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Evaluation of the Tobacco Heating System 2.2

Evaluation of the Tobacco Heating System 2.2. Part 7: Sy toxicological assessment of a mentholated version reveale reduced cellular and molecular exposure effects compared mentholated and non-mentholated cigarette smoke

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Highlights

- Systems toxicology assessment of candidate modified risk tobacco product (THS2.2M).
- Systems toxicology analyses were used to compare THS2.2M with a reference cigarette.
- Assessment of exposure effects using proteomics, transcriptomics, and lipidom
- Exposure showed lower effects of THS2.2M aerosol on rat nose and lung tissue
- Molecular insights on inflammation or cell stress complemented apical endpoint

Abstract

Modified risk tobacco products (MRTPs) are being developed with the aim of reduc

smoking-related health risks. The Tobacco Heating System 2.2 (THS2.2) is a candi
MRTP that uses the *heat-not-burn* principle. Here, systems toxicology approaches
engaged to assess the respiratory effects of mentholated THS2.2 (THS2.2M) in a 9
rat inhalation study (OECD test guideline 413). The standard endpoints were
complemented by transcriptomics and quantitative proteomics analyses of respirat
nasal epithelium and lung tissue and by lipidomics analysis of lung tissue. The adap
response of the respiratory nasal epithelium to conventional cigarette smoke (CS) i
squamous cell metaplasia and an inflammatory response, with high correspondenc
between the molecular and histopathological results. In contrast to CS exposure, the
adaptive tissue and molecular changes to THS2.2M aerosol exposure were much v
and were limited mostly to the highest THS2.2M concentration in female rats. In the
exposure induced an inflammatory response, triggered cellular stress responses, a
affected sphingolipid metabolism. These responses were not observed or were mu
after THS2.2M aerosol exposure. Overall, this system toxicology analysis complem
reconfirms the results from classical toxicological endpoints and further suggests p
reduced health risks of THS2.2M.

Keywords

Modified risk tobacco product; Systems toxicology; Transcriptomics; Proteomics;
Lipidomics; Subchronic inhalation toxicity

Abbreviations

THS2.2, Tobacco Heating System 2.2; THS2.2M, mentholated THS2.2; HPHC, ha
and potentially harmful constituent; CS, cigarette smoke; MRTP, modified risk tob
product; FDR, false-discovery rate; iTRAQ, isobaric tag for relative and absolute
quantitation; MRC, mentholated reference cigarette; LM, low menthol; HM, high m

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2 part of Philip Morris International group of companies.

This article belongs to a special issue

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Evaluation of the Tobacco Heating System 2.2. Part 8: 5-Day randomized reduced exposure clinical study in Poland

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ABSTRACT

The Tobacco Heating System (THS) 2.2, a candidate Modified Risk Tobacco Product (MRTP), is designed to heat tobacco without burning it. Tobacco is heated in order to reduce the formation of harmful and potentially harmful constituents (HPHC), and reduce the consequent exposure, compared with combustible cigarettes (CC). In this 5-day exposure, controlled, parallel-group, open-label clinical study, 160 smoking, healthy subjects were randomized to three groups and asked to: (1) switch from CCs to THS 2.2 (THS group; 80 participants); (2) continue to use their own non-menthol CC brand (CC group; 41 participants); or (3) to refrain from smoking (smoking abstinence (SA) group; 39 participants). Biomarkers of exposure, except those associated with nicotine exposure, were significantly reduced in the THS group compared with the CC group, and approached the levels observed in the SA group. Increased product consumption and total puff volume were reported in the THS group. However, exposure to nicotine was similar to CC at the end of the confinement period. Reduction in urge-to-smoke was comparable between the THS and CC groups and THS 2.2 product was well tolerated.

1. Introduction

The U.S. Family Smoking Prevention and Tobacco Control Act (FSPTCA) defines a Modified Risk Tobacco Product (MRTP) as "any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco related disease associated with commercially marketed tobacco products" (Family Smoking Prevention and Tobacco Control Act). This publication is part of a series of nine publications describing the nonclinical and part of the clinical assessment of a candidate MRTP, Tobacco Heating System (THS) 2.2 regular and a mentholated version (THS 2.2M). The series of publications provides part of the overall scientific program to assess the potential for THS 2.2 to be a reduced risk product. The first publication in this series describes THS 2.2 and the assessment program for MRTPs (Smith et al., 2016). This is followed by six publications, including this one, that describe the nonclinical assessment of THS 2.2 regular and THS 2.2M (Kogel et al., 2016; Oviedo et al., 2016; Schaller et al., 2016a,2016b; Sewer et al., 2016; Wong et al., 2016). The eighth publication in the series describes a clinical study to assess whether the reduced formation of Harmful and Potentially Harmful Constituents (HPHC) for THS 2.2 regular also leads to reduced exposure to HPHCs when the product is used in a clinical setting (Haziza, 2016). A final publication utilizes data gathered from the reduced exposure clinical study on THS 2.2 regular to determine if a systems pharmacology approach can identify exposure response markers in peripheral blood of smokers switching to THS 2.2 (Martin et al., 2016).

Novel tobacco products with the potential to reduce exposure to HPHCs in cigarette smoke, to lower the individual risk of smoking-related diseases, and lessen population harm compared to smoking combustible cigarettes (CC), are important components of current harm reduction strategies. The Tobacco Advisory Group of the Royal College of Physicians opined 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)). The Tobacco Heating System (THS) 2.2, a candidate reduced risk product, was developed by Philip Morris International (PMI) to provide an acceptable alternative to CC smoking and replicate the ritual, taste, sensory characteristics, and nicotine uptake of CC smoking.

Such reduced risk products are regulated in the US under the

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modified risk tobacco product draft guidance, issued by the Food and Drug Administration in 2012, which requires rigorous scientific assessment pre-marketing in order to sell a product with a claim to reduced harm (FDA (Food and Drug Administration), 2012a). THS 2.2 heats the tobacco, rather than burning it, and eliminates combustion to create a far less complex aerosol with lower levels of HPHCs compared with CCs (Borgerding and Klus, 2005; Forster et al., 2015). Heated tobacco products, including earlier THS versions, have been tested in clinical studies in which they reduced exposure to HPHCs and had favorable effects on early, clinically relevant risk markers of disease (Roethig et al., 2010; Sakaguchi et al., 2014; Unverdorben et al., 2010). The first version of the THS (THS 1.0) was launched in limited test markets, namely Switzerland, Japan, Australia, and Germany between 2006 and 2010. Consumer adoption was poor, mostly because of its bulky design and shortcomings in its sensory and taste characteristics. THS 2.2 addressed the shortcomings of THS 1.0 reported by consumers. In addition, the physical and functional characteristics of THS 2.2 were improved by lowering the temperature of the heating element to <350 °C.

The clinical study reported here aimed to demonstrate a reduction in exposure to HPHCs.

In this randomized and controlled clinical study, in a confined setting, we compared the effects of *ad libitum* use of THS 2.2, CC use, and smoking abstinence, on the concentrations of biomarkers of exposure to HPHCs, measured in urine and blood. THS 2.2 and CC were used without restriction. Dual use of CCs and THS 2.2 was not allowed. Additional outcomes included the subjective effects of smoking (product satisfaction, and urge-to-smoke), human puffing topography (HPT), and safety. This study provides a comprehensive insight on the maximum possible reduction in exposure to HPHCs after switching from CC to THS 2.2 and how these reductions compare to what is achieved when subjects stop smoking for the same period of time. Biomarkers of exposure were selected based on a "fit-for-purpose" approach (IOM (Institute of Medicine) 2012) which provides a reasonable representation of the exposure when an individual is using THS 2.2 exclusively along with safety information.

2. Methods

2.1. Participants

Adult Caucasian smokers aged 21–65 years were eligible for participation in the study. Potential participants were eligible if they smoked ≥10 commercially available non-menthol CCs per day with a maximum yield of 1 mg nicotine per cigarette (ISO yield) for the last 4 weeks and had smoked CC for ≥ 3 consecutive years before 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 examinations and a medical check-up. Only candidates not willing to quit smoking in the forthcoming 3 months were allowed in the study, but they had to be ready to accept a 5-day abstention from smoking. Participants with clinically relevant medical conditions, or who potentially required medical interventions (start of treatment, surgery, or hospitalization) and participants with a history of alcohol and/or drug abuse, or who used nicotine–containing products other than their own brand of CC, as well as pregnant or breast-feeding female subjects and females unwilling to use acceptable methods of effective contraception, were excluded. 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 discontinued. They were also referred to a smoking cessation counsellor to receive appropriate medical advice. All participants were compensated for their time and participation.

