Clinical Study Protocol
ZRHM-REXA-08-US

Study title:	A randomized, controlled, open-label, 3-arm parallel group, multi-center study to demonstrate reductions in exposure to selected smoke constituents in apparently healthy smokers switching to the Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol) or observing smoking abstinence, compared to continuing to use menthol conventional cigarettes, for 5 days in confinement and prolonged by 86 days in an ambulatory setting
Short title:	Reduced exposure study using THS 2.2 Menthol with 5 days in a confinement setting followed by 86 days in an ambulatory setting
Registration number:	Not assigned
Study number	ZRHM-REXA-08-US
Product name:	Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol)
Sponsor:	Philip Morris Products S.A. Quai Jeanrenaud 5 2000 Neuchâtel Switzerland
Version number:	Final 5.0
Revision date:	14 April 2014
Authors:	[redacted] PhD, Manager Clinical Science [redacted] MEng MSc, Biostatistician [redacted], MD, Medical Safety Officer

Confidentiality Statement

SYNOPSIS

Sponsor:

Philip Morris Products S.A.
Quai Jeanrenaud 5
2000 Neuchâtel
Switzerland

Product Name:

Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol)

Study Title:

A randomized, controlled, open-label, 3-arm parallel group, multi-center study to demonstrate reductions in exposure to selected smoke constituents in apparently healthy smokers switching to the Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol) or observing smoking abstinence, compared to continuing to use menthol conventional cigarettes, for 5 days in confinement and prolonged by 86 days in an ambulatory setting

Study Number:

ZRHM-REXA-08-US

Short Study Title:

Reduced exposure study using THS 2.2 Menthol with 5 days in a confinement setting and 86 days in an ambulatory setting

Primary Objectives:

The primary objectives of this study are:

- To demonstrate the reduction of primary biomarkers of exposure (BoExp) (Table S1) to harmful and potentially harmful constituents (HPHCs) (except Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) (Total NNAL)) in a confinement setting in smokers switching from menthol conventional cigarettes (mCC) to THS 2.2 Menthol as compared to smokers continuing to smoke mCC, and
- To demonstrate the reduction of Total NNAL in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC (Table S1).

Secondary Objectives:

The secondary objectives of this study are:

- To evaluate self-reported nicotine/tobacco product use including dual-use in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to smoking abstinence (SA).
- To determine the reduction of secondary BoExp in a confinement setting and in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
- To describe the levels of primary and secondary BoExp over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To determine the levels of nicotine over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to describe their levels over the entire exposure period.
- To describe the pharmacokinetic (PK) profiles of the nicotine and cotinine in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
- To describe the change in Cytochrome P450 1A2 (CYP1A2) enzymatic activity in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To determine the changes in lung functions in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC
- To monitor the safety profiles during the study.
- To monitor selected risk markers in a confinement and ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.

Exploratory Objectives:

The exploratory objectives of this study are:

- To describe the following parameters in a confinement and/or ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA:
  - Excretion of mutagenic material in urine.

- Subjective effect of smoking.
- Cytochrome P450 2A6 (CYP2A6) activity.

- To describe the following parameters over the course of the study in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC:
  - Product evaluation.
  - Smoking puffing behavior.
- To describe the following parameter over the course of the study in smokers switching from mCC to THS 2.2 Menthol:
  - Potential combustion occurrences in tobacco plugs.
- To describe the changes in levels of oxysterols on Day 6 and Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol, and in smokers switching from mCC to SA.
- To describe the product use over the course of the study according to the product preference of the subject.
- To describe the smokers' mental state for the intention to quit at Screening, on Day -2, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
- To describe the changes and reductions in the levels of urinary BoExp and risk markers measured in 4-hour urine fraction on Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To evaluate in smokers switching from mCC to THS 2.2 Menthol, and smokers continuing smoking mCC the relationship between[1] :
  - Primary and secondary BoExp and nicotine equivalents (NEQ).
- To determine the concordance between the results for BoExp and risk markers in 24-hour urine and in 4-hour urine fraction [2].
- To monitor the BoExp in subjects quitting smoking according to the time since they quitted [2].

[1] The reporting of the objective will be the subject of a separate report.

[2] The reporting of the objective will be the subject of an appendix to the main Clinical Study Report.

Study Hypothesis:

The hypothesis to be tested is that the geometric mean level of the BoExp for THS 2.2 Menthol is lower relative to mCC.

For primary BoExp, the hypothesis will be tested on Day 5 for monohydroxylbutenyl-mercapturic acid (MHBMA), 3-hydroxypropylmercapturic acid (3-HPMA), S-phenylmercapturic acid (S-PMA), and carboxyhemoglobin (COHb), and on Day 90 Visit for Total NNAL according to the primary and secondary objectives. For the secondary BoExp, the hypothesis will be tested on Day 5, and if significant, on Day 90.

Study Endpoints:

The primary and secondary BoExp to harmful and potentially harmful constituents (HPHCs) measured in the study are presented in Table S1 below:

Table S1: Biomarkers of Exposure

	Biomarkers of Exposure (BoExp)	HPHCs	Matrix
Primary BoExp on Day 5	monohydroxybutenylmercapturic acid (MHBMA)	1,3-butadiene	Urine
	3-hydroxypropylmercapturic acid (3-HPMA)	acrolein	Urine
	S-phenylmercapturic acid (S-PMA)	benzene	Urine
	carboxyhemoglobin (COHb)	carbon monoxide (CO)	Blood
Primary BoExp on Day 90 Visit	total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL)	4 (methylnitrosamino)-1-(3-pyridyl)-1butanone (NNK)	Urine
Secondary BoExp	carbon monoxide	CO	Exhaled breath
	total 1-hydroxypyrene (1-OHP)	pyrene	Urine
	total N-nitrosonornicotine (NNN)	N-nitrosonornicotine	Urine
	4-aminobiphenyl (4-ABP)	4-aminobiphenyl	Urine
	1-aminonaphthalene (1-NA)	1-aminonaphthalene	Urine
	2-aminonaphthalene (2-NA)	2-aminonaphthalene	Urine
	o-toluidine (o-tol)	o-toluidine	Urine
	2-cyanoethylmercapturic acid (CEMA)	acrylonitrile	Urine
	2-hydroxyethylmercapturic acid (HEMA)	ethylene oxide	Urine
	3-hydroxybenzo(a)pyrene	benzo(a)pyrene	Urine

	3-hydroxy-1-methylpropyl-mercapturic acid (HMPMA)	crotonaldehyde	Urine
	S-benzylmercapturic acid (S-BMA)	toluene	Urine
BoExp to nicotine	nicotine equivalents (NEQ) free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free trans-3'-hydroxycotinine, *trans*-3'-hydroxycotinine-glucuronide	nicotine	Urine
	nicotine	nicotine	Plasma
	cotinine	nicotine	Plasma

Primary endpoints:

- To demonstrate the reduction of primary BoExp to HPHCs (except Total NNAL) in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - MHBMA, 3-HPMA, S-PMA (concentration adjusted for creatinine) in 24-hour urine, and COHb in blood (expressed as % of saturation of hemoglobin) as measured on Day 5.
- To demonstrate the reduction of Total NNAL in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - Total NNAL level (concentration adjusted for creatinine) in 24-hour urine fraction as measured on Day 90 Visit.

Evaluation criterion:

The study will be considered successful if the study demonstrates a 50% reduction or more in MHBMA, 3-HPMA, S-PMA, and COHb at Day 5 and in Total NNAL at Day 90 in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC, using a one-sided test with 2.5% type I error probability.

Secondary endpoints

- To evaluate self-reported nicotine/tobacco product use including dual-use in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Number of mCC or Menthol Tobacco Sticks smoked daily as reported on the usage log during the confinement period and self-reported number of any nicotine/tobacco product use on a daily basis as reported on the product use electronic diary.

- To determine the reduction of secondary BoExp in a confinement setting and in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - BoExp listed as secondary (Table S1) (expressed as quantity excreted or concentration adjusted for creatinine) on Day 5 in 24-hour urine and on Day 90 Visit.
- To describe the levels of primary and secondary BoExp over the entire exposure (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - BoExp listed as primary and secondary (Table S1) from Day 1 to Day 5 and Day 30 Visit, Day 60 Visit and Day 90 Visit as follows:
    - Carbon monoxide (expressed as ppm) in exhaled breath.
    - COHb in blood (expressed as % saturation of hemoglobin).
    - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine) in 24-hour urine.
- To determine the levels of nicotine over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to describe their levels over the entire exposure period.
  - NEQ (expressed as quantity excreted and concentration adjusted for creatinine) (Table S1) in 24-hour urine from Day 1 to Day 5 and on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - Nicotine and cotinine in plasma from Day 1 to Day 4, Day 5, and on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
- To describe the pharmacokinetic (PK) profiles of the nicotine and cotinine in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - Peak (highest concentration along the day) on Day 5.
  - Time to peak (actual time when the peak was observed compared to the time of the first cigarette) on Day 5.
  - Weighted average concentration over 24 hours on Day 5
- To describe the change in CYP1A2 enzymatic activity in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Molar metabolic ratio of paraxanthine/caffeine in plasma on Day 5 and Day 90 Visit.
- To determine the changes in lung functions in smokers switching from mCC to THS 2.2

Menthol as compared to those continuing to smoke mCC.

- Full lung functions: Diffusion capacity for Lung CO (DLCO), rate constant of CO (KCO), forced expiratory volume in 1 second (FEV1), force vital capacity (FVC), vital capacity (VC), total lung capacity (TLC), forced residual volume (FRV), inspiratory capacity (IC), mid expiratory flow (25-75).

- To monitor the safety profiles during the study.
  - Adverse events (AEs)/serious adverse events (SAEs) and device events including THS 2.2 Menthol malfunction/misuse.
  - Respiratory symptoms: cough assessment by visual analog scale (VAS) and Likert scales and one open question.
  - Vital signs.
  - Electrocardiogram (ECG).
  - Clinical chemistry, hematology, and urine analysis safety panel.
  - Physical examination.
  - Concomitant medications.
- To monitor selected risk markers in a confinement and ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Systolic and diastolic blood pressure on Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - High sensitive C-reactive protein (hs-CRP), homocysteine, blood glucose, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), and total cholesterol (TC) in serum on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - Fibrinogen in plasma on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - Hemoglobin A1c (HbA1c) in blood on Day 90 Visit.
  - Apolipoprotein A1 (Apo A1) and apolipoprotein B (Apo B) in serum on Day 90 Visit.
  - Soluble inter-cellular adhesion molecule-1 (sICAM-1) in serum on Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - White blood cell (WBC) and platelet counts in blood on Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - 8-epi-prostaglandin F2α (8-epi-PGF2α) and 11-dehydro-thromboxane B2 (11-DTX-B2) in 24-hour urine on Day 5 and on Day 30 Visit, Day 60 Visit, and Day

90 Visit (expressed as concentration adjusted for creatinine).

- Body weight and waist circumference on Day 90 Visit.

Exploratory Endpoints

- To describe the following parameters in a confinement and/or ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA:
  - Excretion of mutagenic material in urine: Ames Mutagenicity test (YG1024+S9) on Day 5 and Day 90 Visit in 24 hour urine.
  - Subjective effect of smoking: Questionnaire of Smoking Urges (QSU-brief questionnaire); questionnaire Minnesota Nicotine Withdrawal Scale (MNWS)-Revised on Day 5 and Day 90 Visit, and nicotine dependence as assessed by the Fagerström Test for Nicotine Dependence (FTND) questionnaire score on Day 90 Visit.
  - CYP2A6 activity: in plasma on Day 6, and on Day 90 Visit using the molar metabolic ratio of trans-3'-hydroxycotinine/cotinine.
- To describe the following parameters over the course of the study in smokers switching from mCC to the THS 2.2 Menthol as compared to smokers continuing to smoke mCC:
  - Product evaluation: Modified Cigarette Evaluation Questionnaire (MCEQ)
  - Smoking puffing behavior: Human smoking topography (HST) parameters and HST questionnaire.
- To describe the following parameter over the course of the study in smokers switching from mCC to THS 2.2 Menthol:
  - Potential combustion occurrences in tobacco plugs: visual inspection of the tobacco plugs.
- To describe the changes in levels of oxysterols on Day 6 and Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol, and in smokers switching from mCC to SA:
  - Plasma concentration of 6α-hydroxy-5α-cholestanol, 7α-hydroxycholesterol, 5α,6α-epoxycholestanol, 7-ketocholesterol, 7β-hydroxycholesterol, 5β,6β-epoxycholestanol, 24(R)-hydroxycholesterol, 25-hydroxycholesterol, 22(R)-hydroxycholesterol, 4ß-hydroxycholesterol, and 27-hydroxycholesterol in addition to total cholesterol.
- To describe the product use over the course of the study according to the product preference of the subject.
  - Number of mCC or Menthol Tobacco Sticks smoked daily as reported on the usage log during the confinement period and self-reported number of any nicotine/tobacco

product use on a daily basis as reported on the product use electronic diary.

