



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



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ABSTRACT

Smoking conventional cigarettes (CCs) exposes smokers to harmful and potentially harmful constituents (HPHCs). The Tobacco Heating System 2.2 (THS 2.2), a candidate modified risk tobacco product, was developed to reduce or eliminate the formation of HPHCs, while preserving as much as possible the taste, sensory experience, nicotine delivery profile and ritual characteristics of CC. This randomized, controlled, open-label study in confinement for 5 day exposure aimed to demonstrate the reduction in exposure to selected HPHCs, to assess nicotine uptake and subjective effects, in participants switching to THS 2.2 ($n = 80$) compared to participants continuing smoking CCs ($n = 40$) and abstaining from smoking ($n = 40$). The subjects were randomized according to sex and daily CC consumption. The levels of biomarkers of exposure to HPHCs were significantly reduced in participants switching to THS 2.2, compared to CC use. More importantly, the magnitude of exposure reduction observed was close to that which was seen in participants who abstained from smoking for 5 days, while nicotine uptake was maintained. Reduction in urge-to-smoke was comparable between THS and CC groups, however THS 2.2 was slightly less satisfactory than CCs. The new, alternative tobacco product THS 2.2 was well tolerated.

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1. Introduction

Conventional cigarettes (CCs) cause serious diseases and the best way to reduce the adverse health consequences of smoking is to quit tobacco use. Although smoking prevalence has declined in many countries over the last decades, millions of adults continue to smoke CCs. Based on the World Health Organization's own predictions, there will be more than one billion smokers by the year 2025. Furthermore, success rates of currently available approaches to quit CC smoking are low. Recognizing this, the policy of tobacco harm reduction – making less harmful products available to smokers who would otherwise continue smoking – has been put forward and is supported by a multitude of stakeholders, including public health organizations, healthcare professionals and

regulators, to complement existing smoking prevention and cessation strategies. The Tobacco Advisory Group of the Royal College of Physicians opined in 2016 that “if nicotine could be delivered effectively and acceptably to smokers without smoke, most if not all of the harm of smoking could probably be avoided” (Royal College of Physicians, 2016).

In line with the current tobacco harm reduction strategy, Philip Morris International (PMI) is developing reduced risk products which replicate the sensorial, ritual and taste attributes of CCs as much as possible, while delivering a respirable aerosol that provides users with a comparable nicotine delivery to CCs but that is significantly less harmful than CC smoke and, therefore, potentially reduces the risk of smoking related diseases.

One of these products is THS 2.2, a heat-not-burn product which marks an important milestone in the evolution of heat-not-burn technology. The clinical study reported in this paper is part of a global clinical program to assess exposure and disease risk reduction associated with the use of THS, one of PMI's reduced risk

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products, also referred to as a candidate modified risk tobacco product by the U.S. Food and Drug Administration (FDA) (FDA (Food and Drug Administration) (2012a)). The study aimed to demonstrate exposure reduction to a selected set of HPHCs when switching from CCs to THS 2.2, as compared to continued CC use and smoking abstinence (SA) for 5 days.

2. Material and methods

2.1. Participants

Adult healthy Japanese smokers, 23–65 years old, were eligible if they smoked ≥ 10 commercially available non-menthol CCs per day with a maximum yield of 1 mg nicotine per CC (ISO yield) for the last 4 weeks and had smoked CC for ≥ 3 consecutive years prior to enrollment. Study participants were recruited via the clinical site's database and through advertisements. Before participation in the study, all participants provided written informed consent and underwent screening procedures, such as physical examination and medical check-up. Only candidates not willing to quit smoking in the forthcoming 3 months, but ready to accept a 5-day smoking interruption could enroll in the study. Participants with clinically relevant medical conditions, or who potentially required medical interventions (start of treatment, surgery, or hospitalization), participants with a history of alcohol and/or drug abuse, or who used nicotine-containing products other than their own brand of CCs, as well as pregnant or breast feeding female subjects and female unwilling to use acceptable methods of effective contraception, were not allowed to participate. All participants were informed that they were free to withdraw from the study at any time; participants willing to quit smoking after enrolment were encouraged to do so and referred to a smoking cessation counselor to receive appropriate smoking cessation support. Participants were compensated for their participation in the study.

2.2. Study design

This study was a controlled, randomized, 3 arm parallel, single-center study in confinement. The Screening Period covered a maximum of 4 weeks (Day -30 to Day -3) prior to admission and enrollment to the study site on Day -2. All subjects tested THS 2.2 using up to 3 THS Tobacco Sticks prior to enrollment. In female participants, the THS 2.2 product test was performed only after a negative urine pregnancy test. After all inclusion/exclusion criteria had been met, eligible candidates were enrolled and confined under medical supervision until Discharge on Day 6. On Day -1 and Day 0 (Baseline), participants smoked their own preferred brand of CCs and baseline assessments were performed. On Day 0, 160 participants were randomized with stratification by sex and average self-reported daily CC consumption over 4 weeks prior to enrollment (10–19 CC vs. >19 CC per day) in a 2:1:1 randomization ratio to THS 2.2 use ($n = 80$), CC smoking ($n = 40$) or to abstain from smoking ($n = 40$). From Day 1 to Day 5, participants in the THS 2.2 and CC groups used THS 2.2 or their own brand of non-menthol CCs, respectively, and exclusively. Participants in the SA arm were asked to abstain from smoking. The use of nicotine replacement therapy was not allowed. After Discharge on Day 6, or in case of an early discontinuation, participants entered a 7-day Safety Follow-Up Period for recording of spontaneously reported new adverse events (AEs) or serious adverse events (SAEs), and follow-up of any ongoing AEs/SAEs occurred during confinement (Fig. 1). During the designated smoking hours from 06:30 to 23:00, CC smoking was allowed *ad libitum* on Day -1 and Day 0, and depending on the participant's product allocation, exclusive use of THS 2.2 or exclusive CC smoking was allowed *ad libitum* from Day 1 to Day 5.

Twenty-four-hour urine was collected on each day.

The study protocol was approved by the local ethics committee in July 2013, prior to study start and conducted at the Higashi Shinjuku Clinic, located in central Tokyo, Japan, in accordance with the declaration of Helsinki (World Medical Association (WMA), 2008), Good Clinical Practice guidelines as transposed into the Japanese regulations (Ministry of Health and Welfare, 2013) and national regulations. The study was registered on ClinicalTrials.gov with ID: NCT01970982.

2.3. Study investigational products

The test product THS 2.2 was developed and provided by Philip Morris Products S.A. (part of Philip Morris International group of companies). THS 2.2 has three components: the THS tobacco Stick, the holder, and the charger. The THS tobacco stick contains a tobacco plug of processed tobacco cast leaf, which is covered by a paper wrap. The overall appearance of the THS tobacco stick is similar to that of a CC, except it is much shorter. The holder includes a battery, controlling electronics, and the heater element. The THS tobacco stick is inserted into the holder, and an electronically controlled heating blade within the holder heats the tobacco according to a carefully controlled temperature profile <350 °C. The charger recharges the holder. To use THS 2.2, the THS tobacco stick is inserted into the holder, the heating of the THS tobacco stick is initiated by pressing the button on the holder and a LED indicates when the initial heating process is complete. The holder and THS tobacco stick are designed to deliver over approximately 6 min or around 14 puffs. At the end of each product use session, the THS holder requires recharging and for the next use a new THS tobacco stick must be used. The test product THS 2.2 contained 0.5 mg nicotine and 4.9 ± 0.5 mg/stick of glycerin as determined under ISO conditions using machine puffing methods. The reference product in this study were the participant's own preferred brand of non-menthol CCs used in the CC group. CCs were not provided by the Sponsor, and subjects were asked to buy and bring their own CCs to the investigational site.