2.2. Study design

This study was designed as a controlled, randomized, three-arm parallel, single-center study in confinement. The Screening Period covered a maximum of 4 weeks (Day −30 to Day −3) prior to Admission on Day −2 to the study site. Prior to enrollment on Day −2, as the last procedure of the eligibility assessments on that day, all subjects participated in a product trial of THS 2.2 (using up to three THS 2.2 *tobacco sticks*). In female subjects, the THS 2.2 product trial was performed only after pregnancy was excluded by a negative urine pregnancy test. On Day −2, 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, participants smoked their own preferred brand of CCs for Baseline assessments. One hundred sixty participants were randomized with stratification by sex, and average self-reported daily cigarette consumption over 4 weeks, before 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) on Day 1 following the two days of Baseline assessments. From Day 1 to Day 5, participants in the THS and CC groups used, respectively, THS 2.2 or their own brand of non-menthol CCs exclusively. Participants in the SA arm were asked to completely abstain from smoking from Day 1 to Day 5. No participant was allowed to use any supportive medication for smoking abstention. On Day 6 or on the day of early discontinuation, end of study procedures were conducted. 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), serious adverse events (SAEs), or follow-up of any ongoing AEs/SAEs that occurred during confinement (Fig. 1).

From Day −1 to Day 5, for CC, and from Day 1 to Day 5 for THS 2.2, product use was allowed during the designated product use hours from 06:30 to 23:00 *ad libitum*, and 24-h urine was collected on each day. The study was conducted between June and September 2013 at BioVirtus Research Site (Kajetany, Poland). The study protocol was approved by the local ethics committee and adhered to the principles of the Declaration of Helsinki (World Medical Association (WMA), 2013), Good Clinical Practice guidelines (International Conference on Harmonisation, 2014) and national regulations. The study was registered at www.clinicaltrials.gov (NCT01959932).

2.3. Investigational products

The THS 2.2 product was developed and provided by Philip Morris Products S.A. (part of Philip Morris International group of companies). The product is described in part 1 of this series (Smith et al., 2016). Briefly, THS 2.2 has three components: the *tobacco stick*, the *holder*, and the *charger*. The *tobacco stick* (FR1 blend) contains a tobacco plug of processed tobacco cast leaf, which is enclosed in a paper wrap. The overall appearance of the *tobacco stick* is similar to a CC, except it is much shorter. The *holder* includes a battery, controlling electronics, and the heating element. The *tobacco stick* is inserted into the *holder*, and an electronically controlled heating blade heats the tobacco according to a carefully controlled temperature profile to temperatures not exceeding 300 °C. The *charger* recharges the *holder*.

To use THS 2.2, the *tobacco stick* is inserted into the *holder* and

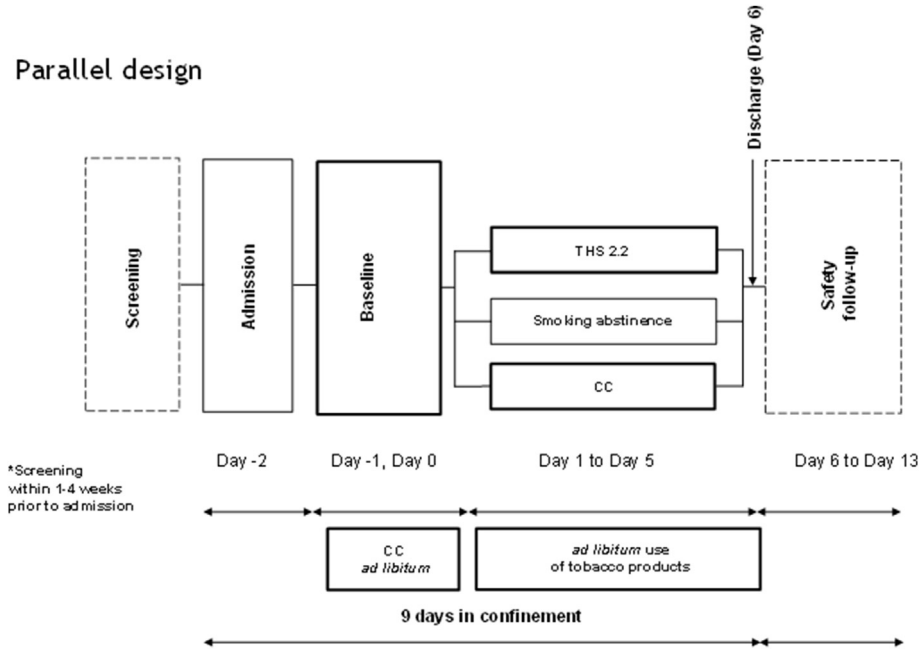

Fig. 1. Study design. Study design schematic representation of the procedures followed by participants. Abbreviations: CC = conventional cigarette use group; THS2.2 = Tobacco Heating System 2.2 use group.

the heating of the *tobacco stick* is initiated by pressing the button on the *holder*. An LED indicates when the initial heating process is complete. The *holder* and *tobacco stick* are designed for a usage period of approximately 6 minutes or for around 14 puffs. The *holder* must be recharged after each usage period of 6 min and a new *tobacco stick* must be used for the next usage cycle. The THS 2.2 test product contained 0.5 mg nicotine as determined under ISO conditions and 56.4 mg/stick of glycerin as aerosol former. The mainstream composition of the aerosol of THS 2.2, and its non-clinical assessment, including neutral red uptake assay, mouse lymphoma, and Ames assays are presented as part of this series, including 8 publications (Schaller et al., 2016a,2016b). A reduction of over 90% for the majority of HPHCs measured, and a decrease in cytotoxicity and mutagenic potential, was observed when compared to the 3R4F reference cigarette. The reference product in this clinical 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.

2.4. Sample size

The sample size was determined based on the expected least squares (LS) mean ratios (THS2.2: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 were randomized at a ratio of 2:1:1 to the THS, CC, and SA group respectively, and were considered sufficient to attain >80% power to show a reduction of ≥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 endpoints using a closed procedure.

2.5. Statistical analysis

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 percent change or relative change as compared to 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 observed on Day 5.

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 log-transformed baseline value of the biomarker. The estimated differences between the study groups and associated CIs were back-transformed to provide relative ratio (THS 2.2/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 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, and precise analytical methods.
4) The HPHC reflects a specific toxic exposure or is 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 (U.S. Department of Health and Human Services, 2012) (carcinogen, cardiovascular toxicant, respiratory toxicant, reproductive and development toxicant, addiction potential).
7) The list of HPHCs includes HPHCs formed at different temperature levels.
8) Most exhibit a variety of elimination half-life times ranging from a few hours up to more than 2 weeks with the majority of elimination half-life times below 24 h.

The study included biomarkers of exposure to the tobacco-specific HPHCs 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N-nitrosonornicotine (NNN), and the biomarkers of exposure of the following 4 HPHCs: 1,3-butadiene, acrolein, benzene, carbon monoxide on which study objectives and sample size were based. In addition to fullfilling criteria 1–8, these 4 biomarkers of exposure were selected as coefficient of variation and mean ratios between a previous prototype of THS and CC were available for each of them from a previous study (Lüdicke et al., 2016). Additionally biomarkers of exposure to other HPHCs were also included (Table 1). Ammonia was not measured as 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, 2014). 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.

In total, 16 HPHCs were evaluated to assess exposure reduction in the THS group compared to the CC and SA groups (Table 1).

In addition, 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, and trans-3'-hydroxycotinine-glucuronide) were also measured to assess nicotine uptake when smokers switched to THS 2.2 use compared to continued CC smoking.

The biomarkers of exposure were assessed in blood, or 24-h urine samples. Creatinine was also measured in 24-h urine for adjustment of the concentration of all urinary biomarkers of exposure. With the exception of COHb and o-toluidine (o-tol), all biomarkers of exposure were determined by liquid chromatography-tandem mass spectrometry. COHb and creatinine were measured by spectrophotometry, and o-tol was measured by gas chromatography–mass spectrometry. All laboratory analyses were carried out using validated methods at Celerion Laboratories (Lincoln, NE, USA and Zurich, Switzerland) except for COHb, which was measured at Synevo Central Lab Sp. z o.o. (Warsaw, Poland).

All bioanalytical assays used were validated and all assays conducted by Celerion Laboratories met the requirements of the FDA Guidance to Industry: Bioanalytical Method Validation (FDA (Food and Drug Administration), 2001). Details on the bioanalytical methods conducted at Celerion Laboratories are reported in a data in brief (Haziza et al., 2016).

