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Original investigation

Reduced Exposure to Harmful and Potentially Harmful Smoke Constituents With the Tobacco Heating System 2.1

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Abstract

Introduction: Heating rather than burning tobacco reduces levels of harmful and potentially harmful constituents, and consumer products using this approach aim to reduce exposure to tobacco toxicants. The Tobacco Heating System (THS) version 2.1 has been enhanced from earlier prototypes with an improved heat control and sensorial experience and thereby user acceptance. Exposure measurements are required to determine whether it may be possible to reduce the individual health risk compared to smoking combustible cigarettes (CCs).

Methods: This controlled clinical study randomly assigned 40 smokers to either a group continuing to use of their own CC brand (*n* = 20) or a group switching to THS 2.1 (*n* = 20) for 5 days. Biomarkers of exposure were measured at baseline and on day 1 through day 5. Product consumption, Human Puffing Topography, the occurrence of adverse events, and an assessment of subjective effects, such as smoking satisfaction and enjoyment of respiratory tract sensations, were also determined.

Results: The group of smokers who switched to THS 2.1 adapted their puffing behavior initially through longer puff duration and more puffs. During the duration of the study, total puff volume returned to baseline levels and the mean daily product consumption increased but with similar nicotine exposure compared to baseline CC use. Biomarkers of exposure to tobacco smoke toxicants which inform product risk assessment were significantly reduced with THS use compared to the CC group. THS 2.1 users experienced less reinforcing effects with THS 2.1 than with their own cigarette brand.

Conclusions: THS 2.1 is a promising alternative to smoking CCs. Notwithstanding possible use adaption through consumption or puffing behavior, the exposure to harmful smoke constituents was markedly reduced with the new heated tobacco platform.

Implications: Exposure markers to harmful and potentially harmful smoke constituents were lowered with the THS 2.1. Heating tobacco instead of burning can offer a potentially lower risk of delivering nicotine compared to CCs.

Introduction

Heating tobacco instead of burning can offer a potentially lower risk of delivering nicotine compared to combustible cigarettes (CCs) because it creates a far less complex aerosol than burned tobacco.

Moreover, tobacco research has consistently demonstrated that harmful and potentially harmful constituents (HPHCs) are reduced or absent in the aerosols of heated tobacco. Heated tobacco products also induce less in vitro toxicology and in vivo pathology in animals

than do CCs and show favorable effects in clinical risk markers (eg, early indicators of disease or injury) in smokers who switch to such an alternative tobacco use.[1,2]

One of these heated tobacco products is the Tobacco Heating System (THS) version 2.1. Early prototypes (eg, THS 1.0) heated the outside of the tobacco stick through contact, and use was limited to 8 puffs per tobacco stick. The peak heating temperature of the tobacco was approximately 550°C.[3,4] THS 1.0 was commercialized in limited test markets in Switzerland, Japan, Australia, and Germany between 2006 and 2010, and the product was distributed through a small number of tobacconists. Consumer feedback highlighted a series of product shortcomings including the bulkiness of the device and dissatisfaction with the taste and flavor, both of which contributed to low adoption levels. As a consequence of these findings, the next versions of THS were developed to improve the sensory experience as well as the appearance of the system to make it more acceptable to current, adult smokers.

A predecessor of THS 1.0 (branded as Accord) with Federal Trade Commission (FTC) nicotine yield of 0.2 mg per cigarette, and operating in a similar manner as THS 1.0, was evaluated in a dose escalation study (5-10-15 Accord cigarettes per day) in which concurrent CC smoking was allowed. Accord did not perform as expected, as the total number of Accord cigarettes plus CCs increased by 24%, while Accord was rated as ineffective at suppressing cravings for cigarettes.[5] This predecessor (also called second-generation electrically heated cigarette smoking system, EHCSS) was investigated in a 12-month study evaluating exposure and cardiovascular risk factors in EHCSS users as compared to cigarette smokers.[6] In this study, the average nicotine uptake decreased by 18%, while the average number of EHCSS cigarettes consumed increased by 95%. Later, in a 1-month study executed with THS 1.0 (ISO nicotine yield 0.3 mg per cigarette), the nicotine uptake decreased by 18% at the end of study as compared to baseline, while the use of THS 1.0 cigarettes increased by more than 50%.[3] These three studies suggest that nicotine delivery may have been too low to satisfy consumers and/or to suppress smoking abstinence symptoms, despite attempts to compensate the nicotine exposure by increasing number of cigarettes per day. Incomplete withdrawal suppression with Accord has also been reported in other publications.[7,8]

