical Pharmacology
2023, 63(10) 1108–1118

DOI: 10.1002/jcph.2293

A Randomized, Controlled Study to Assess Changes in Biomarkers of Exposures Among Adults Who Smoke That Switch to Oral Nicotine Pouch Products Relative to Continuing Smoking or Stopping All Tobacco Use

Jesse Rensch, BA, Jeffery Edmiston, PhD, Jingzhu Wang, MS, Xiaohong Jin, MS, and Mohamadi Sarkar, MPharm, PhD, FCP

Abstract

The purpose of this open-label, randomized, controlled, in-clinic, 5-parallel-group study was to assess biomarkers of exposure (BoE) to select harmful and potentially harmful constituents in adults who smoke (N = 144) switching to oral tobacco products (on!® mint nicotine pouches; test products) compared to continuing smoking cigarettes (CS) and completely quitting all tobacco products (NT). Changes in 20 BoE to select harmful and potentially harmful constituents, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), were evaluated. Adult smokers smoked their usual brand of cigarettes for 2 days (baseline assessments) and then were randomly assigned to ad libitum use of 2, 4, or 8 mg test products, CS, or NT for 7 days. Analysis of covariance was used to assess the Day 7 BoE levels between each group using test products, CS, and NT. The creatinine-adjusted total urinary NNAL and other 18 of 19 BoE levels (except nicotine equivalents [NEs]) were significantly lower ($P < .05$) on Day 7, among all test product groups compared to CS. Geometric least-square means were reduced for all biomarkers of exposure, except NEs, in test product groups by approximately 42%–96% compared to the CS group, and reductions were comparable to the NT group. The geometric least-square means for urinary NE between the test product and the CS groups, although not significantly different, the Day 7 mean change relative to the CS group were 49.9%, 65.8%, and 101% for the 2, 4, and 8 mg test product groups, respectively. The substantial reduction in harmful and potentially harmful constituent exposure suggests complete switching from cigarettes to test products may present a harm reduction opportunity for adults who smoke.

Keywords

biomarkers, biomarkers of exposure, cigarettes, harm reduction, nicotine, tobacco products

The harm caused by tobacco product use is primarily attributable to cigarette smoking, a primary causal factor for morbidity and mortality from serious diseases like lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease.[1–3] Public health authorities, including the Food and Drug Administration (FDA), acknowledge a continuum of risk with combustible cigarettes at the highest end, and non-combustible (smoke-free) products, such as e-vapor, smokeless tobacco, and heat-not-burn, on the lower end of the spectrum.[4–6] The FDA has identified harmful and potentially harmful constituents in cigarette smoke (e.g., carbon monoxide [CO]) that are related to combustion of tobacco.[7] Many of these combustion-related harmful and potentially harmful constituents can be expected to be absent in smoke-free products. A key step in assessing the potential impact on risks of smoking-related diseases involves the reduction in exposure to select harmful and potentially harmful constituents from cigarette smoke.

There is a rapidly growing category of oral tobacco–derived nicotine products[8] like on!® nicotine pouches (referred to as the test products) that are tobacco leaf free and contain pharmaceutical grade tobacco-derived nicotine, flavors, and excipients. Because these products are smoke free and do not contain tobacco leaf, most of the harmful and potentially harmful constituents

Altria Client Services LLC, Richmond, VA, USA

Submitted for publication 25 February 2023; accepted 7 June 2023.

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

are either absent or present at a substantially lower level. Although other studies have demonstrated that completely switching to oral tobacco–derived nicotine products substantially reduces exposure,[9] no such information exists for the test products. Therefore, we conducted this study to confirm that reductions in harmful and potentially harmful constituents as determined by chemical measurements[10] in the test products correspondingly manifest into exposure reductions, as measured by biomarkers of exposure to select harmful and potentially harmful constituents, among adults who smoke who switch completely to the test products.

Our selection of biomarkers of exposure was based on previous publications, including reports by the Institute of Medicine (now called the National Academy of Sciences): "Clearing the Smoke" and "Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease."[11] We selected biomarkers for cigarette smoke constituents or metabolites of these constituents that are representative of the particulate and gas/vapor phase of cigarette smoke, guided by the toxicological relevance, usefulness as surrogates for chemical classes of smoke constituents, and the availability of validated analytical methods. Although biomarkers of exposure are not available for all harmful and potentially harmful constituents identified in the FDA list,[12] we identified biomarkers characterizing exposure to select harmful and potentially harmful constituents classified by the FDA as carcinogens, respiratory toxicants, cardiovascular toxicants, reproductive or developmental toxicants, or addictive constituents (Table S1) found in cigarette smoke.[7] Moreover, these harmful and potentially harmful constituents reflect a range of chemical classes (e.g., carbonyls, aromatic amines, polycyclic aromatic hydrocarbons, nitrosamines). This approach is generally considered reasonable by many researchers,[4,13–15] including the FDA.[16]

In this randomized, controlled study, we investigated changes in biomarkers of exposure among adults who smoked who either continued smoking; completely switched to 2, 4, or 8 mg nicotine levels of the mint-flavored test products; or stopped using all tobacco products. The purpose of this study was to assess biomarkers of exposure to select harmful and potentially harmful constituents in adults who smoke who switch to the test products compared to continuing smoking cigarettes and completely quitting all tobacco products.

Methods

All pertinent study documents were reviewed by an independent institutional review board (Advarra Institutional Review Board, Columbia, MD) prior to study initiation. All participants in this study reviewed, signed, and dated the informed consent form prior to study initiation. This multicenter study was conducted at Quest Pharmaceutical Services (Springfield, MO) and Alliance for Multispecialty Research, LLC (Knoxville, TN, and Lexington, KY). As part of the informed consent, participants were made aware that the products contain nicotine, which is addictive. They were informed that nicotine can harm an unborn baby in people who are pregnant or breast feeding; increase heart rate and blood pressure; aggravate diabetes; and cause dizziness, nausea, and stomach pain.

Ethics Approval

The investigator and all research staff conducted the study in accordance with the ethical standards in the Declaration of Helsinki, Council for International Organizations of Medical Sciences International Ethical Guidelines,[17] and applicable sections of the International Conference on Harmonization Good Clinical Practice Guidelines. The study was registered at https://clinicaltrials.gov (Identifier: NCT05664672).