- To describe the smokers' mental state for the intention to quit at Screening on Day -2, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - By the means of the Prochaska 'Stage of Change' questionnaire.
- To describe the changes and reductions in the levels of urinary BoExp and risk markers measured in 4-hour urine fraction on Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine).
  - 8-epi-PGF2α and 11-DTX-B2 (expressed as quantity and concentration adjusted for creatinine).
- To evaluate in smokers switching from mCC to THS 2.2 Menthol, smokers continuing smoking mCC the relationship between [1]:
  - Primary and secondary BoExp and nicotine equivalents (NEQ) on Day 5 and on Day 90 Visit in 24-hour urine.
- To determine the concordance between the results for BoExp and risk markers in 24-hour urine and in 4-hour urine fraction[2]:
  - MHBMA, 3-HPMA, S-PMA, total NNAL, total 1-OHP, total NNN, 4-ABP, 1-NA, 2-NA, o-tol, CEMA, HEMA, 3-hydroxybenzo(a)pyrene, HMPMA, S-BMA, NEQ, (expressed as quantity excreted in 24-hour urine collection and concentration adjusted for creatinine in 4-hour urine fraction) at baseline and at Day 90 Visit.
  - 8-epi-PGF2α and 11-DTX-B2 (expressed as quantity excreted in 24-hour urine collection and concentration adjusted for creatinine in 4-hour urine fraction) at baseline and at Day 90 Visit.
- To monitor the BoExp in subjects quitting smoking according to the time since they quitted[2]:
  - Carbon monoxide (expressed as ppm) in exhaled breath.
  - COHb in blood (expressed as % saturation of hemoglobin).
  - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine) in 24-hour urine.
  - NEQ (expressed as quantity excreted and concentration adjusted for creatinine).
  - Selected risk markers (hs-CRP, homocysteine, blood glucose, LDL, HDL, TG, TC, fibrinogen, HbA1c, sICAM-1, WBC, platelet count, Apo A1, Apo B, 8-epi-PGF2α, 11-DTX-B2) in respective body matrix when available.

[1] The reporting of the objective will be the subject of a separate report.

[2] The reporting of the objective will be the subject of an appendix to the main Clinical Study Report.

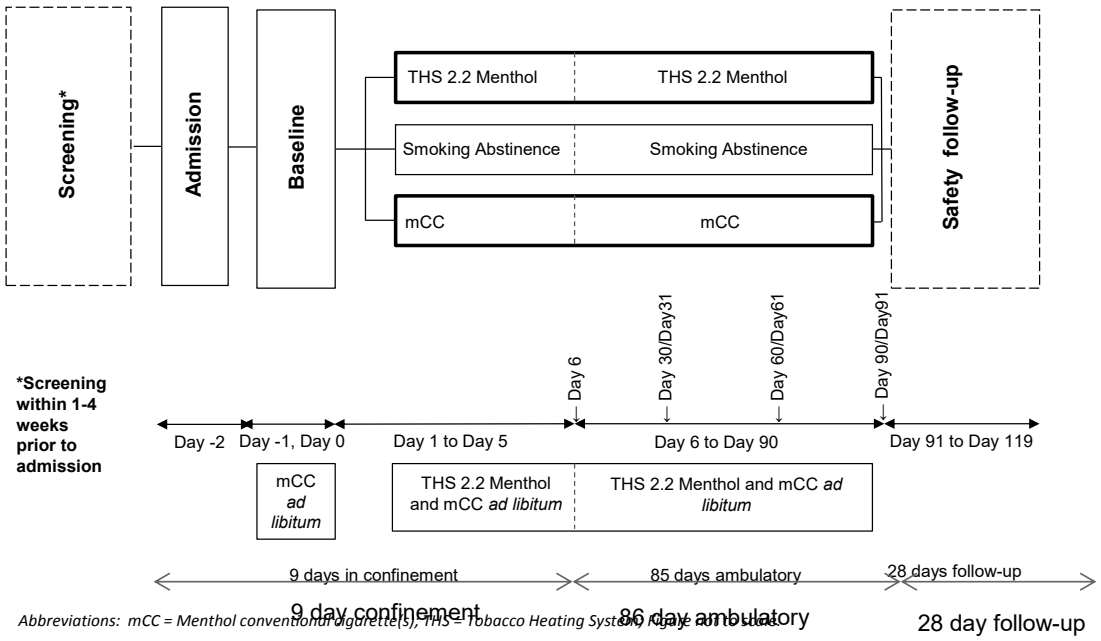
Study Design:

A randomized, controlled, open-label, 3-arm, parallel group study design with a stratified randomization by sex and average daily cigarette consumption over the last 4 weeks as reported during the Screening Visit (smokers smoking 10 to 19 mCC and smokers smoking >19 mCC per day) (Figure S1).

This is an *ad libitum* smoking study. In general, smoking/product use during the confinement period will be allowed from 06:30 AM onwards until around 11:00 PM. During the ambulatory period, there will be no smoking/product use restriction except during the three visits on site (Day 30 Visit, Day 60 Visit, and Day 90 Visit), when product use will be allowed from the time of check-in prior to 08:30 AM to around 11:00 PM on Day 30, Day 60, and Day 90. On Day 31, Day 61, product use will be allowed from 06:30 AM onwards. On Day 91, product use will be allowed after the CYP2A6 activity and full lung function have been performed until time of discharge of Day 91.

During the confinement period, compliance to product/regimen allocation (exclusive use of THS 2.2 Menthol and mCC in THS 2.2 Menthol and mCC arms respectively, and full abstinence from smoking in the SA arm) will be ensured by strict distribution of each Menthol Tobacco Stick/mCC when requested by the subject. During the ambulatory period, the subjects randomized to the THS 2.2 Menthol arm will be instructed to exclusively use THS 2.2 Menthol and subjects randomized to the SA arm will be instructed to abstain from smoking.

Figure S1: Study Design



Abbreviations: mCC = Menthol conventional cigarette(s), THS = Tobacco Heating System, figure not scale

mCC: menthol conventional cigarettes; THS: Tobacco Heating System; figure not scale

- The Screening period covers 4 weeks (Day -30 to Day -3) prior to Admission to the clinic (Day -2):

  A demonstration of the THS 2.2 Menthol will be done by the site staff during the Screening Visit. Subjects will be in a confined setting for 9 days from Day -2 onwards.

- The run-in period (from Admission on Day -2 until 06:29 AM of Day-1):

  Smoking will be allowed *ad libitum* from the time of check-in of the subject until around 11:00 PM. Prior to enrolment on Day -2, as the last procedure of the eligibility assessments, subjects will have a product test of the THS 2.2 Menthol (use of up to three THS Menthol Tobacco Sticks). In female subjects, the THS 2.2 Menthol product test will only be done after pregnancy is excluded by a negative urine pregnancy test. Enrolment takes place after all inclusion and exclusion criteria have been satisfactorily met. Only subjects willing and able to use the product will be enrolled in the study.

- The baseline period (from Day -1, 06:30 AM until Day 1, 06:29 AM):

  All subjects will continue smoking their single preferred brand of mCC *ad libitum*. On Day -1 and Day 0, smoking will be allowed from 06:30 AM onwards until around 11:00 PM. However, on Day 0, smoking will be allowed only after sample for CYP2A6, MNWS and cough questionnaires, and full lung functions have been conducted. Four-hour urine fraction will be collected on Day-1. On Day 0, 24-hour urine will be collected starting in the morning and ending 24 hours later on Day 1.

On Day 0, subjects will be randomized to 1 of the 3 study arms in a 2:1:1 ratio using a stratified randomization

- THS 2.2 Menthol Arm: ~80 subjects, *ad libitum* use of the product.
- mCC Arm: ~40 subjects, *ad libitum* use of their preferred mCC brand.
- SA Arm: ~40 subjects who will abstain from smoking.

Subjects will be informed of their randomized study arm by the study site staff on Day 1 prior to 06:30 AM.

- The exposure period (from Day 1, 06:30 AM until time of discharge on Day 90 Visit (Day 91)):

  The exposure period will include both the exposure period in confinement, and the exposure period in ambulatory setting:

  - The exposure period in the confinement setting (from Day 1, 06:30 AM until time of Discharge on Day 6):

    The exposure period in confinement consists of 5 days of *ad libitum* use of the assigned product from 06:30 AM onwards until around 11:00 PM in THS 2.2 Menthol and mCC arms. Subjects allocated to the SA arm will be asked to abstain from smoking and will not be provided with medication to support SA. Subjects will be provided with psychological support during the period of SA. Use of any tobacco/nicotine-containing product other than the assigned product/regimen will not be allowed.

    Twenty four-hour urine will be collected from Day 1 to Day 5 on site. The end of the 24-hour urine collection from Day 5 will end in the morning on Day 6 prior to Discharge.

    On Day 6, the safety procedures will be conducted before discharge of the subject from the clinic after 9 days in a confined setting. Use of products will be allowed on Day 6 in the THS 2.2 Menthol and mCC arms according to product arm allocation, but only after the sample for CYP2A6 activity, MNWS and cough questionnaires, and full lung function have been performed.

  - The exposure period in ambulatory setting (from time of Discharge on Day 6 until the time of Discharge on Day 90 Visit (Day 91)):

    At the end of the confinement period prior to Discharge on Day 6, subjects will be instructed to continue their assigned product/regimen in an ambulatory setting for 86 days. All subjects in the SA arm will receive smoking cessation counseling and will be able to use nicotine replacement therapy (NRT) if considered necessary by the Investigator or requested by the subject.

Subjects will be required to make three visits (Day 30 Visit, Day 60 Visit, and Day 90 Visit) to the investigational site. Each visit will cover 2 consecutive days on site. For Day 30 Visit and Day 60 Visit, the subject will check-in in the morning prior to 08:30 AM on Day 30 and Day 60, and will check-out on Day 31 and Day 61 respectively. For Day 90 Visit, the subject will checked-in in the morning prior to 08:30 AM on Day 90, and will be discharged on Day 91 after having performed all the safety examination procedures.

Twenty four-hour urine will be collected at each ambulatory visit on Day 30 Visit, Day 60 Visit, and Day 90 Visit at the site. The collection of 24-hour urine will start on Day 30, Day 60, and Day 90 respectively and will end 24-hours later. On Day 90 visit only, the end of 24-hour urine collection will be followed by the collection of a 4-hour urine fraction.

On Day 30, Day 60, and Day 90, subjects in the THS 2.2 Menthol and mCC arms will be allowed to use their assigned product from the time of check-in in the morning prior to 08:30 AM to around 11:00 PM. On Day 31, Day 61, product use will be allowed from 06:30 AM. On Day 91, product use will be allowed only after the sample for CYP2A6 activity and full lung function have been performed until time of discharge of Day 91. The end of the exposure period will be fixed at the time of discharge of Day 91.

During the visits, the use of THS 2.2 Menthol will be strictly forbidden for subjects in the mCC or SA arms.

Subject will not be withdrawn from the study for the use of nicotine/tobacco-containing products other than the assigned product/regimen. Subjects will record in a product use electronic diary any use of CC (menthol or non-menthol), NRT, or other nicotine/tobacco-containing products on a daily basis.

During the confinement and ambulatory settings:

- Subjects in the SA arm will be provided with support including psychological support as requested by the subject or considered necessary by the Investigator/site staff.
- Any subject, who wants to quit smoking during the study taking into account the outcome from the Prochaska ‘Stages of Change’ questionnaire, will be encouraged to do so and will be referred to appropriate medical services. This will not affect subject’s financial compensation and subject will remain in the study.

- <u>The safety follow-up period (from time of Discharge on Day 90 Visit (Day 91) until Day 119)</u>:

  After the time of Discharge on the Day 90 Visit (Day 91), subject will enter a 28-day safety follow-up period during which there will be recording of spontaneously reported new AEs/SAEs and the active follow-up of ongoing AEs/SAEs by the study site. In

general, all AEs will be followed-up until resolved, stabilized (i.e., no worsening of the event), or a plausible explanation for the event has been found. The end of the study (EOS) is defined as the time of Discharge on Day 90 Visit (Day 91) plus 28-day follow-up.