2.7. Cytochrome 1 A2 activity

Because CYP1A2 is an enzyme inducible by polycyclic aromatic amines (PAH), a group of carcinogens found in cigarette smoke (Butler et al., 1992), CYP1A2 activity was measured in this study as an indicator of 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 coffee made from 4.2 g (±10%) regular instant coffee (Nescafé Gold Instant; Nestlé; Deutschland; CAF content: 72 mg/2 g) with 150 ml ± 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. Urine mutagenicity test

The urine mutagenicity test, the Ames test (Ames et al., 1975), was assessed at baseline and Day 5 to provide an estimate of the mutagenic load of urine samples from subjects exposed or not to HPHCs (Gregg et al., 2013). The bacterial strain *Salmonella typhimurium* YG1024, a derivative of the original T98 strain used by Ames, was used, in presence of the metabolic activator S9, as it was shown to be more sensitive to mutagenicity of tobacco smoke (Einisto et al., 1990). The assay was run in accordance with the "OECD guideline for testing of chemicals: bacterial reverse mutation test " (OECD (Organization for Economic Co-operation and Development) 1997)".

2.9. Product use and human puffing topography (HPT)

CC consumption was recorded for all participants from Day −2, and *tobacco sticks* were recorded from Day 1 onwards in participants randomized to the THS group. All products were dispensed individually by the site staff at the participant's request, and dispense of each product was documented in log-books. Smoking abstinence for participants in the SA group was verified by CO breath tests performed 4 times/day (CO < 10 ppm). HPT was performed to measure the average puff duration, inter-puff interval, total puff volume, average puff volume, total number of puffs, and puff frequency 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 THS2.2 and the HPT Sodim® Device and sample holder were validated, according to our internal Quality Management System, to ensure that measurements performed with the device and sample holder are accurate and repeatable. Puffing topography was only assessed in subjects who smoked CCs that were compatible with the HPT device. Therefore, users of slim CCs were excluded from HPT assessments.

2.10. Subjective effects of smoking

The subjective effects of smoking were assessed using self-reported questionnaires that had been validated in the local language. Nicotine dependence was assessed at the Screening Visit using the revised version of the Fagerström Test for Nicotine Dependence (FTND) (Fagerström et al., 2012). Product evaluation was performed using the modified Cigarette Evaluation

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-nitrosonornicotine	N-nitrosonornicotine (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-ABP)	4-aminobiphenyl
1-NA	1-aminonaphthalene (1-NA)	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 matrices; 1 = blood. HPHC = harmful or potentially harmful smoke constituent.
[a] Total NNAL was determined as the molar sum of 4-(methylnitrosamino)-1-(3-pyridy1)-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.

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 smoking is perceived as rewarding and is perceived as providing 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).

2.11. Adverse events, medical history, and concomitant medication

Safety assessment included AEs, SAEs, THS2.2 malfunctions and misuse, vital signs, electrocardiography, spirometry, clinical chemistry, hematology, urinalysis, physical examinations, and use of concomitant medications. AEs were recorded from the signing of 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 (Version Q1 2013) (Uppsala Monitoring Centre, 2012).

3. Results

3.1. Participant disposition and characteristics

The study site screened 329 subjects. 160 were screen failures, 169 tried the THS2.2 during the product test, 9 subjects were enrolled but not randomized; 8 for “abnormal” assessments and 1 for poor vein conditions. (Fig. 2).

A total of 160 participants were randomized, with 80, 41, and 39 participants in the THS, CC, and SA groups, respectively. All of the randomized participants completed the study, except for one participant in the THS group who voluntarily withdrew. There were no marked differences in the age, body mass index, and mean FTND total scores among the three groups. Half of the participants (49.7%) were classified as showing moderate nicotine dependence according to the FTND questionnaires. The three groups were also comparable in terms of the distributions of sex and daily cigarette consumption. Most of the participants smoked cigarettes with an ISO tar yield of 6–8 mg. In each ISO tar yield category, subjects were comparably distributed between the THS, CC, and SA groups (Table 2).

3.2. Number of tobacco sticks and CCs used daily

In the THS group, the mean ± SD number of *tobacco sticks* used daily initially decreased from the number of CCs smoked at baseline (16.0 ± 3.5) to Day 1 (14.9 ± 6.1) and then increased between Day 2 (17.3 ± 6.3) and Day 5 (20.7 ± 8.1). The number of *tobacco sticks* used from Day 2 onwards was greater than the number of CCs smoked at baseline. The mean number of CCs consumed daily initially decreased between baseline (16.2 ± 4.1) and Day 1 (14.5 ± 3.6), but then increased by Day 5 (16.6 ± 3.8) to the baseline level. During the study period, participants in the THS group consumed a greater number of *tobacco sticks* than the number of CCs smoked by participants in the CC group (Table 3).

3.3. Biomarkers of exposure

Levels of biomarkers of exposure to 16 HPHCs and to nicotine are presented in the data in brief Table 1 (Haziza et al., 2016) at Baseline and at Day 5 for the THS, CC and SA groups (all urinary biomarkers of exposure are expressed as concentrations adjusted to creatinine). At baseline, the levels of biomarkers of exposure were comparable in all three groups, except for MHBMA, which was slightly higher in the CC group, but comparable between the THS and SA groups. On Day 5, at the end of the exposure period, the levels of fifteen 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, data in brief, Table 2; Haziza et al., 2016). The reduction was of similar magnitude between the THS and SA groups for each biomarker except for total NNAL, total NNN and 3-HPMA for which the reduction was slightly higher in SA than THS groups.

Significant reductions in the levels of the biomarkers of exposure (urinary biomarkers of exposure expressed as concentrations adjusted to creatinine) were demonstrated in the THS group relative to the CC group on Day 5, with percent reductions of 77% for

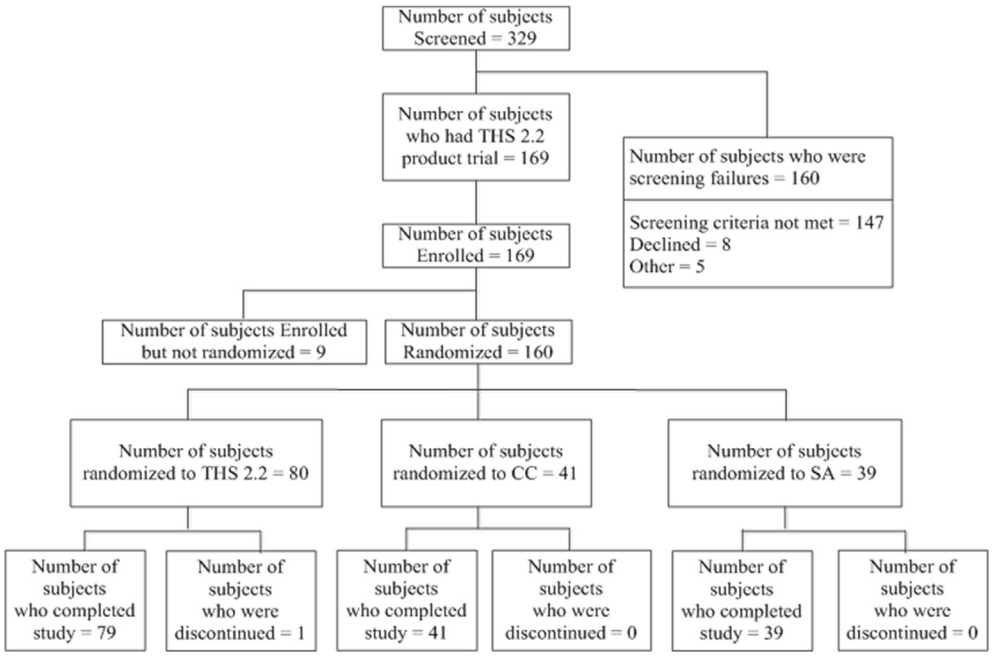

Fig. 2. Disposition of cases. Description of participants' disposition in the course of the study. Abbreviations: CC = conventional cigarette use group; SA = smoking abstinence group; THS2.2 = Tobacco Heating System 2.2 use group.

COHb, 58% for 3-HPMA, 92% for MHBMA, and 94% for S-PMA. Furthermore, reductions of 56% in Total NNAL, 76% in Total NNN, 56% in Total 1-OHP, 85% in 4-ABP, 96% in 1-NA, 88% in 2-NA, 58% in o-toluidine, 87% in CEMA, 68% in HEMA, 77% in 3-HMPMA, and 72% in total-3-OH–B[a]P were observed in the THS group relative to the CC group (Table 4). Comparable levels of reduction were obtained when the urinary biomarkers of exposure were expressed as quantity excreted over 24 h (data in brief, Table 3; Haziza et al., 2016).