Study Participants

This study recruited from the local areas around the clinical sites by radio and online advertisements and enrolled healthy male and female adults who self-affirmed smoking combustible cigarettes, were 21–65 years of age, and were willing to abstain from smoking and use the 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 prior to screening, and a positive urine cotinine test ($\geq$500 ng/mL). Health evaluations included physical exams, measurements of vital signs, and an electrocardiogram. Primary exclusion criteria included any clinically significant medical condition that could jeopardize the safety of the participant or impact the validity of the study results, including women who were pregnant or lactating, and those with poor oral health or dental fixtures that would prevent participants from using the test products. Participants were also excluded if they planned to attempt to quit smoking in the next 30 days from the screening visit or used any tobacco- or nicotine-containing products other than combustible cigarettes within 7 days prior to check-in. Moreover, participants were referred to resources regarding quitting smoking at screening and the end of the study.

Study Products

The 3 test products used in this study are marketed under the on![®] brand name in mint and available in retail stores at 3 nicotine levels: 2, 4, and 8 mg. We selected the mint flavor because, of all the flavors, this is one of the most popular products purchased

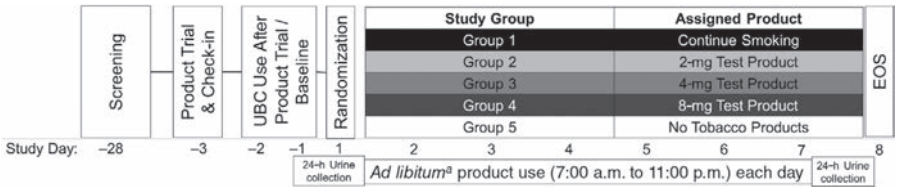

Figure 1. Study design. [a]Participants in the test product groups were required to use at least 1 test product at 3 different time points (11:00 a.m., 3:00 p.m., and 7:00 p.m.) each day for 10 min, to ensure that the test products were used each day.
Abbreviations: EOS: end of study; UBC: usual brand cigarette.

in the marketplace, thereby increasing the likelihood of acceptability among the study participants, while minimizing the complexity of using multiple flavors and additional nicotine levels. The 2, 4, and 8 mg nicotine levels represent the low, middle, and high levels of nicotine in the range of products available in the marketplace (1.5, 2, 3.5, 4, and 8 mg). Moreover, these nicotine levels are the most popular in the marketplace. The test products are oral pouched tobacco products that do not contain cut, ground, powdered, or leaf tobacco. The products contain pharmaceutical-grade tobacco-derived nicotine, microcrystalline cellulose, flavor ingredients, sodium carbonates, and binders. Participants were instructed to place the pouch between the upper lip and gum, on either side of the mouth they chose. The reference product used in this study was the participants' usual brand of cigarettes.

Study Design

This was an open-label, randomized, controlled, 10-day, in-clinic, 5-parallel-group study (Figure 1). Participant screening began approximately 1 month (Day −28) before start of the study. Eligible participants checked into the clinic on Day −3 and completed a product trial using an 8 mg test product for 10 minutes to allow them to become accustomed to the product and to confirm the tolerability of the highest nicotine level. Participants continued to smoke their usual brand of cigarettes through 11:00 p.m. on Day −3 and from 7:00 a.m. to 11:00 p.m. on Days −2 and −1; the baseline measurement for cigarettes per day and biomarkers of exposure was measured on Day −1, and 24-hour urine and whole blood samples were collected on Days −1 and 7.

The primary objective was to compare urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in adults who completely switched from smoking to using the test products with those who continued to smoke cigarettes for 7 days. Secondary objectives included the comparison of an additional 19 biomarkers of exposure: nicotine equivalents (NEs), 2-aminonaphthalene, 4-aminobiphenyl, 2-hydroxyethyl mercapturic acid, 2-cyanoethylmercapturic acid (CEMA), S-phenyl mercapturic acid, 3-hydroxy-1-methylpropylmercapturic acid, 3-hydroxypropylmercapturic acid, 2-hydroxypropyl-mercapturic acid, N-acetyl-S-(2-carbamoylethyl)-l-cysteine, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine, 2-hydroxybutenyl-mercapturic acid, 2-OH-fluorene, 2-naphthol (2-OH-Nap), 1-OH-phenanthrene, 3-hydroxybenzo[a]pyrene, urine mutagenicity, 1-hydroxypyrene in urine, and carboxyhemoglobin (COHb) in blood; and product use behavior on Day 7. All biomarkers of exposure are listed in Table 1 and Table S1.

Participants were randomized into 5 groups (Groups 1–5) on Study Day 1 after completion of 24-hour urine collection and prior to product use. The goal was to recruit approximately 150 participants with the aim of at least 100 participants completing the study with 20 participants minimum per group. Groups were stratified by male or female sex, no more than 60% of either sex in any group, and number of cigarettes per day. The participants were randomized into each of the following groups:

Group 1 – continue smoking (CS): Participants continued to use their usual brand of cigarettes for the duration of the study (Day −3 to Day 7).

Group 2 – 2 mg test product: Participants stopped smoking their usual brand of cigarettes and were instructed to use 2 mg test products ad libitum, except for 3 specific test product use opportunities at 11:00 a.m., 3:00 p.m., and 7:00 p.m. each study day, where they were asked to use the assigned test product for at least 10 minutes to ensure use of the test products.

Group 3 – 4 mg test product: Participants stopped smoking their usual brand of cigarettes and were instructed to use 4 mg test products ad libitum, except for 3 specific test product use opportunities at 11:00 a.m., 3:00 p.m., and 7:00 p.m. each study day, where they were asked to use the assigned test product for at least 10 minutes to ensure use of the test products.

Group 4 – 8 mg test product: Participants stopped smoking their usual brand of cigarettes and were instructed to use 8 mg test products ad libitum (except for 3 specific test product use opportunities at 11:00 a.m.,

Table 1. Participant Demographics (Safety Population)

Parameter	Group 1 CS (n = 29)	Group 2 2 mg test product (n = 28)	Group 3 4 mg test product (n = 30)	Group 4 8 mg test product (n = 30)	Group 5 NT (n = 29)	Overall (n = 146)
Median age (years) (range)	34 (22–51)	33 (23–45)	35 (21–64)	32 (21–49)	35 (24–54)	34 (21–64)
Sex, n (%)						
Male	16 (55.2)	16 (57.1)	17 (56.7)	17 (56.7)	17 (58.6)	83 (56.8)
Female	13 (44.8)	12 (42.9)	13 (43.3)	13 (43.3)	12 (41.4)	63 (43.2)
Race, n (%)						
American Indian or Alaska Native	–	–	–	–	2 (6.9)	2 (1.4)
Black or African American	8 (27.6)	7 (25.0)	13 (43.3)	16 (53.3)	10 (34.5)	54 (37.0)
White	21 (72.4)	20 (71.4)	17 (56.7)	14 (46.7)	17 (58.6)	89 (61.0)
Multiple	–	1 (3.6)	–	–	–	1 (0.7)
BMI (kg/m^2)	30.1 (5.5)	30.4 (6.2)	30.4 (6.3)	28.9 (6.1)	29.8 (5.7)	29.9 (5.9)
Number of cigarettes smoked per day	16.0 (5.5)	16.1 (5.8)	16.2 (5.4)	16.6 (6.4)	16.7 (5.4)	16.3 (5.7)
Number of years of smoking	16.2 (9.2)	16.0 (6.8)	16.7 (10.5)	14.3 (7.9)	15.9 (8.3)	15.8 (8.6)

Data are presented as mean (SD) unless otherwise noted.

