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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)

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Abstract

Introduction: The menthol Tobacco Heating System 2.2 (mTHS) is a newly developed candidate modified-risk tobacco product intended to reduce exposure to the harmful and potentially harmful constituents (HPHCs) of conventional cigarette (CC) smoke. This study examined the impact of switching to mTHS on biomarkers of exposure to HPHCs relative to menthol CCs (mCCs) and smoking abstinence (SA).

Methods: In this three-arm, parallel-group study, 160 Japanese adult smokers (23–65 years; smoking ≥10 mCCs per day) were randomized to mTHS ($n = 78$), mCC ($n = 42$), or SA ($n = 40$) for 5 days in confinement and 85 days in ambulatory settings. Endpoints included biomarkers of exposure to HPHCs, human puffing topography, safety, and subjective effects of smoking measures.

Results: After 5 days of product use, the concentrations of carboxyhemoglobin, 3-hydroxypropylmercapturic acid, monohydroxybutenyl mercapturic acid, and S-phenylmercapturic acid were 55%, 49%, 87%, and 89% lower ($p < .001$), respectively, in the mTHS group than in the mCC group. Other biomarkers of exposure (measured as secondary endpoints) were 50%–94% lower in the mTHS group than in the mCC group on day 5. These reductions in the mTHS group were maintained at day 90, similar to the SA group. Switching to mTHS was associated with changes in human puffing topography (shorter puff intervals and more frequent puffs). The urge-to-smoke and smoking satisfaction levels on day 90 were similar in the mTHS and the mCC groups.

Conclusion: Switching from mCCs to mTHS significantly reduced exposure to HPHCs relative to continuing smoking mCCs with concentrations similar to those observed following SA in Japanese adult smokers.

Implications: This randomized study compared the impact of switching to a modified-risk tobacco product candidate mTHS on biomarkers of exposure to HPHCs of cigarette smoke relative to continuing smoking cigarettes or abstaining from smoking in sequential confinement and ambulatory settings. The study showed that switching to mTHS was associated with significant biomarker

reductions within 5 days in confinement, these reductions being maintained throughout the ambulatory setting up to day 90. The results provide evidence that switching to mTHS reduces real-life exposure to HPHCs in adult smokers.

Introduction

Several harm reduction strategies have been proposed to address the health risks associated with smoking cigarettes, including the development of modified-risk tobacco products (MRTPs) and electronic cigarettes.[1–4] The US Food and Drug Administration (FDA) recently issued draft guidance for industry on regulatory applications for MRTPs.[5] The draft guidance suggests that the applicants should examine several aspects of the product, including health risks, and the MRTP application "must provide scientific evidence to demonstrate that the product significantly reduces harm and the risk of tobacco-related disease to individual users." To achieve this objective, the health risks of MRTPs need to be assessed in a variety of settings, particularly regarding to the formation of harmful and potentially harmful constituents (HPHCs), toxicity in laboratory models, risk in laboratory models, exposure and risk in individual users, and population-level harm.[6]

As previously outlined,[7] an earlier version of the present menthol Tobacco Heating System menthol Tobacco Heating System 2.2 (mTHS 2.2), the Electronically Heated Cigarette Smoking System, was test-marketed in the United States (Accord®) and in Japan (Oasis®) in 1998 and 1999, respectively. Subsequently, THS 1.0 was developed, the tobacco stick still heated externally, at a peak temperature of the tobacco of approximately 550°C. The THS 1.0 was test-marketed in Switzerland, Japan, Australia, and Germany between 2006 and 2010. For both products, nicotine delivery has been shown being too low to satisfy consumers and/or to suppress smoking abstinence (SA) symptoms, despite attempts to compensate for the lower nicotine exposure by increasing the number of product uses.[8–15]

Incomplete withdrawal suppression with Accord® had already previously been reported.[16,17] Consumers also reported shortcomings in the sensory and taste characteristics as well as dislike of the somewhat bulky design.

The development of THS 2.2 addressed the previous shortcomings highlighted by consumers and further enhanced the physical and chemical characteristics of mTHS. The heating temperature is now <350°C, resulting in substantial reduction in exposure to HPHCs, while delivering nicotine comparable to cigarettes. Several studies have not only shown that THS 2.2 and its earlier prototypes reduce exposure to HPHCs in cells and animals,[18–20] but also in smokers.[12–14,21] These previous studies have been largely conducted in confinement and thus may not reflect regular long-term use in ambulatory settings. Accordingly, clinical studies are needed to confirm these earlier studies, and to provide evidence that MRTPs, like the THS, reduce exposure to HPHCs during extended use.

This study was part of a clinical assessment program on both the menthol and the regular THS variants. The regular variant has been assessed first in two 1-week confinement exposure reduction studies, one conducted in Poland (NCT01959932) and one in Japan (NCT01970982). The assessment of the regular variant under ambulatory conditions is also part of a 6-month exposure response study, currently underway in the United States (NCT02396381). The aim of the study reported here was to examine the impact of mTHS on biomarkers of exposure to HPHCs after 5 days of confinement and a further 85 days in an ambulatory setting in Japanese adult smokers to support prior studies in Eastern populations (NCT01780714 [unpublished data] and NCT01780688[21]). To achieve this aim, a three-arm parallel-group study design was used in which mTHS was compared against continuing smoking menthol conventional cigarette (mCC). The effects of switching to mTHS on biomarkers of exposure levels were examined in smokers, and SA was included as a gold standard for reducing the risks of smoking. The ambulatory period was included to examine the exposure–reduction effects of mTHS in real-life conditions.

Methods

This study was performed in accordance with International Conference on Harmonization, Good Clinical Practice, the Declaration of Helsinki guidelines,[22,23] and national regulations, and was approved by the local Institutional Review Board in July 2013 before starting the study. The study was conducted at the Tokyo Heart Center Osaki Hospital and registered at ClinicalTrials.gov (identifier NCT01970995).

Participants

Japanese smokers were recruited via the clinical site's database and via advertisements. Males and females aged 23–65 years with a body mass index of 18.5–32 kg/m^2 were eligible if they smoked ≥10 commercially available mCCs per day (self-reported) in the last 4 weeks (maximum yield of 1 mg nicotine per cigarette), and if they reported to have smoked mCCs for ≥3 years. Other eligibility criteria are listed in Supplementary Table 1.

Smokers of nonmenthol CCs were not eligible for this study to avoid a change in smoking patterns which is likely to result from switching a current smoker of nonmenthol CCs to a menthol product.[24]

Products

The mTHS (2.62 mg/stick of menthol, 1.21 mg/stick of nicotine, and 3.94 mg/stick of glycerin used as aerosol former, obtained under Health Canada Intense smoking regimen, maximum heating temperature 350°C) was used in this study (Supplementary Table 2). Reference products were mCCs of the participant's preferred commercially available brand.