The levels of S-BMA, a biomarker of exposure to toluene, were comparable across all 3 study groups throughout the study including at baseline (data in brief, Tables 1 and 2; Haziza et al., 2016). 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 are abstinent from smoking, an observation reported by other authors as well (Imbriani et al., 1999; Schettgen et al., 2008).

3.4. Exposure to nicotine

Exposure to nicotine was comparable for the THS and CC groups throughout the study period. In the THS and CC groups, geometric mean NEQ values increased from baseline (9.01 mg/g creat for THS and 8.69 mg/g creat for CC) to Day 1 (9.19 mg/g creat for THS and

Table 2
Demographic characteristics by group, at baseline.

Variable and statistic	THS N = 80	CC N = 41	SA N = 39
Age (years)			
Mean ± SD	35.4 ± 9.40	32.6 ± 10.06	33.6 ± 11.51
Range	22 to 59	21 to 59	21 to 60
BMI (kg/m2)			
Mean ± SD	24.46 ± 3.034	25.80 ± 3.228	24.81 ± 2.505
Range	18.9 to 31.6	18.8 to 31.8	20.3 to 31.6
Sex, n (%)			
Male	39 (48.8%)	21 (51.2%)	20 (51.3%)
Female	41 (51.3%)	20 (48.8%)	19 (48.7%)
Daily CC consumption, n (%)			
10 to 19 cigarettes	41 (51.3%)	21 (51.2%)	19 (48.7%)
>19 cigarettes	39 (48.8%)	20 (48.8%)	20 (51.3%)
ISO tar yield, n (%)			
1–5 mg	6 (7.5%)	7 (17.1%)	4 (10.3%)
6–8 mg	43 (53.8%)	26 (63.4%)	29 (74.4%)
9–10 mg	31 (38.8%)	8 (19.5%)	6 (15.4%)
FTND total score[a]			
Mean ± SD	5.0 ± 2.02	5.1 ± 1.83	5.5 ± 1.93
Range	1 to 9	1 to 9	1 to 9

[a] Calculated on a different N; THS = 76, CC = 40, SA = 38. Abbreviations: CC = combustible 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 standardization organization.

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

Visit day	THS N = 80	CC N = 41
Baseline use		
Mean ± SD	16.0 ± 3.45	16.2 ± 4.05
Range	9–33	10–32
Day 1		
Mean ± SD	14.9 ± 6.14	14.5 ± 3.63
Range	5–50	6–22
Day 2		
Mean ± SD	17.3 ± 6.25	15.1 ± 3.79
Range	6–40	8–24
Day 3		
Mean ± SD	18.2 ± 5.94	14.9 ± 3.49
Range	6–38	8–22
Day 4[a]		
Mean ± SD	18.5 ± 6.69	14.3 ± 3.43
Range	9–50	8–21
Day 5[a]		
Mean ± SD	20.7 ± 8.09	16.6 ± 3.79
Range	9–60	10–26

[a] Calculated on a different N; THS = 79. Abbreviations: CC = combustible cigarette use group; THS = Tobacco Heating System 2.2 use group; SD = standard deviation.

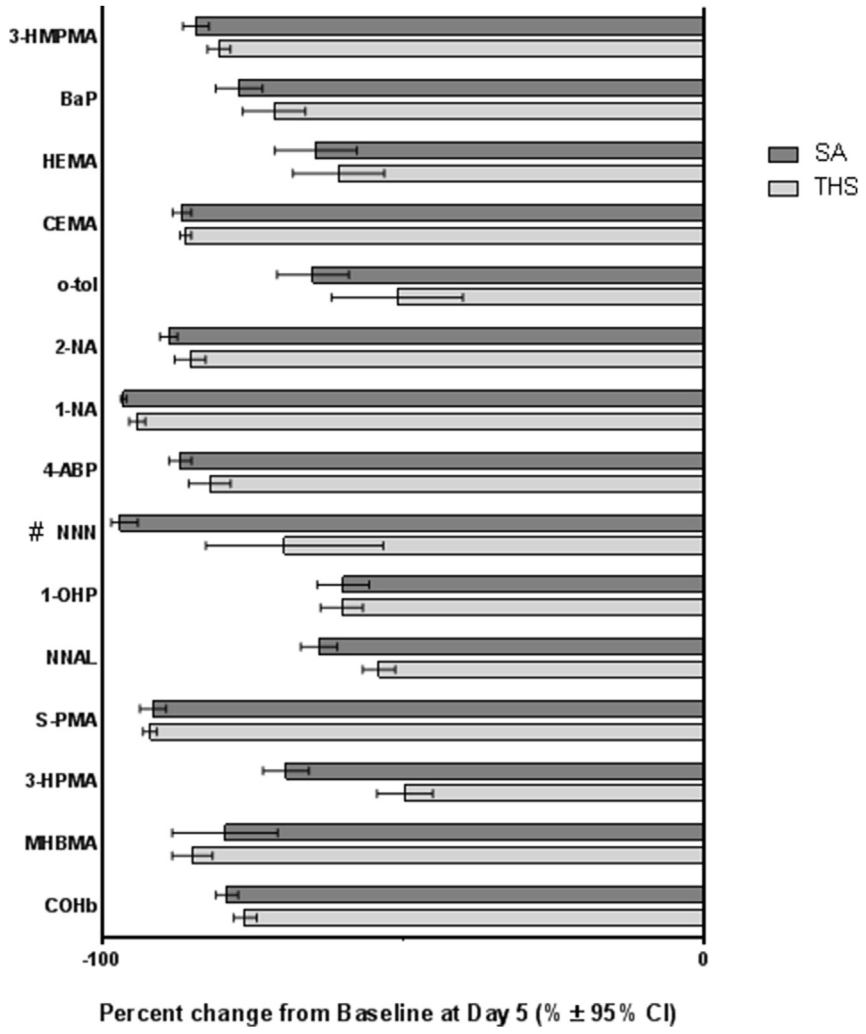

Fig. 3. Disposition of cases. Relative change from baseline were calculated from the geometric means values for each biomarker of exposure. # for total NNN, the median change from Baseline is reported. CC: combustible cigarette; SA: smoking abstinence.

10.27 mg/g creat for CC), decreased slightly from Day 1 to Day 2 (8.66 mg/g creat for THS and 8.91 mg/g creat for CC), to progressively increase to Day 5 (10.60 mg/g creat) for THS and (9.76 mg/g creat) for CC. The values on Day 5 correspond to percent changes from baseline of 22.95% and 14.78% for the THS and CC groups, respectively (Table 4). The NEQ levels in the THS group were similar to those of the CC group on Day 5, with a THS vs. CC ratio of 104.9% (95% CI: 92.0; 119.6). Similar results were obtained with plasma nicotine and cotinine. On Day 5, the geometric mean (95% CI) plasma nicotine (ng/mL) concentrations were similar in the THS and CC groups and were 20.7 (17.46; 24.62) vs. 19.0 (16.52; 21.87) for CC, and plasma cotinine (ng/mL) was 240.0 (211.30; 272.58) vs. 219.7 (190.21; 253.83) for CC.

3.5. Cytochrome 1A2 activity

At baseline, CYP1A2 activity was similar in all three groups. On Day 5, the LS mean CYP1A2 activity following coffee intake was 91.35% and 124.95% in the THS and CC groups respectively with LS mean difference THS-CC of −33.60% (95% CI: −40.59, −26.61). The activity was comparable between the THS and the SA groups (LS mean difference THS-SA: −1.99% (95% CI: −9.10, 5.12).

3.6. Urine mutagenicity test

At Baseline, median Ames mutagenicity test values were 19681 Rev/24 h (min: 0; max: 107,250 Rev/24 h) for THS, 22540 Rev/24 h (min: 3060; max: 113,620 Rev/24 h) for the SA arms, and 15,775 Rev/24 h (min:0; max: 72,216 Rev/24 h) for the CC arm. On Day 5, in the THS and SA arms, the median decrease from baseline for Ames mutagenicity test values was approximately −57% (8823 Rev/24 h; min:0; max: 39,600 Rev/24 h) and −62% (7437 Rev/24 h; min:0; max: 65,400 Rev/24 h), respectively, while in the CC arm, median values increased from Baseline by approximately 29% (21,689 Rev/24 h; min:0; max: 63,840 Rev/24 h).

3.7. Human puffing topography

The baseline values for each assessed HPT parameter were generally comparable in the THS and CC groups while subjects were smoking their own preferred brand of CC and remained unchanged in the CC group between Baseline and Day 1, and between Day 1 and Day 4.