BMI, body mass index; CS, continued smoking; NT, no tobacco product use; SD, standard deviation.

3:00 p.m., and 7:00 p.m. each study day, where they were asked to use the assigned test product for at least 10 minutes to ensure use of the test products).

Group 5 – stop using all tobacco products (no tobacco [NT]): Participants stopped smoking their usual brand of cigarettes and were not allowed access to any tobacco products, including the test products.

Product Use Behavior

Product use behavior was characterized by the clinic staff documenting the number of each product (nicotine pouch or usual brand of cigarettes) used from 7:00 a.m. to 11:00 p.m. each day, the number of test products used per use occasion, and the duration of use of each test product was documented by measuring the time the product was placed in and removed from the mouth.

Biomarkers of Exposure

The biomarkers were measured using validated methods by Celerion (Lincoln, NE) and Analytisch-Biologisches Forschungslabor (ABF, Germany) based on the FDA's Guidance to Industry for Bioanalytical Method Validation.[18] All urinary biomarkers were normalized using urinary creatinine values. Twenty-four-hour urine collections for biomarkers of exposure and blood sampling for COHb were performed on Days −1 and 7. Each 24-hour urine collection was from approximately 7:00 a.m. on the scheduled day to approximately 7:00 a.m. the following day: the 24-hour urine collection began on each scheduled day after the first morning void (and any void prior to 7:00 a.m.) and finished the following morning, with the last void collected at approximately 7:00 a.m. (including first morning void).

Clinical Safety End Points

Clinical safety end points including electrocardiograms, physical examinations (including oral exam, vital signs, hematology, clinical chemistry, and urinalysis) were assessed at the beginning and end of the study. Adverse events (AEs) were coded using the Medical Dictionary for Regulatory Activities Version 2.3.1.

Sample Size Estimation

The sample size estimation, based on total NNAL data, assuming a 2-sided test, 85% power, and $\alpha = 0.017$ Type I error rate to account for the multiplicity adjustment for the comparisons (each of the 3 test product groups vs the CS group), of approximately 20 participants per group was determined to be needed to detect a significant difference between the test product groups and CS group. The sample size calculation used to derive the sample size for this study was based on total NNAL summary statistics from a previous study[9] with a similar study design, where the mean ± standard deviation (SD) of total NNAL on Day 7 was 476.1 ± 296.58 ng/24 hours for the CS group, and 167.3 ± 100.17 ng/24 hours and 176.9 ± 135.17 ng/24 hours for the groups that used a chewable oral tobacco–derived nicotine product with 2 flavors, respectively. Based on the sample size estimation, we planned to randomize n = 30 participants to each group, allowing for potential dropouts and to ensure that at least 20 participants per group complete the study.

Statistical Analysis

Linear mixed models for analysis of covariance were used for comparing the Day 7 biomarkers of exposure values between study groups for the primary end

point (24-hour creatine-adjusted total urinary NNAL [ng/g creatinine]) and secondary end points. In the statistical model, the outcome variable was included as a dependent variable, group and sex as fixed effects, and baseline values of the corresponding biomarkers of exposure as covariates. For the comparisons between the test product groups and CS group, Dunnett's method was used for the multiplicity adjustment and the SAS procedure Proc Mixed was used for statistical analysis (Version 9.4; SAS Institute Inc.). The geometric least-squares mean (GLSM) ratios between the test product groups and the CS group, and the 95% confidence interval for the GLSM ratio of the test product groups and CS group and P values were calculated. The pairwise comparisons of each of the test product groups versus the CS group and the NT group were performed using a Dunnett's test at a 2-sided significance level of 0.05 to adjust for multiplicity. A standard residual analysis using a Proc Mixed procedure was used to examine the validity of the normality assumptions for the study end points. The biomarker results (except urine mutagenicity) that did not present normal residuals on a linear scale were analyzed on a natural log-transformed scale. Square root transformation was used for urine mutagenicity statistical analysis, as mutagenicity data typically have a Poisson distribution.[19] Product use behavior end points were summarized using descriptive statistics by group and study day.

Results

Participant demographics and smoking history are presented in Table 1, with no notable differences among groups. The age of the participants ranged from 21 to 64 years and the sex distribution was generally even (about 55%–59% men and 41%–45% women) and included approximately 25%–53% Black or African American people who smoke. There was a lower proportion of Black or African American people who smoked in the 2 mg test product group (n = 7, 25%) relative to the 8 mg test product group (n = 16, 53.3%). The study participants reported smoking an average of 16–17 cigarettes per day for 14–17 years.

Product Use Behavior

The number of test products used on Day 7 and duration of use are shown in Table S2. On Day 7, participants randomized to the test product groups used a mean ± SD of 10.8 ± 7.7, 11.2 ± 6.91, and 7.6 ± 4.24 pouches per day for the 2, 4, and 8 mg products, respectively. In all test product groups, most participants used 1 pouch per use occasion and the mean ± SD time of use ranged from 25.5 ± 13.0, 24.5 ± 12.4, and 27.3 ± 19.7 minutes for the 2, 4, and 8 mg groups, respectively. The number of test products used over time is shown in Figure S1. The trajectory indicates that the product use behavior was relatively stable over the 7-day period; consistently lower numbers of the 8 mg test products were used compared to the 2 and 4 mg test products, whereas a similar level of usage was observed for the 2 and 4 mg test products.