Study Design and Interventions

The study comprised a 4-week screening period (days −30 to −3), a confinement period (days −2 to 6), an 85-day ambulatory period (days 6–91) (Supplementary Figure 1), and a 28-day safety follow-up period for the recording of spontaneously reported adverse events (AEs) or serious adverse events. On days −1 and 0, participants smoked their own brand of mCCs and underwent baseline assessments. On day 1, the participants were randomized to one of three groups in a 2:1:1 ratio to switch to mTHS (mTHS group), continue smoking mCCs (mCC group), or abstain from smoking (SA group), respectively. Randomization was performed with stratification by sex and daily average mCC consumption (10–19 vs. >19 mCCs/day). Between days 1 and 5, participants in

the mTHS and mCC groups used the allocated product *ad libitum* during the designated smoking hours (06:30 AM to 11:00 PM), while participants in the SA group completely abstained from smoking. During the 85-day ambulatory period, the participants returned to the study site and stayed overnight on the days 30, 60, and 90 visits.

Measurements

Supplementary Table 3 lists the study assessments and when the measurements were taken. Participants in each group were asked to record the use of CCs (menthol or nonmenthol), nicotine replacement therapy, or nicotine-/tobacco-containing products using an electronic diary. Compliance to SA was chemically verified using an exhaled CO breath test during the confinement and ambulatory periods. Twenty-four-hour urine and blood samples were collected daily between days −1 and 5, and on days 30, 60, and 90.

The primary endpoints to assess exposure reduction to HPHCs were: monohydroxybutenyl mercapturic acid (MHBMA), 3-hydroxypropylmercapturic acid (3-HPMA), S-phenylmercapturic acid (S-PMA), total 4 [methylnitrosamino]-1-[3-pyridyl]-1-butanol (total NNAL), and carboxyhemoglobin (COHb). Secondary endpoints to assess exposure reduction to HPHCs were: total 1-hydroxypyrene (total 1-OHP), total N-nitrosonornicotine (NNN), 4-aminobiphenyl (4-ABP), 1-aminonaphthalene (1-NA), 2-aminonaphthalene (2-NA), o-toluidine, 2-cyanoethylmercapturic acid (CEMA), 2-hydroxyethyl mercapturic acid (HEMA), 3-hydroxybenzo(a)pyrene (3-OH-B[a]P), 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA), and nicotine equivalents (NEQ). As various studies have reported overlapping ranges in S-benzylmercapturic acid (S-BMA) levels with only subtle differences observed between smokers and nonsmokers[25–27] and since excretion of S-BMA (BoExp to toluene) did not change across the three arms in this and other studies (NCT01959932 and NCT01970982), S-BMA results are not reported here.

Further details on the endpoints and quantification methods are provided in the Supplementary Materials (Methods and measurements; Supplementary Table 4).

CYP1A2 activity, involved in the activation of heterocyclic and aromatic amines, was measured on days 0, 5, and 90, based on the postdose PX and CAF plasma molar concentrations approximately 6 h (±15 min) after the intake of one Tomerumin® (LionCorp.) caffeine tablet (around 170 mg caffeine) with 150 ± 10 mL water.[28]

Exposure to genotoxic agents was measured by assessing urine mutagenicity on days 0, 5, and 90 by the reverse mutation assay (Ames assay) as revertants/24 h urine.

Spirometry was conducted at least 1 hour after smoking during the screening visit. Spirometry without a bronchodilator was performed prior to product use on days 0 (baseline values) and 6, and at the day 90 visit (day 91) for comparison with the baseline values.

Participant-reported outcomes/subjective effects of smoking were collected using diaries and validated questionnaires (Fagerström Test for Nicotine Dependence, modified Cigarette Evaluation Questionnaire [mCEQ], Questionnaire of Smoking Urges-brief [QSU-brief], visual analog scale for respiratory symptoms, Minnesota Nicotine Withdrawal Scale [MNWS], and Human Puffing Topography [HPT] Questionnaire).

HPT was evaluated using the HPT SODIM® device, model SPA/M (SODIM® Instrumentation, Fleury les Aubrais, France). Parameters measured included the number of puffs, puff volume, total volume, puff duration, and interpuff interval. The topography parameters were not recorded for participants who smoked CCs that were incompatible with the device (eg, slim cigarettes). On days 30, 60, and 90, HPT was assessed over a 4-hour period in the morning.

All clinical laboratory endpoints were measured at independent contract laboratories (Supplementary Table 4), and smoking topography was assessed at Philip Morris International R&D. The laboratories were blinded to the randomization scheme.

Statistical Analysis

The sample size was calculated based on the expected mTHS:mCC ratios of the concentrations of biomarkers of exposure, as observed in previous studies of heated tobacco products (NCT01780714 [unpublished data] and NCT00812279). A sample size of 160 participants randomized 2:1:1 to the mTHS, mCC, and SA groups, respectively, was considered sufficient to attain 80% power to show reductions of ≥50% in the biomarkers of exposure chosen as primary endpoints (total NNAL, COHb, MHBMA, 3-HPMA, and S-PMA) in the mTHS group compared with the mCC group using one-sided tests with 2.5% alpha level.

Additional details on the statistical methods are described in the Supplementary Materials (Supplementary statistical methods).

Results

Participants

The full analysis set comprised 160 participants, randomized as follows: 78 to switching to mTHS, 42 to continuing smoking mCCs, and 40 to SA, of which two, one, and two participants, respectively, voluntarily discontinued. The safety analysis ($n = 175$) contained the 15 subjects who tried the mTHS but were discontinued from enrolment, and thus, not randomized. The disposition of participants is presented in Figure 1.

The participants' characteristics are summarized in Table 1. All three groups were similar in terms of their baseline values. The majority of subjects (53.1%) had a Fagerström Test for nicotine dependence overall classification of moderate nicotine dependence and all subjects smoked noncharcoal filter menthol cigarettes with a maximum yield of 1 mg nicotine and 1–5 mg tar International Organization for Standardization (ISO) per cigarette.

Compliance to the allocated interventions was ensured by a strict distribution of the allocated products during the confinement period. Compliance was also high in the ambulatory period, with 70 (89.7%), 41 (97.6%), and 37 (92.5%) randomized participants in the mTHS, mCC, and SA groups, respectively; these participants were included in the per-protocol (PP) set at day 90. Although no incentives were offered to increase compliance to the allocated product, dual use of mTHS and mCC during the ambulatory period was limited, with an average daily use of fewer than 0.1 mCC in the mTHS group.

