Original Article

A Randomized Control Study in Healthy Adult Smokers to Assess Reduced Exposure to Selected Cigarette Smoke Constituents in Switching to the Novel Heated Tobacco Product DT3.0a

Clinical Pharmacology in Drug Development
2023, 0(0) 1–13

DOI: 10.1002/cpdd.1322

Daisuke Nishihara, Dai Yuki, Takuya Suzuki, Chikako Sakaguchi, Yasufumi Nagata, and Aoi Kakehi

Abstract

This was a randomized, controlled, open-label, confinement study to assess change in exposure to selected cigarette smoke constituents in healthy adult cigarette smokers who switched to using a novel heated tobacco product (direct heating tobacco system, platform 3, generation 3, version a [DT3.0a]). Sixty nonmenthol cigarette smokers were randomized into 1 of the 4 study groups in which subjects switched to a nonmenthol type of tobacco stick used with DT3.0a, switched to a nonmenthol tobacco stick used with an in-market heated tobacco product device (THS), continued to smoke nonmenthol cigarettes, or stopped smoking. Furthermore, 30 menthol cigarette smokers were randomized into 1 of the 2 study groups in which subjects switched to a menthol tobacco stick used with DT3.0a (mDT3.0a) or continued to smoke menthol cigarettes. Fifteen biomarkers of exposure to selected harmful and potentially harmful constituents (HPHCs) were measured during the 5-day exposure period, followed by assessment of nicotine pharmacokinetics with the assigned product. Results indicated that switching to DT3.0a, THS, and mDT3.0a showed significant exposure reductions in most of the selected HPHCs as compared to continuing smoking cigarettes, with reductions being similar in magnitude to reductions observed with smoking cessation. For DT3.0a and mDT3.0a, nicotine pharmacokinetic parameters were not remarkably different from those obtained for cigarettes and the THS except that a longer time to maximum concentration was obtained following use of the mDT3.0a. In conclusion, switching from smoking cigarettes to DT3.0a or THS use reduced exposure to most of the selected HPHCs, and no remarkable differences were observed for the measurements obtained from different flavors of DT3.0a stick.

Keywords

biomarkers of exposure, heated tobacco product, nicotine pharmacokinetics, smoking

Cigarette smoking is a cause of serious diseases including lung cancer, coronary heart disease, emphysema, and chronic bronchitis.[1] It is reported that the cause of smoking-related diseases is exposure to substances in the smoke generated by burning tobacco leaves for a long-term period.[1,2] Thus, there has been a growing interest in noncombustible tobacco products such as heated tobacco products (HTPs), which can reduce exposure to the harmful substances associated with combustion of tobacco.

The US Food and Drug Administration (FDA) has issued a draft Guidance for Industry on the regulatory application of modified-risk tobacco products

Scientific and Regulatory Affairs, Japan Tobacco Inc., Minato-Ku, Tokyo, Japan

Submitted for publication 15 May 2023; accepted 6 August 2023.

Corresponding Author:
Daisuke Nishihara, PhD, Scientific and Regulatory Affairs, Japan Tobacco Inc., 4-1-1 Toranomon Minato-Ku, Tokyo 105-6927, Japan.
(e-mail: daisuke.nishihara@jt.com)

(MRTPs). An MRTP is defined as any tobacco product that is sold or distributed for use to reduce harm or the risk of particular diseases related to marketed tobacco products. In the draft guidance, the FDA recommends the applicant to conduct human studies to assess the reduced health risks related to the use of the candidate tobacco product, including exposure to cigarette smoke constituents (eg, biomarkers of exposure [BoE]) and health outcomes (eg, disease incidence or mortality). In addition, the guidance also recommends including abuse liability information (eg, subjective effects measures, pharmacokinetics [PK] of nicotine) as part of the MRTP application. At this moment, the FDA has authorized 1 manufacturer to market a specific product (IQOS 3 System Holder and Charger, Philip Morris Products S.A.) as an MRTP under the HTP category and has issued an order of "exposure modification" that implies that introduction of this product to the market is expected to benefit the public health (https://www.fda.gov/tobacco-products/advertising-and-promotion/philip-morris-products-sa-modified-risk-tobacco-product-mrtp-applications).

A list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke was established by the FDA (https://www.fda.gov/tobacco-products/rules-regulations-and-guidance/harmful-and-potentially-harmful-constituents-tobacco-products-and-tobacco-smoke-established-list), and BoEs for the selected HPHCs have been suggested for assessing the exposure levels during use of various tobacco/nicotine-containing products.[3] It was reported that exposure to the selected BoEs showed a dose–response relationship with the number of cigarettes smoked[4] and that smoking cessation led to significantly lower exposure to the selected BoEs.[5] For HTPs, a number of clinical studies have been conducted, and reported results suggest a sustained reduction in human exposure to selected HPHCs among those who switched away from combustible cigarettes to some HTPs. The reductions were similar in magnitude to those observed for smoking cessation.[6–13] Thus, in accordance with current knowledge, HTPs are expected to possess consistent reduced toxicant exposure properties. Additionally, regarding the nicotine PK of HTPs, it has been reported that the absorption rate of nicotine is generally similar to that obtained following smoking cigarettes, whereas the amount of absorbed nicotine differs depending on the type of HTP used.[14,15] As new and updated versions of HTPs are being introduced year after year, continuous research is required for these products with novel characteristics. In addition, despite the fact that menthol is one of the most common flavors of HTP tobacco stick, most published clinical studies have assessed nonmenthol regular flavor HTPs and much less is known about how flavor differences impact outcomes.

In 2021, the DT3.0a (direct tobacco heating system, platform 3, generation 0, version a), which electronically heats the inside of a tobacco stick up to a temperature of 295°C, was developed and introduced by Japan Tobacco Inc.[16] The results of the chemical analysis of aerosol generated from the DT3.0a showed that most of the measured selected cigarette smoke constituents were lower levels as compared to the University of Kentucky reference cigarette.[16] In addition, the DT3.0a aerosol registered little response when investigated for mutagenic and cytotoxic effects following in vitro toxicological assays, in contrast to results obtained using the University of Kentucky reference cigarette.[16] Such results would suggest that switching from combustible cigarettes to the DT3.0a potentially reduces exposure to cigarette smoke constituents.

The primary objective of the present study was to evaluate BoEs to the selected HPHCs in healthy adult smokers who either switched to use a nonmenthol-type tobacco stick with a novel HTP (DT3.0a) or an in-market HTP (THS), continued smoking nonmenthol combustible cigarettes, or stopped smoking over a 5-day period. Stopping smoking and switching to THS were set as reference conditions in this study, as these conditions for subjects have been reported to lead to potential reductions in exposure to HPHCs.[6–13] Furthermore, the present study also assessed human exposure in healthy adult smokers who either switched to use a menthol-type tobacco stick with DT3.0a (mDT3.0a) or continued smoking menthol combustible cigarettes. As part of the secondary objectives of this study, nicotine PK parameters of the assigned product were assessed after the completion of the 5-day exposure period.

Methods

Study Design

This was a randomized, controlled, open-label, confinement study carried out at 2 clinics (SOUSEIKAI Fukuoka Mirai Hospital and SOUSEIKAI Hakata Clinic) in Fukuoka, Japan. The study was conducted in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki. Prior to the start of study, the study was approved by Hakata Clinic Institutional Review Board on August 27, 2021, and registered at the UMIN Clinical Trials Registry on September 1, 2021 (UMIN000045304). All participants provided written informed consent to participate in the study.