Biomarkers of Exposure

The descriptive statistics of the biomarkers of exposure measured on Days −1 and 7 and the median change from baseline are presented in Table 2, and statistical comparisons of the GLSM ratio (percentage) at Day 7 are presented in Figure 2. Creatinine-adjusted urine total NNAL was statistically significantly ($P < .05$) reduced in the test product groups compared to the CS group; specifically, NNAL levels were 24%, 25%, and 22% of the CS group at Day 7 for the 2, 4, and 8 mg test product group, respectively. NNAL GLSM levels in the NT group were 16.5% of the CS group and not statistically significantly different from the 2 and 8 mg test product groups (the 4 mg test product group was significantly higher than the NT group; $P < .05$). However, as shown in Table 2, the range of Day 7 mean NNAL values for the test product groups (65–73 ng/g creatinine) were comparable to that observed for the NT group (71 ng/g creatinine).

Of the 19 secondary end point biomarkers of exposure, all except NEs were also statistically significantly reduced ($P < .05$) in the test product groups compared to the group that continued smoking. The reduction for most (16/19) of the additional biomarkers, excluding NEs, CEMA, and 2-OH-Nap, was similar (i.e., not statistically significantly different) to the reduction observed for the NT group (Figure 3 and Figure S2). Since the test products contain nicotine, the NE levels are significantly higher compared to the NT group. While the GLSM values for CEMA in the test product groups at Day 7 were statistically significantly higher compared to the NT group, these values should be viewed in the context of the highly variable baseline values. The average levels for the test products at baseline were 133, 154, and 170 μg/g creatinine and on Day 7 were 17.4, 18.9, and 23 μg/g creatinine for the 2, 4, and 8 mg products, respectively.) The magnitude of change from baseline to Day 7 were generally comparable to the NT group (Figure 3). For 2-OH-Nap, the 8 mg test product group was 62% of the NT group and was significantly lower ($P < .05$).

Adverse Events

A total of 130 participants completed the study, and 16 participants discontinued participation in the study prior to completion. One participant discontinued from the NT group due to an AE (COVID-19 positive); 15 participants discontinued from the other groups due to

Table 2. Biomarker of Exposure on Days −1 and 7 and Median Change from Baseline

Biomarker	Group 1 CS (n = 28)		Group 2 2 mg test product (n = 22)		Group 3 4 mg test product (n = 30)		Group 4 8 mg test product (n = 25)		Group 5 NT (n = 21)	
	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7	Day −1	Day 7
Total NNAL (ng/g Cr)	363 (239)	330 (224)	274 (203)	67.8 (70)[a]	312 (201)	73.2 (43)[a,b]	293 (213)	64.7 (51)[a]	392 (308)	71.3 (62)[a]
Median change		−38.6		−190		−203		−203		−252
NE (mg/g Cr)	11.6 (6.55)	10.4 (5.10)	8.73 (4.42)	5.79 (5.01)[b]	10.3 (4.83)	8.80 (6.66)[b]	10.5 (5.99)	11.8 (8.45)[b]	12.2 (7.34)	0.978 (2.35)[a]
Median change		−0.857		−3.65		−0.482		1.47		−11.1
2-AN (ng/g Cr)	21.7 (11.5)	20.2 (11.3)	17.0 (9.93)	1.07 (0.728)[a]	21.6 (12.0)	1.82 (1.36)[a]	19.8 (11.2)	1.45 (1.35)[a]	20.6 (11.7)	1.29 (1.01)[a]
Median change		−0.419		−16.7		−18.4		−16.1		−20.1
4-ABP (ng/g Cr)	13.8 (6.74)	13.2 (7.44)	10.2 (5.04)	1.42 (0.450)[a]	13.3 (6.29)	1.97 (0.749)[a]	12.8 (6.22)	1.72 (0.985)[a]	12.8 (6.81)	1.92 (1.04)[a]
Median change		−1.12		−9.45		−11.1		−10.9		−11.1
HEMA (μg/g Cr)	5.19 (5.34)	4.63 (4.47)	2.98 (1.69)	0.959 (0.660)[a]	5.64 (5.29)	1.18 (0.758)[a]	5.65 (6.11)	1.55 (1.11)[a]	3.90 (3.42)	1.30 (0.762)[a]
Median change		−0.243		−2.05		−3.29		−2.95		−1.90
CEMA (μg/g Cr)	163 (74.1)	150 (72.1)	133 (65.0)	17.4 (10.3)[a,b]	154 (83.4)	18.9 (10.7)[a,b]	170 (72.9)	23.0 (13.1)[a,b]	160 (94.8)	16.0 (11.6)[a]
Median change		−12.0		−115		−119		−146		−148
S-PMA (μg/g Cr)	4.41 (3.29)	4.14 (3.24)	3.03 (2.24)	0.15 (0.11)[a]	3.90 (2.41)	0.18 (0.11)[a]	3.79 (2.70)	0.19 (0.1)[a]	3.54 (3.18)	0.17 (0.11)[a]
Median change		−0.148		−2.75		−3.29		−3.42		−2.87
HMPMA (μg/g Cr)	371 (161)	345 (159)	303 (121)	53.4 (10.4)[a]	325 (167)	57.1 (25.0)[a]	338 (143)	48.6 (10.3)[a]	353 (198)	55.2 (12.4)[a]
Median change		−19.7		−302		−236		−262		−286
3-HPMA (μg/g Cr)	1250 (605)	1170 (614)	969 (414)	195 (98.9)[a]	1160 (550)	259 (118)[a]	1170 (516)	215 (98.7)[a]	1200 (844)	281 (205)[a]
Median change		−94.0		−844		−949		−998		−814
2-HPMA (μg/g Cr)	52.7 (28.7)	50.9 (31.8)	39.2 (19.4)	9.44 (3.87)[a]	47.3 (28.4)	11.9 (3.97)[a]	45.4 (23.8)	12.5 (7.43)[a]	41.4 (23.6)	11.9 (3.97)[a]
Median change		−3.43		−30.6		−30.7		−28.2		−21.9
AAMA (μg/g Cr)	92.6 (30.5)	87.7 (31.8)	82.6 (36.1)	29.4 (7.0)[a]	95.6 (29.7)	39.1 (12.7)[a]	94.0 (36.4)	36.7 (13.4)[a]	94.3 (46.0)	35.9 (10.4)[a]
Median change		−2.15		−52.4		−55.1		−48.9		−48.6
GAMA (μg/g Cr)	14.4 (6.15)	14.1 (6.48)	13.1 (5.27)	5.93 (1.94)[a]	14.3 (4.29)	7.58 (1.88)[a]	14.7 (5.23)	7.30 (2.24)[a]	14.1 (5.89)	7.26 (2.26)[a]
Median change		0.41		−7.38		−6.13		−6.95		−5.88
2-MHBMA (μg/g Cr)	1.93 (1.53)	1.74 (1.33)	1.24 (1.12)	0.036 (0.034)[a]	1.60 (1.66)	0.049 (0.047)[a]	1.47 (1.37)	0.046 (0.041)[a]	1.26 (1.15)	0.073 (0.080)[a]
Median change		−0.122		−0.891		−1.19		−1.06		−0.658
2-OH-Flu (μg/g Cr)	2.24 (1.11)	2.08 (1.17)	1.71 (0.89)	0.28 (0.14)[a]	2.01 (1.08)	0.36 (0.14)[a]	2.14 (1.09)	0.36 (0.22)[a]	2.08 (1.11)	0.35 (0.20)[a]
Median change		−0.0724		−1.50		−1.61		−1.79		−1.86
2-OH-Nap (μg/g Cr)	11.1 (5.08)	10.6 (5.06)	11.5 (8.46)	5.16 (6.38)[a]	12.6 (5.56)	5.26 (3.89)[a]	13.8 (7.01)	4.80 (4.38)[a,b]	11.8 (7.60)	6.00 (5.56)[a]
Median change		−0.825		−5.98		−7.55		−7.05		−6.56
1-OH-Phe (μg/g Cr)	0.218 (0.107)	0.203 (0.108)	0.162 (0.082)	0.080 (0.040)[a]	0.172 (0.094)	0.080 (0.040)[a]	0.171 (0.090)	0.067 (0.038)[a]	0.191 (0.090)	0.083 (0.043)[a]
Median change		−0.0105		−0.0638		−0.0796		−0.0815		−0.104
3-OH-BaP (pg/g Cr)	216 (155)	246 (215)	139 (111)	30.8 (50.9)[a]	223 (158)	82.5 (105)[a]	172 (139)	57.7 (77.6)[a]	166 (101)	84.8 (174)[a]
Median change		5.37		−117		−102		−94.7		−116
Urine mutagenicity (revertants/24 h)[c]	13,300 (14,000)	10,800 (13,000)	8770 (9480)	1710 (3150)[a]	9170 (11,100)	2400 (3550)[a]	7580 (8210)	1920 (2440)[a]	12,000 (12,700)	3390 (5250)[a]
Median change		−172		−3400		−3450		−2090		−5890
1-OH-Pyr (μg/g Cr)	0.21 (0.15)	0.20 (0.15)	0.15 (0.10)	0.05 (0.05)[a]	0.15 (0.09)	0.05 (0.04)[a]	0.17 (0.11)	0.05 (0.04)[a]	0.17 (0.11)	0.05 (0.04)[a]
Median change		−0.00626		−0.0925		−0.0922		−0.0899		−0.103
Blood COHb (% saturation)[c]	11.8 (4.2)	11.3 (4.3)	10.3 (3.4)	5.0 (1.7)[a]	11.3 (3.2)	4.6 (1.1)[a]	11.1 (5.3)	4.6 (0.9)[a]	11.0 (3.8)	4.2 (1.0)[a]
Median change		−1.05		−6.00		−5.85		−6.10		−7.15