Study Population and Main Criteria for Inclusion:

Female or male smoking, apparently healthy adult subjects without any restriction to races and ethnicities, meeting the following main inclusion criteria:

- Subject is aged with a minimum of 22 years(inclusive).
- Smoking, apparently healthy subject as judged by the Investigator.
- Subject smokes at least 10 commercially available mCC per day (no brand restrictions) for the last 4 weeks, based on self-reporting.
- Subject has smoked for at least the last 3 consecutive years.
- Subject does not plan to quit smoking within the next 6 months.
- Subject is ready to comply with the study protocol (e.g., to accept interruption of smoking for up to 91 days and to use THS 2.2 Menthol).

Investigational Products; Dose; and Mode of Use:

Test Product:

Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol).

Reference Product:

The reference product to the THS 2.2 Menthol during the randomized exposure period is the subject's own preferred commercially available single brand of mCC.

Reference Point: SA.

Sample size:

Approximately one hundred and sixty smokers in the full analysis set (FAS) (~80 in THS 2.2 Menthol arm, ~40 in mCC and ~40 in SA) are required to attain 80% power to show at least a 50% reduction for any of the selected primary BoExp in THS 2.2 Menthol compared to the mCC arm, using a one-sided test with 2.5% type I error probability.

Statistical Methods:

Primary confirmatory endpoint: The hypothesis to be tested is that the geometric mean level of the BoExp for THS 2.2 Menthol is lower relative to mCC.

For BoExp, the hypothesis will be tested on Day 5 for MHBMA, 3-HPMA, S-PMA, and COHb, and on Day 90 Visit for Total NNAL.

The transformed BoExp data will be analyzed by means of a generalized linear model using product arm as covariate adjusting for the following baseline information: sex, average cigarette consumption over the previous 4 weeks, and baseline value of endpoint. The test will be declared significant if the contrast THS 2.2 Menthol versus mCC is significant. Estimates of differences between groups will be back-transformed to provide relative effects.

Descriptive statistics for continuous variables (number of subjects [n], number and percent of subjects with data, mean, standard deviation [SD], median, first and third quartiles, minimum and maximum for continuous data, and the n and absolute and relative [%] frequency for categorical data) will be presented by product arm and overall at each time point, where applicable.

For BoExp, the geometric mean and coefficient of variation will be presented in addition to the mean and SD.

Analyses over time will be descriptive statistics of parameters at each assessment timepoint.

Unless stated otherwise, all statistical tests will be two-sided and conducted at the 5% level, and all quoted confidence intervals (CI) will be two-sided 95% CI.

The per-protocol (PP) population will be the primary analysis for BoExp, and risk markers. The full analysis set will be the primary analysis set for compliance to randomization arm, exposure and questionnaires. Exposure and questionnaires will be described by randomization arm and according to the exclusive THS 2.2 Menthol, dual-use of THS 2.2. Menthol and mCC, mCC exclusive, SA groups.

A sensitivity analysis will be run on the compliant population for the BoExp and risk markers.

The full analysis set will be also a supplemental analysis set for BoExp and risk markers.

Safety will be analyzed using the safety population by randomization arm and according to the exclusive THS 2.2 Menthol, dual-use of THS 2.2 Menthol and mCC, mCC exclusive, SA groups.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

11-DTX-B2	11-dehydro-thromboxane B2
1-NA	1-aminonaphthalene
1-OHP	1-hydroxypyrene
2-NA	2-aminonaphthalene
3-HPMA	3-hydroxypropylmercapturic acid
4-ABP	4-aminobiphenyl
8-epi-PGF2α	8-epi-prostaglandine F2 α
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
B	Blood sample required
BMI	Body mass index
BoExp	Biomarker(s) of exposure
BP	Blood Pressure
BUN	Blood urea nitrogen
CAF	Caffeine
CC	Conventional cigarette
CD	Compact Disc
CDC	Centers for Disease Control and Prevention
CEMA	2-cyanoethylmercapturic acid
CFR	Code of Federal Regulations
CI	Confidence interval
CO	Carbon monoxide
COHb	Carboxyhemoglobin
CRO	Contract Research Organization
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events and Common

	Toxicity Criteria
CTMS	Clinical Trial Management System
CV (statistics)	Coefficient of variation
CYP1A2	Cytochrome P450 1A2
CYP2A6	Cytochrome P450 2A6
DMP	Data Management Plan
DLCO	Diffusion capacity of lung for carbon monoxyde
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ePRO	Electronic patient reported outcome
EOS	End of study
FAS	Full analysis set
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FTND	Fagerström Test for Nicotine Dependence
FVC	Forced vital capacity
FRV	Forced residual volume
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GVP	Gas vapor phase
HbA1c	Hemoglobin A1c
HDL	High density lipoprotein
HEMA	2-hydroxyethyl mercapturic acid
HIV	Human immunodeficiency virus
HMPMA	3-hydroxy-1-methylpropylmercapturic acid
HPHCs	Harmful and potentially harmful constituents
hs-CRP	High sensitive C-reactive protein
HST	Human smoking topography
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IC	Inspiratory capacity
IOM	Institute of Medicine
IP	Investigational Product
IRB	Institutional Review Board

ISO	International Organization for Standardization
IU	International unit
IV	Intravenous
IWRS	Interactive web and voice response system
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LLN	Lower limit of the normal range
LLOQ	Lower limit of quantification
mCC	Menthol conventional cigarette
MCEQ	Modified Cigarette Evaluation Questionnaire
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MEF	Mid expiratory flow
MHBMA	Monohydroxybutenyl mercapturic acid
MNWS	Minnesota Nicotine Withdrawal Scale (revised version)
MR	Mean ratios
mRNA	messenger-ribonucleic acid
MRTP	Modified risk tobacco product
n	Number of subjects
NEQ	Nicotine equivalents
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-nitrosonornicotine
NRT	Nicotine replacement therapy
NSAID	Nonsteroidal anti-inflammatory drugs
o-tol	o-toluidine
PI	Principal Investigator
PK	Pharmacokinetic(s)
PMI	Philip Morris International
PP	Per-protocol
PT	Preferred term
PX	Paraxanthine
QC	Quality control

QSU	Questionnaire of Smoking Urges (brief version)
RBC	Red blood cell (count)
RNA	Ribonucleic acid
SA	Smoking abstinence
SAE	Serious adverse event
SAP	Statistical analysis plan
S-BMA	S-benzylmercapturic acid
SD	Standard deviation
SDTM	Standards Consortium's Study Data Tabulation Model
SES	Socio-Economic Status (questionnaire)
SHM	Sample Handling Manual
sICAM-1	Soluble inter-cellular adhesion molecule
SMAR	Smoking article
SMP	Safety management plan
SOC	System Organ Class
SOP	Standard operating procedure
S-PMA	*S*-phenylmercapturic acid
SQ	Smoking Questionnaire
T_0	Start time of the first product use
TC	Total cholesterol
TG	Triglycerides
THS 2.2	Tobacco Heating System 2.2
TLC	Total lung capacity
TPM	Total particulate matter
U	Urine sample required
[redacted]	[redacted]
ULN	Upper limit of the normal range
ULOQ	Upper limit of quantification
USB	Universal Serial Bus
UV	Ultraviolet
VAS	Visual analog scale
VC	Vital capacity
WBC	White blood cell (count)
WHO	World Health Organization

Explanation of Terms

The following special terms are used in this protocol:

Baseline period	06:30 AM at Day -1 until 06:29 AM of Day 1.
Charger:	The function of the Charger (Model 4) is to recharge the Holder after use. It contains a battery with sufficient capacity to recharge the Holder approximately 20 times. It is a convenient size to carry around, and can itself be recharged from a mains power source.
Day 30 Visit, Day 60 Visit, and Day 90 Visit	Day 30 Visit, Day 60 Visit, and Day 90 Visit start on Day 30, Day 60, and Day 90 respectively at the time of check-in of the subject on site prior to 08:30 AM until the check-out of the day after respectively on Day 31, Day 61, and Day 91.
End of study	End of Study is defined as the time of discharge on Day 91 of the subject plus 28 days of safety follow-up.
Enrolment	On Day -2 for eligible subjects after all applicable inclusion and exclusion criteria have been satisfactorily met and the subject is willing and ready to use the THS 2.2 Menthol (the trial of THS 2.2 Menthol is the last assessment prior to enrolment).
Exposure period in confinement	06:30 AM of Day 1 until time of Discharge on Day 6.
Exposure period in the ambulatory setting	From the time of discharge on Day 6 until the time of Discharge on Day 90 Visit (Day 91).
mCC	The term ‘menthol conventional cigarette’ refers to manufactured and commercially available menthol cigarettes and excludes hand-rolled cigarettes, cigars, pipes, bidis, and other nicotine-containing products.
mCC incompatible with HST SODIM® device	All mCCs that are incompatible with the HST SODIM® device (e.g., slim mCC).

Randomization	Assignment of the subject randomization number in the Interactive Web and Voice Response System. This can be done any time on Day 0, however, subjects are **not** to be informed of their randomization group prior to Day 1.
Run-in period	Admission to site on Day -2 until 06:29 AM of Day -1.
Safety follow-up	After the time of Discharge on Day 90 Visit (Day 91), a 28-day safety follow-up will be done for the recording of spontaneously reported new adverse events (AEs)/serious adverse events (SAEs) and the active follow-up of ongoing AEs/SAEs by the site. In general, any AE will be followed-up until resolved, stabilized (i.e., no worsening of the event) or a plausible explanation for the event has been found.
Screening failure	Subjects who do not meet the entry criteria from the time of informed consent form signature to the time of enrolment will be considered a screening failure.
THS Menthol Tobacco Stick	The Menthol Tobacco Stick (product code C3 Menthol) contains tobacco which, when heated, generates an aerosol. It is custom-designed to be used with the Holder.
THS Tobacco Stick Holder (Holder)	The function of the Holder (Model 4.2) is to heat the Tobacco Stick, delivering an aerosol to the user. The electrical heating is powered from an internal battery which delivers power for about 6 minutes (allowing complete use of a single Menthol Tobacco Stick)
Time of Discharge	Time of Discharge on Day 6: time when the subject is released from the site (confinement period) after all the procedures of the day of Discharge (Day 6) have been conducted prior to entering into the ambulatory period Time of Discharge on Day 90 Visit (Day 91): time when the subject is released from the site and enters the 28-day safety follow-up period
Tobacco Heating Device	The Device comprises everything in THS 2.2 Menthol, except the Menthol Tobacco Stick

Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol)	THS 2.2 Menthol comprises the following components: Menthol Tobacco Stick, Holder, Charger, a Cleaning Tool, a mains power supply, and a USB cable.

1 ETHICS AND REGULATIONS

1.1 Institutional Review Board Approval

Prior to the start of the study, the clinical study protocol, together with its associated documents (informed consent form [ICF], subject information sheet, subject recruitment procedures [e.g., advertisements], written information to be provided to the subjects, Investigator's Brochure [IB], available safety information, the Investigator's curriculum vitae [CV] and/or other evidence of qualifications and any other documents requested by an Institutional Review Board [IRB]), will be submitted for review and approval to the relevant IRB according to the appropriate provisions found in 21 Code of Federal Regulations (CFR) part 50 (Informed Consent of Human Subjects) and 21 CFR part 56 (Institutional Review Boards). The IRB shall be appropriately constituted and perform its functions in accordance with the International Conference on Harmonization (ICH) Tripartite Guidance for Good Clinical Practice (GCP) and local requirements, as applicable.

In accordance with GCP and 21 CFR part 56 (IRB review and approval of clinical investigation), a written confirmation of the IRB approval should be provided to the Sponsor. This should identify the study (Investigator's name, study number, and title) and the documents that have been approved by the IRB, with dates and version numbers, as well as the date of approval. The composition of the IRB, including the name and occupation of the chairperson, will be supplied to the Sponsor together with a GCP compliance statement.

The written approval from the IRB will be filed in the Investigator file, and a copy will be filed in the Study Master File at the Sponsor or designated organization. The study must not start at a site before the Sponsor has obtained written confirmation of favorable opinion/approval from the concerned IRB.

Any substantial change or addition to this protocol will require a written protocol amendment that must be approved by the Sponsor and the Investigator. All amendments will be submitted to the IRB, and substantial amendments will only be implemented after approval by the IRB.

These requirements for approval should in no way prevent any action from being taken by the Investigator or designee or by the Sponsor in order to eliminate immediate hazards to the subjects. If such a change to the protocol is felt to be necessary by the Investigator or designee, and is implemented for safety reasons, the Sponsor and the IRB should be informed immediately.

Relevant safety information will be submitted to the IRB during the course of the study in accordance with national regulations and requirements.

1.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (2008) and are consistent with ICH/GCP applicable regulatory principles.