A study design schematic is shown in Figure S1. Eligible adult nonmenthol and menthol smokers checked into the clinics in the evening of Day −2. Sixty nonmenthol smokers were randomly assigned into 1 of the

4 study groups (switching to a DT3.0a, switching to a THS, continuing smoking their own brand of combustible cigarettes [CCs] or stopping smoking [SS]), and 30 menthol smokers were randomly assigned into 1 of the 2 study groups (switching to an mDT3.0a or continuing smoking their own brand of menthol combustible cigarettes [mCCs]) in equal ratios). Such separated randomizations by nonmenthol and menthol smokers were employed to minimize the impact of changing flavors during switching from subjects' usual brands of cigarettes to HTPs in the study. The randomization was performed separately for nonmenthol and menthol smokers and stratified by site, sex, and visiting cohorts in order to be well balanced. The subjects were randomly assigned into the study groups by using the electronic data capture system, and the investigators and site staff were blinded to the randomization scheme.

On Day −1, subjects were requested to smoke their own brand of cigarettes ad libitum within ±10% of their self-reported daily consumption (the site staff provided cigarettes one by one at the request of the subjects, and staff counted the number of cigarettes smoked by each subject). From Day 1 to Day 5 (the exposure assessment period), all investigational tobacco products were dispensed by the site staff one by one, and the subjects could use their assigned investigational tobacco product from 9:00 a.m. to 10:30 p.m. in supervised smoking areas designated separately by assigned product. Subjects in the DT3.0a, THS, and mDT3.0a groups used their assigned HTPs ad libitum. Subjects in the CC and mCC groups continued to smoke their own brand of cigarettes ad libitum within ±10% of their self-reported daily consumption. Subjects in the SS group abstained from smoking and were not permitted to access the smoking areas. On Day 6, subjects in study groups except the SS group used 1 stick of their assigned investigational tobacco products for nicotine PK assessment. Subjects in all groups were discharged on Day 6 after completing safety assessment procedures.

Subjects

Male and female Japanese healthy smokers aged 21–65 years were eligible to participate in the study if they smoked 11 or more cigarettes/day on average at screening, had exclusively smoked commercially available cigarettes for at least 12 months before screening, and had a positive urinary cotinine result with a cutoff of 200 ng/mL (COT200 Rapid Test Cassette, Technicon International, Inc.).

Before enrollment, the health status of all participants was confirmed by physical examination, medical history, vital signs, electrocardiogram, and clinical laboratory testing. The main exclusion criteria were pregnant or breastfeeding females; males having a body mass index (BMI) of less than 18.5 or greater than 27.7 kg/m^2 and females having a BMI of less than 16.8 or greater than 26.1 kg/m^2; participants who had donated 200 mL or more of blood within 2 weeks or had donated 400 mL or more of blood within 12 weeks (men) or 16 weeks (women) prior to Day 1; and participants who had any clinically relevant abnormal findings, including positive results for severe acute respiratory syndrome coronavirus 2 by polymerase chain reaction, as judged by the investigators. Subjects were also excluded if they had used any prescription drugs, over-the-counter medications (including smoking-cessation medications), or dietary supplements within 2 weeks prior to the exposure assessment period.

Sample Size Determination

The sample size was 15 subjects in each study group. The sample size was determined on the basis of the expected least squares mean ratios of the levels of the selected 15 BoEs (Table 1), as observed previously in smokers who had switched to HTPs for 5 days in previous studies of similar design.[13] For attaining 80% power in the DT3.0a, THS, SS, and mDT3.0a groups as compared to the CC or mCC groups using 2-sided tests with 5% alpha level (Bonferroni correlation, $\alpha = 0.05/4$), a sample size of 12 subjects in each study group was considered sufficient (among the 15 BoEs measured in the present study, calculation of differences in total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL] required the greatest number of subjects).

Investigational Tobacco Products

We assessed 3 HTPs (DT3.0a, mDT3.0a, THS) available on the Japanese market as investigational tobacco products. The HTPs were acquired from the market and provided to the subjects free of charge, while subjects' own brand of cigarettes were purchased and brought to the clinic by the subjects themselves.

DT3.0a (device/regular-type tobacco stick: Ploom X/MEVIUS Rich for Ploom X and Ploom S) and mDT3.0a (device/menthol type tobacco stick: Ploom X/MEVIUS Menthol Cold for Ploom X and Ploom S) developed by Japan Tobacco Inc., consists of a rechargeable holding device and a specially designed tobacco stick. The tobacco stick consists of flavors added to a tobacco leaf blend covered with paper and a filter on the tip and has a shape resembling a cigarette. The heater in the device heats the tobacco stick for approximately 5 minutes (the temperature inside the tobacco stick during use is up to 295°C) to generate tobacco vapor before being inhaled. Aerosol emissions for the tobacco sticks of DT3.0a and mDT3.0a used in this study were reported by Hashizume et al.[16] As an

Table 1. Biomarkers of Exposure to Selected Harmful and Potentially Harmful Constituents

Acronym	Biomarker of exposure	HPHC
3-HPMA	3-Hydroxypropyl-mercapturic acid	Acrolein
3-OH-BaP	3-Hydroxy-benzo[a]pyrene	Benzo[a]pyrene
Total 1-OHP	Total 1-hydroxypyrene	Pyrene[a]
S-PMA	S-Phenylmercapturic acid	Benzene
MHBMA	Monohydroxybutenyl-mercapturic acid	1,3-Butadiene
eCO[b]	Exhaled carbon monoxide	Carbon monoxide
Total NNAL	Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
Total NNN	Total N-nitrosonornicotine	N-nitrosonornicotine
CEMA	2-Cyanoethylmercapturic acid	Acrylonitrile
4-ABP	4-Aminobiphenyl	4-Aminobiphenyl
1-AN	1-Aminonaphthalene	1-Aminonaphthalene (1-AN)
2-AN	2-Aminonaphthalene	2-Aminonaphthalene (2-AN)
3-HMPMA	3-Hydroxy-1-methylpropylmercapturic acid	Crotonaldehyde
HEMA	2-Hydroxyethyl-mercapturic acid	Ethylene oxide
o-Tol	*o*-Toluidine	*o*-Toluidine

[a] Although pyrene is not listed as a harmful and potentially harmful constituent by the US Food and Drug Administration, the biomarker of exposure to pyrene was assessed as an alternative and supportive biomarkers of exposure to benzo[a]pyrene.
[b] The exhaled CO level of subjects was measured by using a piCO+ Smokerlyzer.

in-market HTP, the THS (device/tobacco stick: IQOS 3 DUO/Marlboro Heat Stick Regular) comprises an electronic device that heats specially designed tobacco sticks to approximately 300°C (up to 350°C) via a heating blade. Aerosol emissions of THS have been reported previously.[17]

Baseline Characteristics

The baseline characteristics of subjects, including sex, age, BMI, smoking history, the nominal tar value of the subject's usual brand of cigarette (value printed on each cigarette package), self-reported daily consumption of cigarettes, and score on the Fagerström Test for Nicotine Dependence[18] were recorded at screening.