The disposition of THS 2.2 (tobacco sticks, charger and holders) was managed by the site as per Investigational Product handling manual. Subjects received one charger and two holders. Sticks were distributed one by one for each product use on request of the subject and used sticks were returned to the site staff. Distributed and returned sticks were recorded in an accountability log.

The holders were cleaned following the instructions provided in the THS 2.2 User Guide which included a brief cleaning after each product use experience and a full cleaning using the heat-generated self-clean procedure of THS 2.2 followed by a manual cleaning process using the special cleaning kit provided after each 20 sticks.

2.4. Sample size estimation

The sample size was determined based on the expected least squares (LS) mean ratios (THS/CC) of the concentrations of biomarkers of exposure adjusted for creatinine (except for COHb), as observed in previous studies with heated tobacco products (ClinicalTrials.gov ID: NCT00812279; ID: NCT01780714). A total of 160 participants randomized at a ratio of 2:1:1 to the THS, CC and SA groups respectively, were considered sufficient to attain >80% power to show a reduction of $\geq 50\%$ in the concentrations of carboxyhemoglobin (COHb), 3-hydroxypropylmercapturic acid (3-HPMA), monohydroxybutenyl mercapturic acid (MHBMA), and S-phenylmercapturic acid (S-PMA) in the THS group relative to the CC group, with a one-sided probability of 2.5% for type I error. The overall type I error was preserved by simultaneously testing the

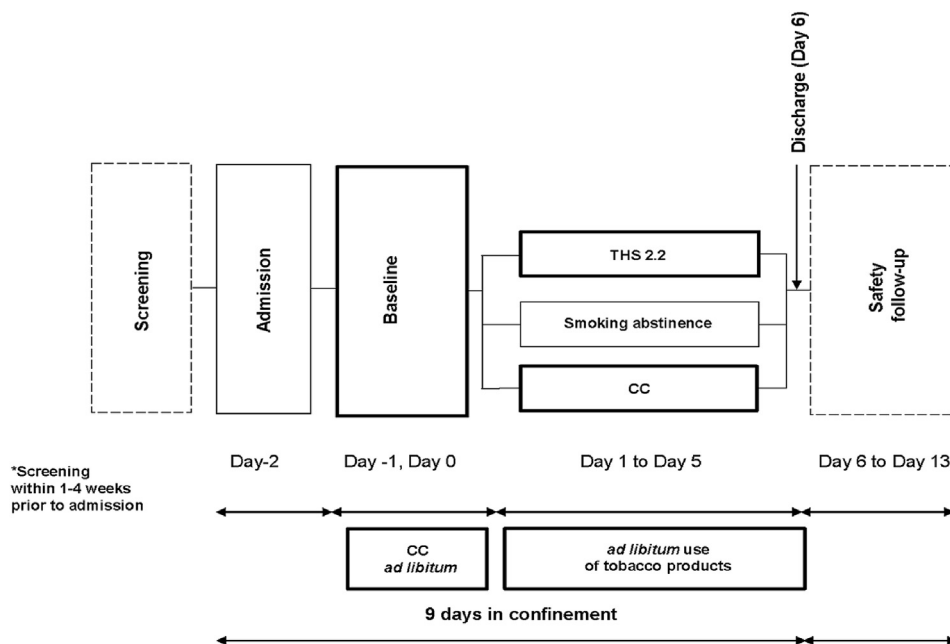


Fig. 1. Study Design. Study design schematic representation of the procedures followed by participants. Abbreviations: CC = conventional cigarette use group; THS 2.2 = Tobacco Heating System 2.2 use group.

endpoints using a closed procedure.

2.5. Statistical methodology

The biomarkers of exposure were analyzed in all randomized participants who used the allocated product at least once after randomization and with at least one valid value for a biomarker of exposure. Statistics were derived for each biomarker of exposure and the change from baseline according to study group and study day. Descriptive summary statistics included the number of participants (n), number and percent of participants with missing data, arithmetic mean, arithmetic standard deviation (SD), median, first and third quartiles, minimum, maximum, geometric mean and associated 95% confidence intervals (CI) and geometric coefficient of variation (CV) for each study group stratified by sex and CC use for 4 weeks before enrollment.

Inferential analysis was performed on the endpoints related to the primary objective including S-PMA, MHBMA, COHb, and 3-HPMA as observed on Day 5. Analysis of covariance was conducted on log-transformed variables to estimate the ratios between the study groups (one sided type I error of 2.5%) with adjustment for sex, CC use over the 4 weeks before enrollment, and the baseline value of the biomarkers of exposure. The estimated differences between the study groups and associated CIs were back-transformed to provide relative effects (THS/CC). A similar statistical approach was applied for the other endpoints. All statistical analyses were performed using Statistical Analysis Software (SAS), version 9.3 (SAS Inc., Cary, NC, USA).

2.6. Biomarkers of exposure

Biomarkers of exposure to selected HPHCs, were measured throughout the study from Day -1 to Day 5. The HPHCs assessed in this study were selected based on the following criteria:

1) HPHCs recommended for mandated lowering in cigarette smoke as defined by the WHO (WHO Study Group et al., 2008)

and the draft guidance of the U.S. Food and Drug Administration (FDA) Center for Tobacco Products (CTP) on "Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke" (FDA (Food and Drug Administration), 2012b).

- 2) The HPHC is specific to cigarette smoke with other sources being minor or non-existent.
- 3) The biomarker of exposure to a HPHC is easily detectable using validated, reliable, reproducible, fit-for-purpose analytical methods.
- 4) The HPHC reflects a specific toxic exposure or be a reliable surrogate of exposure to HPHCs.
- 5) The list of HPHCs includes HPHCs from both the gas and particulate phases.
- 6) The list of HPHCs includes a broad variety of chemical classes and organ toxicity classes as defined by the FDA (carcinogen, cardiovascular toxicant, respiratory toxicant, reproductive and development toxicant, addiction potential) (FDA (Food and Drug Administration), 2012b).
- 7) The list of HPHCs includes HPHCs formed at different temperature levels.
- 8) The HPHCs selected for measurement vary in their elimination half-life times, ranging from a few hours up to more than 2 weeks.

The study included biomarkers of exposure to the tobacco-specific HPHCs 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N-nitrosornicotine (NNN), as well as biomarkers of exposure to 1,3-butadiene, acrolein, benzene, carbon monoxide, pyrene, benzo(a)pyrene, 4-aminobiphenyl, 1-aminonaphthalene, 2-aminonaphthalene, o-toluidine, acrylonitrile, ethylene oxide, toluene and crotonaldehyde. In total, 16 HPHCs were evaluated to assess exposure reduction in the THS group compared to the CC and SA groups (Table 1).

Biomarkers of exposure to nicotine (nicotine and cotinine in plasma and nicotine equivalents measured in 24-h urine: free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free trans-3'-hydroxycotinine, trans-3'-hydroxycotinine-

Table 1
List of biomarkers of exposure and corresponding harmful and potentially harmful constituents.