The Investigator or designee agrees to conduct the clinical study in compliance with the protocol agreed with the Sponsor and approved by the IRB. The Principal Investigator and the Sponsor must sign the protocol (and protocol amendments, if applicable) to confirm this agreement. A copy of the Declaration of Helsinki (2008) is located in the Investigator's Study File.

1.3 Subject Information and Consent

1.3.1 Study Consent/Subject Information Sheet for Study Participation

Before or at the Screening Visit, the Investigator or designee will ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study, and the Investigator or the designee will answer all questions the subject might have to his/her full satisfaction. The subject will have sufficient time for consideration of his/her participation in the study and will be notified that he/she is free to discontinue his/her participation at any time. Once the subject has received all necessary information, and if he/she agrees to participate, this will be documented in the ICF which includes both the subject information sheet and informed consent by the date, time and signature of both the subject and the person who conducted the inform consent discussion during the Screening Visit. No study-specific procedures will be performed before the ICF has been signed (including date and time).

The original, dated and signed ICF(s) must be kept by the Investigator and filed in the Investigator study file at the site or with the subject's files and a copy must be given to the subject.

The subject will be informed that additional data analysis not mentioned in the protocol or the Statistical Analysis Plan (SAP) might be performed with the collected data at a later time. Any additional analysis performed will be covered by data confidentiality, as for the main analysis described in this protocol.

1.3.2 Informed Consent Form/Subject Information Sheet for Long-term Bio-Banking

In addition to the ICF for the participation in the study, each subject will be asked for consent to the additional bio-banking:

- One separate ICF to obtain consent for serum/plasma and urine collection and long-term storage for BoExp and risk markers.
- One separate ICF to obtain consent for collection and long-term storage of blood for further transcriptomics (pharmacogenomics) analysis, of samples from nasal epithelial, and buccal collections.

Each subject will be given full and adequate oral and written information about the nature, purpose, possible risks and benefits of bio-banking, and the Investigator will answer all questions the subject might have to his/her full satisfaction. The subject will be notified that he/she is free to discontinue his/her participation at any time. Once the subject has received all necessary information, and if he/she agrees to participate, this will be documented by the date, time and signature of both the subject and the Investigator and personnel who conducted the informed consent discussion. The subject's consent to collection and storage of any samples in a bio-bank or for transcriptomics/nasal epithelial collection/buccal collection is not a requirement for study participation and the subject's participation in the study (1.3.1) does not depend on their providing consent for sample bio-banking.

1.3.2.1 Bio-bank for Biomarkers of Exposure and Risk Markers

Subjects will be provided with information and will be asked for their consents for samples (serum/plasma/urine) that will be collected and stored in a bio-bank for subsequent analysis of biomarkers of exposure (BoExp) and/or risk markers following completion of this study. No genetic or transcriptomics testing will be done on these samples.

1.3.2.2 Bio-bank for Transcriptomics/Nasal Epithelial Collection/Buccal Collection

Subjects will be provided with information and asked for their consent to collect and store different types of samples in the same ICF:

- Samples of blood for transcriptomics (pharmacogenomics)
- Samples from nasal epithelial collection (molecular profiling)
- Samples from buccal collection (molecular profiling).

Blood samples for transcriptomics will be collected for long-term bio-banking in order to further study the variation of the ribonucleic acid (mRNA and miRNA) in smokers using THS 2.2 Menthol as compared to smokers continuing to smoke mCC or smokers switching to smoking abstinence (SA). In-house data from an exploratory study to assess the reduction of exposure to HPHCs (ZRHX-EX-01 study) in smokers switching to THS 2.1 as compared to smokers continuing smoking CC shows that using THS 2.1, the earlier

version of THS 2.2 results in significant variation of RNA characteristics as compared to smoking CC.

Nasal epithelial and buccal samples will be collected for long-term bio-banking in order to provide biological material for molecular profiling. The changing molecular profiles will provide insight into the biological processes that take place in mCC, THS 2.2 Menthol and SA arms. Given the similarities in the cell types along the respiratory tract, the biological processes identified in the nasal and buccal tissues may reflect those that give rise to lung diseases that are etiologically linked to smoking (Boyle et al., 2010.; **Error! Reference source not found.**).

1.3.3 Amendment to the Informed Consent Form

If a protocol amendment is required, or if new information regarding the risk profile of the IP becomes available, an amendment may be required to the ICF. If revision of the ICF is necessary, the Investigator will, with the support of the Sponsor, ensure that the documents have been reviewed and approved by a relevant IRB before subjects are required to re-sign the ICF (including date and time).

1.4 Good Clinical Practice and Regulatory Requirements

The procedures set out in this clinical study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Sponsor, its authorized representative, and Investigator and designee abide by the principles of the ICH guidelines on GCP. These guidelines apply specifically to pharmaceutical development, but nevertheless provide a robust and ethical framework for conducting clinical studies of tobacco products. The study will also be conducted in accordance with the general ethical principles outlined in the Declaration of Helsinki (2008).

In addition, the Investigator or designee will carry out the clinical study in accordance with applicable national and local laws of the pertinent regulatory authorities.

2 INTRODUCTION

2.1 Background

2.1.1 Smoking-Related Diseases and Harm Reduction Strategy

Cigarette smoking causes pulmonary, cardiovascular diseases and other serious diseases in smokers (U.S. Department of Health and Human Services, 2010). There is no safe cigarette and the best way for smokers to reduce the adverse health consequences of smoking is to quit. Despite the risks which are attributable to smoking, some smokers cannot refrain from smoking or decide to continue smoking. To those smokers who are not able or not willing to quit, Philip Morris International (PMI) is developing alternative approaches by developing products with the potential to reduce the risks of tobacco-related diseases. These products are now referred by the United States Food and Drug Administration (FDA) as modified risk tobacco products (MRTPs) (FDA, 2012a).

2.1.2 Description of the Product and Scientific Findings

Thousands of chemicals - “smoke constituents” - are formed when tobacco is burned or combusted. More than 5,300 smoke constituents have been identified (Rodgman and Perfetti, 2009), and more than 100 of them have been categorized as harmful and potentially harmful constituents (HPHCs) (FDA, 2011).

PMI’s focus has been the development of products that do not combust tobacco but which replicate the “smoking experience” as much as possible. Our approach limits pyrolysis and combustion, by heating tobacco at significantly lower temperatures than conventional cigarette (CC). PMI believes that such products present the best opportunity for reducing harm because they produce vastly lower levels of HPHCs and are more likely to be accepted by smokers as substitutes for CCs. Important to this effort has been providing nicotine in a way that closely parallels CC.

The product developed by PMI, and to be assessed in this study, is the Tobacco Heating System 2.2 Menthol (THS 2.2 Menthol). With this product, the heating of the tobacco is maintained below 400°C, a temperature much lower than what is observed for CC, which can reach 900°C. The THS 2.2 Menthol is composed of the ‘THS Tobacco Stick Holder’, dedicated special Menthol Tobacco Sticks made of conventional tobacco, a Charger, and different accessories. The energy of the THS Tobacco Stick Holder is sufficient to maintain approximately a 6 minute session. Unlike CC, the Menthol Tobacco Sticks do not burn down during their consumption and their lengths remain constant after use.

The non-clinical assessment of earlier development of THS 2.2 Menthol described in the investigator’s brochure (PMI, 2013a) supports the initiation of the clinical studies. No new

or increased toxicological hazard in the product's aerosol was detected compared with CC smoke. Further details on the clinical and non-clinical data are provided in the Investigators' Brochure Edition 2 (PMI, 2013a)

Several clinical studies have been conducted on THS 1.0 (Menthol and non-menthol), an earlier development version of THS 2.2, in Europe, Asia, Africa and the United States. All studies showed reductions in exposure to the majority of measured HPHCs from both aerosol fractions, total particulate matter (TPM)and gas vapor phase (GVP), in subjects who used the THS 1.0 as compared to subjects continuing smoking CC, both, in controlled and ambulatory conditions. No clinical studies were conducted with THS 2.2 Menthol.

THS 2.1 non-Menthol, the predecessor of THS 2.2, was tested in two exploratory clinical studies to measure the nicotine plasma kinetic profile (PK) (ZRHX-PK-02 study) and to assess the reduction of exposure to HPHCs when switching from CC to THS 2.1 (ZRHX-EX-01 study). The observed nicotine plasma PK profile for THS 2.1 was similar to CC as well, there were significant reductions in the exposure to the majority of selected HPHCs (PMI, 2013a).

Clinical studies conducted so far revealed no safety concern for either of the previous versions of THS 2.2 tested.

2.2 Purpose of the Study

The overall goal of the study is to provide information on the reduction in the levels of selected BoExp to HPHCs (except for BoExp to nicotine) and to obtain safety information in apparently healthy subjects using the THS 2.2 Menthol as compared to smokers continuing smoking their preferred brand of mCC in confinement setting for 5 days followed by an ambulatory setting of 86 days. Smokers who will be asked to abstain from smoking will be used as a reference point. The smokers in the THS 2.2 Menthol and mCC arms will be allowed to use the product they are allocated to *ad libitum*.

Additional purpose of the study aim is to understand the effect of using THS 2.2 Menthol on selected variables and their potential association to the reduced exposure to HPHCs (e.g. cytochrome P450 1A2 [CYP1A2] and cytochrome P450 2A6 ([CYP2A6] enzymatic activity, pharmacokinetic [PK] profile of nicotine and cotinine, product evaluation, product use and subjective effects related to smoking, human smoking topography, risk markers).

2.3 Anticipated Benefits and Risks

2.3.1 Anticipated Benefits

Advice on health risks associated with smoking and smoking cessation advice will be provided on Screening, on Admission Day (Day -2), on Day 6 (Day of Discharge), Day 30 Visit, Day 60 Visit, and at Day 90 Visit. The advice will follow the recommendations of the U.S. Public Health Service (U.S. Department of Health and Human Services, 2008). Subjects who are motivated to quit smoking during the study in the THS 2.2 and mCC arms will be referred to appropriate stop smoking services for continuing support and counseling at a higher level. Subjects who participate in this study will also benefit from repeated, detailed health check-ups, which may help to uncover undiagnosed medical conditions.

2.3.2 Anticipated Foreseeable Risks due to Study Procedures

- Risks related to blood sampling, e.g. excessive bleeding, fainting, hematoma, paresthesia, or infection.
- Risks related to chest X-rays, e.g. a small increase of risk to develop cancer later in life.
- Risks related to drug application as part of testing procedures (i.e., spirometry with short-acting bronchodilator at Screening and at Day 6 and Day 90 Visit) per study protocol and scientifically accepted standards.

2.3.3 Anticipated Foreseeable Risks due to Investigational Product (THS 2.2 Menthol/mCC)

- Change in smoking habits due to study requirements and related concomitant symptoms, (e.g., craving, withdrawal symptoms).
- All risks related to study procedures, IP, or support for SA will be explained in detail to the subjects. Mitigation will include:
  - Close monitoring and medical evaluation of potential safety signals throughout the study and follow-up.
  - Using accepted research and scientific standards, (e.g., blood samples not to exceed local blood donation standards).
  - Management and follow-up of adverse events (AEs)/serious adverse events (SAEs).

2.3.4 Unforeseeable Risks

Substantial body of evidence already exists on the predecessors of THS 2.2 Menthol and no safety concerns were reported. The possibility of unforeseeable events/risks will be explained at Screening and at Admission. Mitigation will include close monitoring and medical supervision to detect any unforeseeable risk or safety signals at the earliest possibility.

3 STUDY OBJECTIVES

3.1 Primary Objectives

The primary objectives of this study are:

- To demonstrate the reduction of primary biomarkers of exposure (BoExp) (Table 1) to HPHCs (except Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) (Total NNAL)) in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC, and
- To demonstrate the reduction of Total NNAL in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.

3.2 Secondary Objectives

The secondary objectives of this study are:

- To evaluate self-reported nicotine/tobacco product use including dual-use in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to smoking abstinence (SA).
- To determine the reduction of secondary BoExp in a confinement setting and in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
- To describe the levels of primary and secondary BoExp over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To determine the levels of nicotine over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to describe their levels over the entire exposure period.
- To describe the pharmacokinetic profiles of the nicotine and cotinine in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
- To describe the change in CYP1A2 enzymatic activity in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To determine the changes in lung functions in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.

- To monitor the safety profiles during the study.
- To monitor selected risk markers in a confinement and ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.