Another heat-not-burn product (Eclipse, FTC nicotine yield 0.2 mg per cigarette), with a heating source made of carbon, was tested in a clinical study. Eclipse was successful in maintaining nicotine blood levels and craving control to same levels as with CCs and to decrease concurrent CCs to the same extent nicotine inhaler did, but the exposure to carbon monoxide significantly increased with Eclipse,[9] which was confirmed in subsequent publications.[10,11] A broader review of products "that potentially reduce toxicant exposure" (p. 9), including heat-not-burn products as well as evidentiary requirements and regulatory contexts, is given by Hatsukami et al.[12]; Rees et al. review clinical trial methods for assessing "potential reduced exposure products" (p. 3196) and provide an overview of studies that entail switching from CC to heat-not-burn products.[13]

THS 2.1 tested in this study has evolved to become slimmer and less bulky, and the heating blade is now inserted directly into the THS tobacco stick rather than heating the tobacco stick from the outside, which results in a more consistent heating of the tobacco at lower temperatures (<400°C). Consequently, further substantial lowering of HPHC levels are achieved in the THS 2.1 aerosol compared with THS 1.0, as well as an improved sensorial experience to address the consumer feedback received for previous versions. By

design, the use of THS 1.0 was limited to 8 puffs per cigarette, while THS 2.1 offers an operating window of 6 minutes and 14 puffs, more in line with the smoking ritual usually observed with traditional cigarettes. This evolution of the electronic device from THS 1.0 to THS 2.1, in addition to lowering the operating temperature (from 550°C with THS 1.0 to <400°C with THS 2.1, thus decreasing the emission of HPHCs generated at temperatures above 400°C), represents a significant improvement of the product. These new product features provide smokers not willing to quit tobacco use with an alternative tobacco product having the potential for lower risk of delivering nicotine than a cigarette and a better consumer's acceptance due to a more sustained delivery of nicotine than previous versions and improvements in taste, smell, and smoking ritual.

The aim of using THS to reduce human exposure to tobacco toxicants, and eventually to reduce the health risks associated with tobacco use, requires evidence on several levels. Chemical and toxicological investigations are necessary to demonstrate the potential to reduce exposure and risk. Clinical research, including measurements of exposure, aims to establish a relationship between THS use and exposure reduction and constitutes an important initial step to determine whether a product can reduce an individual's health risk. Moreover, exposure itself can be influenced by factors such as quantity and frequency of use, puffing style (human smoking topography), and subjective responses (urge-to-smoke and withdrawal relief, and perceived nicotine effect) to the alternative tobacco product.

Typically, switching studies are conducted to establish the modification in exposure of tobacco products. These include randomized clinical evaluations where subjects are required to switch from their current product to a new test product or to stop smoking. The study designs are flexible and can accommodate shorter or longer term exposure, *ad libitum* versus controlled use settings, and are fundamental for the assessment of the reduction in exposure to the HPHCs from the tobacco product.[13] In this controlled clinical study, we examined whether the levels of selected biomarkers of exposure were reduced in smokers who switched from CCs to THS 2.1 as compared to smokers that continued to smoke CCs. The study was carried out for 1 week in confinement and allowed *ad libitum* product use. Subjective effects and Human Puffing Topography (HPT) were also evaluated.

Methods

Participants

Subjects were recruited via the clinical site's database and through advertisements. Of all 42 current smokers enrolled and having had a trial of THS 2.1 prior to randomization, 40 subjects aged 23–65 years were randomly assigned to either continue to use their own brand of CCs (*n* = 20) or to switch to THS 2.1 (*n* = 20) for a period of 5 days. The two other subjects (enrolled as backup subjects to assure that the determined sample size was reached) were excluded from participation prior to randomization. All participants reported smoking for at least the last three consecutive years and had smoked daily 10 commercially available nonmenthol CCs (≤1 mg nicotine per CC, ISO yield) for at least 4 weeks prior to the start of the study. Smokers of menthol CCs were not eligible for this study to avoid inducing changes in smoking patterns which are likely to result from switching current smoker of menthol CCs to a nonmenthol product, as the prevalence of switching between menthol and nonmenthol cigarettes is low.[14]

All subjects underwent medical tests, which included providing their medical history; a physical examination of height and body weight; measuring vital signs of systolic and diastolic blood pressure, pulse rate, and respiratory rate; and an electrocardiogram to show that they were in good health and were able to safely participate in the study. They also underwent a clinical laboratory examination, which included hematology, clinical chemistry, and urinalysis. Volunteers provided written informed consent for participation in the study and were paid for their participation. Subjects were free to leave the study at any time, including when they chose to quit smoking.