Acronym	Biomarker of exposure	HPHC
Total NNAL ^a	Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
Total NNN	Total N-nitrososornicotine	N-nitrososornicotine (NNN)
MHBMA	Monohydroxybutenyl mercapturic acid	1,3-butadiene
3-HPMA	3-hydroxypropylmercapturic acid	Acrolein
S-PMA	S-phenylmercapturic acid	Benzene
COHb	Carboxyhemoglobin ¹	Carbon monoxide
Total 1-OHP ^b	Total 1-hydroxypyrene	Pyrene
Total 3-OH-B[a]P	3-hydroxy-benzo(a)pyrene	Benzo(a)pyrene
4-ABP	4-aminobiphenyl	4-aminobiphenyl
1-NA	1-aminonaphthalene	1-aminonaphthalene
2-NA	2-aminonaphthalene	2-aminonaphthalene
o-tol	o-toluidine	o-toluidine
CEMA	2-cyanoethylmercapturic acid	Acrylonitrile
HEMA	2-hydroxyethylmercapturic acid	Ethylene oxide
3-HMPMA	3-hydroxy-1-methylpropylmercapturic acid	Crotonaldehyde
S-BMA	S-benzylmercapturic acid	Toluene

Biomarkers of exposure matrix is 24 h urine if not otherwise stated. Other matrixes; 1 = blood. HPHC = harmful or potentially harmful smoke constituent. a = Total NNAL was determined as the molar sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its O-glucuronide conjugate. b = Total 1-OHP was determined as the molar sum of 1-hydroxypyrene and its glucuronide and sulfate conjugates.

glucuronide) were assessed to verify a comparable exposure to nicotine when smokers use THS 2.2 or smoke CCs.

Biomarkers of exposure to selected HPHCs, were measured in blood collected from Day 0 to Day 5, or 24-h urine samples collected throughout the study from Day -1 to Day 5. Creatinine was also measured in 24-h urine for adjustment of the concentration of all urinary biomarkers of exposure. With the exception of the samples collected for carboxyhemoglobin (COHb), samples were processed and stored at -20 °C pending biomarker analysis.

Nicotine, cotinine, trans-3'-hydroxycotinine, were measured in plasma and following a solid phase extraction, the extracts were injected onto a qualified LC-MS/MS instrument.

O-toluidine, 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl, S-phenylmercapturic acid, total 1-hydroxypyrene, total 3-hydroxy benzo[a]pyrene, total NNAL, and total NNN were measured in urine after hydrolysis. In addition to the total assays, nicotine equivalents were also measured with the direct analysis of nicotine, cotinine, trans-3'-hydroxycotinine, nicotine-N-glucuronide, cotinine-N-glucuronide, and trans-3'-hydroxycotinine-O-glucuronide in urine. A direct analysis of urinary concentrations of MHBMA, 3-HPMA, HMPMA, CEMA, S-BMA, were performed. All analysis were conducted with a qualified LC-MS/MS instrument. All bioanalytical assays used were validated to meet the requirements of the FDA Guidance to Industry: Bioanalytical Method Validation (FDA (Food and Drug Administration), 2001).

Urinary creatinine was measured spectrophotometrically using a College of American Pathology (CAP)/Clinical Laboratory Improvement Amendments (CLIA) validated assay.

All laboratory analyses were carried out at Celerion Laboratories (Lincoln NE, USA) except for COHb which was assessed by Tokiwa Chemical Industries Co. (Tokyo, Japan). Details on the bioanalytical methods conducted at Celerion Laboratories are reported in (Haziza et al., under revision).

2.7. Cytochrome 1 A2 activity

Because CYP1A2 is an enzyme inducible by polycyclic aromatic amines, some HPHCs found in cigarette smoke (Butler et al., 1992), CYP1A2 activity was measured in this study as an indicator of overall exposure. Cytochrome P450 (CYP) 1A2 enzymatic activity was measured on Day 0 and on Day 5. It was based on the post-dose paraxanthine (PX) and caffeine (CAF) plasma molar concentrations approximately 6 h (± 15 min) after the intake of one Tomerumin[®]

(LionCorp.) caffeine tablet (around 170 mg caffeine) with 150 ml \pm 10 ml water. CYP1A2 activity was assessed by measuring PX and CAF concentrations and calculating the PX/CAF molar metabolic ratio (Faber and Fuhr, 2004).

2.8. Product use and human puffing topography

CC and THS tobacco stick consumption was recorded for all participants from Day -2 until discharge. All products were dispensed and recorded one at a time by the site staff at the participant's request. Smoking abstinence for participants in the SA group was verified by CO breath tests performed 4 times/day (CO < 10 ppm). Human puffing topography (HPT) was performed to measure parameters such as puff duration, inter-puff interval, puff volume, and total volume for each CC used at baseline in all participants, and on Days 1 and 4, in both the CC and THS groups. HPT was performed using the HPT SODIM[®] device, model SPA/M (SODIM[®] Instrumentation, Fleury les Aubrais, France).

The sample holders for the HPT Sodim[®] Device were specifically designed for compatibility with THS 2.2 and the HPT Sodim[®] Device and sample holder were validated according to PMI's internal quality management system to ensure that measurements performed with the device and sample holder are accurate and repeatable. Furthermore puffing topography was only assessed in subjects who smoked CCs that were compatible with the HPT device. Users of slim CCs were excluded from HPT assessments.

2.9. Subjective effects

The subjective effects were assessed using self-reported questionnaires validated for use in the local language. Nicotine dependence was assessed at the Screening Visit using the revised version of the Fagerström Test for Nicotine Dependence (FTND) (Fagerstrom et al., 2012). Product evaluation was performed using the modified Cigarette Evaluation Questionnaire (mCEQ) (Cappelleri et al., 2007) on Day -1 in all participants, and from Days 1–5 in the THS and CC groups. The following domains of the mCEQ were evaluated: Smoking Satisfaction (satisfying, tastes good, and enjoyment of smoking); Psychological Reward (calms down, makes more alert, reduces irritability, helps concentration, reduces hunger); Aversion (dizziness, nausea); Enjoyment of Respiratory Tract Sensations (single-item assessment); and Craving Reduction (single-item assessment). The urge-to-smoke, which evaluates how

rewarding smoking is perceived and provides relief from the urge to smoke, was assessed in all participants on a daily basis from Day –1 to Day 5 using the 10-item brief version of the Questionnaire of Smoking Urges (QSU-brief) (Cox et al., 2001).

2.10. Adverse events, medical history, concomitant medication, product malfunction and misuse

Safety assessment included AEs, SAEs, THS 2.2 malfunctions and misuse, vital signs, electrocardiography, spirometry, clinical chemistry, hematology, urinalysis, physical examinations and use of concomitant medications. AEs were recorded from the time of signing the informed consent form until the end of the study (end of the Safety Follow-Up Period). AEs, concomitant diseases, and medical/surgical history were coded using the Medical Dictionary for Regulatory Activities (MedDRA version 16.0). Prior and concomitant medications were coded according to the World Health Organization enhanced drug dictionary (Uppsala Monitoring Centre, Q1 2013).

3. Results

3.1. Participants

The study site screened 267 subjects, 101 were screen failures, and 166 tried THS 2.2 during the product test phase. The 166 participants who tried THS 2.2 were enrolled and included in the safety population. Among these participants, 6 were not randomized; either due to participants' personal reasons, or due to randomization quota already being met. One hundred sixty participants were randomized, with 80, 40, and 40 participants in the THS 2.2, CC, and SA groups, respectively. One hundred fifty eight participants completed the study with 2 participants in the SA group who voluntarily withdrew from the study (Fig. 2).