Exposure Assessments

As shown in Table 1, the study examined the following 15 BoEs (3-hydroxypropyl-mercapturic acid [3-HPMA], 3-hydroxy-benzo[a]pyrene [3-OH-BaP], total 1-hydroxypyrene [1-OHP], S-phenylmercapturic acid [S-PMA], monohydroxybutenyl-mercapturic acid [MHBMA], exhaled carbon monoxide [eCO], total NNAL, total N-nitrosonornicotine [NNN], 2-cyanoethylmercapturic acid [CEMA], 4-aminobiphenyl [4-ABP], 1-aminonaphthalene [1-AN], 2-aminonaphthalene [2-AN], 3-hydroxy-1-methylpropylmercapturic acid [3-HMPMA], 2-hydroxyethyl-mercapturic acid [HEMA], and o-toluidine [o-Tol]). These BoEs were selected as reliable surrogate markers of exposure to HPHCs and pyrene. They have been widely measured in clinical studies to evaluate exposure from tobacco product use.[3,19–21] The eCO level of subjects was measured using a piCO+ Smokerlyzer (Bedfont Scientific Ltd.) at the same time each evening (between 5:00 p.m. and 7:00 p.m.) on Day −1, Day 3, and Day 5. Each eCO measurement took place between 30 and 45 minutes after the subject's previous use of any investigational tobacco product. The 24-hour urine samples for measuring 14 BoEs (3-HPMA, 3-OH-BaP, total 1-OHP, S-PMA, MHBMA, total NNAL, total NNN, CEMA, 4-ABP, 1-AN, 2-AN, 3-HMPMA, HEMA, and o-Tol) were pooled from the morning on Day −1, Day 3, and Day 5 to the following morning. Creatinine was also measured in 24-hour urine for adjustment of the concentration of all urinary BoEs. All bioanalyses were carried out using validated methods at Celerion. The details of these methods have been published previously.[13,14]

Nicotine uptake was assessed by measuring nicotine equivalents (the molar sum of nicotine and the 5 major metabolites) in the same 24-hour urine samples used for BoE assessment. The subject's product consumption on each day from Day 1 to Day 5 was recorded for all subjects, except those who were assigned to the SS group, by counting the number of consumed cigarettes or HTP tobacco sticks.

Questionnaires

Subjective effects were assessed by using self-reported Japanese versions of questionnaires including the Questionnaire on Smoking Urges–Brief (QSU-Brief),[22] modified Cigarette Evaluation Questionnaire (mCEQ),[23] and Minnesota Nicotine Tobacco Withdrawal Scale (MNWS;

http://www.med.uvm.edu/behaviorandhealth/research/minnesota-tobacco-withdrawal-scale). These were selected as the most widely used questionnaires among the various methods available to research the subjective effects in relation to tobacco product use. Within 15 minutes after the last use of their own brand of cigarette on Day −1, and after the last use of investigational products on Days 3 and 5, subjects (except in the SS group) answered the questionnaires in the following order: QSU-Brief, mCEQ, and MNWS. The QSU-Brief items were rated on a 7-point scale (ranging from 1 [strongly disagree] to 7 [strongly agree]), and the following factors/scores were calculated: Factor 1 (Desire to Smoke, sum of items 1, 3, 6, 7, and 10); Factor 2 (Negative Reinforcement, sum of items 2, 4, 5, 8, and 9); and Total score (sum of all items). The mCEQ items were rated on a 7-point scale (ranging from 1 [not at all] to 7 [extremely]), and the following subscales scores were evaluated: Smoking Satisfaction (average of items 1, 2, and 12); Psychological Reward (average of items 4–8); Aversion (average of items 9 and 10); Enjoyment of Respiratory Tract Sensations (item 3); and Craving Reduction (item 11). The MNWS items were rated on a 5-point scale (ranging from 0 [none] to 4 [severe]), and the total score (sum of all items) were calculated.

Nicotine PK Assessment

On Day 6, subjects in the study groups (except the SS group) used a cigarette or stick of their assigned investigational tobacco products, the same product for the exposure assessment period, at the specified time for the nicotine PK assessment, after abstaining from the use of any tobacco products for at least 10 hours. A baseline blood sample was taken (−10 minutes) and then subjects used the assigned investigational tobacco product ad libitum. Blood samples for plasma nicotine analysis were obtained at 2, 4, 6, 8, 10, 15, 30, 60, 90, 120, and 240 minutes relative to the start of product use. Plasma nicotine was analyzed by liquid chromatography–tandem mass spectrometry using validated analytical methods with appropriate quality controls at Celerion. The PK parameters of the maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}), and area under the plasma concentration–time curve from time zero to the last quantifiable concentration ($AUC_{0–tlast}$) were calculated by Phoenix WinNonlin, version 8.0 (Certara LP).

Safety

Safety outcomes, including an assessment of adverse events (AEs), were recorded. These comprised any abnormal clinical findings from vital signs, clinical laboratory tests, and physical examinations throughout the study period following randomization.

Statistical Analysis

The primary study end points were differences in the selected 15 BoE levels between the intervention study groups (DT3.0a, THS, and SS groups) and the control group (CC group) and between the intervention study group (mDT3.0a group) and the control group (mCC group). The levels of BoE were ln-transformed, and analysis of covariance was performed with the end points observed at the end of exposure (Day 5) by study group and site, with the baseline (Day −1) value as a covariate (2-sided significance level with 5% alpha level, with Bonferroni-correlation for multiplicity due to 4-way comparison, $\alpha = 0.05/4$). The estimated differences between the study groups and associated confidence intervals (CIs; $\alpha = 0.05/4$) were back-transformed to provide relative values (DT3.0a/CC, THS/CC, SS/CC, and mDT3.0a/mCC).

Descriptive statistics of the daily product consumption and nicotine equivalents results including percentage changes in values from baseline or Day 1 and end points observed on Day 5 ([end point − baseline or Day 1]/baseline × 100%) were presented. For the questionnaires, the descriptive statistics of changes in values were calculated from baseline and end points noted on Day 5. Descriptive statistics were presented to describe the PK parameters (C_{max}, T_{max}, and $AUC_{0–tlast}$) from the plasma nicotine concentrations for each study group.

Statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc.).

Results

Subject Demographics

In total, 289 participants were screened, and 92 subjects were enrolled into the study in accordance with the planned sample size (Figure S2). Two of these subjects who were to be assigned to the SS group discontinued the study before the exposure assessment period started. The reasons for discontinuation were withdrawal of consent/participation and failure to comply with the protocol. The remaining 90 subjects (15 in each study group) completed the study, either with use of investigational tobacco products or as part of the SS group according to assignment. Demographic characteristics of the subjects are summarized in Table S1. Overall, there were no notable differences in subject characteristics, including age, sex, BMI, the tar values of the subject's usual brand of cigarettes, daily cigarette consumption per day, and the Fagerström Test for Nicotine Dependence score.