Study Design

The enrollment of eligible subjects took place on day –2 after the inclusion/exclusion criteria had been assessed. On day –2, the subjects entered the clinic and were confined under medical supervision until discharge on day 6. Baseline assessments were carried out on day –1 and day 0, including the determination of exposure biomarkers from 24-hour urine samples. The 40 subjects were randomized into two parallel groups (THS 2.1 and CC groups) using a stratification based on sex and daily cigarette consumption (10–19 CC vs. >19 CC per day).

Between study days 1 and 5, subjects in the THS 2.1 group used THS 2.1 exclusively and *ad libitum* during the designated smoking hours from 06:30 to 23:00 hours. Subjects randomized to the CC study arm continued to smoke their own brand of CC. HPT was assessed in a nested substudy at baseline and study days 1 and 4. The study was conducted at a single center in Poland and was approved by an independent ethics committee (The Bioethics Committee of the District Medical Chamber in Warsaw). The study was registered on the clinicaltrials.gov Web site (NCT01780714), as were two studies on THS menthol (NCT01989156; NCT01970995).

Study Investigational Products

The test product THS 2.1 contains 0.3 mg nicotine and 50 mg glycerol as aerosol former determined under smoke chemistry ISO conditions (12 puffs). It was developed by Philip Morris International and provided by the Sponsor. THS 2.1 has three components: the THS tobacco stick, the holder, and the charger. The THS tobacco stick has a tobacco plug containing processed tobacco cast leaf, which is covered by a paper wrap. The holder includes a battery, controlling electronics, and the heater element. The THS tobacco stick is inserted into the holder and heats the tobacco via an electronically controlled heating blade. The charger recharges the holder (Figure 1). The THS
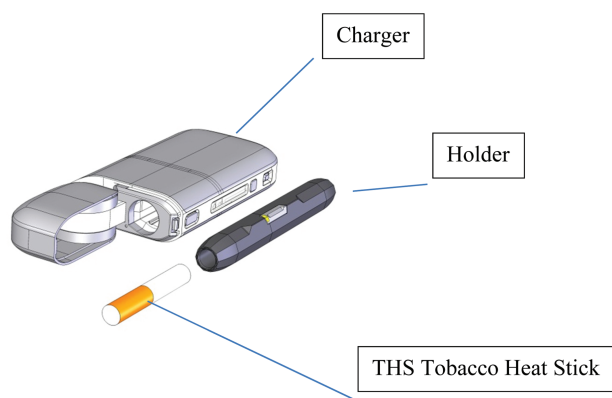

Figure 1. Tobacco Heating System.

tobacco sticks were preheated for 30 seconds in the THS holder and the energy capacity of the holder was sufficient to maintain a product use session for up to 6 minutes. At the end of each product use session, the THS holder required recharging.

The reference product in this study was nonmenthol CCs of the subject's own preferred commercially available brand. CCs were not provided by the Sponsor, and subjects were asked to buy and bring their own CCs to the investigational site.

Outcome and Assessment

Assessment of Biomarkers of Exposure

Biomarkers of exposure were measured in both blood samples and 24-hour urine samples during the study on day –1 through day 6. The samples were stored at 4°C–8°C during the day of sample collection. With the exception of the samples collected for carboxyhemoglobin (COHb), samples were processed and stored at –20°C pending biomarker analysis. Biomarkers of exposure were assessed for the following tobacco-specific HPHCs: nicotine, *N*-nitrosonornicotine (NNN),[15] and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); as well as the following tobacco-related HPHCs: 1,3-butadiene, 2-naphthylamine, 4-aminobiphenyl, carbon monoxide, acrylonitrile, acrolein, benzene, pyrene, and o-toluidine (o-tol) (Supplementary Table 1).

Nicotine, cotinine, trans-3′-hydroxycotinine, caffeine, and paraxanthine were measured in plasma using assays validated to meet the FDA Guidance to Industry: Bioanalytical Method Validation (2001). Clinical samples were assayed with the addition of stable label internal standards to the aliquoted sample. Both assays employed a solid-phase extraction to concentrate and purify the target analytes. The extracts were injected onto a qualified LC-MS/MS instrument with positive ions detected in multiple-reaction monitoring mode.

The following biomarker assays were validated as "fit-for-purpose" to meet the applicable portions of the same Guidance to Industry. Total o-toluidine [o-tol], 2-aminonaphthalene [2-NA], 4-aminobiphenyl [4-ABP], S-phenylmercapturic acid [S-PMA], total 1-hydroxypyrene [1-OHP], monohydroxybutenyl mercapturic acid [MHBMA], total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [total NNAL] (determined as the molar sum of 4-(methylnitrosamino)-1-(3-pyridy1)-1-butanol and its *N*- and *O*-glucuronide conjugates), 2-cyanoethylmercapturic acid [CEMA], 3-hydroxypropylmercapturic acid [3-HPMA], and total *N*-nitrosonornicotine [NNN] (determined as the molar sum of *N*-nitrosonornicotine and its *N*-glucuronide) were measured in urine.