No difference in terms of age, body mass index, and mean FTND total scores was observed between the study groups at Baseline. Overall, the majority of enrolled participants (55.4%) had a moderate FTND score. The distribution of participants between the 3 groups regarding sex and daily cigarette consumption was comparable (Table 2).

Table 2
Demographic characteristics by group, at baseline.

Variable and statistic	THS	CC	SA
Randomized, n	80	40	40
Completed, n	80	40	38
Sex n (%)			
Male	40 (50.0%)	20 (50.0%)	20 (50.0%)
Female	40 (50.0%)	20 (50.0%)	20 (50.0%)
Age (years)			
Mean \pm SD	37.6 \pm 11.7	37.2 \pm 11.7	35.9 \pm 10.6
Range	23–64	23–65	23–64
BMI (kg/m ²)			
Mean \pm SD	22.7 \pm 2.7	22.9 \pm 2.7	22.7 \pm 2.8
Range	18.6–30.8	18.6–28.7	18.7–28.8
CC consumption n (%)			
10 to 19 cig/day	44 (55.0%)	22 (55.0%)	21 (52.5%)
>19 cig/day	36 (45.0%)	18 (45.0%)	19 (47.5%)
FTND total score			
Mean \pm SD	4.6 \pm 1.7	4.0 \pm 1.7	4.3 \pm 1.7
Range	1–8	1–8	1–9

Abbreviations: CC = conventional cigarette use group; SA = smoking abstinence group; THS = Tobacco Heating System 2.2 use group; SD = standard deviation; BMI = body mass index; FTND = Fagerström Test for Nicotine Dependence, ISO = International Organization for Standardization.

3.2. Number of THS Tobacco Sticks and CCs used daily

In the THS group, the mean number of THS tobacco sticks (mean \pm SD) used daily initially decreased from the number of CCs smoked at Baseline (10.3 \pm 3.4) to Day 1 (8.3 \pm 3.0), increasing afterwards with product use comparable to Baseline on Day 5 (9.9 \pm 3.9). The mean number of CCs consumed in the CC group daily was 10.5 \pm 3.0 at Baseline and increased from 10.6 \pm 3.1 on Day 1–12.5 \pm 3.5 on Day 5. Over the study period, participants in the THS 2.2 group consumed on average about 2 THS tobacco sticks less compared to the number of CCs smoked in the CC group on a day by day basis (Table 3).

3.3. Biomarkers of exposure to selected HPHCs

Levels of biomarkers of exposure to 16 HPHCs and to nicotine are presented in the Supplementary Table 1 at Baseline and at Day 5

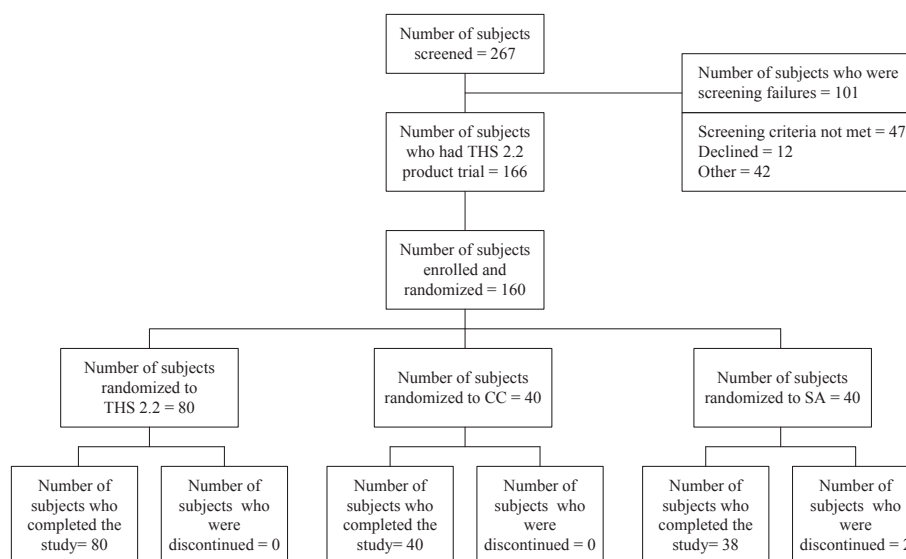


Fig. 2. Disposition of Cases. Description of participants' disposition in the course of the study. Abbreviations: CC = conventional cigarette use group; SA = smoking abstinence group; THS 2.2 = Tobacco Heating System 2.2 use group.

Table 3
Daily product consumption; number of THS tobacco sticks and cigarettes.

Visit day	THS N = 80	CC N = 40
Baseline use		
Mean ± SD	10.3 ± 3.43	10.5 ± 2.97
Range	2–19	4–19
Day 1		
Mean ± SD	8.3 ± 3.03	10.6 ± 3.02
Range	1–15	3–19
Day 2		
Mean ± SD	9.5 ± 3.61	11.9 ± 3.38
Range	1–18	6–19
Day 3		
Mean ± SD	9.9 ± 3.87	12.0 ± 3.25
Range	1–20	5–18
Day 4		
Mean ± SD	9.9 ± 3.84	11.8 ± 2.90
Range	1–19	6–17
Day 5		
Mean ± SD	9.9 ± 3.93	12.5 ± 3.52
Range	1–19	6–20

Abbreviations: CC = conventional cigarette use group; SA = smoking abstinence group; THS = Tobacco Heating System 2.2 use group; SD = standard deviation.

for the THS, CC and SA groups. At baseline, the levels of biomarkers of exposure were comparable in all three groups. On Day 5, at the end of the exposure period, the levels of 15 biomarkers of exposure (COHb, S-PMA, MHBMA, 3-HPMA, total NNN, total NNAL, total 1-OHP, 4-ABP, 1-NA, 2-NA, o-toluidine, CEMA; HEMA, 3-HMPMA, and total 3-OH-B[a]P) were reduced in both THS and SA groups as compared to Baseline (Fig. 3, Supplementary Table 2). The reduction was of similar magnitude between the THS and SA groups for each biomarker except for total NNN, 3-HPMA, total NNAL, total 3-OH-B[a]P and HMPMA for which the reduction was slightly higher in SA than THS groups.

Substantial reductions in urinary concentrations adjusted for creatinine, of approximately 76% (ratio 23.09%) in MHBMA, 47% (ratio 52.86%) in 3-HPMA, 84% (ratio 15.68%) in S-PMA, and 52% (ratio 47.10%) in COHb (in blood) were demonstrated after 5 days of switching to THS use as compared to continuing smoking. Furthermore reductions of 50% (ratio 49.03%) in total NNAL, 69% (ratio 30.06%) in total NNN, 53% (ratio 46.44%) in total 1-OHP, 81% (ratio 18.21%) in 4-ABP, 95% (ratio 4.44%) in 1-NA, 82% (ratio 17.62%) in 2-NA, 49% (ratio 50.52%) in o-toluidine, 78% (ratio 21.21%) in CEMA, 53% (ratio 46.50%) in HEMA, 62% (ratio 37.71%) in 3-HMPMA and 70% (ratio 29.99%) in total 3-OH-B[a]P were observed in the THS group relative to the CC group (Table 4).

Despite the fact that S-BMA is suitable to detect toluene in environmental and occupational studies (Lovreglio et al., 2010), in this study it could not discriminate between smokers and smokers who stop smoking, an observation reported by other authors as well (Supplementary Tables 1 and 2) (Imbriani et al., 1999; Schettgen et al., 2008).