3.3 Exploratory Objectives

The exploratory objectives of this study are:

- To describe the following parameters in a confinement and/or ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA:
  - Excretion of mutagenic material in urine.
  - Subjective effect of smoking.
  - Cytochrome P450 2A6 (CYP2A6) activity.
- To describe the following parameters over the course of the study in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC:
  - Product evaluation.
  - Smoking puffing behavior.
- To describe the following parameter over the course of the study in smokers switching from mCC to THS 2.2 Menthol:
  - Potential combustion occurrences in tobacco plugs.
- To describe the changes in levels of oxysterols on Day 6 and Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol, and in smokers switching from mCC to SA.
- To describe the product use over the course of the study according to the product preference of the subject.
- To describe the smokers' mental state for the intention to quit at Screening, on Day -2, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
- To describe the changes and reductions in the levels of urinary BoExp and risk markers measured in 4-hour urine fraction on Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
- To evaluate in smokers switching from mCC to THS 2.2 Menthol, and smokers continuing smoking mCC the relationship between[1] :
  - Primary and secondary BoExp and nicotine equivalents (NEQ).

- To determine the concordance between the results for BoExp and risk markers in 24-hour urine and in 4-hour urine fraction [2].
- To monitor the BoExp in subjects quitting smoking according to the time since they quitted [2].

[1] The reporting of the objective will be the subject of a separate report.

[2] The reporting of the objective will be the subject of an appendix to the main Clinical Study Report.

3.4 Study Endpoints

The primary and secondary BoExp to HPHCs measured in the study are presented in Table 1:

Table 1: Biomarkers of Exposure

	Biomarkers of Exposure (BoExp)	HPHCs	Matrix
Primary BoExp on Day 5	monohydroxybutenylmercapturic acid (MHBMA)	1,3-butadiene	Urine
	3-hydroxypropylmercapturic acid (3-HPMA)	acrolein	Urine
	S-phenylmercapturic acid (S-PMA)	benzene	Urine
	carboxyhemoglobin (COHb)	carbon monoxide (CO)	Blood
Primary BoExp on Day 90 Visit	total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (Total NNAL)	4 (methylnitrosamino)-1-(3-pyridyl)-1butanone (NNK)	Urine
Secondary BoExp	carbon monoxide	CO	Exhaled breath
	total 1-hydroxypyrene (total 1-OHP)	pyrene	Urine
	total N-nitrosonornicotine (NNN)	N-nitrosonornicotine	Urine
	4-aminobiphenyl (4-ABP)	4-aminobiphenyl	Urine
	1-aminonaphthalene (1-NA)	1-aminonaphthalene	Urine
	2-aminonaphthalene (2-NA)	2-aminonaphthalene	Urine
	o-toluidine (o-tol)	o-toluidine	Urine
	2-cyanoethylmercapturic acid (CEMA)	acrylonitrile	Urine
	2-hydroxyethylmercapturic acid (HEMA)	ethylene oxide	Urine
	3-hydroxybenzo(a)pyrene	benzo(a)pyrene	Urine
	3-hydroxy-1-methylpropyl-mercapturic acid (HMPMA)	crotonaldehyde	Urine
	S-benzylmercapturic acid (S-BMA)	toluene	Urine

	Biomarkers of Exposure (BoExp)	HPHCs	Matrix
BoExp to nicotine	nicotine equivalents (NEQ) free nicotine, nicotine-glucuronide, free cotinine, cotinine-glucuronide, free trans-3'-hydroxycotinine, *trans*-3'-hydroxycotinine-glucuronide	nicotine	Urine
	nicotine	nicotine	Plasma
	cotinine	nicotine	Plasma

3.4.1 Primary Endpoints

- To demonstrate the reduction of primary BoExp to HPHCs (except Total NNAL) in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - MHBMA, 3-HPMA, S-PMA (concentration adjusted for creatinine) in 24-hour urine, and COHb in blood (expressed as % of saturation of hemoglobin) as measured on Day 5.
- To demonstrate the reduction of Total NNAL in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - Total NNAL level (concentration adjusted for creatinine) in 24-hour urine fraction as measured on Day 90 Visit.

Evaluation criterion:

The study will be considered successful if the study demonstrates a 50% reduction or more in MHBMA, 3-HPMA, S-PMA, and COHb at Day 5 and in Total NNAL at Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC, using a one-sided test with 2.5% type I error probability.

3.4.2 Secondary Endpoints

- To evaluate self-reported nicotine/tobacco product use including dual-use in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Number of mCC or Menthol Tobacco Sticks smoked daily as reported on the usage log during the confinement period and self-reported number of any nicotine/tobacco product use on a daily basis as reported on the product use electronic diary.

- To determine the reduction of secondary BoExp in a confinement setting and in an ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - BoExp listed as secondary (expressed as quantity excreted or concentration adjusted for creatinine) (Table 1) as measured in 24-hour urine on Day 5 and on Day 90 Visit.
- To describe the levels of primary and secondary BoExp over the entire exposure (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - BoExp listed as primary and secondary (Table 1) from Day 1 to Day 5 and Day 30 Visit, Day 60 Visit and Day 90 Visit as follows:
    - Carbon monoxide (CO) (expressed as ppm) in exhaled breath.
    - COHb in blood (expressed as % saturation of hemoglobin).
    - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine) in 24-hour urine.
- To determine the levels of nicotine over the entire exposure period (confinement and ambulatory periods) in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to describe their levels over the entire exposure period.
  - NEQ (expressed as quantity excreted and concentration adjusted for creatinine) (Table 1) in 24-hour urine from Day 1 to Day 5 and on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - Nicotine and cotinine in plasma from Day 1 to Day 4, Day 5, and on Day 30 Visit, Day 60 Visit, and Day 90 Visit.
- To describe the PK profiles of the nicotine and cotinine in a confinement setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.
  - Peak (highest concentration along the day) on Day 5.
  - Time to peak (actual time when the peak was observed compared to the time of the first cigarette) on Day 5.
  - Weighted average concentration over 24 hours on Day 5.
- To describe the change in CYP1A2 enzymatic activity in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Molar metabolic ratio of paraxanthine (PX)/caffeine (CAF) in plasma on Day 5 and Day 90 Visit.

- To determine the changes in lung function in smokers switching from mCC to THS 2.2 Menthol as compared to those continuing to smoke mCC.
  - Full lung functions: Diffusion capacity for lung CO (DLCO), rate constant of CO (KCO), forced expiratory volume in 1 second (FEV1), force vital capacity (FVC), vital capacity (VC) expressed as L, total lung capacity (TLC), forced residual volume (FRV), inspiratory capacity (IC), mid expiratory flow (25-75).
- To monitor the safety profiles during the study.
  - AEs/ SAEs, and device events including THS 2.2 Menthol malfunction/misuse.
  - Respiratory symptoms: cough assessment by visual analogue scale (VAS) and Likert scales and one open question.
  - Vital signs.
  - Electrocardiogram (ECG).
  - Clinical chemistry, hematology, and urine analysis safety panel.
  - Physical examination.
  - Concomitant medications.
- To monitor selected risk markers in a confinement and ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Systolic and diastolic blood pressure on Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - High sensitive C-reactive protein (hs-CRP), homocysteine, blood glucose, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC) in serum on Day 30 Visit, Day 60 Visit, and Day 90 Visit
  - Fibrinogen in plasma on Day 30 Visit, Day 60 Visit, and Day 90 Visit
  - Hemoglobin A1c in blood on Day 90 Visit.
  - Apolipoprotein A1 (Apo A1) and Apolipoprotein B (Apo B) in serum on Day 90 Visit.
  - Soluble inter-cellular adhesion molecule-1 (sICAM-1) in serum on Day 6, on Day 30 Visit, Day 60 Visit and Day 90 Visit.
  - White blood cell (WBC) and platelet count in blood on Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit.
  - 8-epi-prostaglandin F2α (8-epi-PGF2α) and 11-dehydro-thromboxane B2 (11-DTX-B2) in 24-hour urine on Day 5 and on Day 30 Visit, Day 60 Visit, and Day 90 Visit (expressed as concentration adjusted for creatinine).

- Body weight and waist circumference on Day 90 Visit.

3.4.3 Exploratory Endpoints

- To describe the following parameters in a confinement and/or ambulatory setting in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA:
  - Excretion of mutagenic material in urine: Ames Mutagenicity test (YG1024+S9) on Day 5 and Day 90 Visit in 24 hour urine.
  - Subjective effect of smoking: Questionnaire of Smoking Urges (QSU-brief questionnaire); questionnaire Minnesota Nicotine Withdrawal Scale (MNWS)-Revised on Day 5 and Day 90 Visit, and nicotine dependence as assessed by the Fagerström Test for Nicotine Dependence (FTND) questionnaire score on Day 90 Visit.
  - CYP2A6 activity: in plasma on Day 6, and on Day 90 Visit using the molar metabolic ratio of trans-3'-hydroxycotinine/cotinine.
- To describe the following parameters over the course of the study in smokers switching from mCC to the THS 2.2 Menthol as compared to smokers continuing to smoke mCC:
  - Product evaluation: MCEQ
  - Smoking puffing behavior: HST parameters and HST questionnaire.
- To describe the following parameter over the course of the study in smokers switching from mCC to THS 2.2 Menthol:
  - Potential combustion occurrences in tobacco plugs: visual inspection of the tobacco plugs.
- To describe the changes in levels of oxysterols on Day 6 and Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol , and in smokers switching from mCC to SA:
  - Plasma concentration of 6α-hydroxy-5α-cholestanol, 7α-hydroxycholesterol, 5α,6α-epoxycholestanol, 7-ketocholesterol, 7β-hydroxycholesterol, 5β,6β-epoxycholestanol, 24(R)-hydroxycholesterol, 25-hydroxycholesterol, 22(R)-hydroxycholesterol, 4ß-hydroxycholesterol, and 27-hydroxycholesterol in addition to total cholesterol.
- To describe the product use over the course of the study according to the product preference of the subject.
  - Number of mCC or Menthol Tobacco Sticks smoked daily as reported on the log during the confinement period and self-reported number of any nicotine/tobacco product use on a daily basis as reported on the product use electronic diary.
- To describe the smokers' mental state for the intention to quit at Screening on Day -2, Day 30 Visit, Day 60 Visit, and Day 90 Visit:

- By the means of the Prochaska 'Stage of Change' questionnaire.

- To describe the changes and reductions in the levels of urinary BoExp and risk markers measured in 4-hour urine fraction on Day 90 Visit in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC and to SA.
  - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine).
  - 8-epi-PGF2α and 11-DTX-B2 (expressed as quantity and concentration adjusted for creatinine).
- To evaluate in smokers switching from mCC to THS 2.2 Menthol, smokers continuing smoking mCC the relationship between [1]:
  - Primary and secondary BoExp and nicotine equivalents (NEQ) on Day 5 in 24-hour urine and on Day 90 Visit.
- To determine the concordance between the results for BoExp and risk markers in 24-hour urine and in 4-hour urine fraction [2]:
  - MHBMA, 3-HPMA, S-PMA, total NNAL, total 1-OHP, total NNN, 4-ABP, 1-NA, 2-NA, o-tol, CEMA, HEMA, 3-hydroxybenzo(a)pyrene, HMPMA, S-BMA, NEQ, (expressed as quantity excreted in 24-hour urine collection and concentration adjusted for creatinine in 4-hour urine fraction) at baseline and at Day 90 Visit.
  - 8-epi-PGF2α and 11-DTX-B2 (expressed as quantity excreted in 24-hour urine collection and concentration adjusted for creatinine in 4-hour urine fraction) at baseline and at Day 90 Visit.
- To monitor the BoExp in subjects quitting smoking according to the time since they quitted [2]:
  - Carbon monoxide (expressed as ppm) in exhaled breath.
  - COHb in blood (expressed as % saturation of hemoglobin).
  - Urinary BoExp (expressed as quantity excreted and concentration adjusted for creatinine) in 24-hour urine.
  - NEQ (expressed as quantity excreted and concentration adjusted for creatinine).
  - Selected risk markers (hs-CRP, homocysteine, blood glucose, LDL, HDL, TG, TC, fibrinogen, hemoglobin A1c, sICAM-1, white blood cell, platelet count, Apo A1, Apo B, 8-epi-PGF2α, 11-DTX-B2) in respective body matrix when available.

[1] The reporting of the objective will be the subject of a separate report.

[2] The reporting of the objective will be the subject of an appendix to the main Clinical Study Report.