Product Consumption and Nicotine Uptake

Daily product consumption during the exposure assessment period for each study group (except the SS group)

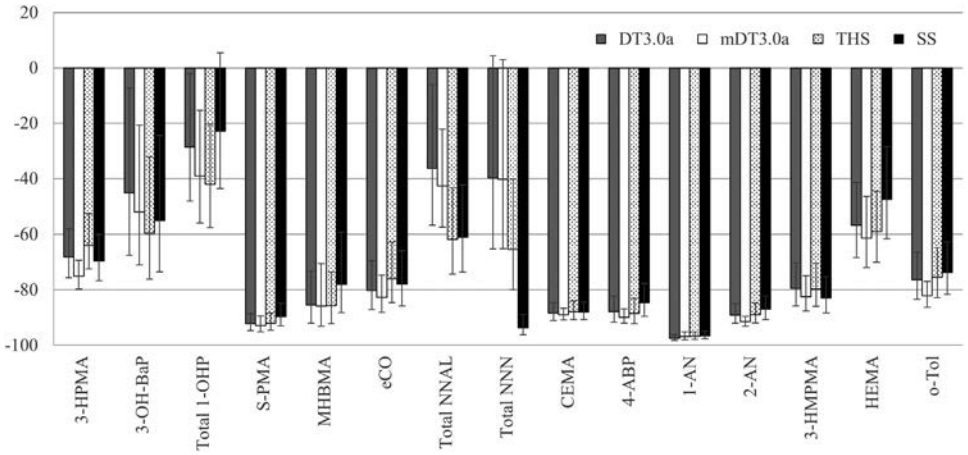

Figure 1. Biomarkers of exposure level at final time point during exposure assessment period, in comparison with continuing smoking (percentage reduction compared to the CC/mCC group). Percentage reduction values are presented as geometric least squares mean ratios (percentage) and confidence intervals ($\alpha = 0.05/4$) from analysis of covariance model performed on ln-transformed Day 5 values with ln-transformed baseline value, study group and site as fixed effect factors on Day 5. 1-AN, 1-aminonaphthalene; 1-OHP, 1-hydroxypyrene; 2-AN, 2-aminonaphthalene; 3-HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA, 3-hydroxypropyl-mercapturic acid; 3-OHBaP, 3-hydroxy-benzo[a]pyrene; 4-ABP, 4-aminobiphenyl; CEMA, 2-cyanoethylmercapturic acid; DT3.0a, direct heating tobacco system, platform 3, generation 3, version a; eCO, exhaled carbon monoxide; HEMA, 2-hydroxyethyl-mercapturic acid; mCC, menthol combustible cigarettes; mDT3.0a, menthol direct heating tobacco system, platform 3, generation 3, version a; MHBMA, monohydroxybutenyl-mercapturic acid; NNAL, 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol; NNN, N-nitrosonornicotine; o-Tol, o-toluidine; S-PMA, S-phenylmercapturic acid; SS, stopping smoking; THS, tobacco heating system.

is shown in Table 2. In the CC and mCC groups, the mean number of cigarettes smoked ranged from 12.9 to 13.3 cigarettes/day and from 13.8 to 14.2 cigarettes/day, respectively, confirming that each subject maintained ±10% of their self-reported daily cigarette consumption as required in the protocol. Following switching to HTPs over 5 days, product consumption was generally stable with % change (from Day 1 to Day 5) of 11.9%, 9.2%, and −0.4% for the DT3.0a, THS, and mDT3.0a groups, respectively.

Table 2 shows the nicotine uptake levels from baseline to Day 5, estimated by urinary nicotine equivalents levels. Over the exposure assessment period, nicotine uptake was generally stable in the CC and mCC groups (14.1% and 1.8% from baseline to Day 5, respectively), and decreased in the SS group (−89.3% from baseline to Day 5). Following switching to HTPs over 5 days, nicotine uptake was generally stable with percentage changes (from baseline to Day 5) of 7.7%, −15.4%, and −1.2% for the DT3.0a, THS, and mDT3.0a groups, respectively.

Overall, product consumption and nicotine uptake in the DT3.0a and mDT3.0a groups were generally stable over the exposure assessment period.

Biomarkers of Exposure

Reductions of BoE levels in the DT3.0a, mDT3.0a, THS, and SS groups on Day 5 relative to the respective comparator groups (the CC or mCC groups) are presented in Figure 1, with statistical data also shown in Table S2.

In the SS group, the reductions (percentage) in 15 BoEs relative to the CC group ranged from −22.9% to −96.6%, and there were statistically significant differences between the SS group and the CC group for 14 BoE levels (exception: total 1-OHP). In the DT3.0a, THS, and mDT3.0a groups, the reductions (percentage) in 15 BoEs relative to the respective comparator groups (the CC or mCC groups) ranged from −28.7% to −97.5%, −42.0% to −96.8%, and −39.0% to −96.9%, respectively. Except for total NNN in the DT3.0a and mDT3.0a groups, a significant reduction in all BoEs was observed on Day 5 in subjects who switched to the HTPs as compared to the CC or mCC groups.

The reductions (percentage) relative to the CC group on Day 5 were similar in magnitude in the HTP and SS groups for each BoE except total NNN, based on overlaps of 95% CIs of the reductions. For total 1-OHP in the SS group, although there was no statistically significant difference compared with the CC group, the reductions (percentage) in the SS group (95% CI, −43.6% to 5.50%) were of similar magnitude to those in the DT3.0a group (95% CI, −48.1% to −2.10%), THS group (95% CI, −57.7% to −20.4%), and mDT3.0a group (95% CI, −56.0% to −15.3%). For total NNN in the DT3.0a group (95% CI, −65.3% to 4.40%) and mDT3.0a group (95% CI, −65.2% to 2.90%), the reductions relative to the CC group were slightly less than

Table 2. Product Consumption and Nicotine Uptake Levels During Exposure Assessment Period

Product consumption (sticks per day of the HTPs and cigarettes per day of the cigarettes)							Nicotine uptake levels (nicotine equivalents, mg/mg creatinine)						
	Study groups							Study groups					
Mean (SD)	DT3.0a (n = 15)	THS (n = 15)	SS (n = 15)	CC (n = 15)	mDT3.a (n = 15)	mCC (n = 15)	Mean (SD)	DT3.0a (n = 15)	THS (n = 15)	SS (n = 15)	CC (n = 15)	mDT3.0a (n = 15)	mCC (n = 15)
Baseline[a]	12.6 (2.1)	12.4 (2.2)	12.5 (2.0)	12.7 (4.2)	13.8 (2.5)	14.6 (6.8)	Baseline[a]	6.6 (2.9)	7.5 (3.9)	6.5 (4.9)	6.7 (3.9)	6.5 (2.7)	6.5 (3.0)
Day 1	10.7 (4.0)	11.9 (3.7)	–	12.9 (4.2)	10.6 (2.1)	14.2 (7.0)							
Day 2	11.5 (3.8)	12.3 (3.5)	–	13.1 (4.2)	9.47 (1.9)	13.9 (6.7)							
Day 3	11.6 (3.2)	13.3 (4.1)	–	13.3 (4.4)	10.0 (1.8)	14.1 (6.7)	Day 3	6.8 (3.3)	6.6 (2.9)	0.9 (0.6)	7.3 (3.8)	6.0 (3.2)	6.6 (3.8)
Day 4	11.1 (3.5)	12.4 (4.5)	–	12.9 (4.5)	9.73 (2.6)	13.8 (6.8)							
Day 5	11.3 (3.5)	12.7 (4.0)	–	13.2 (4.1)	10.5 (3.0)	14.1 (6.5)	Day 5	6.8 (3.3)	6.2 (3.0)	0.4 (0.2)	7.2 (3.4)	6.5 (3.8)	6.8 (3.8)
Change from Day 1 on Day 5 (%)	11.9 (31.4)	9.15 (21.3)	–	3.36 (8.6)	−0.4 (24.3)	0.4 (6.2)	Change from baseline on Day 5 (%)	7.7 (42.2)	−15.4 (15.7)	−89.3 (11.7)	14.1 (30.2)	−1.2 (34.4)	1.8 (17.4)

CC, combustible cigarettes; DT3.0a, direct heating tobacco system, platform 3, generation 3, version a; HTPs, heated tobacco products; mCC, menthol combustible cigarettes; mDT3.0a, menthol direct heating tobacco system, platform 3, generation 3, version a; SD, standard deviation; SS, stopping smoking; THS, tobacco heating system.