Biomarker of exposure data are presented as mean (standard deviation), and change data are presented as median. Data are from the creatinine-adjusted urine biomarker of exposure population, except as noted below.

AAMA, N-acetyl-S-(2-carbamoylethyl)-l-cysteine; 4-ABP, 4-aminobiphenyl; 2-AN, 2-aminonaphthalene; CEMA, 2-cyanoethylmercapturic acid; COHb, carboxyhemoglobin; Cr, creatinine; CS, continued smoking; GAMA, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine; HEMA, 2-hydroxyethyl mercapturic acid; HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 2-HPMA, 2-hydroxypropyl-mercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; 2-MHBMA, 2-hydroxybutenyl-mercapturic acid; NE, nicotine equivalent; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NT, no tobacco product use; 3-OH-BaP, 3-hydroxybenzo(a)pyrene; 2-OH-Flu, 2-OH-fluorene; 2-OH-Nap, 2-Naphthol; 1-OH-Phe, 1-OH-phenanthrene; 1-OH-Pyr, 1-hydroxypyrene; S-PMA, S-phenyl mercapturic acid.

[a] Indicates that the least-squares mean compared on Day 7 is statistically significantly different ($P < .05$) from Group 1 (CS).

[b] Indicates that the least-squares mean compared on Day 7 is statistically significantly different ($P < .05$) from Group 5 (NT).

[c] Data from the biomarker of exposure population (whereas, all other data are from the creatinine-adjusted urine biomarker of exposure population); the number of participants in each group in this population: Group 1, n = 26; Group 2, n = 21; Group 3, n = 28; Group 4, n = 24; Group 5, n = 18.

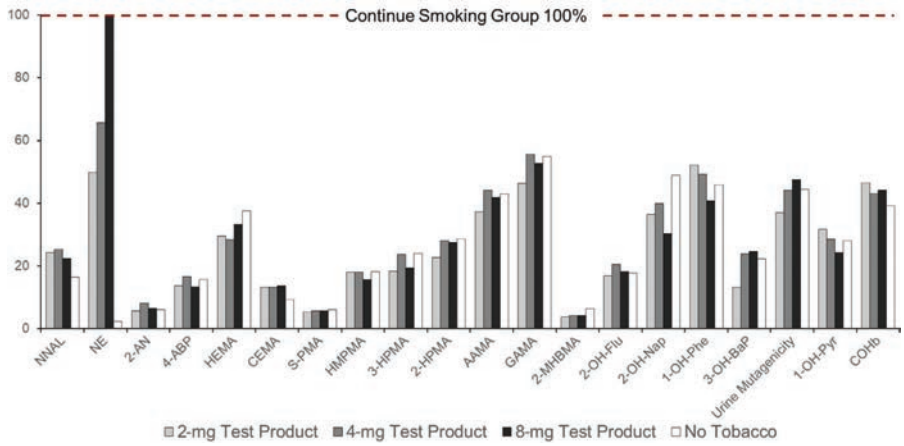

Figure 2. Biomarkers of exposure statistical comparison geometric least squares mean ratio (percentage) at Day 7. AAMA, N-acetyl-S-(2-carbamoylethyl)-l-cysteine; 4-ABP, 4-aminobiphenyl; 2-AN, 2-aminonaphthalene; CEMA, 2-cyanoethylmercapturic acid; COHb, carboxyhemoglobin; GAMA, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine; HEMA, 2-hydroxyethyl mercapturic acid; HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 2-HPMA, 2-hydroxypropyl-mercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; 2-MHBMA, 2-hydroxybutenyl-mercapturic acid; NE, nicotine equivalent; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 3-OH-BaP, 3-hydroxybenzo(a)pyrene; 2-OH-Flu, 2-OH-fluorene; 2-OH-Nap, 2-Naphthol; 1-OH-Phe, 1-OH-phenanthrene; 1-OH-Pyr, 1-hydroxypyrene; S-PMA, S-phenyl mercapturic acid.