Biomarkers of Exposure

Table 2 lists the biomarkers of exposure measured as the primary and secondary endpoints at baseline, and on days 5 and 90 (geometric means and 95% confidence intervals [CI]). At baseline, the biomarkers of exposure assessed as part of the primary objective were comparable in all three groups, except for MHBMA concentrations, which were approximately 11% higher in the mCC group than in the mTHS and SA groups. The concentrations of COHb, 3-HPMA, MHBMA, and S-PMA on day 5 were approximately 55% (95% CI: 52.0, 57.9), 49% (95% CI: 42.8, 55.1), 87% (95% CI: 83.4, 89.0), and 89% (95% CI: 87.0, 90.7) lower ($p < .001$), respectively, in the mTHS group than in the mCC group. The total NNAL

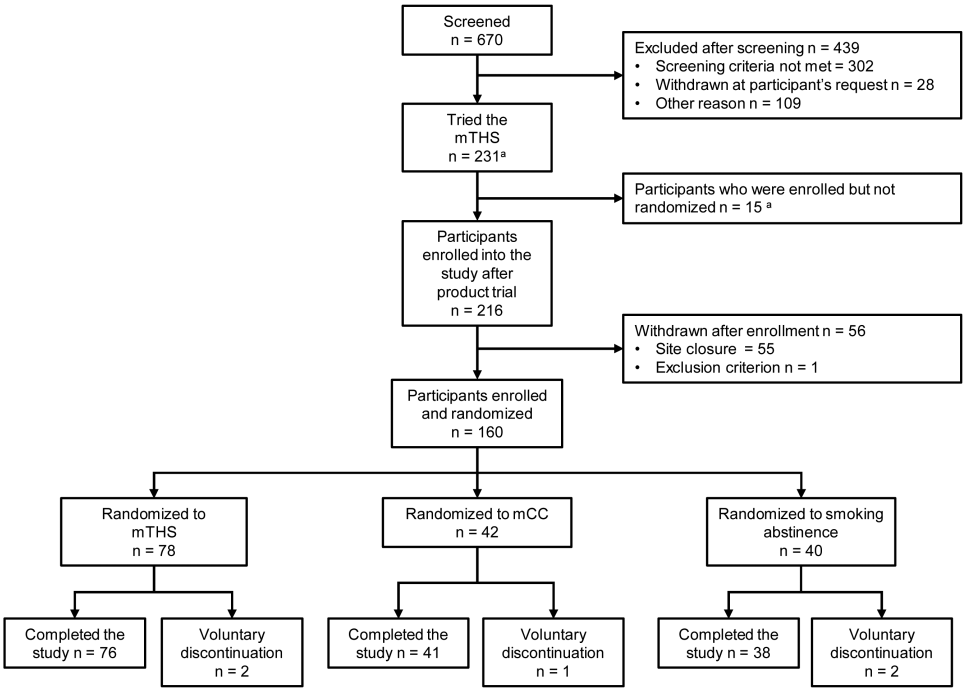

Figure 1. Participant disposition. mCC = menthol cigarettes; mTHS = menthol Tobacco Heating System 2.2. [a]Included in prerandomization safety analyses. The study was conducted between August 2013 and July 2014 at the Tokyo Heart Center Osaki Hospital, Tokyo, Japan.

concentration on day 90 was 77% (95% CI: 68.9, 82.6) lower ($p < .001$) in the mTHS group than in the mCC group (Figure 2). The reductions achieved with switching to mTHS in these endpoints were generally consistent with those observed in the SA group.

The biomarkers of exposure measured as secondary endpoints were 50%–94% lower in the mTHS group than in the mCC group on day 5. The reductions on day 5 were maintained through to day 90 (–41% to –94% mTHS vs. mCC) and the concentrations observed in the mTHS group approached those observed in the SA group. Day 5 nicotine exposure as assessed by the LS mean urinary NEQ concentration adjusted for creatinine was approximately 16% (95% CI: –1.1, 36.0) higher in the mTHS group than in the mCC group (Figure 2). This difference progressively reduced over time and on day 90 the NEQ concentration was comparable between the mTHS group and the mCC group (LS mean mTHS:mCC ratio: 104%; 95% CI: 66.7, 163.2). In contrast, in the SA group, the NEQ concentrations decreased from baseline to day 5 by 96.2% and remained stable until day 90.

CYP1A2

At baseline, CYP1A2 activity was similar in all three groups. On day 5, the LS mean CYP1A2 activity after product use was 28.04% lower in the mTHS group than in the mCC, and was comparable between the mTHS group and the SA group (LS mean ratio mTHS:SA 102.43%). On day 90, the LS mean CYP1A2 activity after product use decreased further in the mTHS group and was 30.91% lower in this group than in the mCC group, and was comparable between the mTHS group and the SA group (geometric LS mean ratio mTHS:SA 92.48%).

Ames

The Ames values showed high variability and a number of outliers. Between baseline and day 5, mean Ames assay values decreased from 14508 to 9237 rev/24 h in the SA group and from 17294 to 7500 rev/24 h in the mTHS group. This reduction in mutagenicity was sustained during the ambulatory period (8137 and 6761 rev/24 h in the SA and mTHS groups, respectively, at day 90). In the mCC group, the mean Ames assay values on days 5 and 90 were comparable to the baseline value. Similar trends were observed in terms of median values (Table 3).

Subjective Effects of Smoking

The results of the questionnaires assessing subjective effects are presented in Figure 3. As can be seen, the baseline scores of all questionnaires were comparable for the three study groups. The MNWS and QSU-brief scores were very high in the SA group during the confinement period but decreased all along the study, to reach scores even lower than those observed in the other study groups.

The mCEQ scores for the Craving Reduction, Enjoyment of Respiratory Tract Sensations, Psychological Reward, and Smoking Satisfaction subscales were lower in the mTHS group than in the mCC group from days 1 until 30. There was a negligible difference in the aversion subscale. From day 30 onwards, the subscale scores were comparable between the mTHS and mCC groups, remained stable afterwards and were similar to the baseline scores.

The QSU-brief total scores remained fairly stable in the mTHS and mCC groups throughout the confinement and ambulatory periods, albeit the scores were slightly higher in the mTHS group than in the mCC group. This difference was consistent with a baseline imbalance between the two groups, as confirmed by the ANCOVA results at day 90 (LS mean difference mTHS – mCC: 0.24; 95% CI: –0.25, 0.72). The QSU-brief total score increased markedly between days 1 and 5 in the SA group, but decreased thereafter, as expected, until the end of the study (LS mean difference mTHS – SA: 1.06; 95% CI: 0.56, 1.55).

The changes in the mean MNWS withdrawal scores were similar to those found in QSU-brief total scores.