[a]All subjects were requested to smoke their own brand of cigarettes ad libitum within ±10% of their self-reported daily consumption during the baseline period (Day −1).

those in the THS group (95% CI, −80.0% to −40.2%), and those in the SS group, which showed the largest reduction (95% CI, −96.3% to −89.0).

Levels of 15 BoEs at baseline, Day 3, and Day 5 for the DT3.0a, THS, SS, CC, mDT3.0a, and mCC groups are provided in Table S3. From baseline to Day 5, an apparent general decrease in all BoE levels was observed in the DT3.0a, THS, SS, and mDT3.0a groups.

Subjective Effects

Table 3 shows the mean changes of the subjective effects scores (QSU-Brief: Factor 1, Factor 2, and Total score; mCEQ: Smoking Satisfaction, Psychological Reward, Aversion, Enjoyment of Respiratory Tract Sensations, and Craving Reduction; and MNWS: total score) from baseline to Day 5. Except for Factor 1 of the QSU-Brief administered to the THS group and "Aversion" subscale of the mCEQ administered to the DT3.0a, THS, and mDT3.0a groups, all groups showed a decrease in the mean subscale scores on Day 5 as compared to the baseline.

For most of the subjective effects scores, the mean changes in scores from baseline to Day 5 for the DT3.0a, THS, and mDT3.0a groups were not remarkably different from those for the CC and mCC groups. However, based on overlaps of the 95% CIs of the mean changes, it is noteworthy that a decrease in the mCEQ subscale of "Smoking Satisfaction" on Day 5 was apparent for the DT3.0a (95% CI, −2.89 to −1.47) and THS groups (95% CI, −3.02 to −1.20) when comparing to the CC group (95% CI, −0.69 to 0.20). In addition, the mCEQ subscale of Enjoyment of Respiratory Tract Sensations markedly decreased on Day 5 for the THS group (95% CI, −3.21 to −1.19) in comparison to the CC group (95% CI, 0.51–0.64).

Nicotine PK

Nicotine PK parameters calculated following use of each investigational tobacco product are summarized in the Table 4. The mean nicotine concentration–time profiles following a single use of assigned investigational tobacco product are also shown in Figure 2, in which the overall shapes of the curves can be seen to be similar among the study groups. The mean C_{max} and $AUC_{0-tlast}$ were 30.1 ng/mL and 34.4 ng • h/mL for the CC group, 24.7 ng/mL and 29.3 ng • h/mL for the mCC group, 22.4 ng/mL and 29.2 ng • h/mL for the DT3.0a group, 18.7 ng/mL and 26.6 ng • h/mL for the THS group, and 25.7 ng/mL and 34.5 ng • h/mL for the mDT3.0a group, respectively. These C_{max} and $AUC_{0-tlast}$ for the DT3.0a, THS, and mDT3.0a groups were not markedly different from those for the respective comparator groups (the CC or mCC group) based on overlap of the 95% CIs of the mean values.

Table 3. Changes of Subjective Effects Scores from Baseline to Day 5

		Study groups				
		DT3.0a (n = 15)	THS (n = 15)	CC (n = 15)	mDT3.0a (n = 15)	mCC (n = 15)
QSU-Brief – Factor 1	Mean (95% CI)	−0.9 (−7.0 to 5.1)	0.3 (−4.1 to 4.6)	−3.1 (−5.1 to −1.2)	−4.3 (−6.5 to −2.0)	−4.1 (−7.3 to −0.9)
QSU-Brief – Factor 2	Mean (95% CI)	−3.2 (−6.1 to −0.3)	−1.5 (−6.7 to 3.7)	−3.1 (−5.4 to −0.8)	−4.8 (−9.0 to −0.6)	−3.6 (−7.0 to −0.2)
QSU-Brief – Total score	Mean (95% CI)	−4.1 (−12.8 to 4.6)	−1.3 (−10.7 to 8.1)	−6.3 (−10.1 to −2.5)	−9.1 (−15.0 to −3.3)	−7.7 (−14.1 to −1.2)
mCEQ – Smoking satisfaction	Mean (95% CI)	−2.2 (−2.9 to −1.5)	−2.1 (−3.0 to −1.2)	−0.2 (−0.7 to 0.2)	−1.9 (−3.0 to −0.7)	−0.6 (−1.2 to 0.1)
mCEQ – Psychological reward	Mean (95% CI)	−1.0 (−1.7 to −0.2)	−1.4 (−2.0 to −0.7)	−0.4 (−1.0 to 0.1)	−1.7 (−2.5 to −0.8)	−0.6 (−1.1 to 0.0)
mCEQ – Aversion	Mean (95% CI)	0.2 (−0.4 to 0.7)	0.0 (−0.3 to 0.3)	0.1 (−0.4 to 0.5)	−0.2 (−0.9 to 0.4)	−0.6 (−1.3 to 0.0)
mCEQ – Enjoyment of respiratory sensations	Mean (95% CI)	−1.4 (−2.5 to −0.3)	−2.2 (−3.2 to −1.2)	0.1 (−0.5 to 0.6)	−1.9 (−2.8 to −0.9)	−0.5 (−1.3 to 0.3)
mCEQ – Craving reduction	Mean (95% CI)	−1.9 (−3.0 to −0.8)	−2.0 (−2.8 to −1.2)	−0.7 (−1.6 to 0.1)	−1.5 (−2.8 to −0.3)	−0.9 (−1.9 to 0.0)
MNWS – Total score	Mean (95% CI)	−1.6 (−4.1 to 0.9)	−4.0 (−6.1 to −1.9)	1.0 (−4.0 to 6.0)	−2.9 (−5.0 to −0.7)	−2.7 (−6.2 to 0.8)

CI, confidence interval; CC, combustible cigarettes; DT3.0a, direct heating tobacco system, platform 3, generation 3, version a; mCC, menthol combustible cigarettes; mCEQ, modified Cigarette Evaluation Questionnaire; mDT3.0a, menthol direct heating tobacco system, platform 3, generation 3, version a; MNWS, Minnesota Nicotine Tobacco Withdrawal Scale; QSU-Brief, Questionnaire on Smoking Urges–Brief; SS, stopping smoking; THS, tobacco heating system.

Table 4. Nicotine PK Parameters

		Study groups				
		DT3.0a (n = 15)	THS (n = 15)	CC (n = 15)	mDT3.0a (n = 15)	mCC (n = 15)
C_{max} (ng/mL)	Mean (95% CI)	22.4 (16.1–28.7)	18.7 (14.3–23.2)	30.1 (21.0–39.2)	25.7 (18.7–32.8)	24.7 (19.0–30.4)
$AUC_{0-tlast}$ (ng • h/mL)	Mean (95% CI)	29.2 (19.5–38.9)	26.6 (17.1–36.1)	34.4 (24.3–44.6)	34.5 (26.8–42.1)	29.3 (22.1–36.5)
T_{max} (min)	Mean (95% CI)	5.9 (3.7–8.1)	3.7 (3.0–4.3)	4.7 (3.6–5.9)	6.6 (5.6–7.6)	4.6 (3.8–5.4)

$AUC_{0-tlast}$, the area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration; C_{max}, maximum observed plasma concentration; CC, combustible cigarettes; CI, confidence interval; DT3.0a, direct heating tobacco system, platform 3, generation 3, version a; mCC, menthol combustible cigarettes; mDT3.0a, menthol direct heating tobacco system, platform 3, generation 3, version a; SS, stopping smoking; THS, tobacco heating system; T_{max}, time to reach maximum observed plasma concentration.