An acidic hydrolysis was employed for the measurement of the aromatic amines and S-PMA. total 1-OHP, total NNAL, and NNN were hydrolyzed enzymatically. In addition to the total assays, nicotine equivalents (Neq) were also measured by the direct analysis of nicotine, cotinine, trans-3′-hydroxycotinine, nicotine-*N*-glucuronide, cotinine-*N*-glucuronide, and trans-3′-hydroxycotinine-*O*-glucuronide in urine. The Neq reported here were calculated from the concentrations determined by direct analysis of nicotine and its five predominant metabolites. For MHBMA, 3-HPMA, and CEMA, a direct analysis of urinary concentrations was performed. Clinical sample aliquots, supplemented with stable label internal standards, were extracted either by a validated liquid–liquid or solid-phase extraction approach. The extracts were injected onto a qualified LC-MS/MS instrument and detected in multiple-reaction monitoring mode.

Urinary creatinine was measured spectrophotometrically using CAP/CLIA validated assays. For COHb assessment on day 5, the

evening blood sample (20:00 hours) was used for analysis. COHb was measured by spectrophotometry.

A detailed description of the bioanalytical methods used is available in the Supplementary Material.

Adverse Events, Medical History, and Concomitant Medication

Adverse events (AEs) and medical history were coded using the Medical Dictionary for Regulatory Activities (MedDRA version 15.0). Medications were coded according to the World Health Organization enhanced drug dictionary (March 2012).[16]

Subjective Effects of Smoking Assessment

The Fagerström Test for Nicotine Dependence (FTND) Questionnaire was used to assess nicotine dependence during the screening visit.[17] The Modified Cigarette Evaluation Questionnaire (MCEQ) was used to assess the degree to which subjects experienced the reinforcing effects of smoking on a daily basis from day −1 to day 5.[18] The following domains of the MCEQ were evaluated: Smoking Satisfaction (satisfying, tastes good, enjoyment of smoking); Psychological Rewards (calms down, makes more alert, reduces irritability, helps concentration, and reduces hunger); Aversion (dizziness, nausea); Enjoyment of Respiratory Tract Sensations (single-item assessment); and Craving Reduction (single-item assessment).

Product Use and HPT Assessment

From day −2 onward, CC consumption was recorded for all subjects, and from day 1 onward for subject randomized to the THS 2.1 group, the consumption of THS tobacco sticks was also recorded. All products were dispensed upon subject request, one at a time, by the study site staff and documented in the product accountability log.

HPT parameters, including puff duration, interpuff interval, puff volume, number of puffs, and total volume, were measured in a substudy for each CC used at the 2 days of baseline, and on days 1 and 4 in both the CC and THS 2.1 groups using the SODIM smoking device, model SPA/M (SODIM Instrumentation, Fleury-les-Aubrais, France). Thirty subjects (16 subjects for the THS 2.1 group and 14 subjects for the CC group) participating in the main study were eligible and provided baseline data for HPT assessment.

The sample holders for the HST Sodim Device were specifically designed for compatibility with THS 2.1 and the HST Sodim Device and sample holder were validated according to our internal Quality Management System to ensure that measurements performed with the devices and sample holders are accurate and repeatable. Furthermore, smoking topography with the HST device was only done in subjects who smoked CCs that were compatible with the HST device and the device was tested/validated for. Therefore, users of, for example, slim CCs and CCs including charcoal filters were excluded.

Sample Size

Sample size determination was based on mean THS:CC ratios of the concentrations of biomarkers of exposure adjusted for creatinine, as observed in previous studies with heated tobacco products.[19] A sample size of 20 subjects in each group was considered sufficient to attain more than 80% power to show a reduction of 60% or more in COHb, 3-HPMA, MHBMA, and S-PMA biomarkers of exposure in the THS 2.1 group compared with the CC group, using a 5% testwise type I error probability.