No change in the levels of creatinine was observed at baseline and after 5 days of exposure (Supplementary Table 3).

3.4. Exposure to nicotine

Nicotine exposure as assessed by NEQ was lower at Day 1 in the THS group compared to the CC group (4.55 vs. 5.32 mg/g creat) before increasing afterwards and reaching similar levels of NEQ between THS 2.2 users and participants who continued to smoke CC (THS 2.2 vs. CC ratio 97.72%) at Day 5. In both groups, NEQ values increased from baseline to Day 5 with percent changes from baseline of 16.94% and 9.49% for the THS and CC groups, respectively. In agreement with the results for NEQ, plasma nicotine and

concentration profiles were comparable for the THS and CC groups, with similar mean nicotine concentrations reported on all days except on Day 1 when the nicotine plasma concentration in the THS group (10.46 ng/mL) was lower than that of the CC group (14.68 ng/mL). Nicotine concentrations increased from Day 1 to Day 5 for both the THS and CC groups with a remaining negligible difference between the groups of about 6.01% on Day 5.

Plasma cotinine concentration profiles were comparable for the THS and CC groups, with similar concentrations reported at baseline (140.37 and 147.32 ng/mL, respectively) and Day 5 (161.00 and 164.30 ng/mL, respectively). Mean plasma cotinine concentrations increased by approximately 16.14% and 6.63% from Baseline to Day 5 in the THS and CC groups respectively.

3.5. Cytochrome 1A2 activity

At baseline, CYP1A2 activity was similar in all three groups. On Day 5, the CYP1A2 activity following coffee intake was 56.56% and 76.50% in the THS and CC groups, respectively, with difference THS-CC of –21.65% (95% CI: –25.49, –17.81). In the THS group, CYP1A2 activity reduced from baseline to Day 5 by –27.36% (95% CI: –30.51, –24.22) to levels comparable to the SA group (LS mean difference THS-SA: 2.29% (95% CI: –1.62, 6.19)).

3.6. Human puffing topography

The baseline values for each assessed HPT parameter were similar in the THS and CC groups while subjects were smoking their own preferred brand of CC and were generally stable in the CC group between baseline and Day 4 with the exception of total number of puffs and total puff volume which decreased slightly in CC arm on Day 1 and average inter puff interval which increased from baseline to Day 4.

In the THS group, total puff volume and average puff volume were 18% and 25% lower on Day 1 relative to the CC group. From Day 1 to Day 4 both parameters increased with similar values for total puff volume and a remaining difference of 15% for average puff volume between the CC and THS group.

In contrast, on Day 1, the total number of puffs and the puff frequency were 11% and 18% higher in the THS group relative to the CC group respectively. In addition the total puff duration was 11% longer in the THS group compared to the CC group on Day 1. These differences further increased on Day 4 with the total number of puffs and the puff frequency being about 19% and 27% higher and the total puff duration 23% longer in the THS relative to CC group.

No notable differences were observed in average puff duration and total smoking duration on both Days 1 and 4 between participants who switched to THS 2.2 use and participants who continued to smoke CC (Table 5).

3.7. Modified cigarette evaluation questionnaire subscales

The mCEQ results showed that on Day 5, compared to baseline, smoking satisfaction was lower for participants who switched to THS 2.2 use compared to participants who continued to smoke CC, with differences of –0.69. Aversion, craving reduction, and enjoyment of respiratory tract sensation, and psychological reward were comparable in the THS and CC groups on Day 5 (Table 6). Descriptive statistics for each sub-item of the mCEQ (mean scores) are provided on Day 5 in Fig. 4 for the THS and CC groups.

3.8. Urge-to-smoke symptoms (QSU-Brief)

The mean urge-to-smoke total scores were comparable in all study groups at baseline, with scores of 4.13, 4.13, and 3.98 in the

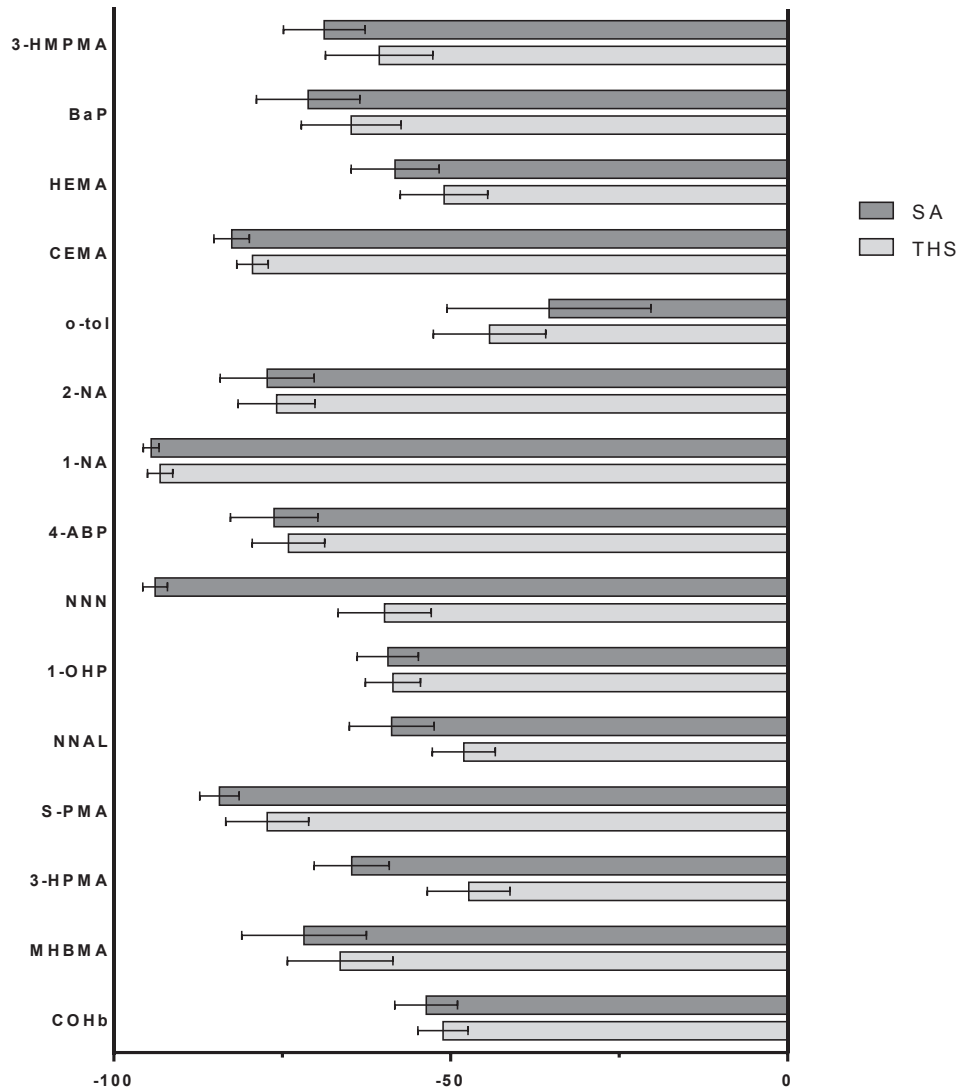


Fig. 3. Biomarkers of exposure relative (%) change from baseline to end of exposure (Day 5) per study group. Relative change from baseline. Results presented are the geometric means (95% confidence interval) values for each biomarker of exposure. Abbreviations: SA = smoking abstinence group; THS = Tobacco Heating System 2.2 use group.