4 INVESTIGATIONAL PLAN

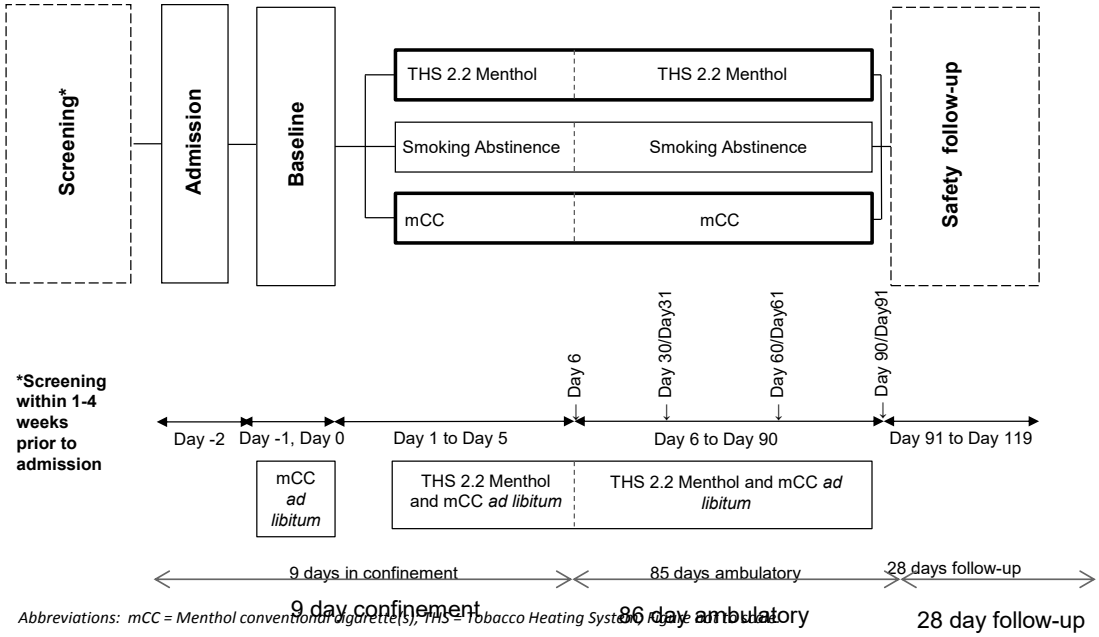
4.1 Overall Study Design and Plan

A randomized, controlled, open-label, 3-arm, parallel group study design with a stratified randomization by sex and average daily cigarette consumption over the last 4 weeks as reported during the Screening Visit (smokers smoking 10 to 19 mCC and smokers smoking >19 mCC per day) (Figure 1).

This is an *ad libitum* smoking study. In general, smoking mCC or use of THS 2,2 Menthol during the confinement period will be allowed from 06:30 AM onwards until around 11:00 PM. During the ambulatory period, there will be no smoking/product use restriction except during the three visits on site (Day 30 Visit, Day 60 Visit, and Day 90 Visit), when product use will be allowed from the time of check-in prior to 08:30 AM to around 11:00 PM on Day 30, Day 60, and Day 90. On Day 31, Day 61, product use will be allowed from 06:30 AM onwards. On Day 91, product use will be allowed after the sample for CYP2A6 activity and the full lung function have been performed until time of discharge of Day 91.

During the confinement period, compliance to product/regimen allocation (exclusive use of THS 2.2 Menthol and mCC in THS 2.2 Menthol and mCC arms respectively, and full abstinence from smoking in the SA arm) will be ensured by strict distribution of each Menthol Tobacco Stick/mCC when requested by the subject. During the ambulatory period, the subjects randomized to the THS 2.2 Menthol arm will be instructed to exclusively use THS 2.2 Menthol and subjects randomized to the SA arm will be instructed to abstain from smoking.

Figure 1. Study Design



Abbreviations: mCC = Menthol conventional cigarette(s), THS = Tobacco Heating System, d = day, amb = ambulatory

mCC: menthol conventional cigarettes; THS: Tobacco Heating System; figure not scale

- <u>The Screening period covers 4 weeks (Day -30 to Day -3) prior to Admission to the clinic (Day -2):</u>

  A demonstration of the THS 2.2 Menthol will be done by the site staff during the Screening Visit. Subjects will be in a confined setting for 9 days from Day -2 onwards.

- <u>The run-in period (from Admission on Day -2 until 06:29 AM of Day-1):</u>

  Smoking will be allowed *ad libitum* from the time of check-in of the subject until around 11:00 PM. Prior to enrolment on Day -2, as the last procedure of the eligibility assessments, subjects will have a product test of the THS 2.2 Menthol (use of up to three THS Menthol Tobacco Sticks). In female subjects, the THS 2.2 Menthol product test will only be done after pregnancy is excluded by a negative urine pregnancy test. Enrolment takes place after all inclusion and exclusion criteria have been satisfactorily met. Only

subjects willing and able to use the product will be enrolled in the study. Subjects who do not meet eligibility criteria on Day -2 will be considered as screening failure until the time of enrolment.

- The baseline period (from Day -1, 06:30 AM until Day 1, 06:29 AM):

  All subjects will continue smoking their single preferred brand of mCC *ad libitum*. On Day -1 and Day 0, smoking will be allowed from 06:30 AM onwards until around 11:00 PM. However, on Day 0, smoking will be allowed only after sample for CYP2A6, MNWS and cough questionnaires, and full lung functions have been conducted.

  Four-hour urine fraction will be collected on Day-1. On Day 0, 24-hour urine will be collected starting in the morning and ending 24 hours later on Day 1.

  On Day 0, subjects will be randomized to 1 of the 3 study arms in a 2:1:1 ratio using a stratified randomization. The full analysis set (FAS) population will be as follows:

  - THS 2.2 Menthol Arm: ~80 subjects, *ad libitum* use of the product.
  - mCC Arm: ~40 subjects, *ad libitum* use of their preferred mCC brand.
  - SA Arm: ~40 subjects who will abstain from smoking.

  Subjects will be informed of their randomized study arm by the study site staff on Day 1 prior to 06:30 AM.

- The exposure period (from Day 1, 06:30 AM until time of Discharge on Day 90 Visit (Day 91)):

  The exposure period will include both the exposure period in confinement, and the exposure period in ambulatory setting:

- The exposure period in confinement setting (from Day 1, 06:30 AM until time of Discharge on Day 6):

  The exposure period in confinement consists of 5 days of *ad libitum* use of the assigned product from 06:30 AM onwards until around 11:00 PM in THS 2.2 Menthol and mCC arms. Subjects allocated to the SA arm will be asked to abstain from smoking and will not be provided with medication to support SA. Subjects will be provided with psychological support during the period of SA. Use of any tobacco/nicotine-containing product other than the assigned product/regimen will not be allowed.

  Twenty four-hour urine will be collected from Day 1 to Day 5 on site. The end of the 24-hour urine collection from Day 5 will end in the morning on Day 6 prior to Discharge.

  On Day 6, the safety procedures will be conducted before discharge of the subject from the clinic after 9 days in a confined setting. Use of products will be allowed on Day 6 in the THS 2.2 Menthol and mCC arms according to product arm allocation, but only after

the sample for CYP2A6 activity, MNWS and cough questionnaires, and full lung functions have been performed.

- The exposure period in ambulatory setting (from time of Discharge on Day 6 until the time of Discharge on Day 91):

  At the end of the confinement period prior to Discharge on Day 6, subjects will be instructed to continue their assigned product/regimen in an ambulatory setting for 86 days. All subjects in the SA arm will receive smoking cessation counseling and will be able to use nicotine replacement therapy (NRT) if considered necessary by the Investigator or requested by the subject (no other medication supportive for smoking cessation will be allowed).

  Subjects will be required to make three visits (Day 30 Visit, Day 60 Visit, and Day 90 Visit) to the investigational site. Each visit will cover 2 consecutive days on site. For Day 30 Visit and Day 60 Visit, the subject will check-in in the morning prior to 08:30 AM on Day 30 and Day 60, and will check-out on Day 31 and Day 61 respectively. For Day 90 Visit, the subject will checked-in in the morning prior to 08:30 AM on Day 90, and will be discharged on Day 91 after having performed all the safety examination procedures.

  Twenty four-hour urine will be collected at each ambulatory visit on Day 30 Visit, Day 60 Visit, and Day 90 Visit at the site. The collection of 24-hour urine will start on Day 30, Day 60, and Day 90 respectively and will end 24-hours later. On Day 90 visit only, the end of 24-hour urine collection will be followed by the collection of a 4-hour urine fraction.

  On Day 30, Day 60, and Day 90, subjects in the THS 2.2 Menthol and mCC arms will be allowed to use their assigned product from the time of check-in in the morning prior to 08:30 AM to around 11:00 PM. On Day 31, Day 61, product use will be allowed from 06:30 AM. On Day 91, product use will be allowed only after the sample for CYP2A6 activity and full lung function have been performed until time of discharge of Day 91. The end of the exposure period will be fixed at the time of discharge of Day 91.

  During the visits, the use of THS 2.2 Menthol will be strictly forbidden for subjects in the mCC or SA arms.

Subject will not be withdrawn from the study for the use of nicotine/tobacco-containing products other than the assigned product/regimen. Subjects will record in a product use electronic diary any use of CC (menthol or non-menthol), NRT, or other nicotine/tobacco-containing products on a daily basis.

During the confinement and ambulatory settings:

- Subjects in the SA arm will be provided with support including psychological support as requested by the subject or considered necessary by the Investigator/site staff.

- Any subject, who wants to quit smoking during the study (which takes into account the outcome from the Prochaska 'Stage of Change' questionnaire), will be encouraged to do so and will be referred to medical services. This will not affect subject's financial compensation and subject will remain in the study. No NRT will be provided during the confinement period.

- The safety follow-up period (from time of Discharge on Day 90 Visit (Day 91) until Day 119):

  After the time of Discharge on the Day 90 Visit (Day 91), subject will enter a 28-day safety follow-up period during which there will be recording of spontaneously reported new AEs/SAEs and the active follow-up of ongoing AEs/SAEs by the study site. In general, all AEs will be followed-up until resolved, stabilized (i.e., no worsening of the event), or a plausible explanation for the event has been found. The end of the study (EOS) is defined as the time of Discharge on Day 90 Visit (Day 91) plus 28-day follow-up.

4.2 Rationale for Study Design and Control Groups

The minimum age of 22 years in the inclusion criteria was selected based on:

- The legal age of smoking in the location of the chosen sites is 19 years.
- To account for the 3 years of smoking history.

This clinical study aims to demonstrate reductions in exposure to selected HPHCs in apparently healthy smokers switching to the THS 2.2 Menthol, a candidate MRTP, as compared to using mCCs (see IB Edition 2). The main reference in this study will be smokers who continue to smoke mCC. Smokers who stop smoking (the SA arm) will be used as a reference point for the maximum possible reduction in exposure to HPHCs.

The confinement period will provide information on maximum possible exposure reductions in a well-controlled environment and will allow full control of daily cigarette consumption. The ambulatory period will provide a perspective of product usage in the real world setting, where smoking of a few CC (menthol and non-menthol) in addition to THS 2.2 Menthol and SA is expected. It will provide information on reduction in selected BoExp and related changes in selected risk markers when THS 2.2 Menthol is used in a real world setting.

The choice of HPHCs to be assessed in this study is derived from the World Health Organization (WHO) (Ashley et al., 2008) and the draft guidance on Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke (FDA, 2011).

In the WHO list, 9 HPHCs (acrolein, CO, 1-3 butadiene, benzene, NNN, NNK, acetaldehyde, benzo[a]pyrene, and formaldehyde) with evidence of carcinogenicity, respiratory and cardiac

toxicity were recommended to be measured as priority in the smoke chemistry for mandated lowering (Ashley et al., 2008).

Exposure to 4 HPHCs (acrolein, CO, 1,3-butadiene, and benzene) among these 9 priority HPHCs will be assessed by measuring their respective BoExp as primary endpoints after 5 days of exclusive use of THS 2.2 Menthol, mCC or SA. The following characteristics apply to these primary BoExp:

- They are several-fold higher in smokers than in smokers abstinent from smoking (Lindner et al., 2011).
- They exhibit, on average, an elimination half-life of ≤24 hours. Therefore, the 5 days of exposure are sufficient to reach the maximum reduced levels with the THS 2.2 Menthol and SA arms (4 to 5 times the half-life will lead to less than 5% of the original exposure levels of assessed biomarkers on Day 5).

Total NNAL was selected as primary endpoints at Day 90 Visit of THS 2.2 Menthol use as:

- This biomarker is tobacco specific (Goniewicz et al., 2009), and exhibits, on average, an elimination half-life of 10 to 15 days. Therefore, the 91 days of exposure are sufficient to reach the steady state with the THS 2.2 Menthol and SA arms (4 to 5 times the half-life will lead to less than 5% of the original exposure levels on Day 90 Visit).