In the THS group, values for total puff volume, average puff volume, and total number of puffs were within the same ranges from Baseline to Day 4. In contrast, average puff duration, total puff

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

Biomarkers	Ratio[a] % and CI THS (N = 81)/CC (N = 41)
NEQ (mg/g creat)	104.9 (92.0; 119.6)
Nicotine[b,c] (ng/mL)	112.9(91.3; 139.5)
Cotinine[b,c](ng/mL)	111.0 (90.8; 135.7)
Total NNAL (pg/mg creat)	43.5 (39.3; 48.2)
Total NNN (pg/mg creat)	24.1 (17.7; 32.8)
COHb[c] (%)	23.5 (22.0; 25.0)
MHBMA (pg/mg creat)	8.4 (6.8; 10.2)
3-HPMA (ng/mg creat)	41.6 (37.7; 46.0)
S-PMA (pg/mg creat)	6.0 (5.2; 6.9)
Total 1-OHP (pg/mg creat)	44.3 (39.8; 49.4)
4-ABP (pg/mg creat)	14.9 (12.8; 17.4)
1-NA (pg/mg creat)	3.7 (3.1; 4.5)
2-NA (pg/mg creat)	11.5 (10.0; 13.3)
o-tol (pg/mg creat)	41.7 (36.0; 48.3)
CEMA (ng/mg creat)	13.2 (11.5; 15.0)
HEMA (pg/mg creat)	32.0 (27.1; 37.8)
3- HMPMA (ng/mg creat)	22.5 (20.1; 25.3)
Total 3-OH-B[a]P (fg/mg creat)	27.5 (23.2; 32.6)

[a] Ratio: Geometric least squares mean ratio (%) and confidence intervals from an ANCOVA model conducted on log-transformed Day 5 values with log-transformed baseline value (urinary biomarker of exposure expressed as concentration adjusted to creatinine), study arm, sex and CC consumption reported at screening as fixed effect factors (THS/CC) on Day 5.
[b] Weighted average concentration over 24 h (Cavg); also for nicotine and cotinine the ratio is calculated on the weighted average concentration over 24 h.
[c] Measured between 8:00 PM and 10:00PM. Abbreviations: CI = Confidence interval. CC = combustible cigarette group; THS = Tobacco Heating System 2.2 group.

duration, and puff frequency all progressively increased from baseline to Day 1 and then to Day 4.

The average puff duration was about 26% and 32% longer relative to CC on Day 1 and Day 4 respectively, with values of 2.0s for THS vs 1.6s for CC on Day 1 and 2.1s for THS vs 1.6s for CC on Day 4. The total puff duration was about 36% and 37% longer relative to CC on Day 1 and Day 4 respectively. The puff frequency was about 31% higher and 32% higher on Day 1 and Day 4 respectively for THS vs CC (Table 5).

3.8. Subjective effects of smoking

3.8.1. Modified cigarette evaluation questionnaire subscales

The mCEQ average results of the whole 5 day exposure period showed that, adjusted for baseline, smoking satisfaction, craving reduction, enjoyment of respiratory tract sensation and psychological reward, were lower for participants who switched to THS 2.2 use compared to participants who continued to smoke CC, with differences of −1.26,-1.12, −1.00, −0.72, respectively with 95% CIs excluding 0. Aversion was comparable between THS and CC (Table 6).

3.8.2. Urge-to-smoke symptoms (QSU-brief)

The mean urge-to-smoke total scores were comparable in all groups at baseline, with scores of 3.69, 3.49, and 3.49 in the THS, CC, and SA groups, respectively. The mean urge-to-smoke total scores remained stable and were comparable between the THS and CC groups throughout the study, ranging from 2.9 to 3.3 in the THS group, and from 3.2 to 3.5 in the CC group. Considering all time-points of assessment, the QSU-brief total score was comparable between the THS and CC groups with the difference between THS and CC of −0.3 (95% CI: −0.75, 0.12) (Fig. 4).

In the SA group, as expected, the urge-to-smoke total score increased significantly from 3.49 at baseline to 5.3 on Day 1, corresponding to an increase of 1.8 (95% CI: 4.82, 5.76). From Day 3 onwards, the urge-to-smoke started to decrease but remained above the baseline value on Day 5. The LS mean difference in QSU-brief total score between the THS group and the SA group was −1.8 (95% CI: −2.27, −1.39).

Table 5
Human puffing topography parameters per cigarette in THS and CC groups.

Variable and Day	THS N = 56	CC N = 27	THS/CC Ratio (%) N = 83
Total Puff Volume (mL)			
Baseline[a]	774.6 (774.7; 819.6)	899.3 (764.4; 1034.2)	
Day 1[b]	792.8 (725.3; 860.3)	819.2 (728.8; 909.7)	109.5 (94.7; 126.7)
Day 4[c]	810.5 (752.4; 868.7)	845.8 (757.2; 93,452)	105.2 (92.5; 119.7)
Average Puff Volume (mL)			
Baseline[a]	53.4 (49.8; 57.1)	53.6 (46.8; 60.5)	
Day 1[b]	50.6 (46.2; 55.0)	49.2 (43.7; 54.8)	101.5 (90.6; 113.)
Day 4[c]	52.9 (84.7; 57.1)	49.4 (44.2; 54.6)	105.5 (95.5; 116.5)
Average Puff Duration (s)			
Baseline[a]	1.6 (1.5; 1.8)	1.5 (1.3; 1.8)	
Day 1[b]	2.0 (1.8; 2.2)	1.5 (1.3; 1.8)	126.0 (115.3; 137.9)
Day 4[c]	2.1 (1.9; 2.4)	1.5 (1.3; 1.8)	132.2 (120.2; 145.5)
Total Puff Duration (s)			
Baseline[a]	23.8 (22.1; 25.5)	25.9 (23.2; 28.6)	
Day 1[b]	32.0 (29.5; 34.7)	26.2 (23.2; 29.3)	136.0 (119.8; 154.5)
Day 4[c]	33.2 (30.1; 36.4)	26.6 (23.6; 29.8)	137.3 (119.3; 158.1)
Total Number of Puffs			
Baseline[a]	15.1 (14.1; 16.1)	17.1 (15.3; 19.0)	
Day 1[b]	16.2 (15.4; 17.0)	17.2 (15.3; 19.1)	103.0 (94.2; 112.7)
Day 4[c]	15.7 (15.1; 16.5)	17.7 (15.9; 19.7)	97.1 (89.4; 105.6)
Puff frequency (puffs/min)			
Baseline[a]	3.5 (3.2; 3.9)	4.4 (3.7; 5.2)	
Day 1[b]	5.0 (4.7; 5.4)	4.6 (3.9; 5.3)	131.3 (116.9; 147.5)
Day 4[c]	5.2 (4.9; 5.6)	4.8 (4.0; 5.7)	132.3 (118.2; 148.1)

All values are Mean and 95% CI, except THS/CC mean ratio (%); adjusted geometric least squares means ratio, and 95% confidence intervals.
Abbreviations: CC = combustible cigarette use group; THS = Tobacco Heating System 2.2 use group.
[a] Calculated on a different N; THS = 56.
[b] Calculated on a different N; THS = 79.
[c] Calculated on a different N; THS = 78.

Table 6
Analysis of change from baseline in mCEQ.

Subscale	THS N = 79	CC N = 41	(THS − CC) difference N = 120
Smoking Satisfaction	−1.51	−0.25	−1.26 (−1.68, −0.85)
Aversion	0.15	−0.10	0.25 (0.04, 0.46)
Craving Reduction	−1.53	−0.41	−1.12 (−1.58, −0.66)
Enjoyment of Respiratory Tract Sensation	−1.23	−0.23	−1.00 (−1.36, −0.64)
Psychological Reward	−1.13	−0.41	−0.72 (−1.06, −0.39)