THS, CC, and SA groups, respectively and remained stable and comparable between the THS and CC groups throughout the study, with the minimum and maximum values ranging from 1.0 to 6.7 in the THS group, and from 1.0 to 5.8 in the CC group (Fig. 5).

Considering all of the time-points, the LS mean difference in the QSU-brief total score between the THS and CC groups was 0.13 (95% CI: -0.19, 0.45).

In the SA group, as expected, the urge-to-smoke total score increased significantly from 3.98 at baseline to 4.64 on Day 1, corresponding to an increase of 0.66 (95% CI: 4.20, 5.08). From Day 3 onwards, the urge-to-smoke started to decrease but remained above the baseline value on Day 5 (Fig. 5). The LS mean difference in QSU-brief total score between the THS group and the SA group was -0.65 (95% CI: -0.98, -0.32).

3.9. Safety

The safety population consisted of 166 participants comprised out of the 160 randomized participants and the 6 participants who were exposed to THS from the product test on Day -2, but not randomized. Overall, there were 11 AEs reported by 10 of the 166

participants (6.0%) in the safety population, all of which were mild in intensity. The incidence and frequency of AEs were comparable in the THS (6 AEs in 6/80 participants [7.5%]), the CC (4 AEs in 3/40 participants [7.5%]), and the SA groups (1 AE in 1/40 participants [2.5%]). The most frequent AEs reported in the THS 2.2 or CC group were blood triglycerides increased, neutrophil count decreased, blood potassium decreased, protein urine present, and white blood cell count decreased. The only AE reported after SA was hemoglobin decreased (Table 7).

There were no SAEs and none of the randomized subjects were discontinued from the study because of an AE. None of the AEs reported were assessed as being related to THS 2.2 or CC use.

4. Discussion

The study demonstrated reductions in biomarkers of exposure to selected HPHCs by switching from CC smoking to THS 2.2 use. The confinement setting allowed the investigation of the exposure reduction to HPHCs achievable under ideal, confined conditions and monitored product compliance after 5 days of THS 2.2 use compared to CC smoking, using SA as a benchmark. To assess the

Table 4
Biomarkers of exposure, ratios of THS relative to CC (%).

Biomarkers of exposure	Ratio THS/CC (% and CI)
NEQ (mg/g creat)	104.89 (92.03; 119.55)
Nicotine ^a (ng/mL)	112.91 (91.36; 139.54)
Cotinine ^a (ng/mL)	96.14 (70.73; 130.67)
Total NNAL (pg/mg creat)	49.03 (41.95; 57.30)
Total NNN (pg/mg creat)	30.06 (23.74; 38.06)
COHb ^b (%)	47.10 (44.30; 50.08)
MHBMA (pg/mg creat)	23.09 (18.41; 28.95)
3-HPMA (ng/mg creat)	52.86 (45.67; 61.17)
S-PMA (pg/mg creat)	15.68 (13.09; 18.78)
Total 1-OHP (pg/mg creat)	46.44 (41.32; 52.19)
4-ABP (pg/mg creat)	18.21 (15.29; 21.69)
1-NA (pg/mg creat)	4.44 (3.80; 5.18)
2-NA (pg/mg creat)	17.62 (14.72; 21.08)
o-tol (pg/mg creat)	50.52 (42.28; 60.38)
CEMA (ng/mg creat)	21.21 (18.10; 24.86)
HEMA (pg/mg creat)	46.50 (39.53; 54.69)
3- HMPMA (ng/mg creat)	37.71 (31.57; 45.05)
Total 3-OH- B[a]P (fg/mg creat)	29.99 (24.84; 36.20)

Geometric least squares mean ratio (%) (95% confidence intervals) from an ANCOVA model conducted on log-transformed Day 5 values with log-transformed baseline value, study arm, sex and CC consumption reported at screening as fixed effect factors (THS/CC) on Day 5. a) Weighted average concentration over 24 h (Cavg); for nicotine and cotinine the ratio is calculated on the weighted average concentration over 24 h, b) Measured between 8:00 p.m. and 10:00 p.m. Abbreviations: CC = conventional cigarette group; THS = Tobacco Heating System 2.2 group.

Table 5
Human puffing topography parameters.

Variable and day	THS N = 80	CC N = 40	THS/CC(%)
Total puff volume (mL)			
Baseline	738.71 ± 321.26	801.53 ± 294.08	
Day 1	623.68 ± 318.70	753.60 ± 282.90	81.58 (68.90; 96.58)
Day 4	705.25 ± 330.46	742.09 ± 299.67	99.24 (85.90; 114.64)
Average puff volume (mL)			
Baseline	49.86 ± 18.84	51.02 ± 16.13	
Day 1	39.42 ± 19.47	50.84 ± 15.49	74.78 (66.42; 84.20)
Day 4	42.20 ± 19.30	49.22 ± 16.14	85.45 (75.93; 96.16)
Average puff duration (s)			
Baseline	2.01 ± 0.80	1.76 ± 0.56	
Day 1	2.07 ± 0.93	1.82 ± 0.54	99.14 (91.36; 107.60)
Day 4	2.10 ± 1.00	1.80 ± 0.60	102.64 (93.42; 112.78)
Total puff duration (s)			
Baseline	30.54 ± 14.68	27.31 ± 8.21	
Day 1	33.62 ± 18.43	26.86 ± 8.32	110.91 (96.69; 127.22)
Day 4	36.68 ± 19.58	27.07 ± 8.83	123.45 (108.14; 140.93)
Total number of puffs			
Baseline	15.47 ± 5.30	16.15 ± 4.84	
Day 1	16.44 ± 5.63	15.13 ± 4.62	111.04 (100.84; 122.27)
Day 4	17.68 ± 5.63	15.50 ± 4.61	118.57 (109.35; 128.57)
Puff frequency (puffs/min)			
Baseline	3.79 ± 1.09	4.10 ± 1.16	
Day 1	4.26 ± 1.47	3.84 ± 1.19	117.76 (107.74; 128.72)
Day 4	4.55 ± 1.73	3.77 ± 1.11	126.61 (114.73; 139.72)

Comparative assessment of human puffing topography parameters. Parameters are per THS tobacco stick/cigarette. All values are mean and standard deviation, except THS/CC mean ratio; adjusted geometric least squares means ratio (%), and 95% confidence intervals. Abbreviations: CC = conventional cigarette use group; THS = Tobacco Heating System 2.2 use group.

potential of THS 2.2 as a suitable alternative for CC smokers, this study included endpoints to investigate the adaptation to and acceptance of THS 2.2 through the assessment of HPT and subjective effects.

The study demonstrated that switching to THS 2.2 leads to reduction in COHb, S-PMA, MHBMA, and 3-HPMA, 4 biomarkers of exposure to the following HPHCs: carbon monoxide, benzene, 1–3 butadiene, and acrolein respectively, after 5 days of use in a controlled setting relative to smoking CC. Furthermore, reductions in an additional 11 biomarkers of exposure were observed in

subjects using THS 2.2 for 5 days compared to subjects continuing to smoke CC. Biomarkers of exposure to HPHCs were compared between THS 2.2, the participant's own brand of non-menthol CCs, and smoking abstinence. Overall, the reduction in exposure to HPHCs assessed in this study were comparable to those observed in the smoking abstinence group. The more pronounced differences in total NNN levels in the THS arm compared to the SA arm compared to those of total NNAL is likely related to the half-life of total NNAL which is longer (10–18 days) (Goniewicz et al., 2009) than that of total NNN. Considering that it is normally assumed that complete elimination is achieved following 4 to 5 half-life, only initial and progressive decrease for is observed for total NNAL when maximal magnitude of reduction is probably achieved for total NNN after 5 days of THS exposure. Another possible explanation might be related to a direct evaporative transfer of both compounds (Rodgman and Perfetti, 2013) which could occur at higher rate for NNK vs NNN when tobacco is heated to temperature below pyrolysis (Forster et al., 2015).