withdrawal of consent (1 from the CS group, 3 from the 2 mg test product group, 5 from the 8 mg test product group, and 6 from the NT group). In general, the use of the test products under the study conditions appeared to be well tolerated by the healthy adults who smoke, and there were no serious AEs reported. Among the 146 randomized participants, 56 (38%) experienced a total of 86 AEs; the majority of AEs (76) were mild in severity, and 10 were moderate. Headache was the most frequently reported event, experienced by a total of 23 participants (16%); all remaining AEs were experienced by 5 or fewer participants ($\leq$3.4%) each. Of the AEs that were reported as moderate in severity in the test product groups, the investigator considered 4 events (2 of headache in the 4 mg group; 1 each of mouth irritation and nausea in the 8 mg group) to be related to the study products.

Discussion

In summary, we report statistically significant reductions in biomarkers of exposure to harmful and potentially harmful constituents classified as carcinogens, respiratory toxicants, cardiovascular toxicants, and reproductive or developmental toxicants as well as urine mutagenicity compared to continuing to smoke. These reductions, on average, were comparable to tobacco abstinence. The exposure to nicotine was, on average, trending proportionate to the nicotine levels in the test products. Since biomarkers of exposure incorporate actual human use and account for absorption, distribution, metabolism, and excretion factors, such measurements provide a more accurate estimate compared to product chemistry analysis. Therefore, these observations confirm that absence or significantly low levels of harmful and potentially harmful constituents observed during chemical characterization of the products[10] indeed result in substantial reductions in exposure to select harmful and potentially harmful constituents when people who smoke switch to these products. This study indicates that chemical analysis provides a reasonable estimate of exposure to harmful and potentially harmful constituents.

The findings in our study are biologically plausible since chemical characterization of many of the harmful and potentially harmful constituents, specifically nicotine-derived nitrosamine ketone, 2-aminonapthalene, acrylonitrile, acrolein, benzene, benzo[a]pyrene, 1,3-butadiene, and CO in the test products were below the limit of quantitation (measurements conducted in ISO 17025 accredited laboratories using validated methods for the matrices). Thus, the corresponding biomarkers of exposure to these harmful and potentially harmful constituents (total NNAL, aminonaphthalene, CEMA, 3-hydroxypropylmercapturic acid, S-phenyl mercapturic acid, 3-OH BaP, 3-hydroxy-1-methylpropylmercapturic acid, and COHb) are substantially lower relative to the CS group and comparable to the NT group. Additionally, similar levels of reduction in urine mutagenicity were observed between the test product and CS groups and were comparable to the NT group. Since urine mutagenicity is often considered a general biomarkers of exposure to genotoxic agents,[20] our study findings suggest overall reduction in levels of

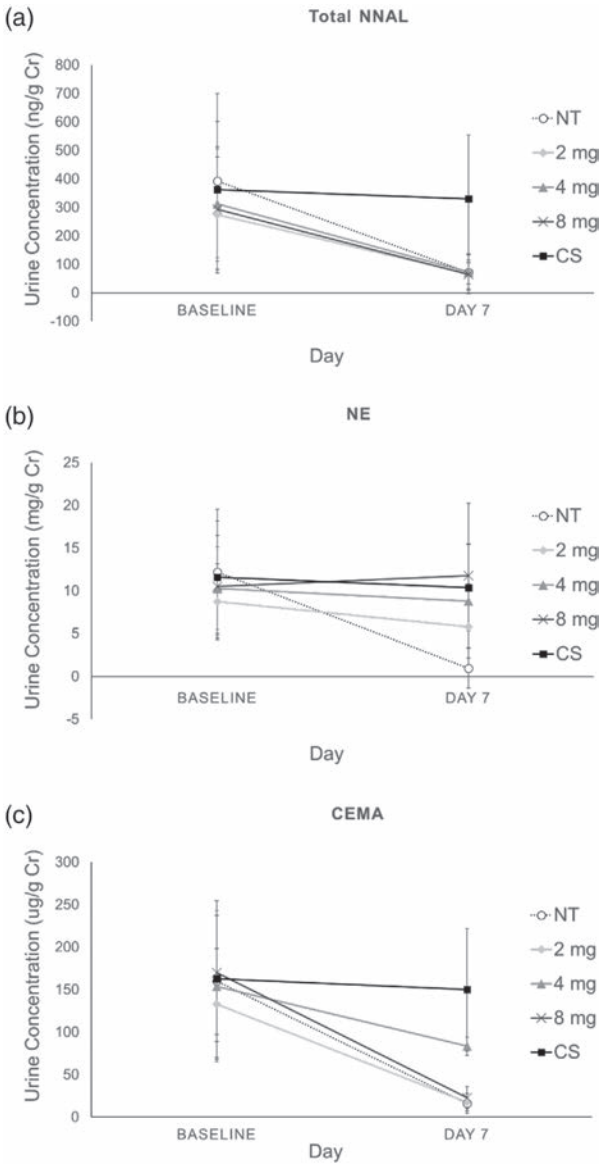

Figure 3. Baseline and Day 7 excretion of (a) NNAL, (b) NE, and (c) CEMA biomarkers. CEMA, 2-cyanoethylmercapturic acid; Cr, creatinine; CS, continue smoking; NE, nicotine equivalent; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

genotoxic compounds among adults who switch to the test products relative to continuing to smoke cigarettes.

Importantly, despite the higher number of test products used (approximately 10–11 pouches/day) of the 2 and 4 mg test products relative to the 8 mg product (approximately 8 pouches/day), the reductions in biomarkers of exposure were comparable across the 3 test products. These observations suggest that the biomarkers of exposure to these harmful and potentially harmful constituents were not impacted by the nicotine content. Our chemical analysis of the test products indicated that although the levels of 4-aminophenyl were measurable in the test products, they were approximately 98% lower compared to the levels found in cigarette smoke,[10] which corresponds to the range of reduction in the urinary 4-aminophenyl levels among the test products compared to the CS group as well as the NT groups. Many of the biomarkers of exposure to harmful and potentially harmful constituents did not reduce completely to nondetectable levels either due to relatively long half-life (e.g., NNAL has a half-life of approximately 11–14 days) or due to exposure from other sources (e.g., food[21]) or environment (e.g., benzene[22]) or endogenous levels already

present (e.g., CO and acrolein[14]). This phenomenon is observed from the significant levels of these biomarkers reported among nonsmokers.[23] CEMA, a metabolite of acrylonitrile, has been investigated as a potential biomarker to distinguish between the use of combustible versus noncombustible tobacco products.[24] Although the levels of this metabolite among test products were statistically significantly higher compared to the NT group, the average levels were comparable (Figure 3), suggesting that these differences have little biological relevance. Moreover, marked variability has been reported for urinary CEMA,[25] and the authors suggest a number of potential sources for this phenomenon, including interindividual variation such as body mass index, diet, metabolism and polymorphisms, individual smoking and inhalation behavior, sample collection and storage, and instrument precision. Importantly, the CEMA levels among the test product groups were lower compared to the CS group.