Some risk markers have been selected in order to evaluate biological changes in THS 2.2 Menthol arm as compared to mCC arm using SA arm as a reference point to verify if the trend of changes upon THS 2.2 Menthol use follow the same trajectory as SA. Among the ones selected, some are well-known to be affected by smoking and to be reversible upon SA as follows:

- CYP1A2 activity, the enzyme which mainly metabolizes caffeine, is decreased as soon as 5 days of SA and after 5 days of use of another candidate MRTP (Faber et al., 2004) and data on file from a previous study (YVD-CS01-EU study).
- 11-DTX-B2 (a major stable metabolite of thromboxane A2, which elicits mainly platelet aggregation) was decreased after 1 week of SA (Benowitz et al., 1993) and after 5 days of use of another candidate MRTP (YVD-CS01-EU study).
- Blood pressure, hs-CRP, fibrinogen, homocysteine, fasting blood glucose, LDL, HDL, TG, TC, hemoglobin A1c (HbA1c), waist circumference, sICAM-1, WBC count, Apo A1, Apo B, and 8-epi-PGF2α will be evaluated as risk markers (Eliasson et al., 2001; Vasan et al., 2006) for cardiovascular monitoring purposes. According to the literature, some of these risk markers are known to be sensitive to smoking cessation: the levels of HDL increase when the levels of sICAM-1, WBC count, and 8-epi-PGF2α decrease following 1 to 3 months of smoking cessation (Pilz et al., 2000; Eliasson et al., 2001).

- Body weight as a mean increase of 4.5 kg is observed after 12 months of smoking abstinence with the most weight gain occurring within the first 3 months of quitting (Aubin et al., 2012).
- Oxysterol will be measured in the study to verify that the reduction observed in animal models of disease upon smoking cessation and switching to candidate MRTP (in house data) is also observed in humans.
- CYP2A6 activity, the enzyme involved in nicotine metabolism will be assessed in this study to evaluate if the use of THS 2.2 Menthol impacts the activity of this enzyme.
- Lung function including Diffusion capacity for lung CO (DLCO), Forced expiratory volume in 1 second (FEV1), force vital capacity (FVC), vital capacity (VC), total lung capacity (TLC), forced residual volume (FRV), inspiratory capacity (IC), maximum expiratory flow (25-75) will be assessed in this study as it has been shown that some of these parameters may be improved early on after smoking cessation and are markers correlating with early but still reversible changes occurring in distal airway upon smoking (Verbank et al, 2006; Sansores et al., 2001).

Other parameters such as human smoking topography, product evaluation, and subjective effects related to smoking including smoking urges and withdrawal symptoms will be evaluated.

Twenty four-urine will be collected in this study as it is a well-established method to measure the levels of excretion of BoExp. Four-hour urine fraction will also be collected to better understand its accordance with 24-hour urine in the perspective of using such fraction in future ambulatory studies thereby minimizing operational and subject constraints.

As part of the characterization of the study population it is important to measure variables that have been shown to be related to nicotine dependence and product reinforcing value. Based on prior tobacco research these factors include age, gender, ethnicity, educational and socio-economic status, tobacco use history, expectations of the effects of the products tested, nicotine exposure, health and mental health status and use of psychoactive substances. In order to capture, these data, subjects will be asked questions about their socio-economic status at screening. Such data would allow comparing populations across studies.

All subjects will be asked to provide their own mCC according to their anticipated needs for the whole confinement period in order to minimize any changes in their smoking behavior.

4.3 Appropriateness of Measurements

The laboratory measures to be utilized in this study were selected based on the following criteria: 1) the availability of a validated analytical method; 2) measure is known to be directly or indirectly affected by smoking; 3) measure is readily reversible after smoking cessation/abstinence; 4) timeframe of reversibility of measure in the perspective of the study duration; 5) practicality/acceptability by subjects; 6) robustness of the method (rapid, simple, accurate).

All questionnaires utilized for this study, except the cough, the socio-economic questionnaire, the smoking questionnaire, and the HST questionnaires, are available as validated questionnaires.

4.4 Study Duration

The entire study duration per subject will be 123 to 150 days, including a Screening period of up to 28 days prior to baseline (Day -30 to Day -3), a 9-day confinement setting (Day -2 to time of Discharge of Day 6) followed by a 86-day ambulatory setting (from the time of Discharge on Day 6 to the time of Discharge on Day 91), and a 28-day safety follow-up period (until Day 119). The EOS is defined as the time of Discharge of Day 90 Visit (Day 91) of the last subject plus 28-day follow-up.

5 STUDY POPULATION

5.1 Selection of Study Population

In total, 160 female or male smoking, apparently healthy adult subjects who smoke per day at least 10 mCC for the last 4 weeks will be randomized in this study. All races/ethnicities will be considered eligible for the study.

The maximum number of mCC smoked per day is not limited. Subjects must have a smoking history of at least 3 years of consecutive smoking prior to the Screening Visit. There will be no brand restrictions as long as the cigarettes are mentholated. Subjects can smoke different brands until Admission to the clinic. From Admission to the clinic onwards, however, they must restrict themselves to one preferred mCC brand. The smoking status will be verified with a urinary cotinine test (cotinine ≥200 ng/mL). Each sex and each of the smoking strata (those smoking 10 to 19 mCC and those smoking >19 mCC per day as reported by the subject in the 4 weeks prior to the Screening Visit) should have a quota applied to ensure they represent at least 40% of the population.

5.1.1 Inclusion Criteria

At the Screening Visit/Day of Admission, each subject must meet the following criteria:

Inclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
1. Subject has signed the ICF and is able to understand the information provided in the Subject Information Sheet and ICF.	Administrative	X	
2. Subject is at a minimum 22 years of age(inclusive).	Safety	X	
3. Smoking, apparently healthy subject as judged by the Investigator based on all available assessments from the Screening period/Day of Admission (e.g., safety laboratory, spirometry, vital signs, physical examination, ECG, chest X-ray, and medical history).	Safety	X	X

Inclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
4. Subject smokes at least 10 commercially available mCCs per day (no brand restrictions), for the last 4 weeks, based on self-reporting. Furthermore, the subject has been smoking for at least the last 3 consecutive years. The smoking status will be verified based on a urinary cotinine test (cotinine ≥200 ng/mL).	Effect	X	X
5. The subject does not plan to quit smoking within the next 6 months as assessed by the Prochaska 'Stage of Change' questionnaire.	Safety	X	X
6. The subject is ready to comply with study protocol (e.g. readiness to accept interruptions of smoking for up to 91 days and to use THS 2.2 Menthol *).	Effect	X	X

*readiness to use THS 2.2 Menthol will only be asked at Day of Admission based on the product trial.

5.1.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria must not be enrolled into the study:

Exclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
1. As per Investigator judgment, the subject cannot participate in the study for any reason (e.g., medical, psychiatric and/or social reason).	Safety	X	X
2. A subject who is legally incompetent, physically or mentally incapable of giving consent (e.g., emergency situation, under guardianship, subject in a social or sanitary establishment, prisoners or subjects who are involuntarily incarcerated).	Administrative	X	
3. The subject has clinically relevant diseases which required medications (including but not limited to gastrointestinal, renal, hepatic, neurological, hematological, endocrine, oncological, urological, immunological, pulmonary, and cardiovascular disease or any other medical condition (including safety laboratory as per CTCAE), which in the opinion of the Investigator would jeopardize the safety of the subject.	Safety	X	X
4. Subject who has forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) <0.7 and FEV_1 <80% predicted value at post-bronchodilator spirometry (GOLD, 2013).	Safety	X	
5. Subject with asthma condition (FEV_1/FVC < 0.75 and reversibility in FEV_1 > 12% (or > 200 mL) from pre	Safety	X	

Exclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
to post-bronchodilator values).			
6. Subjects with renal insufficiency as defined by serum creatinine levels of >1.3 mg/dL for females and >1.5 mg/dL for males.	Safety	X	
7. The subject has a body mass index (BMI) <18.5 or ≥35 kg/m^2.	Safety	X	X
8. As per Investigator judgment, the subject has medical conditions which require or will require in the course of the study, a medical intervention (e.g., start of treatment, surgery, hospitalization) which may interfere with the study participation and/or study results.	Effect	X	X
9. Any subject with an history of adverse events linked to caffeine or caffeine containing drugs (e.g., Vivarin), such as but not limited to hypersensitivity or allergy.	Safety	X	X
10. The subject has used nicotine-containing products other than commercially available mCC (either tobacco-based products or NRT), as well as electronic cigarettes and similar devices, within 4 weeks prior to assessment.	Effect	X	X
11. The subject has received medication (prescription or over-the-counter) within 14 days or within five half-lives of the drug (whichever is longer) prior to the Admission Day (Day -2), which has an impact on CYP1A2 or CYP2A6 activity.	Effect		X

Exclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
12. If a subject has received any medication (prescribed or over-the-counter) within 14 days prior to Screening or prior to the Admission Day (Day -2), it will be decided at the discretion of the Investigator if these can potentially interfere with the study objectives or subject's safety.	Effect	X	X
13. Concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) or acetylsalicylic acid.	Effect	X	X
14. The subject has a positive alcohol test and/or the subject has a history of alcohol abuse that could interfere with the subject's participation in the study.	Administrative	X	X
15. The subject has a positive urine drug test.	Administrative	X	X
16. Positive serology test for human immunodeficiency virus (HIV)1/2, hepatitis B or hepatitis C.	Safety	X	
17. Donation or receipt of whole blood or blood products within 3 months prior to Admission.	Safety	X	X
18. The subject is a current or former employee of the tobacco industry or of their first-degree relatives (parent, sibling, child).	Administrative	X	
19. The subject is an employee of the investigational site or any other parties involved in the study or of their first-degree relatives (parent, sibling, and child).	Administrative	X	
20. The subject has participated in a clinical study within 3 months prior to the Screening Visit.	Safety	X	

Exclusion Criteria	Rationale	Screening	Day of Admission (Day -2)
21. For women only: Subject is pregnant (does not have negative pregnancy tests at Screening and at Admission) or is breast feeding.	Safety	X	X
22. For women only : Subject does not agree to use an acceptable method of effective contraception*	Safety	X	X

* e.g., Intrauterine device, intrauterine system, established use of oral/injectable/implantable/transdermal hormonal methods, barrier methods of contraception (condoms, occlusive caps) with spermicidal foam/gel/film/suppository, vasectomized partner(s), or true abstinence (periodic abstinence and withdrawal are not effective methods) from Screening until the end of the safety follow-up period. Hysterectomy, tubal ligation, bilateral oophorectomy or post-menopausal status are reasons for not needing to use birth control. Post-menopausal status is defined as women who have not experienced menses for greater than 12 months. If a woman claims she's post-menopausal, but has had her menses within 12 months, a follicle stimulating hormone test must be performed and must be within acceptable limits.

5.1.3 Removal of Subjects from the Study

Subjects will be informed that they are free to withdraw from the study at any time. Subjects should be questioned for the reason of premature withdrawal, although they are not obliged to disclose it. This needs to be fully documented in the Source Document and electronic Case Report Form (eCRF).

When a subject withdraws or is removed from the study (both confinement and ambulatory periods), the whole safety examination procedure planned at the day of Discharge on Day 6 (Section 9.5) must be performed as soon as possible after the time of withdrawal unless the subject has withdrawn their informed consent to do so.

After the time of withdrawal, the subject will enter into the 28-day period of safety follow-up. Subjects withdrawn or removed from the study cannot re-enter the study.

Subjects must be withdrawn from the study for any of the following reasons:

- Withdrawal of informed consent.
- Any AE or condition (including clinically relevant changes in a laboratory parameter) which at the discretion of the Investigator no longer justifies the subject's participation in this study.

- Positive pregnancy testing (any invasive procedures, including the drawing of blood MUST NOT be performed after diagnosis of pregnancy, see Section 8.5).
- The Sponsor or Investigator terminates the study. If the Sponsor or the Investigator decides to prematurely terminate the study, the subject will be promptly informed and will follow the safety procedures for early termination as described in Section 9.5. The head of the medical institution should report the fact and the reason in writing to the IRB.
- Withdrawal is considered to be in the best interest of the subject or the other subjects.

Subjects may be discontinued from the study for any of the following reasons:

- Lost to follow-up.
- Concomitant treatment with non-authorized medication as defined in the context of this study (in general, any concomitant medication should be discussed with the Contract Research Organization [CRO] Medical Monitor on an ongoing basis).
- During the confinement period, if a subject uses any mCC or nicotine/tobacco-containing product other than the product/regimen he/she is assigned to, he will be withdrawn from the study.
- Noncompliance to the study procedures based on the judgment of the Investigator.