Statistical Analysis

Analyses of the biomarkers of exposure were performed on randomized subjects who had record of at least one post-randomization product use and at least one valid biomarker assessment. Statistics were derived for each biomarker and the change from baseline according to study arm and day. Inferential analysis was performed on the endpoints observed at the end of exposure (ie, day 5 or the last available measurement). Analysis of covariance (ANCOVA) was conducted to estimate differences between study arms (testwise $\alpha = 5\%$) adjusting for sex, average daily cigarette consumption at baseline (the last available value prior to day 1), and the baseline log-transformed level of the biomarker. If the residuals from the ANCOVA analysis were not normally distributed based on the Shapiro–Wilk test ($\alpha = 5\%$), robust 95% confidence intervals (CIs) were derived by resampling (bootstrap 5000 samples). Estimates of differences between study arms and associated CIs were back-transformed to provide relative effects (THS 2.1/CC). The MCEQ was summarized for the individual items and each domain score. Descriptive statistics were presented for the individual items and domain scores by study arm for the MCEQ, stratified by gender and baseline cigarette consumption. Analysis was performed via a mixed model on observed data. All statistical analyses were performed using Statistical Analysis Software (SAS), version 9.1.3.

Results

Participants

Of 42 enrolled subjects who tried THS 2.1 prior to randomization, 40 were randomized in accordance with the determined sample size and completed the study. There was no difference between either group in terms of sex, age, body mass index, daily cigarette consumption, or per ISO tar yield category. Sixteen subjects had a FTND classification of moderate or severe in the THS 2.1 group compared with 11 subjects in the CC group (Table 1).

Product Consumption

At baseline, the average number of CCs smoked was similar in the THS 2.1 (18.9 ± 4.5) and CC (18.2 ± 4.0) groups. The mean number of THS tobacco sticks consumed daily increased over the course of the study to 27.2 on day 5. In the CC group, the mean number of CCs consumed daily increased to 20.1 on day 5 (Supplementary Table 2).

Biomarkers of Exposure

At baseline, levels of biomarkers of exposure (nicotine, cotinine, Neq, total NNN, total NNAL, o-tol, total 1-OHP, 2-NA, and 4-ABP) to particulate smoke constituents (nicotine, NNN, NNK, o-tol, pyrene, 2-NA, and 4-ABP) were not different between THS 2.1 and CC (Supplementary Table 3). Similarly, no difference was observed for exposure markers (3-HPMA, COHb, and CEMA) to gas-phase smoke constituents (acrolein, carbon monoxide, and acrylonitrile), or for exposure markers (S-PMA and MHBMA) to both particulate and gas-phase smoke constituents (benzene and 1,3-butadiene) (Supplementary Table 3).

On day 5, a significant reduction in all biomarkers of exposure, not associated with nicotine exposure (ie, plasma nicotine, cotinine, and Neq), was observed, in those who switched to THS 2.1 compared with the CC group (Table 2). The greatest change was observed for S-PMA (93%, 95% CI 90.6–94.9), followed by 2-NA (89.1%, 95% CI 86.5–91.3), and MHBMA (88.5%, 95% CI 84.7–91.4). Due to

Table 1. Subject Demographics at Baseline

Variables	CC group	THS 2.1 group	Total[a]
Number of subjects (*n*)	20	20	42
Age (y)			
Mean ± *SD*	37.8 ± 8.3	37.6 ± 9.0	38.5 ± 9.0
Range	27–56	24–53	24–56
Sex, *n* (%)			
Male	9 (45.0%)	10 (50.0%)	19 (45.0%)
Female	11 (55.0%)	10 (50.0%)	23 (55.0%)
BMI (kg/m^2)			
Mean ± *SD*	23.5 ± 2.6	24.1 ± 2.3	24.0 ± 2.6
Range	19.0–28.8	19.9–29.9	19.0–29.9
Daily cigarette consumption, *n* (%)			
10–19 cig/d	10 (50.0%)	10 (50.0%)	20 (48.0%)
>19 cig/d	10 (50.0%)	10 (50.0%)	22 (52.0%)
ISO tar yields, *n* (%)			
3–5 mg	2 (10.0%)	3 (15.0%)	6 (14.3%)
6–8 mg	15 (75.0%)	13 (65.0%)	29 (69.0%)
9–10 mg	3 (15.0%)	4 (20.0%)	7 (16.7%)
ISO nicotine yields, *n* (%)			
≤0.6 mg	13 (65.0%)	10 (50.0%)	25 (59.5%)
>0.6 mg[b]	7 (35.0%)	10 (50.0%)	17 (40.5%)
FTND total score[c]			
Mean ± *SD*	6.5 ± 2.0	6.1 ± 1.6	6.3 ± 1.7
Range	3–10	3–9	3–10

BMI = body mass index; CC = combustible cigarette; FTND = Revised Fagerström Test for Nicotine Dependence Questionnaire; *SD* = standard deviation; THS = Tobacco Heating System.

[a]Safety population (*n* = 42) includes two subjects that were exposed to THS 2.1 but were not randomized to the THS 2.1 or the CC group.