Given that NEs (a measure of nicotine and 5 of its major metabolites) approximates 85% of nicotine uptake, the average NE excretion observed among the groups that were randomized to the 2, 4, and 8 mg test products reflects the amount of nicotine in the test products. The NE excretion for the 8 mg product was not proportionately higher compared to the 2 and 4 mg products, because the participants in this group consumed fewer pouches than the other 2 groups (Figure S1). The nicotine exposure followed a trend consistent with the nicotine content in the 3 test products (at 49.9%, 65.8%, and 101% of the CS group for the 2, 4, and 8 mg test products, respectively; Figure 2). The 24-hour urinary NE levels reflect daily nicotine exposure from use of the 3 test products, which further informs the abuse potential of these products. The nicotine pharmacokinetics and subjective effects have been previously reported in 2 separate randomized controlled clinical studies.[26,27] In the first study, flavor varieties were evaluated using the 4 mg nicotine pouches, and in the second study, all nicotine levels including the test products were assessed. The authors report that, based on these studies, the on![®] nicotine pouches likely have lower or comparable abuse potential than cigarettes.[26,27] However, the outcome measures were based on a controlled use of a single pouch. In the current study, we report nicotine exposure from daily ad libitum use of test products over a 7-day period, which further enhances the existing body of evidence on abuse liability assessments of the test products. The proportionate average increase in nicotine exposure associated with the 2, 4, and 8 mg test products, while not significantly different compared to smoking cigarettes, confirms the findings from the pharmacokinetic studies that the abuse liability will not be higher than cigarettes. Abuse liability should be viewed in the context of its role in reducing smoking-related harm.[28] Some degree of abuse liability has been proposed to support overall population tobacco harm reduction.[29,30] A smoke-free product with substantially lower abuse liability than cigarettes likely will not be adopted or used extensively and may not encourage existing adults who smoke to switch from cigarettes. The findings reported in this study suggest that adults who smoke are able to get nicotine levels from the test products that could facilitate complete switching to the test products. Complete switching from smoking to use of the test products would result in sustained long-term reduction in exposure to harmful and potentially harmful constituents, which should reduce risks to smoking-related harm.

Our results concur with a previous study where similar results of significant reductions in exposure to many of the same biomarkers associated with harmful and potentially harmful constituents were observed when switching from cigarettes to other oral tobacco–derived nicotine products.[9,31] The results from our study are also consistent with other studies assessing similar biomarkers of exposure among adults switching from cigarettes to Snus[13] or novel oral products that contain ground tobacco (e.g., lozenges, meltable strips, or toothpicks[32,33]). Since the products used in those studies contained tobacco leaf, the reductions in the biomarkers to some of the harmful and potentially harmful constituents (e.g., nicotine-derived nitrosamine ketone) were not as comparable to smoking abstinence as the test products. A clear pattern is established from the literature and our study: Chemical measurement of harmful and potentially harmful constituents reasonably characterizes exposure to harmful and potentially harmful constituents for oral tobacco products. If the products do not contain or have substantially lower levels of harmful and potentially harmful constituents, then exposure is reduced to the similar extent. This pattern is consistent regardless of format (pouch, gums, discs, etc.) and for products that are tobacco leaf free, exposure to harmful and potentially harmful constituents (except for nicotine) is not impacted by nicotine content or variable consumption levels.

The results of this study should be considered in the context of its strengths and limitations. Because the study was conducted in a confined clinic setting, the compliance to the study products was assured by study staff for strict adherence to the study protocol. The likelihood of secondhand or other sources of exposure was minimized by separately confining the study participants smoking cigarettes from those using the test products. The restricted in-clinic environment allowed for accurate characterization of biomarkers of exposure levels in 24-hour urine samples that are

difficult to assess in an ambulatory setting. However, the controlled clinical environment might be viewed as a limitation. The ad libitum use behavior in the clinic may not reflect typical use patterns under real-world conditions; moreover, the participants were instructed to use a minimum of 3 pouches per day, which may not align with actual use behavior under naturalistic settings. Indeed, the usage occasions and durations of use in this study were greater than those observed in another actual use study[34] of the test products. Nonetheless, despite the higher consumption patterns observed in our study, the reductions in exposure to most harmful and potentially harmful constituents is encouraging and suggests the harm reduction potential of the test products regardless of consumption patterns. Another potential limitation may be that the duration of 7 days may not be long enough to stabilize test product use behavior; however, participants used the test products consistently over the study duration with no notable differences associated with study day (Figure S1). Another limitation of the study was that the selected biomarkers of exposure did not measure all possible harmful and potentially harmful constituents. The selected biomarkers represented a range of harmful and potentially harmful constituents that covered the majority of types of tobacco and cigarette smoke harmful and potentially harmful constituents on the FDA's Established List.[12] In addition, we included biomarkers that represent classes of compounds to address the overall exposure to harmful and potentially harmful constituents when adults who smoke switch to the test products. Finally, the study population may not fully represent the population of adults who smoke in the United States. The study population was a sample of adults who smoke who reported smoking only 10 to 30 cigarettes per day. However, the exposure reduction (except for nicotine) when switching to the test products is comparable to no tobacco use, regardless of differences in the number of cigarettes smoked, metabolism, or other background differences.