Table 1. Participant Characteristics at Baseline and Product Consumption During the Study Periods

Variables	mTHS	mCC	SA	Total
N	78	42	40	160
Age (years)				
Mean ± *SD*	37.1 ± 10.58	37.4 ± 11.23	37.0 ± 9.96	37.2 ± 10.54
Range	23–65	23–64	23–55	23–65
BMI (kg/m^2)				
Mean ± *SD*	22.85 ± 2.963	22.44 ± 2.876	22.48 ± 3.386	22.65 ± 3.03
Range	18.7–32.7	18.9–28.4	18.5–31.8	18.5–32.7
Sex, *n* (%)				
Male	45 (57.7%)	25 (59.5%)	22 (55.0%)	92 (57.5%)
Female	33 (42.3%)	17 (40.5%)	18 (45.0%)	68 (42.5%)
Daily mCC consumption, cigarettes/day, *n* (%)				
10–19	40 (51.3)	23 (54.8)	21 (52.5)	84 (52.5)
>19	38 (48.7)	19 (45.2)	19 (47.5)	76 (47.5)
ISO tar yield, mg, *n* (%)				
1–5 mg	46 (59.0%)	22 (52.4%)	23 (57.5%)	91 (56.9%)
6–8 mg	21 (26.9%)	14 (33.3%)	12 (30.0%)	47 (29.4%)
9–10 mg	7 (9.0%)	4 (9.5%)	2 (5.0%)	13 (8.1%)
>10 mg	4 (5.1%)	2 (4.8%)	3 (7.5%)	9 (5.6%)
FTND total score				
Mean ± *SD*	4.3 ± 1.78	4.3 ± 1.81	4.7 ± 2.08	4.4 ± 1.86
Range	1–9	1–8	0–9	0–9
Number of tobacco sticks/CCs used per day, mean ± *SD* (*n*)[a]				
Confinement period				
Day 0 (CCs)	13.1 ± 3.83 (76)	12.5 ± 3.87 (42)	12.8 ± 3.95	—
Day 1	11.4 ± 3.91 (76)	11.0 ± 4.01 (42)	—	—
Day 2	12.0 ± 4.14 (76)	12.5 ± 4.16 (41)	—	—
Day 3	12.1 ± 3.76 (76)	12.1 ± 4.17 (41)	—	—
Day 4	12.4 ± 3.84 (76)	11.3 ± 3.96 (41)	—	—
Day 5	13.9 ± 4.33 (76)	13.6 ± 4.68 (41)	—	—
Ambulatory period				
Days 6–30	11.7 ± 5.95 (74)	13.8 ± 4.16 (41)	—	—
Days 30–60	12.7 ± 6.25 (71)	14.9 ± 5.70 (41)	—	—
Days 60–90	12.7 ± 6.48 (70)	15.2 ± 5.04 (41)	—	—

BMI = body mass index; FTND = Fagerström Test for Nicotine Dependence (Revised Version); ISO = International Organization for Standardization; mCC = menthol cigarettes; mTHS = menthol Tobacco Heating System 2.2; SA = smoking abstinence; *SD* = standard deviation.
[a]Per-protocol set.

Human Puffing Topography

The results of the HPT assessments are presented in Figure 4. At baseline, the total smoking duration was not different in the mTHS and mCC groups, and remained constant during the confinement period in the mCC group but decreased in the mTHS group from days 1 to 4. During the ambulatory period, the total smoking duration decreased in both groups between days 4 and 90. At baseline, the total number of puffs drawn by the participants was slightly higher in the mTHS group than in the mCC group and remained constant during the confinement period in both groups. On day 90, the total number of puffs drawn by the participants remained higher in the mTHS group than in the mCC group. The average puff interval at baseline was comparable in the mTHS and mCC groups, and decreased during the confinement period in the mTHS group but not in the mCC group. During the ambulatory period, the average inter puff interval decreased further in the mTHS group until day 90 with only a slight decrease in the mCC group. The average puff intervals at days 4 and 90 were shorter in the mTHS group than in the mCC group. The total puff volume and average puff volume decreased in the mTHS group from Baseline to day 1 before recovering slightly until day 4. Overall, the total puff volume was comparable in the mTHS and mCC group on day 90, but the average puff volume was lower in the mTHS group than in the mCC group.

Safety

Postrandomization, 60/160 (37.5%) participants experienced 93 AEs, of which 21 AEs occurred in 15 participants (9.4%) in confinement and 72 AEs occurred in 52 participants (32.5%) in the ambulatory period. Only one AE in the SA group during confinement and one in the mCC group during the ambulatory period were classified as moderate; none as severe. One AE (diarrhea) was considered to be related to mTHS and six were related to study procedures.

Supplementary Table 5 lists the most common AEs in each group. AEs that occurred in ≥5% of participants in any group included decreased hemoglobin, decreased neutrophils, increased blood triglycerides, nasopharyngitis, and vertigo.

There were no clinically relevant abnormalities in vital signs, electrocardiograms, spirometry, or physical examinations, apart from an increased bodyweight of 2.5 kg (95% CI: 1.57, 3.46) at day 90 in the SA group compared with no change in the mTHS and mCC groups.

Table 2. Geometric Means (95% CI) of Biomarkers of Exposure at Baseline, Day 5, and Day 90 (Per-Protocol Population)