Statistical analysis conducted on urinary biomarkers of exposure expressed as quantity excreted, showed a comparable magnitude of reduction to when biomarkers of exposure were expressed as concentration adjusted to creatinine (data not reported). These data along with the creatinine values which did not show any variation of excretion between baseline and after 5 days

of exposure suggest that adjustment of the concentrations of urinary biomarker of exposure to creatinine does not bring additional variability.

Cigarette smoke contains thousands of chemicals, and over a hundred are classified as HPHCs (Rodgman and Perfetti, 2013). In 2012, the US FDA published an abbreviated list of 20 HPHCs, from which 18 constituents in cigarette smoke were recommended to be measured and reported (FDA (Food and Drug Administration), 2012b). The present study assessed 16 HPHCs in addition to nicotine, including 14 of the HPHCs requested by the FDA and 9

Table 6
Analysis of change from baseline in mCEQ.

Subscale	THS	CC	(THS–CC) difference
	N = 80	N = 40	
Smoking satisfaction	–1.15	–0.47	–0.69 (–1.04, –0.34)
Aversion	–0.16	–0.17	0.01 (–0.19, 0.21)
Craving reduction	–0.92	–0.75	–0.17 (–0.59, 0.25)
Enjoyment of respiratory tract sensation	–0.55	–0.21	–0.34 (–0.74, 0.06)
Psychological reward	–0.75	–0.57	–0.18 (–0.42, 0.07)

Adjusted LS means and 95% CIs from an ANCOVA model with study arm, sex, CC consumption reported at Screening Visit, and study arm × day fitted as fixed effect factors with baseline fitted as a covariate. Day fitted as a repeated factor. Abbreviations: CC = conventional cigarette use group; THS = Tobacco Heating System 2.2 use group. CI = confidence interval; mCEQ = modified cigarette evaluation questionnaire.

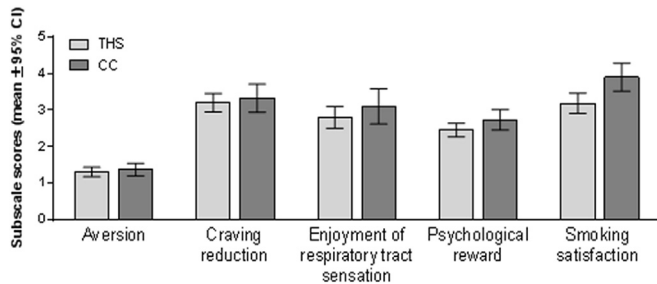


Fig. 4. mCEQ Subscale mean score for each sub items on Day 5. Mean score for each sub items of the mCEQ questionnaire at Day 5 in the THS and CC groups.; Abbreviations: Tobacco Heating System (THS) 2.2, conventional cigarette (CC), confidence intervals (CI).

HPHCs that the World Health Organization recommended to be lowered in cigarette smoke (FDA (Food and Drug Administration), 2012b; WHO Study Group et al., 2008). We also measured exposure to pyrene (as total 1-OHP), an indicator of exposure to polycyclic aromatic hydrocarbons (PAH), and exposure to the aromatic amine o-toluidine (o-tol), as both, PAH and o-toluidine are strong carcinogens, associated with colon and bladder cancer (IARC (International Agency for Research on Cancer), 2012). Ethylene oxide (HEMA) was assessed because inhalation of ethylene oxide is irritating to mucous membranes including those associated with the respiratory system (U.S. Department of Health and Human Services et al., 1990). In summary, the group of biomarkers of exposure assessed in this work covers HPHCs of multiple chemical and organ toxicity classes, present in both the gas

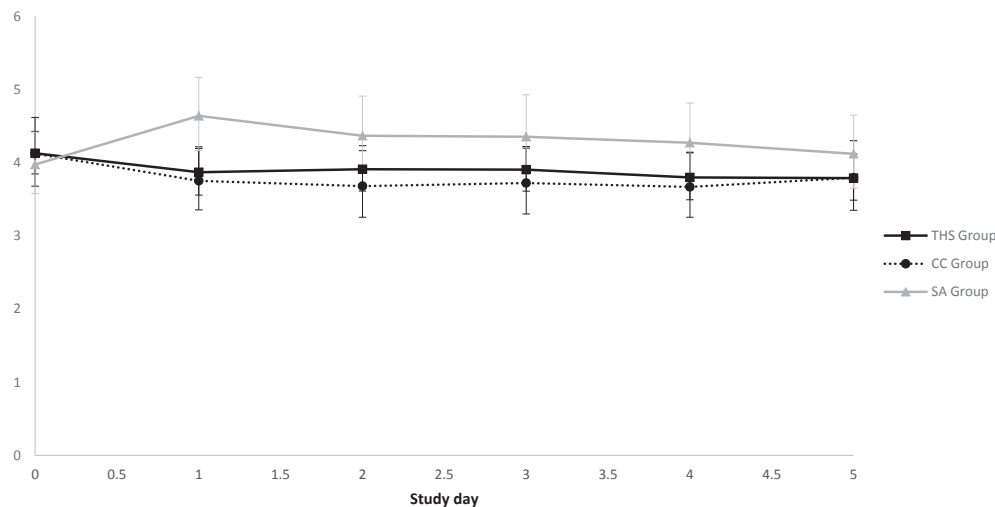


Fig. 5. Questionnaire on smoking urges mean total score. Comparison of the urge-to-smoke total scores derived from the QSU-brief. Results are presented as the mean (95% confidence interval). Abbreviations: CC = conventional cigarette use group; THS = Tobacco Heating System 2.2 use group; SA = smoking abstinence group.

Table 7
Adverse events.

Adverse events	THS N = 80	CC N = 40	SA N = 40	Overall safety N = 166
Total AEs (%)	6 (7.5%)	3 (7.5%)	1 (2.5%)	10 (6.0%)
Blood triglycerides increased	2 (2.5%)	2 (5.0%)	0	4 (2.4%)
Neutrophil count decreased	2 (2.5%)	1 (2.5%)	0	3 (1.8%)
Blood potassium decreased	1 (1.3%)	0	0	1 (0.6%)
Hemoglobin decreased	0	0	1 (2.5%)	1 (0.6%)
Protein urine present	1 (1.3%)	0	0	1 (0.6%)
White blood cell count decreased	0	1 (1.25%)	0	1 (0.6%)

Adverse events occurred during the study. Terms coded using MedDRA® version 16.0. Abbreviations: N = number of subjects Percentages were calculated using the N of subjects in the column headers. AE = adverse event; CC = conventional cigarette use group; SA = smoking abstinence group; THS = Tobacco Heating System 2.2 use group; Overall Safety = Participants exposed at least once to THS 2.2.

and particulate phases, with different half-life times and found at different formation temperatures and, therefore, should provide relevant evidence that THS 2.2, as a heat-not-burn-product, reduces HPHC exposure.