[b]Only three subjects in the THS group and three subjects in the CC group smoked CC brands with an ISO nicotine yield of 0.5 mg or below.

[c]Eight subjects in the CC group and three subjects in the THS 2.1 group did not provide FTND scores.

a single high NNN baseline value in the THS 2.1 group, the sensitivity of the reduction on day 5 to this value was assessed through reanalysis without the respective subject; the corresponding percent reduction of the THS 2.1/CC geometric mean ratio of NNN on day 5 was 87.7% (95% CI 84.2–90.4), being very close to the result contained in Table 2 based on all subjects. The biomarkers associated with nicotine exposure show a very consistent result over the 5 days of exposure when comparing those who switched to THS 2.1 to those that continued smoking CC. The THS 2.1 group was exposed to 85%–89% of the nicotine across the three biomarkers of exposure associated with nicotine exposure (ie, plasma nicotine, cotinine, and Neq). This resulted from the values of Neq and plasma cotinine increasing slightly in the CC group over the exposure period, while for the THS 2.1 group, all of the parameters slightly decreased.

HPT Assessment

The mean puff volumes in the THS 2.1 group were consistently 12%–14% greater than those of the CC group on both days 1 and 4. The mean number of puffs was also greater in the THS 2.1 group on day 1, but not on day 4. The longer puff duration and slightly increased puff volumes consequently initially led to a higher total puff volume in the THS 2.1 group on day 1 compared to baseline. Total puff volume came back near to baseline levels by day 4 in the THS group due to a reduction in the number of puffs and a slight reduction in puff volume (Table 3).

Subjective Effects of Smoking

Significantly less reinforcing effects of smoking were reported for the THS 2.1 group compared with the CC group for many of the MCEQ subscale scores on most study days (Supplementary Table 4). In particular, greater Smoking Satisfaction was reported in the CC group compared with the THS 2.1 group on day 1: both groups reported a fall from an identical baseline level of 4.9 to 4.6 (95% CI 4.1–5.2) for CC versus 2.7 (2.0–3.3) for THS 2.1, although this increased to 3.4 (2.8–3.9) for THS 2.1 on day 5 compared with 4.8 (4.1–5.5) for CC. A greater Enjoyment in Respiratory Tract Sensations was also reported in the CC group, with a value of 3.6 (2.7–4.5) versus 2.1 (1.4–2.8) for THS 2.1 on day 1. Moreover, higher Psychological Rewards and greater Craving Reductions were documented by the CC group compared with the THS 2.1 group after 5 days of exposure.

Safety Results

Four subjects in the THS 2.1 group experienced five AEs, while 13 AEs were experienced by 10 subjects in the CC group. No AEs led to discontinuation of product use, and all were classed as mild or moderate. Commonly reported AEs included mild investigational laboratory abnormalities such as elevated blood triglycerides and COHb levels. No serious AEs were reported in this study.

Discussion

The present study compared biomarkers of exposure to 11 selected HPHCs in smokers continuing to smoke their own brand of CC with those of smokers who switched to THS 2.1 in a controlled clinical setting. Exposure markers provide direct, quantitative evidence of the presence of HPHCs, or their metabolites, in the body. In 2012, the US Food and Drug Administration's (FDA) Center

for Tobacco Products (CTP) established an abbreviated list of 18 HPHCs to be measured in smoke.[20] The present study assessed nine of these HPHCs requested by the FDA. In addition, we measured pyrene (measured with 1-OHP) as representative compound for the polycyclic aromatic hydrocarbons as well as the aromatic amine o-toluidine.

Switching from the subject's own preferred brand of CC to THS 2.1 for 5 days significantly reduced selected biomarkers of exposure compared with those who continued to smoke CC. In the THS 2.1 arm, COHb, 3-HPMA, MHBMA, S-PMA, total NNAL, 1-OHP,

Table 2. ANCOVA Comparison of Biomarkers of Exposure Between THS 2.1 and CC on Day 5