	mTHS	mCC	SA
Total NNAL (pg/mg Cr)[a,b]			
Baseline	85.64 (72.96, 100.51)	84.77 (68.88, 104.33)	79.54 (61.76, 102.42)
Day 5	37.90 (32.29, 44.48)	85.94 (70.93, 104.13)	29.58 (22.24, 39.35)
Day 90	23.23 (19.34, 27.91)	95.03 (77.31, 116.82)	13.95 (9.00, 21.60)
Total NNN (pg/mg Cr)			
Baseline	4.45 (3.38, 5.86)	3.97 (2.87, 5.47)	4.13 (2.84, 6.00)
Day 5	1.20 (0.97, 1.49)	4.10 (2.94, 5.73)	0.15 (0.12, 0.18)
Day 90	1.40 (1.13, 1.73)	4.28 (3.03, 6.05)	0.26 (0.17, 0.40)
COHb (%)[b]			
Baseline	5.11 (4.75, 5.49)	5.17 (4.70, 5.70)	5.15 (4.72, 5.62)
Day 5	2.48 (2.40, 2.57)	5.55 (5.06, 6.08)	2.50 (2.38, 2.64)
Day 90	2.97 (2.88, 3.06)	5.73 (5.24, 6.25)	3.04 (2.84, 3.26)
MHBMA (pg/mg Cr)[b]			
Baseline	653.78 (530.04, 806.39)	737.29 (554.67, 980.04)	614.87 (451.06, 838.16)
Day 5	81.71 (75.52, 88.41)	622.58 (454.60, 852.64)	80.72 (70.92, 91.88)
Day 90	141.74 (120.62, 166.57)	785.27 (576.82, 1069.04)	136.83 (114.40, 163.66)
3-HPMA (ng/mg Cr)[b]			
Baseline	667.53 (599.28, 743.54)	642.20 (552.68, 746.21)	691.14 (587.29, 813.34)
Day 5	304.68 (284.63, 326.14)	591.33 (507.72, 688.69)	186.71 (163.39, 213.36)
Day 90	386.37 (356.30, 418.97)	695.58 (602.43, 803.13)	276.13 (242.11, 314.93)
S-PMA (pg/mg Cr)[b]			
Baseline	1058.84 (857.94, 1306.79)	1096.79 (823.05, 1461.57)	1027.37 (751.76, 1404.03)
Day 5	118.36 (107.37, 130.48)	1096.47 (805.13, 1493.22)	102.51 (85.19, 123.34)
Day 90	145.58 (121.67, 174.18)	1157.25 (848.59, 1578.17)	144.07 (109.87, 188.92)
Total 1-OHP (pg/mg Cr)[c]			
Baseline	153.98 (138.85, 170.75)	164.33 (143.20, 188.58)	148.01 (127.26, 172.14)
Day 5	46.36 (41.68, 51.55)	122.90 (104.71, 144.26)	41.14 (35.42, 47.78)
Day 90	85.47 (76.64, 95.33)	167.38 (146.23, 191.58)	88.21 (75.53, 103.01)
4-ABP (pg/mg Cr)			
Baseline	9.33 (8.44, 10.32)	8.75 (7.44, 10.29)	7.99 (6.57, 9.71)
Day 5	1.97 (1.76, 2.21)	9.50 (8.15, 11.07)	2.16 (1.87, 2.50)
Day 90	2.07 (1.82, 2.36)	9.62 (8.12, 11.39)	2.35 (1.90, 2.89)
1-NA (pg/mg Cr)			
Baseline	61.45 (55.12, 68.52)	57.24 (49.04, 66.80)	53.48 (44.92, 63.68)
Day 5	3.14 (2.85, 3.46)	53.27 (45.86, 61.89)	2.85 (2.50, 3.26)
Day 90	3.55 (2.96, 4.26)	55.34 (46.21, 66.26)	4.22 (3.20, 5.55)
2-NA (pg/mg Cr)			
Baseline	15.49 (13.82, 17.37)	15.32 (13.13, 17.87)	13.64 (11.43, 16.28)
Day 5	1.97 (1.80, 2.15)	14.23 (12.18, 16.62)	2.04 (1.82, 2.28)
Day 90	2.34 (2.11, 2.59)	14.84 (12.63, 17.44)	2.63 (2.20, 3.15)
o-tol (pg/mg Cr)			
Baseline	128.19 (112.28, 146.36)	136.04 (107.42, 172.27)	120.54 (96.23, 150.98)
Day 5	51.64 (45.52, 58.59)	127.28 (103.27, 156.88)	48.82 (40.94, 58.21)
Day 90	68.35 (53.91, 86.67)	125.64 (96.13, 164.20)	77.86 (56.72, 106.88)
CEMA (ng/mg Cr)			
Baseline	75.32 (66.47, 85.36)	75.19 (62.27, 90.80)	76.74 (63.97, 92.05)
Day 5	12.43 (11.12, 13.90)	68.17 (56.39, 82.40)	11.78 (9.84, 14.10)
Day 90	7.91 (6.74, 9.29)	83.98 (69.17, 101.95)	8.41 (5.99, 11.81)
HEMA (pg/mg Cr)			
Baseline	3203.95 (2699.53, 3802.62)	3148.47 (2465.16, 4021.17)	3201.31 (2477.20, 4137.07)
Day 5	1137.96 (995.50, 1300.81)	2235.37 (1742.88, 2867.03)	1113.73 (923.72, 1342.83)
Day 90	1741.53 (1510.19, 2008.30)	3739.46 (2858.39, 4892.12)	1633.12 (1286.77, 2072.69)
3-HMPMA (ng/mg Cr)			
Baseline	300.07 (266.94, 337.32)	298.73 (256.46, 347.96)	298.08 (258.32, 343.96)
Day 5	124.47 (115.36, 134.30)	286.80 (251.37, 327.21)	113.48 (99.38, 129.59)
Day 90	154.30 (137.07, 173.70)	299.41 (260.62, 343.97)	158.57 (132.95, 189.14)
3-OH-B[a]P (fg/mg Cr)			
Baseline	83.73 (70.69, 99.18)	82.00 (67.42, 99.71)	71.96 (59.20, 87.47)
Day 5	20.72 (18.61, 23.07)	75.10 (62.60, 90.08)	17.84 (15.45, 20.58)
Day 90	30.02 (25.29, 35.65)	86.92 (71.78, 105.27)	28.88 (22.56, 36.98)

Table 2. *Continued*

	mTHS	mCC	SA
NEQ (mg/g Cr)[d]			
Baseline	5.71 (5.08, 6.41)	5.56 (4.64, 6.65)	5.40 (4.43, 6.59)
Day 5	6.16 (5.55, 6.83)	5.22 (4.35, 6.27)	0.16 (0.12, 0.20)
Day 90	6.85 (5.96, 7.88)	6.33 (5.11, 7.84)	0.37 (0.18, 0.78)

The bioanalytical procedures are described in the Supplementary Materials.

CEMA = 2-cyanoethylmercapturic acid; COHb = carboxyhemoglobin; Cr = creatinine; HEMA = 2-hydroxyethylmercapturic acid; 3-HPMA = 3-hydroxypropylmercapturic acid; 3-HMPMA = 3-hydroxy-1-methylpropylmercapturic acid; MHBMA = monohydroxybutenyl mercapturic acid; mTHS = menthol Tobacco Heating System 2.2; mCC = menthol cigarettes; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 3- NNN = N-nitrosonornicotine; 1-OHP = 1-hydroxypyrene; 4-ABP = 4-aminobiphenyl; 1-NA = 1-aminonaphtalene; 2-NA = 2-aminonaphthalene; o-tol = o-toluidine; 3-OH-B[a]P = 3-hydroxy(a)benzopyrene; NEQ = nicotine equivalent; SA = smoking abstinence; S-PMA = S-phenylmercapturic acid.

[a]Total NNAL was determined as the molar sum of 4-(methylnitrosamino)-1-(3-pyridy1)-1-butanol and its O-glucuronide conjugate.

[b]Primary endpoint.

[c]1-OHP was determined as the molar sum of 1-hydroxypyrene and its glucuronide and sulfate conjugates.