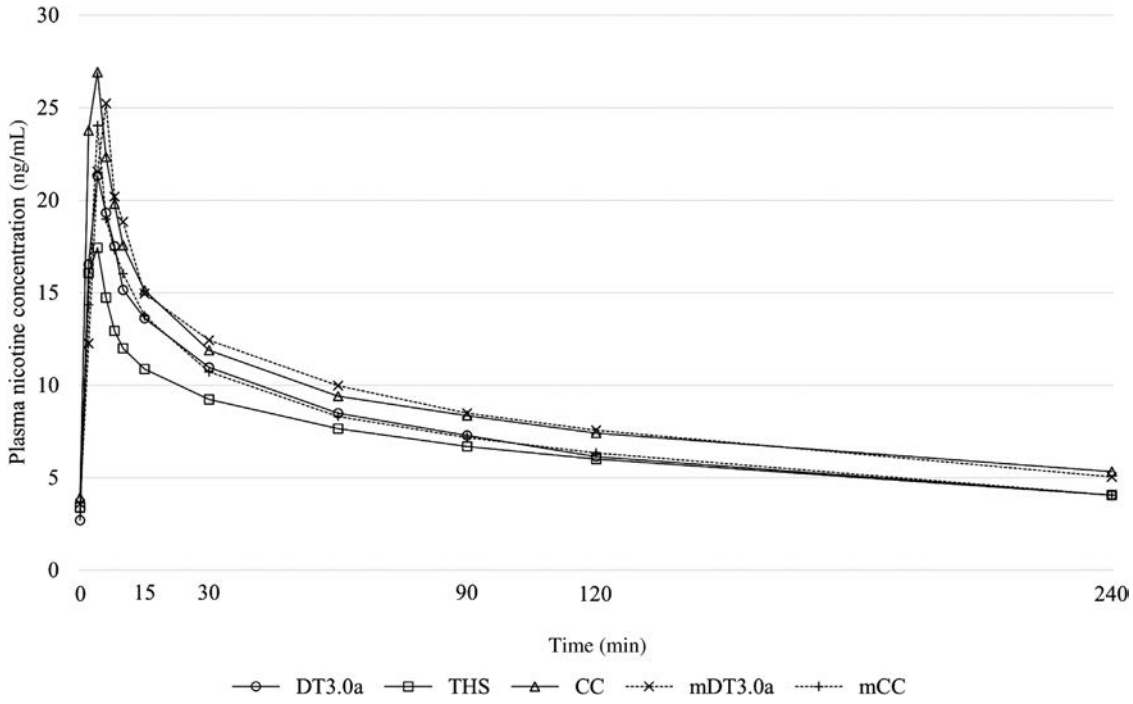

Figure 2. Plasma nicotine concentration-time curve. CC, combustible cigarettes; DT3.0a, direct heating tobacco system, platform 3, generation 3, version a; mCC, menthol combustible cigarettes; mDT3.0a, menthol direct heating tobacco system, platform 3, generation 3, version a; SE, standard error; SS, stopping smoking; THS, tobacco heating system.

The mean T_{max} values were 4.72 minutes for the CC group, 4.59 minutes for the mCC group, 5.91 minutes for the DT3.0a group, 3.65 minutes for the THS group, and 6.59 minutes for the mDT3.0a group. While no marked difference in T_{max} was observed between the DT3.0a and THS groups and CC group based on overlap of the 95% CIs, T_{max} for the mDT3.0a group (95% CI, 5.62–7.56 minutes) was longer than that for the mCC group (95% CI, 3.81–5.37 minutes) without overlap of 95% CIs.

Safety

No exposure period AEs were reported in the study. Before the exposure assessment period, mild AEs (headache, vomiting and nausea) occurred in 1 subject who was to be assigned to the SS group. Although all AEs were resolved during the day, the investigator decided to discontinue this subject due to a failure to comply with the protocol. There were no other AEs, no clinically significant abnormalities in clinical and physiological laboratory values, or any deaths or serious AEs in the study.

Discussion

There has been a growing interest in the research of novel tobacco and nicotine products, which can reduce the harmful substances associated with combustion of tobacco in cigarettes. Scientific evidence that shows a reduction of human exposure to tobacco toxicants is a key component to gain early insight into the potential for reduced risk of novel tobacco and nicotine products. Here, we report data from a randomized, controlled,

open-label, confinement study in healthy adult Japanese smokers conducted to evaluate the effect of switching from a cigarette to nonmenthol and menthol variants of the novel HTP/DT3.0a product on BoE. In addition, BoE levels were assessed in subjects who switched to using an in-market HTP/THS, or who completely abstained from any tobacco product use. This study was conducted under highly controlled and short-term confinement conditions to assess exposure associated with the exclusive use of the assigned investigational tobacco products. Furthermore, randomization was performed for nonmenthol and menthol smokers separately to assign them into the nonmenthol and menthol variants of the DT3.0a (DT3.0a and mDT3.0a) groups, respectively, to mitigate possible impacts of flavor change on the results.

In the present study, exposure to most of the measured 15 BoEs were significantly reduced, following switching to novel (DT3.0a and mDT3.0a products) and in-market HTPs (THS) for 5 days. Except for total NNN, relative BoE reduction (percentage) in the DT3.0a and mDT3.0a groups compared to the CC and mCC groups ranged from − 36.3% to − 97.5%. The reductions were similar in magnitude with those observed in the SS (− 22.9% to − 96.6%) and THS groups (− 42.0% to − 96.8%). Similarly, previous studies have shown that switching from CC to HTPs led to significant reductions in exposure to HPHCs in comparison with continuing smoking and the magnitude of these reductions was similar to that observed for smoking cessation.[6–13]

For total 1-OHP, all HTP groups showed marked reductions relative to the CC and mCC groups (−28.7% to −39.0%). However, the SS group showed a small reduction in total 1-OHP relative to the CC group (−22.9%), and no statistically significant reduction was observed between the SS and CC groups. In the SS group, mean total 1-OHP decreased from baseline (234 pg/mg creatinine) to Day 3 (138 pg/mg creatinine) but increased on Day 5 (198 pg/mg creatinine, as shown in Table S3). A similar change in total 1-OHP was also observed in another published confinement study with less dietary restriction, in which subjects were provided several foods that could have resulted in increased polycyclic aromatic hydrocarbon exposure such as fried chicken and grilled fish.[12] In the present study, to minimize influences on the BoE results from factors other than use of the assigned tobacco products, all subjects were provided with the same diet without grilled or smoked foods, and subjects were not permitted to access smoking areas designated for using the other investigational tobacco products. Nevertheless, this change of the total 1-OHP values in the present study was observed in the SS group, and moreover, a similar change was also observed in the DT3.0a group (262, 169, and 204 pg/mg creatinine from baseline to Day 5, as shown in Table S3) but not observed in other groups. Total 1-OHP has been measured as a substitute BoE for benzo[a]pyrene; however, recently 3-OH-BaP has become widely measured as a BoE for benzo[a]pyrene. In addition, pyrene is not included in the list of HPHCs established by the FDA. Considering such inconsistent changes in total 1-OHP during confinement, it is worth reconsidering use of total 1-OHP in assessments for exposure to cigarette smoke constituents in further studies.