Biomarkers of exposure	Percent reduction of THS 2.1/CC geometric LS mean (95% CI)	N (THS 2.1)	N (CC)
COHb (%)	76.7 (74.3–78.9)**	20	20
3-HPMA (μg/g creat)[a]	72.1 (67.4–76.1)**	20	20
MHBMA (μg/g creat)[a]	88.5 (84.7–91.4)**	20	20
S-PMA (μg/g creat)[a]	93.0 (90.6–94.9)**	20	20
Total NNAL (ng/g creat)[a]	66.5 (56.6–74.2)**	20	20
1-OHP (ng/g creat)[a]	57.2 (49.2–63.9)**	20	20
Total NNN (ng/g creat)[a]	88.2 (84.8–90.8)**	20	20
4-ABP (ng/g creat)[a]	59.4 (48.0–68.4)**	20	20
2-NA (ng/g creat)[a]	89.1 (86.5–91.3)**	20	20
o-tol (ng/g creat)[a]	42.3 (30.0–52.5)**	20	20
CEMA (ng/g creat)[a]	85.3 (82.7–87.4)**	20	20
Neq (mg/g creat)[a]	12.7 (0.3–23.6)	20	20
Nicotine (ng/mL)[b]	4.1 (–20.0–23.4)	19	20
Cotinine (ng/mL)[b]	8.3 (–6.9–21.4)	19	20

1-OHP = 1-hydroxypyrene; 2-NA = 2-aminonaphthalene; 3-HPMA = 3-hydroxypropylmercapturic acid; 4-ABP = 4-aminobiphenyl; ANCOVA = analysis of covariance; CC = combustible cigarette; CEMA = 2-cyanoethylmercapturic acid; CI = confidence interval; COHb = carboxyhemoglobin; LS mean = least squares mean; MHBMA = monohydroxybutenyl mercapturic acid; Neq = nicotine equivalents; o-tol = o-toluidine; S-PMA = S-phenylmercapturic acid; THS = Tobacco Heating System; Total NNAL = total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; Total NNN = total *N*-nitrosonornicotine.

[a]In 24-h urine.

[b]In plasma.

**Reduction in biomarkers of exposure achieved statistical significance with *P*-values < .001.

total NNN, 4-ABP, 2-NA, and CEMA were reduced from baseline from –45% to –88% compared with a change from –2% to +68% for CC. The largest change was seen in S-PMA, amounting to a reduction of 88% after switching to THS 2.1, followed by a reduction of >80% for 2-NA, CEMA, and total NNN. The reduction of o-tol (–23%) was lower than expected. This was investigated and the most likely explanation for the limited reduction in o-tol is that the general population is known to be exposed ubiquitously to o-tol, which is detected in μg/kg amounts in meat and dairy products and in upper ng/kg amounts in salads, vegetables, eggs, alcoholic beverages, cereals, and fish. Even higher concentrations in the upper μg/kg range were reported for ice cream powders, food colorings, and soft drink concentrates. o-Toluidine occurs also in the aroma of black tea.

Although the varying levels of tobacco-specific nitrosamines (TSNAs) delivered from currently available CCs are attributed to differences in tobacco blend, filler, and curing/manufacturing process, the substantial reduction of TSNAs with THS 2.1 compared to CC is explained differently. A recent study by Forster et al.[21] demonstrated that the evaporative transfer of NNK present in cured tobacco to the mainstream aerosol occurs at low temperatures but to the most extend above 120°C to 140°C, although no clear linearity could be shown. Furthermore, the authors showed that the percentage of TSNAs released from the tobacco into the aerosol at the temperatures assessed in this study (100°C to 200°C) was very low (<10%) compared to the available amounts in the used tobacco rod.

Although the core operating temperature of THS 2.1 is slightly above the temperature range characterized by Foster et al.[21] for TSNAs, the circular temperature distribution profile in the tobacco plug of THS 2.1, with temperatures decreasing rapidly from the inner core of the tobacco plug to well below 100°C on the outer surface, contributes significantly to the reduced release and direct transfer of TSNAs into the aerosol of THS 2.1 compared to CC. In addition, the amount of tobacco contained in THS is significantly less than in CC which adds to the reduced amount of TNSAs released with THS 2.1. Therefore, it can be concluded that although variations in levels of TSNAs in the tobacco blend and filler used in THS may play a role, the significantly lower temperature, the circular temperature distribution profile, and the significantly reduced amount of tobacco in THS 2.1 compared to CC would be the most significant contributors to the reduction in TSNAs.

The puffing topography data and the number of products used suggest that THS 2.1 users altered the way they consumed THS 2.1

Table 3. Human Puffing Topography Parameters (Mean ± *SD*) in CC and THS 2.1 Groups