[d]NEQ was determined as the molar sum of nicotine, cotinine, and trans-3′-hydroxycotinine plus their respective glucuronide conjugates.

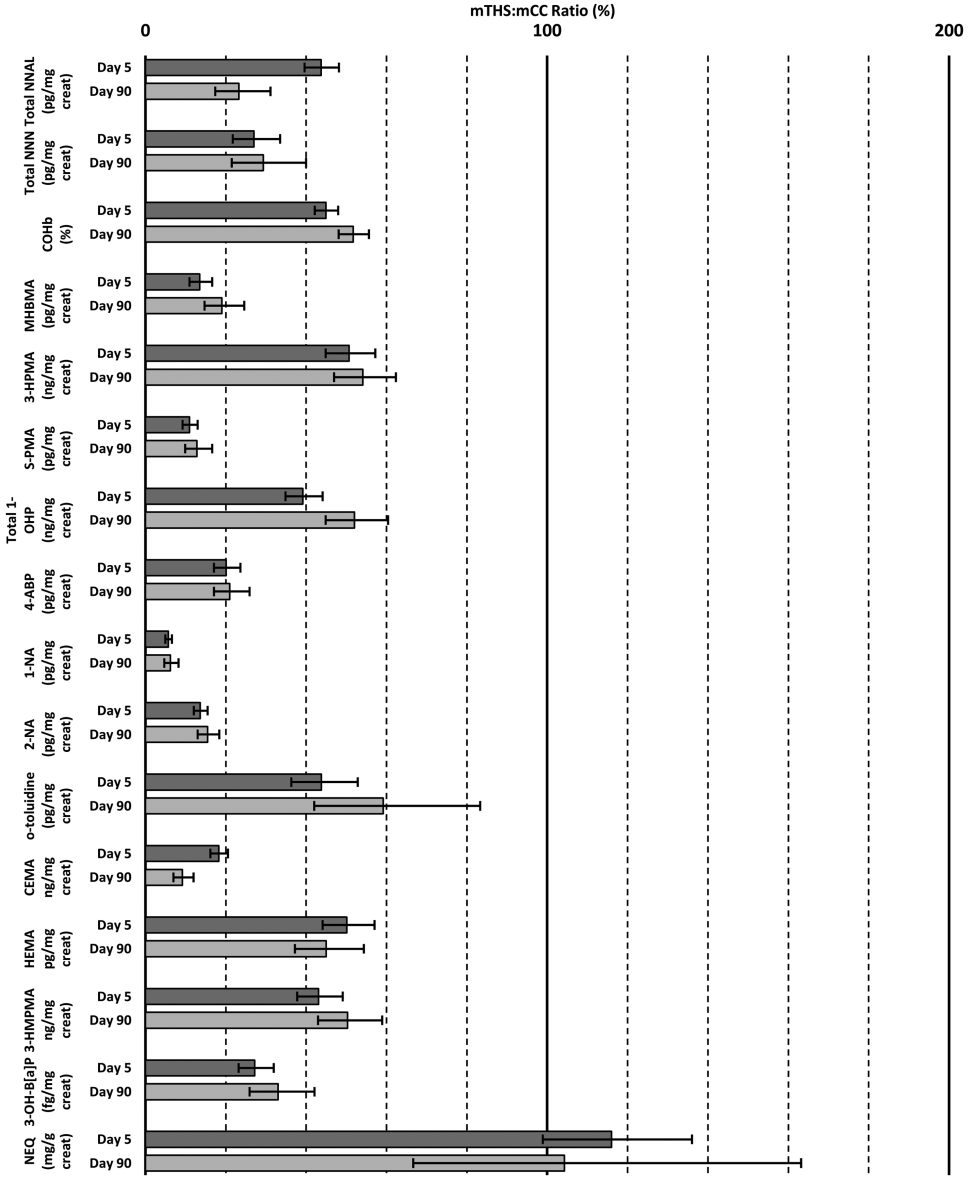

Figure 2. mTHS:mCC ratios (%) and 95% confidence intervals calculated at day 5 (dark grey) and day 90 (light gray) for the PP population. Supplementary Table 4 contains the full list of biomarkers of exposure and abbreviations. PP = per-protocol.

Table 3. Results of the Ames Test

Group	Timepoint	*N*	Mean	*SD*	Min	Median	Max
mTHS	Baseline	65	17 294	12 543	0	13 944	51 505
	Day 5	73	7500	8886	0	4856	47 872
	Day 90	70	6761	6689	0	5400	47 872
mCC	Baseline	38	15 132	10 702	2332	13 236	51 400
	Day 5	40	13 477	7826	0	13 579	39 633
	Day 90	40	17 204	12 258	0	13 193	47 824
SA	Baseline	35	14 508	10 212	0	14 000	44 541
	Day 5	37	9237	10 000	0	6386	44 541
	Day 90	37	8137	8523	0	4977	35 588

Results are expressed as revertants in 24-hour urine samples. mTHS = menthol Tobacco Heating System 2.2; mCC = menthol cigarettes; SA = smoking abstinence; *SD* = standard deviation.