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

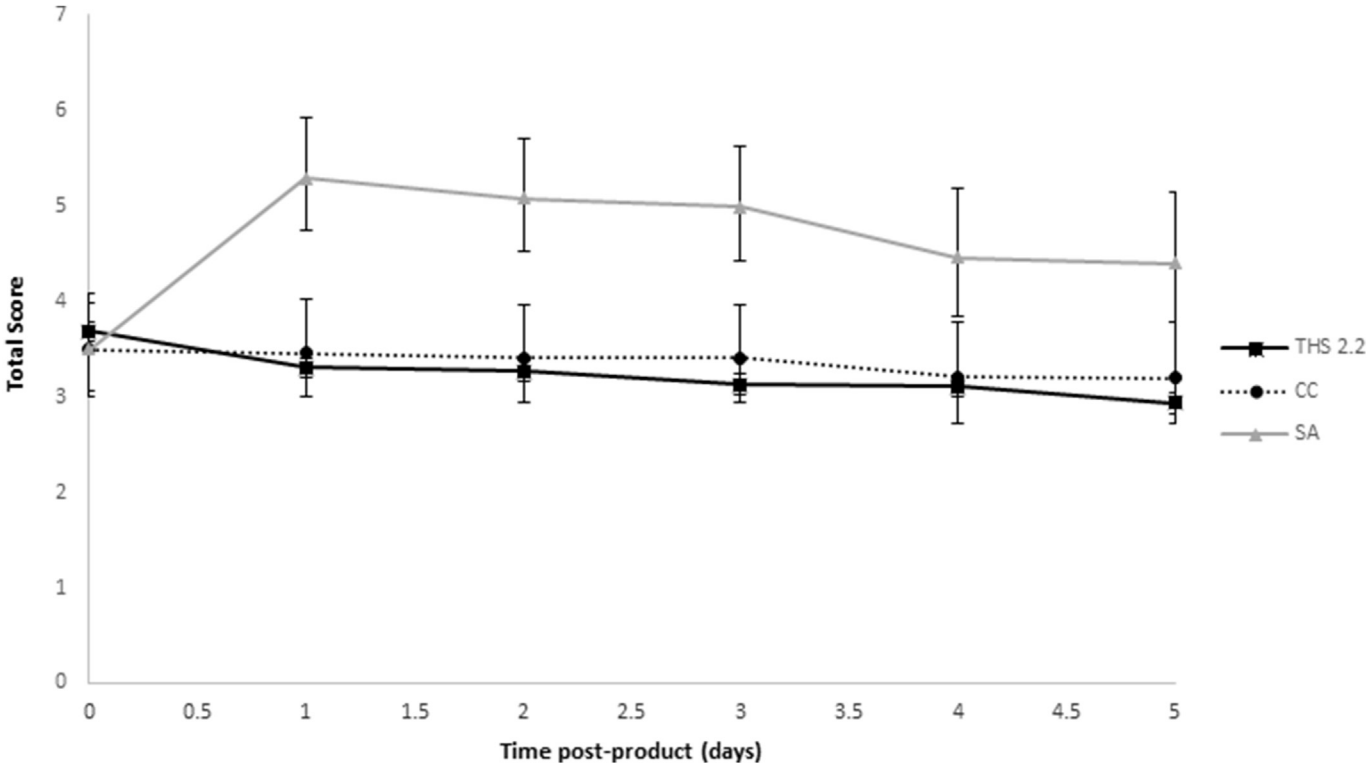

Fig. 4. Questionnaire on smoking urges mean total score. Comparison of the relief from smoking total scores derived from the QSU-brief. Results are presented as the mean (95% confidence interval). Abbreviations: CC = combustible cigarette use group; SA = smoking abstinence group; THS = Tobacco Heating System 2.2 use group.

3.9. Safety

The safety population consisted of 169 participants. This included all 160 randomized participants and 9 individuals who were enrolled and exposed to THS 2.2 from the product test on Day −2 but were not randomized. Overall, 227 AEs were reported by 112/169 participants. There were no SAEs and none of the randomized subjects were discontinued from the study because of an AE. Most of the AEs were classified as mild or moderate in severity. Only one severe AE was reported. This severe AE occurred in the CC group and was not considered to be related to CC use or to study procedures. The incidence of AEs with frequency >5% were similar in the THS (50/80 participants [62.5%]), CC (29/41 [70.7%] participants), and SA (24/39 [61.5%]) groups. The 9 participants who were exposed, but not randomized, reported at least one AE each. The most frequent AEs in the THS and CC groups were headache, oropharyngeal pain, syncope, polyuria, and spirometry abnormal. The most frequent AEs in the SA group were headache, back pain, influenza-like illness, spirometry abnormal, abdominal distension, hypertriglyceridemia, polyuria, hypertension, and vertigo. A descriptive summary of AE with frequency > than 5% is provided in Table 7.

4. Discussion

The present study demonstrated that switching to THS 2.2 leads to a 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, reduction in an additional 11 biomarkers of exposure was observed in subjects using THS 2.2 for 5 days compared to subjects continuing to smoke CC. Biomarkers of exposure to HPHCs were assessed and 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 was comparable to that observed in the smoking abstinence group.

The reduction of the 15 biomarkers of exposure in the THS group vs the CC group ranged from 56% to 96%. For the biomarkers of exposure which were measured, a similar magnitude of reductions

Table 7
Adverse events.

Adverse Events	THS (N = 80)	CC (N = 41)	SA (N = 39)	Overall Safety (N = 169)
Total AEs (%)	50 (62.5%)	29 (70.7%)	24 (61.5%)	112 (66.3%)
AEs (%) over 5%				
Headache	24 (30.0%)	16 (39.0%)	13 (33.3%)	56 (33.1%)
Syncope	6 (7.5%)	4 (9.8%)	0	10 (5.9%)
Spirometry abnormal	4 (5.0%)	3 (7.3%)	2 (5.1%)	12 (7.1%)
Abdominal distension	0	0	2 (5.1%)	2 (1.2%)
Oropharyngeal pain	7 (8.8%)	3 (7.3%)	0	10 (5.9%)
Hypertriglyceridaemia	3 (3.8%)	2 (4.9%)	2 (5.1%)	7 (4.1%)
Polyuria	6 (7.5%)	4 (9.8%)	2 (5.1%)	12 (7.1%)
Back pain	3 (3.38%)	2 (4.9%)	3 (7.7%)	8 (4.7%)
Hypertension	1 (1.3%)	1 (2.4%)	2 (5.1%)	6 (3.6%)
Influenza like illness	0	1 (2.4%)	3 (7.7%)	4 (2.4%)
Vertigo	3 (3.8%)	0	2 (5.1%)	5 (3.0%)

Description of total adverse events occurred during the study and summary of adverse events with frequency > than 5%. 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 = combustible 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.

were found in a study where smokers of up to 20 CC at baseline switched for 5 days to smoking abstinence (Theophilus et al., 2015) as well as in other studies on smoking abstinence (Carmella et al., 2012; Sarkar et al., 2008).

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 (Food and Drug Administration, 2012a,2012b). The present study assessed 16 HPHCs, including 14 of the HPHCs requested by the FDA and 9 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 polycyclic aromatic hydrocarbon, and exposure to the aromatic amine o-toluidine (o-tol), as both are strong carcinogens, associated with colon and bladder cancer (IARC (International Agency for Research on Cancer), 2012) as well as exposure to ethylene oxide (HEMA), 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). 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 from 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 environmental exposure, its short half-life and lack of a specific metabolite, as well as the lack of a robust, simple and validated method suitable for a clinical setting, exposure to formaldehyde was not measured in this study.

The study showed that switching from CCs to THS 2.2 for 5 days reduced biomarkers of exposure to HPHCs to values generally lower than 50% or more, and approached exposure reduction observed in the SA group. Although the magnitude of reduction for total NNAL, total NNN, 3-HPMA, and 3-HMPMA, were slightly lower in the THS group relative to SA, the levels found in both the THS and SA group were considerably lower when compared to the levels of biomarkers of exposure found in the CC group. For total NNAL, total NNN, and 3-HMPMA, it is likely that these low levels can be detected in urine of THS groups because of the direct transfer of NNN, NNK, and crotonaldehyde which is reported to occur when tobacco is heated at low temperature between 100 °C and 200 °C (Forster et al., 2015; Rodgman and Perfetti, 2013). For 3-HPMA, it is described in the literature that it could be formed from the degradation of glycerin (Qadariyah et al., 2011). The exposure reduction observed in the SA group was overall preserved in participants using THS 2.2. In conclusion, the elimination of combustion provided by the heat-not-burn technology, effectively minimized the exposure to HPHCs usually found in CC smokers.

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. These active metabolites (N-acetoxy derivatives) can react with DNA to form covalent heterocyclic amine-DNA adducts, which are strong carcinogens associated with colon and bladder cancer (Matsuda et al., 2015; Gunes and Dahl, 2008; MacLeod et al., 1997). In addition, the CYP1A2 expression itself is induced to a large extent by PAH which are found in cigarette smoke (Butler et al., 1992). The 72% reduction of total-3-OH–B[a]P, a PAH; after 5 days of THS use compared to CC, likely explains the ~30% reduction in CYP1A2 activity in the THS group, similar to the reduction in the SA group. Thus, the reduction in enzymatic activity of CYP1A2 when subjects use THS for 5 days, as observed upon smoking cessation, is not only an additional indicator of reduced exposure to HPHCs but also reflects a favorable biological change associated with the lower level of active and carcinogenic metabolites. This may support the potential of THS to lower the risk of tobacco-related diseases.