	Baseline	Day 1	Day 4
THS 2.1 group (*n* = 16)			
Puff duration (s)	1.9 ± 0.6	2.5 ± 0.7	2.7 ± 0.8
Interpuff interval (s)	14.4 ± 3.3	9.8 ± 2.6	10.51 ± 4.5
Puff volume (mL)	54.8 ± 11.2	61.3 ± 16.4	59.5 ± 14.4
Puff number	15.2 ± 3.7	16.0. ± 5.8	14.0 ± 5.1
Total volume (mL)	811.4 ± 185.8	921.0 ± 251.1	800.0 ± 257.9
CC group (*n* = 14)			
Puff duration (s)	2.1 ± 0.5	2.1 ± 0.5	2.0 ± 0.5
Interpuff interval (s)	15.2 ± 4.4	16.0 ± 5.5	17.2 ± 6.0
Puff volume (mL)	54.6 ± 14.2	54.0 ± 13.8	53.0 ± 13.9
Puff number	14.9 ± 3.6	14.8 ± 4.4	14.4 ± 3.8
Total volume (mL)	782.8 ± 166.4	762.3 ± 169.0	729.0 ± 148.9

CC = combustible cigarette; *SD* = standard deviation; THS = Tobacco Heating System.

compared to CC product use. A higher total puff volume was initially observed in the THS group, mainly due to an increased volume and number of individual puffs. However, the total puff volume in the THS group decreased with study duration returning back to levels observed at baseline. At the same time, the use of THS tobacco sticks increased by 27% over the study period, suggesting that the adaptation in product use behavior was affected more through product consumption than alterations in puffing behavior. With 85% of subjects in the THS 2.1 group switching from CC brands with an ISO nicotine yield at or above 0.6 mg nicotine/CC, these results represent an expected adaptation and compensatory effect of switching to a new product with a lower nicotine content than the subjects' usual brand.

For the CC group, product use increased by 12%, while HPT parameters remained stable over the entire product use period with the exception of a slight drop of total puff volume from baseline to day 4. The increase in CC use is likely related to a change of the subjects' daily routine and related smoking behavior by being confined at the study site for the duration of the study.

THS 2.1 was perceived as less rewarding in terms of sensory and physical effects than CCs, with Smoking Satisfaction and the Enjoyment of Respiratory Tract Sensation being particularly reduced on day 1 in the THS 2.1 group compared with the CC group. These differences in sensorial characteristics compared with the subjects' preferred CC brand are likely to have contributed to the observed change in consumption pattern.

One of the strengths of this study is its randomized, controlled design allowing for *ad libitum* product use. It provides an indication of the maximum possible short-term exposure reductions achievable with exclusive use of THS 2.1.

While very encouraging results with respect to HPHCs reduction were observed, some other performance features may need to be further improved. A nonstatistically significant decrease in nicotine uptake (Neq, Table 2) was observed in THS users (12.7% vs. CC) despite subtle changes in puffing topography (Table 3) and a more than 40% increase in THS 2.1 tobacco stick consumption versus baseline cigarette smoking. These results are consistent with the observed lower satisfaction, enjoyment, rewards, and craving reductions from THS 2.1 versus CC.

The study was conducted under highly controlled conditions, that is, subjects were not allowed to use other tobacco products than the allocated products, but they were free to use them as they wished, during an extended daily time window (6:30–23:00). This design allowed to investigate the intrinsic product performance (ie, exclusive use of the product) with regard to reduction of exposure to HPHCs by limiting the influence of confounding factors on HPHCs levels. This is an important step for understanding the THS 2.1 contribution to the exposure to HPHCs, as the majority of key HPHCs can also arise from sources other than cigarette smoke.

One important limitation of the study is the lack of a smoking abstinence arm, which could have provided a benchmark for the clinical relevance of the observed HPHC exposure reductions.

The study design has also limitations in terms of product evaluation. It is known from market research that changes in product use behavior usually require more than 1 week of adoption to a new product. Given the nature of the product, consumer tests would provide better ways to investigate product acceptance in terms of ritual, taste, and smell than clinical studies. The consumption of THS 2.1 in addition to other tobacco products or to other nicotine-containing products/e-cigarettes (dual use) was not allowed in the study. The

amount of dual use and the associated behavioral trajectories and the consequences on exposure to HPHCs would require specific longer-term studies in real-world settings.

In summary, this study provides evidence that reduction of exposure to HPHCs was achieved despite the increase in consumption and adaptation in puffing behavior associated with exclusive use of THS 2.1. While some improvements in product performance may need to be considered and while results to be confirmed under real-life conditions, our findings suggest that THS 2.1 can offer an alternative for cigarette smokers. Additionally, THS 2.1 was well tolerated and had a safety profile comparable with that of CCs. Further studies with a larger sample size, longer duration, and in ambulatory, more real-world conditions are needed to assess how the encouraging reductions in biomarkers of exposure observed are sustained in a less controlled setting and how product use behavior with THS 2.1 changes over time.

Supplementary Material

Supplementary Material and Supplementary Tables 1–4 can be found online at http://www.ntr.oxfordjournals.org

Declaration of Interests

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

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