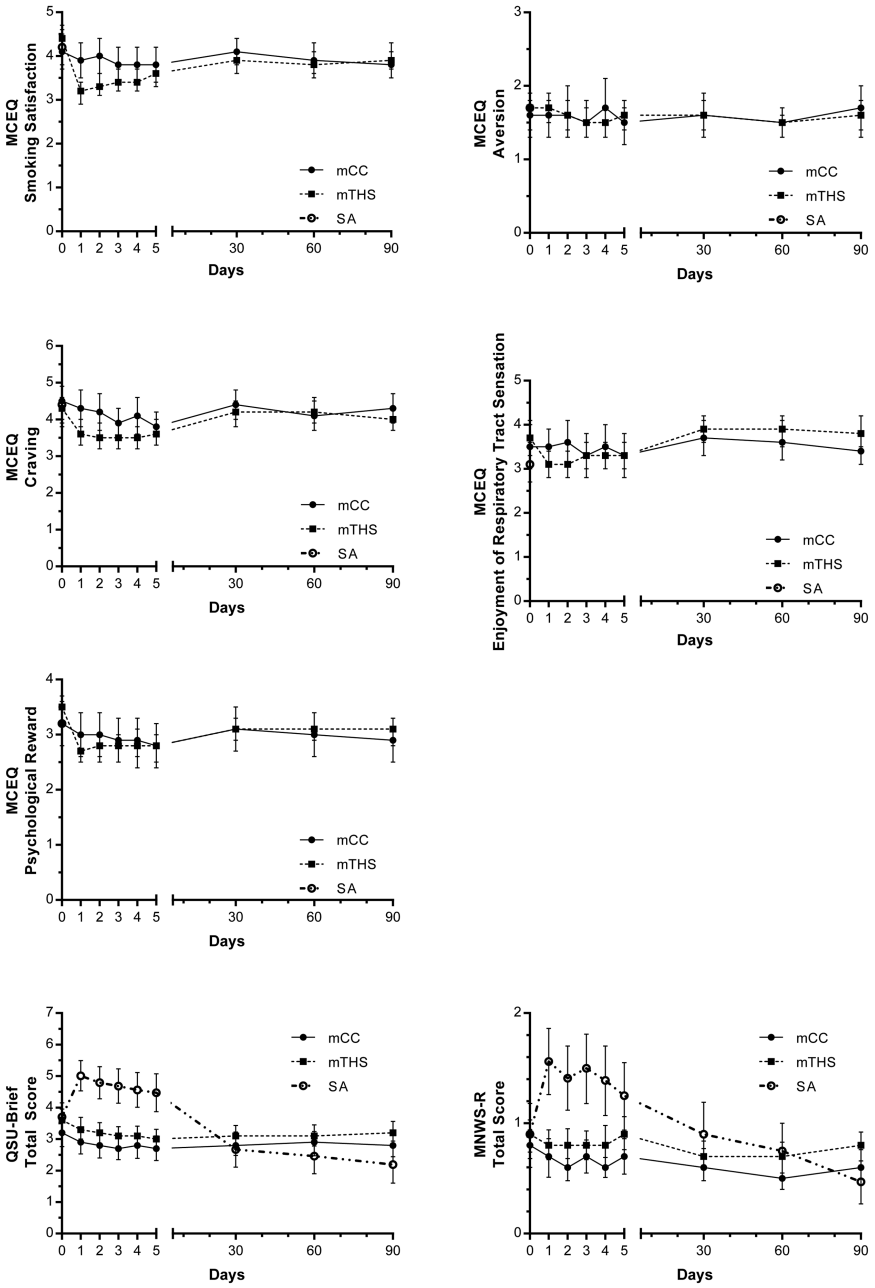

Figure 3. Subjective effects of smoking (means and 95% CIs). mCEQ = modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine Withdrawal Scale; mTHS = menthol Tobacco Heating System 2.2; mCC = menthol cigarette; QSU = Questionnaire on Smoking Urges; SA = smoking abstinence.

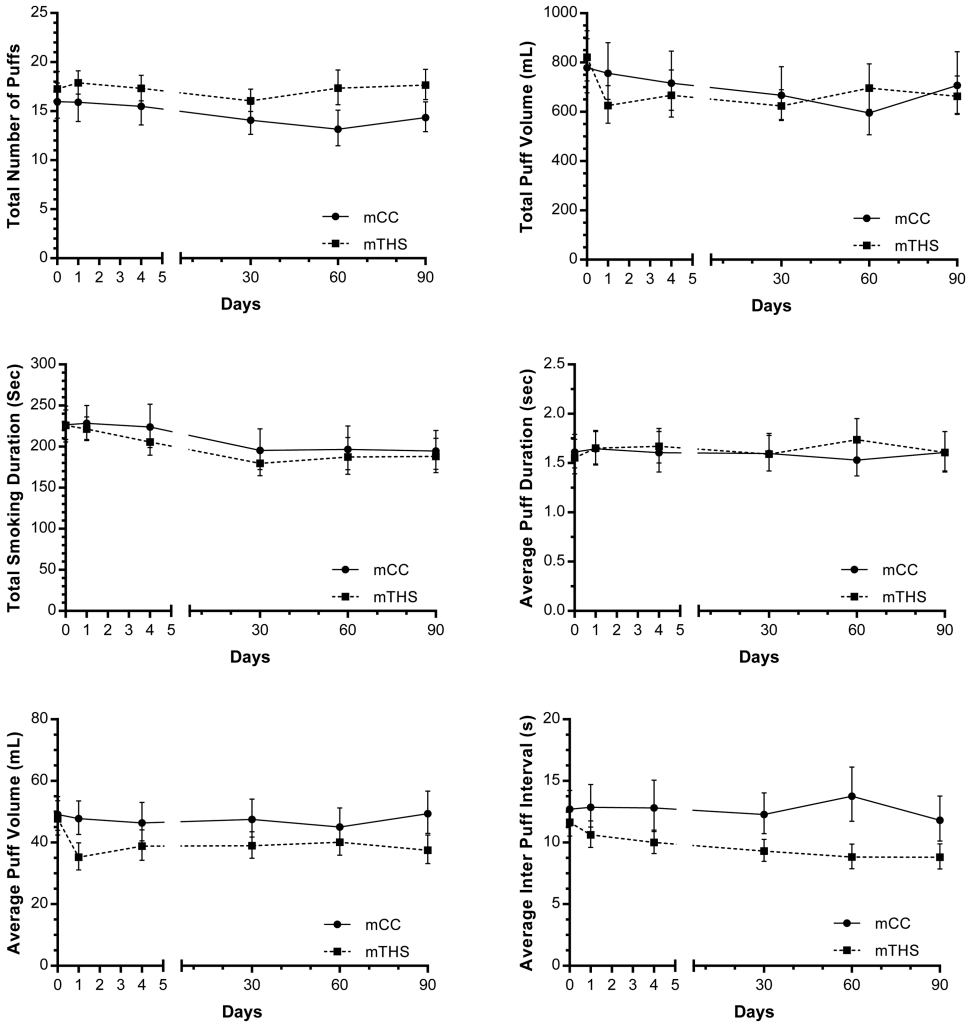

Figure 4. Human puffing topography (geometric means and 95% CIs). mTHS = menthol Tobacco Heating System 2.2; mCC = menthol cigarette; SA = smoking abstinence.

Discussion

The mTHS was developed to reduce or eliminate the formation of HPHCs in the aerosol through heating and not burning tobacco, while preserving the taste, sensory experience, nicotine delivery profile, and ritual characteristics of mCC as much as possible.

This study was conducted as part of the global clinical program for THS and was designed to demonstrate exposure reduction to HPHCs contained in cigarette smoke relative to continuing to smoke mCCs. The SA group was included to provide a benchmark of the reduction in exposure possible with smoking cessation.

The study showed that switching from mCCs to mTHS was associated with clear reductions in systemic exposure to HPHCs relative to continuing to smoke mCCs, with the reductions in the exposure profiles in the mTHS group following similar patterns to those observed in the SA group. The reductions were apparent within 5 days of product use in confinement and were maintained for a further 85 days in ambulatory settings in the mTHS group, consistent with the lower concentrations of HPHCs in the THS aerosol relative to the CC aerosol.[18–21] In view of the fact that product use was strictly monitored during confinement, relatively high COHb concentration of approximately 2.5% were found on day 5 in both the mTHS and in the SA groups after having decreased from about 5.1% at baseline. As reference ranges of 1–3% have been reported,[29] the observed levels appear still normal and possibly reflect environmental exposure.