Conclusions

The harmful effects of cigarettes primarily arise from exposure to harmful and potentially harmful constituents present in smoke. However, adults who smoke who are unable or unwilling to quit could potentially reduce harm by switching to oral tobacco–derived nicotine like the test products. The results of this biomarkers of exposure study demonstrates that the exposure to select harmful and potentially harmful constituents (except for nicotine) are substantially reduced when adults who smoke cigarettes switch to the test products relative to those who continue smoking and are comparable to stopping smoking. The reduction in biomarkers of exposure was not discernable between nicotine levels, suggesting that other products in the on![®] nicotine pouch portfolio may result in similar reductions in exposure. These products are not risk free because they contain nicotine, which is addictive, and the best option for adults who smoke should be to quit using all tobacco products. However, our results clearly demonstrate the harm reduction opportunity of on![®] nicotine pouches. Results of this study are encouraging, and support the notion that, when adults who are unable or unwilling to quit cigarettes completely switch to these nicotine pouches, and maintain switching over a long time period, they may sustain the reduction in exposure to most harmful and potentially harmful constituents and thus likely experience reductions in risk of smoking-related diseases.

Acknowledgments

Scientific writing support was provided by Meghan L. Thompson, PharmD, PhD.

Conflicts of Interest

The authors are employees of Altria Client Services LLC.

Funding

This study was funded by Altria Client Services LLC.

Data Availability Statement

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

References

1. U.S. Department of Health and Human Services. *The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General*. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.
2. Dresler CM, Leon ME, Straif K, et al. Reversal of risk upon quitting smoking. *Lancet*. 2006;368(9533):348-349.
3. Doll R, Peto R, Wheatley K, et al. Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ*. 1994;309(6959):901-911.
4. Hatsukami DK, Joseph AM, Lesage M, et al. Developing the science base for reducing tobacco harm. *Nicotine Tob Res*. 2007;9 suppl 4:S537-553.
5. Cappelleri JC, Bushmakin AG, Baker CL, t al. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. *Addict Behav*. 2007;32(5):912-923.
6. Gottlieb S, Zeller M. A nicotine-focused framework for public health. *N Engl J Med*. 2017;377(12):1111-1114.
7. FDA. *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*. U.S. Department of Health and Human Services; 2012.
8. Bandi P, Asare S, Majmundar A, et al. Relative harm perceptions of E-cigarettes versus cigarettes, U.S. adults, 2018–2020. *Am J Prev Med*. 2022;63(2):186-194.

9. Edmiston J, Liu J, Wang J, Sarkar M. A randomized, controlled study to assess biomarkers of exposure in adult smokers switching to oral nicotine products. *J Clin Pharmacol*. 2022;62(11):1445-1458.
10. Wagner KA, Danielson TL, Flora JW, et al. A comparison of harmful and potentially harmful constituents in on!® nicotine pouches to harmful and potentially harmful constituents in cigarettes and swedish snus. Paper presented at: the 75th Tobacco Science Research Conference; September 11–14, 2022; New Orleans, LA.
11. IOM (Institute of Medicine, now called National Academy of Sciences). *Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease*. IOM; 2010.
12. FDA. Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke; Established List. Center for Tobacco Products. *Rockville, MD*. 2012. Docket No. FDA-2012-N-0143. 2012–7727. 20034–20037.
13. Sarkar M, Liu J, Koval T, et al. Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro Snus. *Nicotin Tob Res*. 2010;12(2):105-116.
14. 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.
15. Gale N, McEwan M, Camacho OM, et al. 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.
16. FDA. *Scientific Review of IQOS Modified Risk Application – Technical Project Lead Summary*, Page 12. 2020.
17. Macrae DJ. The Council for International Organizations and Medical Sciences (CIOMS) guidelines on ethics of clinical trials. *Proc Am Thorac Soc*. 2007;4(2):176-178, discussion 178–179.
18. FDA. Guidance for Industry. Bioanalytical Method Validation. May, 2001. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf
19. Kirkland D. *Statistical Evaluation of Mutagenicity Test Data*. Cambridge University Press; 2008.
20. DeMarini DM, Brooks LR, Bhatnagar VK, et al. Urinary mutagenicity as a biomarker in workers exposed to benzidine: correlation with urinary metabolites and urothelial DNA adducts. *Carcinogenesis*. 1997;18(5):981-988.
21. Strickland P, Kang D, Sithisarankul P. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. *Environ Health Perspect*. 1996;104 suppl 5:927-932.
22. Scherer G, Renner T, Meger M. Analysis and evaluation of trans,trans-muconic acid as a biomarker for benzene exposure. *J Chromatogr B Biomed Sci Appl*. 1998;717(1-2):179-199.
23. Scherer G. Biomonitoring of inhaled complex mixtures–ambient air, diesel exhaust and cigarette smoke. *Exp Toxicol Pathol*. 2005;57 suppl 1:75-110.
24. Bhandari D, Zhang L, Zhu W, et al. Optimal cutoff concentration of urinary cyanoethyl mercapturic acid for differentiating cigarette smokers from nonsmokers. *Nicotine Tob Res*. 2022;24(5):761-767.
25. Minet E, Cheung F, Errington G, et al. Urinary excretion of the acrylonitrile metabolite 2-cyanoethylmercapturic acid is correlated with a variety of biomarkers of tobacco smoke exposure and consumption. *Biomarkers*. 2011;16(1):89-96.
26. Rensch J, Liu J, Wang J, et al. Nicotine pharmacokinetics and subjective response among adult smokers using different flavors of on!™ nicotine pouches compared to combustible cigarettes. *Psychopharmacology (Berl)*. 2021;238(11):3325-3334.
27. Liu J, Rensch J, Wang J, et al. Nicotine pharmacokinetics and subjective responses after using nicotine pouches with different nicotine levels compared to combustible cigarettes and moist smokeless tobacco in adult tobacco users. *Psychopharmacology (Berl)*. 2022;239(9):2863-2873.
28. Carter LP, Stitzer ML, Henningfield JE, et al. Abuse liability assessment of tobacco products including potential reduced exposure products. *Cancer Epidemiol Biomarkers Prev*. 2009;18(12):3241-3262.
29. Abrams DB, Glasser AM, Pearson JL, et al. Harm minimization and tobacco control: reframing societal views of nicotine use to rapidly save lives. *Annu Rev Public Health*. 2018;39:193-213.
30. Abrams DB, Glasser AM, Villanti AC, et al. Managing nicotine without smoke to save lives now: evidence for harm minimization. *Prev Med*. 2018;117:88-97.
31. Azzopardi D, Haswell LE, Frosina J, et al. Assessment of biomarkers of exposure and potential harm, and physiological and subjective health measures in exclusive users of nicotine pouches and current, former and never smokers. *Biomarkers*. 2023;28(1):118-129.
32. 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.
33. 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.
34. Becker E, McCaffrey S, Lewis J, et al. Characterization of Ad Libitum Use Behavior of on!™ Nicotine Pouches. *Am J Health Behav*. 2023. Accepted for publication.

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.