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 lead to a reduction in CYP1A2 gene and protein expression to levels approaching those of cessation (Lo Sasso et al., 2016).

Along with these results, the similar magnitude of decrease in mutagenic load of urine samples from subjects in both THS and SA groups, add to the evidence of reduced exposure to HPHCs. The high variability of this test as exhibited by the large range covered by the minimum and maximal values for each median estimate is in agreement with other published data (Miura et al., 2015; Roethig et al., 2008; Sakaguchi et al., 2014; Sarkar et al., 2010). This high variability is well known and could be explained by the high sensitivity of the test to factors other than tobacco smoke such as diet, but also by the complexity of the assay as a cellular test.

The reduction in exposure to HPHCs was not found to be related to differences in product consumption, as daily product use increased in the THS group and was overall higher than in the CC group. It was anticipated that switching to THS 2.2 would require a period of adaptation for the participants concerned as the THS users were using a new product with a different nicotine yield, taste, and sensory characteristics compared to their own brand of CC. The topography and consumption results, as well as the results observed for subjective effects measures support this interpretation. Adjustment of individuals to the new product occurred during the study, not only through an increase in daily *tobacco stick* use, compared to CC daily consumption, but also through changes in HPT parameters. In summary, the HPT assessment indicated that participants using THS 2.2, had a comparable total puff volume, took a similar number of puffs of a similar volume, but adapted to the product by taking longer puffs with an increased average puff duration and total puff duration, while shortening the inter-puff

interval and increasing the puff frequency compared to those smoking CC. These changes in product use behavior allowed participants in the THS group to titrate nicotine closely to their baseline levels before the product switch and to comparable levels as observed in the CC group at the end of the 5-day exposure period.

Whilst the urge-to-smoke scores were comparable between groups throughout the study, the subscales of mCEQ showed that craving reduction, enjoyment of respiratory tract sensation, psychological reward, and smoking satisfaction were all lower for THS 2.2 compared to CC throughout the 5 days of exposure. By contrast, aversion was higher for THS than CC from Day 1 (the first Day of switching) with the difference between the two groups becoming progressively smaller up to Day 5. However, it is likely that the duration of the study was not sufficient to show the completion of an adaptation process and the study should be taken with the limitations inherent to the design.

One limitation of this 5 day study is that it could not capture a completed reduction of the biomarkers of exposure with a long half-life such as Total NNAL which exhibit a half-life of about 10–18 days (Goniewicz et al., 2009; Hecht et al., 1999). Nevertheless, the 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 confinement setting limits the generalizability of the results to real-world conditions of use and the interpretation of subjective effects related to THS 2.2. Indeed, the adaptation of smokers to a new tobacco product is likely to take longer than 5 days.

For these reasons, longer studies in ambulatory settings need to be conducted to evaluate how the reductions would be sustained with less control over product use. Yet this study allowed an assessment of comparative exposure under optimal conditions, by its randomized controlled design, the *ad libitum* product use in a confined setting, and by avoiding dual use.

A strength of the study was that all 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, toxicity grades, half-lives, gas and particulate phases, and formation temperatures, providing indication that THS 2.2 reduces exposure to a broad spectrum of HPHCs. In conclusion, this study showed that *ad libitum* use of THS 2.2 for 5 days reduced biomarkers of exposure to 15 HPHCs as compared with continuing CCs, and that decreases in the THS group approached those observed after smoking abstinence. The observed reductions in biomarkers of exposure to HPHCs occurred despite an increase in *tobacco stick* use and changes in puffing behavior compared with CC use. Although the scores for product evaluation and the sensory experience for THS 2.2 were lower than those for CCs, urge-to-smoke reduction and aversion reduction over time indicate that THS 2.2 offers a suitable alternative to CCs, although it may take longer than 5 days to complete adaptation to THS 2.2. THS 2.2 was well tolerated and had a safety profile comparable with CCs.

Conflict of interest statement

The work reported in this publication involved a candidate Modified Risk Tobacco Product developed by Philip Morris International (PMI) and was solely funded by PMI. All authors are (or were) employees of PMI R&D or worked for PMI R&D under contractual agreements.

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References

Ames, B.N., et al., 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31, 347–364.
Borgerding, M., Klus, H., 2005. Analysis of complex mixtures – cigarette smoke. Exp. Toxicol. Pathol. 57, 43–73.
Butler, M.A., et al., 1992. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2, 116–127.
Cappelleri, J.C., et al., 2007. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. Addict. Behav. 32, 912–923.
Carmella, S.G., et al., 2012. Correction to effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. Chem. Res. Toxicol. 25, 763.
Cox, L.S., et al., 2001. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob. Res. 3, 7–16.
Einisto, P., et al., 1990. Sensitivity of Salmonella typhimurium YG1024 to urine mutagenicity caused by cigarette smoking. Mutat. Res. 245, 87–92.
Euler, D.E., et al., 1996. Effect of cigarette smoking on pentane excretion in alveolar breath. Clin. Chem. 42, 303–308.
Faber, M.S., Fuhr, U., 2004. Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. Clin. Pharmacol. Ther. 76, 178–184.
Fagerström, K., et al., 2012. The Fagerström Test for Nicotine Dependence as a predictor of smoking abstinence: a pooled analysis of varenicline clinical trial data. Nicotine Tob. Res. 14, 1467–1473.
Family Smoking Prevention and Tobacco Control Act. Public Law No. 111–131 (June 22, 2009).
FDA (Food and Drug Administration), 2001. Guidance for Industry. Analytical Procedures and Methods Validation for Drugs and Biologics.
FDA (Food and Drug Administration), 2012a. Guidance for Industry - Modified Risk Tobacco Product Applications - Draft Guidance.
FDA (Food and Drug Administration), 2012b. Guidance for Industry - Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act - Draft Guidance.
Forster, M., et al., 2015. An experimental method to study emissions from heated tobacco between 100-200 degrees C. Chem. Cent. J. 9, 20.
Goniewicz, M.L., et al., 2009. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Cancer Epidemiol. Biomark. Prev. 18, 3421–3425.
Gregg, E.O., et al., 2013. Urinary biomarkers of smokers' exposure to tobacco smoke constituents in tobacco products assessment: a fit for purpose approach. Biomarkers 18, 467–486.
Gunes, A., Dahl, M.L., 2008. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics 9, 625–637.
Haziza, C., De La, Bourdonnaye, G., Skiada, D., Ancerewicz, J., Baker, G., Picavet, P., Luedicke, F., 2016. Evaluation of the Tobacco Heating System 2.2. Part 8: 5-day randomized reduced exposure clinical trial in Poland. Regul. Toxicol. Pharmacol. 81, S17–S26 (PMID: 27816672).
Hecht, S.S., et al., 1999. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res. 59, 590–596.
IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. Chemical agents and related occupations. IARC Monogr. Eval. Carcinog. Risks Hum. 100 (Pt F), 9–562.
Imbriani, M., et al., 1999. [Significance of urinary concentrations of S-benzyl-N-acetylcysteine (S-BMA) in subjects exposed to toluene] Italian. G. Ital. Med. del Lav. Ergon. 21, 329–333.
International Conference on Harmonisation, 1996. ICH Harmonised Tripartite Guideline for Good Clinical Practice. Available from. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf (Accessed on 14 August 2014).
IOM (Institute of Medicine), 2012. Scientific Standards for Studies on Modified Risk Tobacco Products. The National Academies Press, Washington, DC.
Kogel, U., Titz, B., Schlage, W.K., Nury, C., Martin, F., Oviedo, A., Lebrun, S., Elamin, A., Guedj, E., Trivedi, K., Ivanov, N.V., Vanscheeuwijck, P., Peitsch, M.C., Hoeng, J., 2016. Evaluation of the Tobacco Heating System 2.2. Part 7: systems