Despite the high variability associated with the Ames test, there was a clear trend in the decrease in carcinogens of tobacco smoke after switching to mTHS as compared with those continuing mCC, with a similar trend in the SA group. These findings provide additional evidence of reduced exposure when switching to mTHS, because the Ames assay is indicative of exposure to genotoxic agents. The reduction in urine mutagenicity as soon as 5 days after switching to mTHS is in agreement with the published half-life of smoking-related urine mutagenicity (approximately 7–23 hours).[30] The source of the high variability of the urine mutagenicity values is likely due to (1) the relative high sensitivity of this assay to diet, as previously reported,[31] even in nonsmokers, (2) individual metabolic differences, and (3) variability of the cellular-based assay itself.[32]

CYP1A2 is involved in the activation of carcinogenic heterocyclic and aromatic amines.[33] These active metabolites (N-acetoxy derivatives) can react with DNA to form covalent heterocyclic amine-DNA adducts.[34] The extent of tumor induction resulting from these DNA adducts is dependent on the amount of aromatic or heterocyclic amines converted to the reactive, carcinogenic metabolites, which

is in turn dependent on CYP1A2 activity and likely to enhance the risk of tobacco-related cancers.[35] The induction of CYP1A2 activity is largely driven by polycyclic aromatic hydrocarbons present in cigarette smoke.[36] This study has shown that exposure to polycyclic aromatic hydrocarbon such as B[a]P was reduced by about 77% at the end of the exposure period, which likely explains the approximately 31% reduction in CYP1A2 activity in the mTHS group, similar to the reduction in the SA group, as compared with mCC. This further supports the potential of mTHS to lower the risk of certain tobacco-related cancers. In line with these data from human exposure, in a previously reported study conducted in Apoe$^{-/-}$ mice for 8 months, cigarette smoke induced both gene, and protein expression of CYP1A2 in the liver (the main site of CYP1A2 expression), while exposure to THS aerosol did not. Furthermore, switching to THS aerosol following cigarette smoke exposure led to a reduction in CYP1A2 gene and protein expression to levels approaching those of cessation.[37]

Several other MRTPs are under development and have been evaluated in randomized studies. In a study by Ogden et al.[38,39] adult smokers were switched to tobacco-heating cigarettes, snus, and ultralow machine yield tobacco-burning cigarettes. They noted that switching to these products achieved meaningful reductions in exposure to many potentially harmful constituents of cigarette smoke. Likewise, Miura et al.[40] compared a noncombustion inhaler-type product to CCs containing 1 mg tar, and to SA for 4 weeks in a residential setting in Japanese adult male smokers. They reported that switching to the noncombustion inhaler-type product achieved significant reductions in 14 biomarkers of exposure, and that the concentrations of these biomarkers (except for nicotine and NNK) after 29 days were similar to those in the SA group. Sakaguchi et al.[41] compared the effects of a prototype heated cigarette with those of CCs containing 10 mg tar for 4 weeks in a residential setting in Japanese adult smokers. They also reported that switching to the heated cigarette markedly lowered the biomarkers of exposure to nicotine, benzene, 1,3-butadiene, acrolein, hydrogen cyanide, crotonaldehyde, NNK, pyrene, and 4-aminobiphenyl, but not carbon monoxide compared with continuing CCs.

Clinical studies can indicate adult smoker acceptance of the product through measures such as nicotine pharmacokinetics and smoker satisfaction questionnaires. A recently reported nicotine pharmacokinetic study showed that the THS replicates the nicotine delivery profile of cigarettes, indicating that it has the potential to be an effective substitute for CCs.[21] Since other factors such as taste, flavor, and sensory experience are also important for adult smoker's longer-term acceptance, additional tools to measure subjective effects such as mCEQ, QSU-brief, and MNWS were applied in this study. The results indicate that the mTHS delivered similar levels of acceptability than the smoker's usual cigarettes, at 5 and 90 days after switching, and could substitute for the accustomed nicotine concentrations at the beginning of the study. This is also reflected in the high compliance level in the mTHS group during the ambulatory period. As compliance with SA was also high, cultural effects may as well have exerted a favorable influence in the present Japanese study population. Other than in typical smoking cessation studies, where essentially abstinence is ascertained, the present design included regular site visits with comprehensive assessments, including physical examinations as well as urine and blood sampling. This may also have contributed to the high compliance levels.

The switch to mTHS led to relatively small changes in smoking topography. Smaller and more frequent puffs with a shorter inter puff interval and a lower average puff volume were taken with the mTHS than with mCC to achieve a comparable total puff volume on day 90. The number of tobacco sticks used per day in the mTHS group on day 90 was slightly lower than the number of mCCs used per day in the mCC group. The mCEQ, MNWS, and QSU-brief questionnaire scores observed in the mTHS and mCC groups were very close, with a maximum difference of 0.5 points between the two groups, and a stabilization of the different scores noticeable after a few days of confinement. The findings on the mCEQ, QSU-brief, and MNWS, the changes in puffing topography and the number of products used per day indicate that smokers found the mTHS an acceptable alternative to combustible cigarettes.

The incidence of AEs was relatively low and only one (diarrhea) was considered related to the mTHS.

There are limitations of this study that warrant mentioning, including the potential for dual use in the mTHS group and the opportunity to resume mCC smoking in the SA group in the ambulatory period, which could have confounded the results. However, these limitations are addressed by the study design, comprising sequential confinement, and ambulatory periods, and by the fact that the exposure reduction seen under very controlled conditions was sustained in the ambulatory, more real-life environment. Furthermore, cigarette smoking in the SA group was low, as demonstrated by subjective (participant-reported) and objective (biomarkers of exposure) data.

Overall, the study design and methodology supports the robustness of the finding that switching to mTHS led to significant reductions in biomarker levels within 5 days in confinement, which were maintained throughout the ambulatory setting up to day 90, thus providing evidence that switching to mTHS reduces real-life exposure to HPHCs in adult smokers.

Conclusions

In conclusion, the results of this study indicate that switching from mCCs to mTHS was associated with significant reductions in biomarkers of exposure to HPHCs relative to continuing mCCs in Japanese smokers. The impact of switching to the mTHS on biologically relevant risk markers is described in a separate publication.

Supplementary Material

Supplementary Tables 1–5 and Figure 1 can be found online at http://www.ntr.oxfordjournals.org

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Declaration of Interests

All authors are employees of Philip Morris Products S.A.

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