While the SS and THS groups showed statistically significant reductions in total NNN relative to the CC group (−93.7% and −65.4%, respectively), the total NNN reduction in the DT3.0a and mDT3.0a groups was not statistically significant relative to the CC and mCC groups (−39.8% and −40.2%, respectively). A decreasing directional trend in total NNN levels was observed in all HTP groups from baseline to Day 5, but mean total NNN on Day 5 in the DT3.0a and mDT3.0a groups (1.88 and 4.19 pg/mg creatinine, respectively) was higher than that found in the THS group (1.53 pg/mg creatinine, as shown in Table S3). This may be explained by noting that the amounts of NNN in a vapor generated under the same machine vaping condition were 18.8 ng/stick for the DT3.0a product and 37.2 ng/stick for the mDT3.0a product, whereas the NNN concentration was 17.2 ng/stick for the THS product.[18] In addition, it has been reported that the amounts of constituents generated from HTPs are known to be varied depending on individual puffing behavior.[24,25] In the present study, it was confirmed that the amounts of constituents, including NNN, generated by the DT3.0a and mDT3.0a products varied depending on puffing topography (data not shown). Thus, relatively higher amounts of NNN emissions from the DT3.0a and mDT3.0 as compared to the THS and the fact that variations in NNN emissions depend on individual puffing patterns potentially explain nonsignificant reductions of total NNN exposure in the DT3.0a and mDT3.0a groups.

Daily product consumption was generally stable in all study groups (within ±11.9% change from Day 1 to Day 5) except for the SS group. Nicotine uptake levels were also stable, within ±15.4% change from baseline to Day 5 in all study groups except the SS group. Previous studies also reported that product consumption and nicotine uptake were generally stable after switching to HTPs from cigarettes.[7–12,14,26–30]

In the draft guidance, the FDA recommends applicants to acquire information relating to the abuse liability of candidate MRTP. Although there are no predetermined abuse liability testing methods for tobacco products, the subjective effects of product use are thought to be one of the informative elements

for assessment.[31] In this study, subjective effects were assessed by using the QSU-Brief, mCEQ, and MNWS questionnaires, which have been used in clinical studies in relation to tobacco product use. The mean subjective effects scores from baseline to Day 5 in all HTP groups were generally stable or slightly decreased. When comparing the results between groups, mean changes of the scores in all HTP groups were not markedly different from those in the CC and mCC groups, expect that Smoking Satisfaction (in the DT3.0a and THS groups) and Enjoyment of Respiratory Tract Sensations (in the THS group) decreased as compared to the CC group. This would suggest that, from the perspective of subjective effects results in the present study, abuse liability of HTPs use did not exceed that of cigarette smoking.

In addition to the subjective effects, nicotine PK is one of the potential factors of abuse liability assessment, and the FDA recommends applicants to include it as a part of the assessment for MRTP. Nicotine PK parameters (C_{max}, $AUC_{0–tlast}$, and T_{max}) in the HTP groups were not markedly different from those in the CC and mCC groups, based on the overlaps of the 95% CIs of the mean values, except that T_{max} was delayed in the mDT3.0a group in comparison to the mCC group. A similar trend was observed in the DT3.0a group, where mean T_{max} was slightly longer than in the CC and THS groups. It has been reported previously that T_{max} following use of the THS product was detected immediately after product use.[32] The THS product is designed to deliver 14 puffs per tobacco stick within a maximum of 6 min. Since the mean T_{max} of the THS group in the present study was 3.65 minutes, it can be assumed that the subjects tended to finish THS product use before 6 minutes when the subjects took 14 puffs of the THS. In contrast, the DT3.0a and mDT3.0a products have no puff count limit and can be continuously used up to approximately 5 minutes, which may lead to a relatively longer T_{max} for the DT3.0a and mDT3.0a groups. However, the relationship between T_{max} and product use duration still remains unclear, as product use duration was not measured in the present study. Results obtained in the present study would suggest that the abuse liability of the DT3.0a and mDT3.0a products did not exceed those of cigarettes and THS in the context of nicotine PK, since nicotine deliveries from the DT3.0a and mDT3.0a products were not higher or faster than those of the comparators. However, further detailed analysis is required to confirm these interpretations, during which nicotine PK of DT3.0a/mDT3.0a product use could be conducted in conjunction with capturing product use behavior (eg, product use duration) as well as employing a crossover design, which will mitigate impacts of between-subjects difference on results.

No adverse events nor clinically significant findings were observed in this study in relation to investigational tobacco product use, suggesting that no safety concerns are expected during short-term use of these HTPs.

We also compared outcome measures between the nonmenthol and menthol flavors of the DT3.0a (DT3.0a and mDT3.0a) groups as an exploratory approach. For all measured BoEs, the magnitude of reductions was not remarkably different between the DT3.0a and mDT3.0a groups, based on overlap of 95% CIs of the reductions. Overall, product consumption and nicotine uptake in the DT3.0a and mDT3.0a groups were stable over the exposure assessment period. No remarkable difference was observed between the DT3.0a and mDT3.0a groups for all subjective effects scores and nicotine PK parameters, based on overlap of 95% CIs of the mean changes. With respect to product safety, no exposure period AEs was observed for either of the DT3.0a or mDT3.0a groups. Overall, the present study showed no remarkable differences in any outcome measures on comparison between nonmenthol and menthol flavors of the DT3.0a (DT3.0a and mDT3.0a groups).

Limitations

Among the 15 BoEs measured in the present study, total NNAL has a longer half-life (10–18 days) than the other BoEs with half-lives of approximately 2 days or shorter. A larger decrease in total NNAL levels was reported as results of longer duration of switching from smoking to HTPs up to 360 days.[11,33,34] Therefore, the magnitude of total NNAL reduction might be limited in a short-term switching design of the present study. While the current confinement study design provides benefits to assess reduction in exposure when smokers exclusively switch to the assigned products while minimizing confounding factors as much as possible, results must be interpreted within the context of the study's limitations. For example, the study duration was short, while it is known that a user of tobacco products needs time to adapt their use behavior to a new product. Although consumption was relatively stable in HTP groups in this study, the possibility still remains that some of the subjects might not have fully adapted to their assigned HTPs. In addition, the study does not provide insight into the impact of flavor choices that may be made in the real world, where smokers can choose any flavor variants of HTP when switching from cigarettes. In the future, it would be beneficial to evaluate whether reduced HPHC exposure is associated with reduced risk of smoking-related diseases by evaluating candidate biomarkers of potential harm, as well as gathering scientific evidence of actual risk reduction under more real-world conditions.

Conclusions

Significant reductions in exposure to most of the selected HPHCs relative to smoking continuation were indicated from the present study with a 5-day confinement setting performed in adult healthy Japanese smokers. Reductions in exposure to most of the selected HPHCs observed in the novel HTP groups were similar in magnitude with those observed in the SS and in-market HTP/THS groups. Additionally, the result suggested that flavor difference would not have remarkable impacts on these outcome measures, when switching from cigarettes to the DT3.0a product within the same flavor group.

Acknowledgments

The authors are very grateful to Yuji Kumagai, MD, PhD; Lesley Giles, PhD; and employees of JT Scientific & Regulatory Affairs Division.

Conflicts of Interest

All authors are employees of Japan Tobacco Inc.