Four HPHCs present on the FDA's list; ammonia, formaldehyde, acetaldehyde and isoprene, were not measured. Isoprene was not considered because of the high amount of endogenous production (OECD (Organization for Economic Co-operation and Development), 2005), and because of the very short half-life. Isoprene levels in humans have not been reported as being reliable to distinguish smokers and non-smokers (Euler et al., 1996). Acetaldehyde was not measured because of the lack of an established biomarker of exposure. Because of the various sources of exposure to formaldehyde including environment, its short half-life and lack of a specific metabolite, exposure to formaldehyde was also not measured in this study. No known specific biomarker of exposure to ammonia exists in humans or animals and, no evidence for alterations in clinical indices of body ammonia or nitrogen levels after exposure to exogenous ammonia has been reported (U.S. Department of Health and Human Services, 2004). Due to the rapid clearance of ammonia from the body or its metabolization to compounds found endogenously at appreciable levels, ammonia is not a suitable biomarker of exposure in the context of the assessment of a candidate MRTP such as THS 2.2.

The study showed that switching from CCs to THS 2.2 for 5 days reduced biomarkers of exposure to HPHCs to values between 47% and 96%, and approached exposure reduction observed in the SA group. The fact that total NNN, 3-HPMA, total NNAL, 3-HMPMA and total 3-OH-B[a]P were slightly higher in the THS group relative to SA, can likely be explained by the remaining levels of these HPHCs in the THS 2.2 aerosol as evaluated by the smoke chemistry (Schaller et al., 2016). Also, the formation through heating of glycerin might add other possible explanations (Qadariyah et al., 2011). Overall, the observed results strongly support the approach of heating versus burning tobacco to reduce the exposure to HPHCs in smokers using THS 2.2 while allowing users to effectively keep nicotine levels close to CC.

CYP1A2 catalyzes many of the reactions involved in the metabolism of low therapeutic-index drugs and synthesis of cholesterol, steroids, and other lipids (Kroon, 2007). More importantly, CYP1A2 enzymes are monooxygenase involved in the activation of carcinogenic heterocyclic and aromatic amines, strong carcinogens associated with colon and bladder cancer (Gunes and Dahl, 2008; MacLeod et al., 1997). The CYP1A2 expression itself is induced to a large extent by polycyclic aromatic hydrocarbons (PAH) which are found in cigarette smoke (Butler et al., 1992).

The 70% relative reduction of total 3-OH-B[a]P, after 5 days of THS use compared to CC, likely contributes to the 27% reduction from baseline in CYP1A2 activity in the THS group, similar to the reduction in the SA group. This may further support the potential of THS to lower the risk of certain tobacco-related cancers. Corroborating the data from human exposure, a previously reported study conducted in Apoe^{-/-} mice for 8 months, showed that gene and protein expression of CYP1A2 in the liver was induced by cigarette smoke and not by exposure to THS aerosol. In addition, a reduction in CYP1A2 gene and protein expression to levels approaching those of cessation was observed after switching from cigarette smoke exposure to THS aerosol exposure (Lo Sasso et al., 2016). Thus, the reduction in enzymatic activity of CYP1A2 as observed upon smoking cessation is an additional indicator of reduced exposure to HPHCs when subject use THS for 5 days.

Exposure to nicotine was comparable between the THS and CC groups at Baseline. After an initial decrease at Day 1 values increased by Day 5 to similar levels in the THS and CC group. The

change from CC to the THS requires adaptation and CC smokers had therefore to adjust to a new product with a different nicotine yield, taste and sensory characteristics compared to their own brand of CC. The decrease in nicotine levels observed at Day 1 was likely caused by this switch and the start of the adaptation process. This process appears to be accompanied by a change in human puffing topography, with an increase in puff count, puff frequency and puff duration, to compensate for a decrease in average puff volume and initial drop in total puff volume at Day 1. Furthermore a slight initial drop in number of THS tobacco sticks consumed on Day 1, which was lower than the number of average CC smoked at baseline, and recovery back to levels of THS tobacco stick use observed at baseline by Day 3 adds an explanation to the observed nicotine profile.

The results of the QSU-brief showed similar reductions in urge-to-smoke for THS 2.2 relative to CC and were distinct to that which was observed during SA. As expected, smoking abstinence drove an urge-to-smoke increase from Day 1 onwards with an increase of 23% from Baseline. This increase was slightly lower in magnitude but overall consistent with data from the literature, where an increase of approximately 54% was observed in smokers 24 h post smoking abstinence. (West and Ussher, 2010).

The mCEQ scale however showed that over the course of the study, psychological reward and smoking satisfaction were lower for participants who switched to THS 2.2 compared to those who continued to smoke CC, while there were no notable differences in aversion, craving reduction, and enjoyment of respiratory tract sensation. The combination of the results for nicotine uptake and subjective results are indicative of a smooth transitional adaptation towards an acceptance of the product, although due to the short duration of the study, participants with a long history of smoking their own CCs may not have been able to completely adjust to THS 2.2 before the study end. Nevertheless, overall subjective effect measures, daily product consumption numbers together with HPT data as well as nicotine uptake indicate that THS 2.2 offered an acceptable experience for a current CC smokers.

However, the study should be taken with the limitations inherent to the design. The study was too short to fully assess the reduction in exposure to NNK with THS 2.2 use as total NNAL has an apparent half-life of 10–18 days (Goniewicz et al., 2009). Nevertheless, the relative reduction of 56% in the levels of total NNAL, a tobacco specific-nitrosamine for which an association with lung cancer is demonstrated in smokers, is extremely promising as one can expect even further decline, considering the long-half-life of this metabolite, under prolonged use of THS. Finally, the clinic-confined setting, is a limit to the generalization of the results to a more real world use setting, where use of other tobacco and nicotine containing products can occur. For these reasons, longer studies in ambulatory settings need to be conducted to evaluate how the reductions to exposure would be sustained with less control over product use.

Yet, this study allowed an assessment of comparative exposure in optimal conditions, inherent to its randomized controlled design, and the controlled *ad libitum* product use in a confined setting preventing from dual use.

A strength of the study was that all urinary biomarkers of exposure were measured in 24-h urine collection using validated methods. Compared to partial urine or spot urine, 24-h urine collection is considered the most accurate approach to measure excretion of the metabolites generated from exposure to HPHCs. Furthermore the HPHCs measured in this study cover multiple chemical classes, organ toxicity classes, half-lives, gas and particulate phases, and formation temperatures, providing indication that THS 2.2 reduces exposure to a broad spectrum of HPHCs.

5. Conclusions

The study demonstrated that switching from CC smoking to THS 2.2 use resulted in substantial reductions in exposure to 15 selected HPHCs. The kinetics and the magnitude of decrease of biomarkers of exposure levels observed in the THS group were approaching the levels observed in the SA group for the majority of the biomarkers of exposure. Nicotine uptake was similar between the THS and CC groups at the end of the 5 day exposure period after users had started to adapt to a new product, and with a transitional period of changing puffing behavior, were able to achieve their desired nicotine level. The combination of the results of nicotine exposure and subjective effect measures indicated that THS 2.2 offered comparable satisfaction with regards to taste and sensorial experience, to that which was observed in CC smokers. No SAEs or severe AEs were reported during this study, with the total number of AEs being very low and evenly balanced across study groups.

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Conflict of interest statement

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

Clinical trial registration

NCT01970982 (ClinicalTrials.gov).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.yrtph.2016.09.014>.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.yrtph.2016.09.014>.

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