toxicological assessment of a mentholated version revealed reduced cellular and molecular exposure effects compared with cigarette smoke. Regul. Toxicol. Pharmacol. 81, S123–S138 (PMID: 27818347).
Kroon, L.A., 2007. Drug interactions with smoking. Am. J. Health Syst. Pharm. 64, 1917–1921.
Lo Sasso, G., et al., 2016. Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe(-/-) mice - a systems toxicology analysis. Inhal. Toxicol. 28, 226–240.
Lovreglio, P., et al., 2010. Validity of new biomarkers of internal dose for use in the biological monitoring of occupational and environmental exposure to low concentrations of benzene and toluene. Int. Arch. Occup. Environ. Health 83, 341–356.
Lüdicke, F., et al., 2016. Reduced exposure to harmful and potentially harmful smoke constituents with the tobacco heating system 2.1. Nicotine Tob. Res.
MacLeod, S., et al., 1997. Polymorphisms of CYP1A1 and GSTM1 influence the in vivo function of CYP1A2. Mutat. Res. 376, 135–142.
Martin, F., Ivanov, N.V., Haziza, C., Hoeng, J., Peitsch, M.C., 2016. Evaluation of the Tobacco Heating System 2.2. Part 9: application of systems pharmacology to identify exposure response markers in peripheral blood of smokers switching to THS2.2. Regul. Toxicol. Pharmacol. 81, S151–S157 (PMID: 27845159).
Matsuda, K., et al., 2015. 2015 Genome-wide association study identified SNP on 15q24 associated with bladder cancer risk in Japanese population. Hum. Mol. Genet. 24, 1177–1184.
Miura, N., et al., 2015. A study to investigate changes in the levels of biomarkers of exposure to selected cigarette smoke constituents in Japanese adult male smokers who switched to a non-combustion inhaler type of tobacco product. Regul. Toxicol. Pharmacol. 71, 498–506.
OECD (Organization for Economic Co-operation and Development), 1997. Guidelines for the Testing of Chemicals: Bacterial Reverse Mutation Test [test 471]. Available from. http://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf (Accessed on 13 January 2016).
OECD (Organization for Economic Co-operation and Development), 2005. SIDS Initial Assessment Report for SIAM 20: Isoprene. Available from. http://webnet.oecd.org/HPV/UI/handler.axd?id2d19a70d-10ec-4ff9-9b9a-0978d0db8798 (Accessed on 24 June 2016).
Oviedo, A., Lebrun, S., Kogel, U., Ho, J., Tan, W.T., Titz, B., Leroy, P., Vuillaume, G., Bera, M., Martin, F.T., Rodrigo, G., Esposito, M., Dempsey, R., Ivanov, N.V., Hoeng, J., Peitsch, M.C., Vanscheeuwijck, P., 2016. Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with cigarette smoke. Regul. Toxicol. Pharmacol. 81, S93–S122 (PMID: 27818348).
Qadariyah, L., et al., 2011. Degradation of glycerol using hydrothermal process. Bioresour. Technol. 102, 9267–9271.
Rodgman, A., Perfetti, T.A., 2013. The Chemical Components of Tobacco and Tobacco Smoke. CRC Press, Taylor & Francis Inc, United States.
Roethig, H.J., et al., 2008. A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation Electrically Heated Cigarette Smoking System. J. Clin. Pharmacol. 48, 580–591.
Roethig, H.J., et al., 2010. Short term effects of reduced exposure to cigarette smoke on white blood cells, platelets and red blood cells in adult cigarette smokers. Regul. Toxicol. Pharmacol. 57, 333–337.
Royal College of Physicians, 2016. Nicotine without Smoke. Tobacco Harm Reduction. A Report by the Tobacco Advisory Group of the Royal College of Physicians. RCP, London.
Sakaguchi, C., et al., 2014. Exposure evaluation of adult male Japanese smokers switched to a heated cigarette in a controlled clinical setting. Regul. Toxicol. Pharmacol. 69, 338–347.
Sarkar, M., et al., 2008. Evaluation of biomarkers of exposure to selected cigarette smoke constituents in adult smokers switched to carbon-filtered cigarettes in short-term and long-term clinical studies. Nicotine Tob. Res. 10, 1761–1772.
Sarkar, M., et al., 2010. Valuation of biomarkers of exposure in adult cigarette smokers using Marlboro snus. Nicotine Tob. Res. 12, 105–116.
Schaller, J.-P., et al., 2016a. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical Composition, Genotoxicity, Cytotoxicity, and Physical Properties of the Aerosol, 81, pp. S27–S47.
Schaller, J.-P., Pijnenburg, J.P.M., Ajithkumar, A., Tricker, A.R., 2016b. Evaluation of the Tobacco Heating System 2.2. Part 3: influence of the tobacco blend on the formation of harmful and potentially harmful constituents of the Tobacco Heating Systems 2.2 aerosol. Regul. Toxicol. Pharmacol. 81, S48–S58 (PMID: 27793747).
Schettgen, T., et al., 2008. Fast determination of urinary S-phenylmercapturic acid (S-PMA) and S-benzylmercapturic acid (S-BMA) by column-switching liquid chromatography-tandem mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 863, 283–292.
Sewer, A., Kogel, U., Talikka, M., Wong, E., Guedj, E., Ivanov, N.V., Hoeng, J., Peitsch, M.C., 2016. Evaluation of the Tobacco Heating System 2.2. Part 5: miRNA expression from a 90-day rat inhalation study indicates reduced effects of the aerosol on lung tissue compared with cigarette smoke exposure. Regul. Toxicol. Pharmacol. (accepted).
Smith, M., et al., 2016. Evaluation of the Tobacco Heating System 2.2. Part 1 Description of the System and the Scientific Assessment Program, 81, pp. S17–S26.
Theophilus, E.H., et al., 2015. Magnitudes of biomarker reductions in response to controlled reductions in cigarettes smoked per day: a one-week clinical confinement study. Reg. Tox. Pharm. 71, 225–234.
U.S. Department of Health and Human Services, 2012. Docket No. FDA-2012-N-0143: harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed. Regist. 77, 20034–20037.
U.S. Department of Health and Human Services, 2014. The Health Consequences of Smoking - 50 Years of Progress: a Report of the Surgeon General.
U.S. Department of Health and Human Services, et al., 1990. Toxicological Profile for Ethylene Oxide. Available from. http://www.atsdr.cdc.gov/toxprofiles/tp137.pdf (Accessed on 27 January 2015).
Unverdorben, M., et al., 2010. Acute effects of cigarette smoking on pulmonary function. Regul. Toxicol. Pharmacol. 57, 241–246.
Uppsala Monitoring Centre, 2012. WHO Drug Dictionary Enhanced (WHO DDE). Available from. www.umc-products.com (Accessed on 03 November 2014).
WHO Study Group, et al., 2008. The scientific basis of tobacco product regulation: second report of a WHO study group. World Health Organ Tech. Rep. Ser. 1–277, 1–277.
Wong, E., Kogel, U., Veljkovic, E., Martin, F., Xiang, Y., Boue, S., Vuillaume, G., Leroy, P., Guedj, E., Rodrigo, G., Ivanov, N.V., Hoeng, J., Peitsch, M.C., Vanscheeuwijck, P., 2016. Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarettes smoke. Regul. Toxicol. Pharmacol. 81, pp. S59–S81 (PMID: 27793746).
World Medical Association (WMA), 2013. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects.

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Evaluation of the Tobacco Heating System 2.2

Evaluation of the tobacco heating system 2.2. Part 9: Application of systems pharmacology to identify exposure response markers in peripheral blood of smokers switching THS2.2

Florian Martin, Marja Talikka, Nikolai V. Ivanov, Christelle Haziza, Julia Hoeng, Manuel C.

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Highlights

- Blood as a surrogate to probe smoking induced transcriptomic changes.
- Blood-based gene signature applied to smokers switching to THS 2.2 in a clinic study.
- Signature classified switch to THS 2.2 closer to abstinence than continuous smo

Abstract

As part of current harm reduction strategies, candidate modified risk tobacco produ (MRTP) are developed to offer adult smokers who want to continue using tobacco p an alternative to cigarettes while potentially reducing individual risk and population l compared to smoking cigarettes. One of these candidate MRTPs is the Tobacco H System (THS) 2.2 which does not burn tobacco, but instead heats it, thus producing significantly reduced levels of harmful and potentially harmful constituents (HPHC)

compared with combustible cigarettes (CC). A controlled, parallel group, open-labe
study was conducted with subjects randomized to three monitored groups: (1) switc
from CCs to THS2.2; (2) continuous use of non-menthol CC brand (CC arm); or (3) s
abstinence (SA arm) for five days. Exposure response was assessed by measurin
biomarkers of exposure to selected HPHCs. To complement the classical exposure
response measurements, we have used the previously reported whole blood derive
signature that can distinguish current smokers from either non-smokers or former s
with high specificity and sensitivity. We tested the small signature consisting of only
genes on the blood transcriptome of subjects enrolled in the clinical study and show
reduced exposure response in subjects that either stopped smoking or switched to
candidate MRTP, the THS2.2, compared with subjects who continued smoking thei
tobacco product.

Keywords

Smoking; Blood; Exposure response; Gene expression signature; Novel tobacco
system; MRTP

Corresponding author.

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