Funding

This study was funded by Japan Tobacco Inc as the sponsor. Japan Tobacco Inc. also designed the study and created the manuscript.

Data Availability Statement

The data relevant to this article will be shared on request.

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*. Centers for Disease Control and Prevention; 2014.
2. 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.
3. 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.
4. 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.
5. Rostron BL, Corey CG, Chang JT, van Bemmel DM, Miller ME, Chang CM. Changes in cigarettes per day and biomarkers of exposure among US adult smokers in the population assessment of tobacco and health study waves 1 and 2 (2013-2015). *Nicotine Tob Res*. 2020;22(10):1780-1787."
6. Haziza C, de La Bourdonnaye G, Merlet S, et al. Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: a randomized controlled study in confinement. *Regul Toxicol Pharmacol*. 2016;81:489-499.
7. Lüdicke F, Haziza C, Weitkunat R, Magnette J. Evaluation of biomarkers of exposure in smokers switching to a carbon-heated tobacco product: a controlled, randomized, open-label 5-day exposure study. *Nicotine Tob Res*. 2016;18(7):1606-1613.
8. Haziza C, de La Bourdonnaye G, Skiada D, et al. Biomarker of exposure level data set in smokers switching from conventional cigarettes to Tobacco Heating System 2.2, continuing smoking or abstaining from smoking for 5 days. *Data Brief*. 2017;10:283-293.
9. Lüdicke F, Baker G, Magnette J, Picavet P, Weitkunat R. Reduced exposure to harmful and potentially harmful smoke constituents with the tobacco heating system 2.1. *Nicotine Tob Res*. 2017;19(2):168-175.
10. Tran CT, Bosilkovska M, de La Bourdonnaye G, Blanc N, Haziza C. Reduced levels of biomarkers of exposure in smokers switching to the Carbon-Heated Tobacco Product 1.0: a controlled, randomized, open-label 5-day exposure trial. *Sci Rep*. 2020;10(1):19227.
11. Gale N, McEwan M, Eldridge AC, et al. Changes in biomarkers of exposure on switching from a conventional cigarette to tobacco heating products: a randomized, controlled study in healthy Japanese subjects. *Nicotine Tob Res*. 2019;21(9):1220-1227.
12. Yuki D, Takeshige Y, Nakaya K, Futamura Y. Assessment of the exposure to harmful and potentially harmful constituents in healthy Japanese smokers using a novel tobacco vapor product compared with conventional cigarettes and smoking abstinence. *Regul Toxicol Pharmacol*. 2018;96:127-134.
13. Yuki D, Kikuchi A, Suzuki T, et al. Assessment of the exposure to selected smoke constituents in adult smokers using in-market heated tobacco products: a randomized, controlled study. *Sci Rep*. 2022;12(1):18167.
14. Picavet P, Haziza C, Lama N, Weitkunat R, Lüdicke F. Comparison of the pharmacokinetics of nicotine following single and ad libitum use of a tobacco heating system or combustible cigarettes. *Nicotine Tob Res*. 2016;18(5):557-563.
15. Yuki D, Sakaguchi C, Kikuchi A, Futamura Y. Pharmacokinetics of nicotine following the controlled use of a prototype novel tobacco vapor product. *Regul Toxicol Pharmacol*. 2017;87:30-35.
16. Hashizume T, Ishikawa S, Matsumura K, Ito S, Fukushima T. Chemical and in vitro toxicological

comparison of emissions from a heated tobacco product and the 1R6F reference cigarette. *Toxicol Rep*. 2023;10:281-292.
17. Jaccard G, Tafin Djoko D, Moennikes O, Jeannet C, Kondylis A, Belushkin M. Comparative assessment of HPHC yields in the tobacco heating system THS2.2 and commercial cigarettes. *Regul Toxicol Pharmacol*. 2017;90:1-8.
18. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström test for nicotine dependence: a revision of the Fagerström tolerance questionnaire. *Br J Addict*. 1991;86(9):1119-1127.
19. Scherer G. Biomonitoring of inhaled complex mixtures–ambient air, diesel exhaust and cigarette smoke. *Exp Toxicol Pathol*. 2005;57(suppl 1):75-110.
20. Gregg EO, Minet E, McEwan M. Urinary biomarkers of smokers' exposure to tobacco smoke constituents in tobacco products assessment: a fit for purpose approach. *Biomarkers*. 2013;18(6): 467–486.
21. Schick SF, Blount BC, Jacob PR, et al. Biomarkers of exposure to new and emerging tobacco delivery products. *Am J Physiol Lung Cell Mol Physiol*. 2017;313(3):L425-L452.
22. 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.
23. 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.
24. Mariner DC, Ashley M, Shepperd CJ, Mullard G, Dixon M. Mouth level smoke exposure using analysis of filters from smoked cigarettes: a study of eight countries. *Regul Toxicol Pharmacol*. 2011;61(Suppl 3): S39-50.
25. Jones J, Slayford S, Gray A, Brick K, Prasad K, Proctor C. A cross-category puffing topography, mouth level exposure and consumption study among Italian users of tobacco and nicotine products. *Sci Rep*. 2020;10(1):12.
26. 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.
27. Lüdicke F, Picavet P, Baker G, et al. Effects of switching to the tobacco heating system 2.2 menthol, smoking abstinence, or continued cigarette smoking on biomarkers of exposure: a randomized, controlled, open-label, multicenter study in sequential confinement and ambulatory settings (part 1). *Nicotine Tob Res*. 2018;20(2): 161–172.
28. Ludicke F, Picavet P, Baker G, et al. Effects of switching to the menthol tobacco heating system 2.2, smoking abstinence, or continued cigarette smoking on clinically relevant risk markers: a randomized, controlled, open-label, multicenter study in sequential confinement and ambulatory settings (Part 2). *Nicotine Tob Res*. 2018;20(2): 173–182.
29. Ludicke F, Ansari SM, Lama N, et al. Effects of switching to a heat-not-burn tobacco product on biologically relevant biomarkers to assess a candidate modified risk tobacco product: a randomized trial. *Cancer Epidemiol Biomarkers Prev*. 2019;28(11): 1934–1943.
30. Haziza C, de La Bourdonnaye G, Donelli A, et al. Reduction in exposure to selected harmful and potentially harmful constituents approaching those observed upon smoking abstinence in smokers switching to the menthol tobacco heating system 2.2 for 3 months (Part 1). *Nicotine Tob Res*. 2020;22(4): 539–548.
31. Vansickel A, Baxter S, Sherwood N, Kong M, Campbell L. Human abuse liability assessment of tobacco and nicotine products: approaches for meeting current regulatory recommendations. *Nicotine Tob Res*. 2021;24(3): 295–305.
32. Brossard P, Weitkunat R, Poux V, et al. Nicotine pharmacokinetic profiles of the Tobacco Heating System 2.2, cigarettes and nicotine gum in Japanese smokers. *Regul Toxicol Pharmacol*. 2017;89: 193–199.
33. Gale N, McEwan M, Camacho OM, Hardie G, Proctor CJ, Murphy J. Changes in biomarkers after 180 days of tobacco heating product use: a randomised trial. *Intern Emerg Med*. 2021;16(8):2201-2212.
34. Gale N, McEwan M, Hardie G, Proctor CJ, Murphy J. Changes in biomarkers of exposure and biomarkers of potential harm after 360 days in smokers who either continue to smoke, switch to a tobacco heating product or quit smoking. *Intern Emerg Med*. 2022;17(7):2017-2030.

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.