Smoking of CC (menthol and non-menthol) in the THS 2.2 Menthol or SA arms during the ambulatory period will not be considered a reason for withdrawal of the subject from the study. However, the smoking of CCs (menthol and non-menthol) or use of any nicotine/tobacco-containing products including NRT other than the product/regimen the subject is assigned to during the ambulatory period will be documented in the daily product use electronic diary.

Subjects withdrawn prematurely after randomization will not be replaced and will not be allowed to re-enter the study. All subject withdrawals have to be documented properly in the source documentation and the eCRF.

5.1.4 Violation of Selection Criteria

Subjects who are eligible at Screening, but who do not meet the entry criteria at Admission Day (Day -2), will be considered a screening failure until the time of Enrolment and will be replaced by other subjects.

Subjects who violate the entry criteria prior to Enrolment, but who are considered eligible, will be immediately withdrawn from the study when the violation is detected. If subjects are not yet randomized, they can be replaced. If subjects are randomized, they will be withdrawn from the study and will not be replaced.

6 INVESTIGATIONAL PRODUCTS

6.1 Description of Investigational Products

6.1.1 Test Product

THS 2.2 Menthol comprises the following components: Menthol Tobacco Stick, Holder, Charger, a Cleaning Tool, a mains power supply, and a USB cable (see the user guide in Appendix 3):

Charger:	The function of the Charger (Model 4) is to recharge the Holder after use. It contains a battery with sufficient capacity to recharge the Holder approximately 20 times. It is a convenient size to carry around, and can itself be recharged from a main power source.
THS Tobacco Stick Holder (Holder):	The function of the Holder (Model 4.2) is to heat the Tobacco Stick, delivering an aerosol to the user. The electrical heating is powered from an internal battery which delivers power for about 6 minutes (allowing complete use of a single Menthol Tobacco Stick)
THS Menthol Tobacco Stick (Menthol Tobacco Sticks):	The Menthol Tobacco Stick (product code C3 Menthol) contains tobacco which, when heated, generates an aerosol. It is custom-designed to be used with the Holder.

The overall objective of the design is to provide an acceptable experience in which the HPHC level in the aerosol is substantially reduced in comparison with mCC.

The THS 2.2 Menthol will be provided by the Sponsor.

Per cigarette tar, nicotine, and carbon monoxide yields are measured by standardized machined test methods. The most widely used test method is ISO 4387. PMI has developed a modified version of this method, which improves the determination of tar in products with high water content, which is typical for heated tobacco products (PMI, 2012a; PMI, 2012b; PMI, 2013a; PMI, 2013b). Another method is the more intensive smoking method developed by Health Canada (Health Canada, 1999).

Table 2 lists the commonly reported measures (PMI, 2013a):

Table 2. Measured Aerosol Fractions for the Menthol Tobacco Sticks

Constituent (mg/Menthol Tobacco Stick)	ISO [1]	Health Canada Intense regime [2]
Tar/NFDPM	5.0	12.6
Nicotine	0.5	1.20
Carbon monoxide	1.0	0.60

[1] International Organization for Standardization (ISO machine-smoking regimen). The analytical method has been modified to avoid inaccuracies as a result of condensation from high water-content aerosols.

[2] Health Canada Intense machine-smoking regimen (55 mL puff volume, 2-second puff duration, 30-second inter-puff interval) (Health Canada, 1999).

NFDPM: nicotine-free dry particulate matter

6.1.2 Reference Product / Baseline Period Products

During the run-in period (Admission to clinic until 06:29 AM of Day -1) and the baseline period (from 06:30 AM of Day -1 until 06:29 AM of Day 1), all subjects will continue smoking their preferred commercially available single brand of mCC. Subjects will not be allowed to roll their own mCC.

The reference product to the THS 2.2 Menthol during the randomized exposure period will be the subject's own preferred commercially available single brand of mCC.

All eligible subjects will be asked to purchase their own preferred single brand of mCC prior to Admission and provide his/her anticipated amount of mCC for a total of 9 days plus 4 extra packs on Day -2 (Admission Day) to the site staff. The mCCs will not be provided by the Sponsor. In case more mCC are needed during the confinement period, re-supply of mCC can be envisaged.

6.1.3 Packaging and Labeling

At Admission, all study subjects will provide the anticipated amount of mCC in sealed packs to the study staff. The mCC packs provided by the subjects should not be opened and the cellophane wrapper should be intact.

Each pack of mCC provided by the subject will be labeled to identify which subject the cigarettes belong to (labels should be affixed to the cellophane wrapper of the lower part of the pack by site staff). Each pack of mCCs will be labeled to identify necessary information to match the subject with its suppliers.

For the Menthol Tobacco Sticks, the packs and cartons will be labeled with the necessary information.

6.2 Use of Investigational Product(s)

Subjects will never be requested or forced to smoke or to use the THS 2.2. Menthol and will be free to stop using their allocated product (THS 2.2 Menthol or mCC) at any time during the study. The study is designed as an *ad libitum* use study. During the screening period, subjects will be allowed to smoke except during the procedures of the Screening Visit at the discretion of the site.

6.2.1 Run-in Period

Smoking of mCC *ad libitum* will be allowed prior to admission and throughout the day except during the procedures at the discretion of the site. Smoking will be allowed from the time of check-in of the subject until around 11:00 PM. All subjects (except women with a positive pregnancy test at Screening or at Admission) will undergo a THS 2.2 Menthol product test.

Following the confirmation that the subject is ready to use the THS 2.2 Menthol product, subjects will be enrolled.

6.2.2 Baseline Period

During the baseline period, all subjects will be allowed to continue smoking *ad libitum* their single preferred usual brand of mCC. On Day -1, smoking will be allowed from 06:30 AM onwards until around 11:00 PM. However, on Day 0, smoking will be allowed only after sample for CYP2A6 activity, MNWS and cough questionnaires, and full lung functions have been conducted.

6.2.3 Entire Exposure Period

During the exposure confinement period from Day 1 until the time of Discharge on Day 6, subjects will not be allowed to use any nicotine/tobacco-containing products other than their assigned product/regimen. Smoking will be allowed from 06:30 onwards until around 11:00 PM. On Day 6, the use of the products (THS 2.2 Menthol or mCC) will be allowed only after the sample for CYP2A6 activity, cough and MNWS questionnaires, and full lung functions have been done. Smoking will not be allowed in the SA arm.

During the exposure ambulatory period, subjects will be instructed to continue using exclusively their assigned product/regimen. The use of any CCs (menthol or non-menthol) or nicotine/tobacco-containing products other than the product/regimen the subject is assigned to must be documented in the daily product use electronic diary. Subjects in the SA arm will be instructed to abstain from smoking.

In the morning of the Day 91, subjects in THS 2.2 Menthol and mCC arms will not be allowed to use their assigned product until the sample for CYP2A6 activity and full lung functions have been conducted at the clinic. Smoking will not be allowed in the SA arm.

During the study, any subject who wants to quit smoking will be encouraged to do so and referred for further treatment as per the standard of care in the country in which the study is conducted.

6.2.3.1 THS 2.2 Menthol Arm

During the exposure period in confinement, subjects randomized to the THS 2.2 Menthol arm will use exclusively THS 2.2 Menthol from Day 1, 06:30 AM onwards until time of Discharge on Day 6.

At the time of Discharge on Day 6 and on each ambulatory visit, subjects will be instructed to continue using exclusively THS 2.2 Menthol *ad libitum* until time of discharge of Day 90 Visit (Day 91).

6.2.3.2 Menthol Conventional Cigarettes Arm

During the exposure period in confinement, subjects randomized to the mCC arm will continue smoking their mCC from Day 1, 06:30 AM onwards until time of Discharge on Day 6.

On the time of Discharge on Day 6, subjects will be informed that they can continue to smoke their mCC *ad libitum* until the time of Discharge on Day 90 Visit (Day 91).

6.2.3.3 Smoking Abstinence Arm

During the exposure period in confinement, subjects randomized to the SA arm will be instructed to abstain from smoking from Day 1, 06:30 AM onwards until the time of Discharge on Day 6. They will not be provided with medication supportive for smoking abstinence or NRT.

On Day 6 and on each ambulatory visit and at any appropriate occasion, subjects in the SA arm will be instructed to remain abstinent with or without NRT (no other medication supportive for SA will be allowed) until time of discharge of Day 90 Visit (Day 91).

6.2.4 Stopping Rules for Investigational Product

For safety purposes, smoking should be temporarily stopped in the event of any signs suggesting nicotine overexposure, (e.g., gastrointestinal disturbance [nausea, vomiting, diarrhea, stomach or abdominal pain], cold sweats, headache, dizziness, and breathing problems) or any reasons at the discretion of the Investigator.

6.2.5 Safety Follow-up Period

During the safety follow-up period (after the time of Discharge on Day 90 Visit (Day 91) until Day 119), There will be no smoking restrictions. Subjects in the SA arm who wish to continue their SA will be referred for further treatment as per the standard of care in the country in which the study is conducted, if requested by the subject.

6.3 Method for Assigning Subjects to Study Arms

When all the eligibility criteria have been met, randomization will be done through the Interactive Web and Voice Response System (IWRS) on Day 0 at any time during the day. Subjects will be informed of their randomized study arm in the morning of Day 1, prior to 06:30 AM.

Subjects will be randomized to one of the 3 study arms THS 2.2 Menthol:mCC:SA in a 2:1:1 ratio. Stratified randomization will be conducted by sex and by daily average cigarette consumption in the 4 weeks prior to the Screening Visit (those smoking 10 to 19 mCC and those smoking >19 mCC per day) reported by the subject. In each arm, each sex and each of the smoking strata should have a quota applied to ensure they represent at least 40% of the study population.

6.4 Blinding

This is an open-label study; therefore, the subjects and Investigators will be unblinded to subject's arm. However, there will be a limited degree of blinding in the data review and data analysis process. In particular, PMI and CRO personnel will be blinded to the randomized arm as summarized in the following table:

Blinded Study Personnel	End of Blinding Period
PMI and CRO study statisticians	After the SAP finalization or PMI blind database lock(*), whichever comes last.
PMI data manager	After the finalization of PMI blind database review.(*)
PMI safety and clinical scientists	After the finalization of PMI blind database review(*). Can be actively un-blinded before that time point in case of the occurrence of any safety question, when appropriate.

(*) As part of the PMI quality control (QC) activity, data listings will be reviewed by PMI before database lock, with no access to the randomization arm information.

Any PMI and CRO personnel who are not listed in the above table will be unblinded by default.

6.5 Investigational Product Accountability and Compliance

6.5.1 Dispensing Investigational Product

During the confinement period, each mCC will be dispensed from Day -2 onwards to the subjects. Subjects in the THS 2.2 Menthol arm will be provided by the site personnel with Menthol Tobacco Sticks from Day 1 to Day 6 stick by stick. One mCC/Menthol Tobacco Stick will be allowed at a time, as per the study design, and documented in an appropriate log.

On each day of the confinement period, the time of dispense and return for each product has to be documented from Day -2 for mCC and from Day 1 for Menthol Tobacco Sticks onwards. The start of product use on each day will correspond to the time of dispense of the first mCC/Menthol Tobacco Stick. The subject must not take a puff of the Menthol Tobacco Stick during the pre-heating time. The product will not be promoted for commercial distribution or test market.

During the ambulatory period, subjects in the THS 2.2 Menthol arm will be provided THS 2.2 Menthol including anticipated amount of Menthol Tobacco Sticks to cover the period until the next study visit. An additional number of Menthol Tobacco Sticks will be dispensed to the subjects at these visits to cover for any unexpected delay to the visit schedule made by the subject. Extra delivery of THS Menthol Tobacco Sticks in between two visits may be envisaged. Subjects in the mCC arm will buy their mCCs directly from shops and will not be reimbursed.

6.5.2 Storage and Accountability

A person in the site staff designated by the Investigator will be responsible for the storage and accountability of the IPs, in accordance with the Sponsor's requirements.

The THS 2.2 Menthol and mCCs will be stored in a secured storage site with access limited to authorized personnel only. Full accountability of the distributed products will be ensured by the designated site staff.

6.5.2.1 Confinement Period

On each day of the confinement period, study site staff will record on the Accountability Log every occasion from Day -2 to time of Discharge on Day 6 that mCCs are dispensed to a subject by the study site staff and every occasion from Day 1 to time of Discharge on Day 6 that the THS 2.2 Menthol product components (i.e., THS Tobacco Stick Holder, THS Charger, THS accessories) and Menthol Tobacco Sticks are dispensed to a subject.

Subjects will return each butt of mCC immediately after use from Day -2 to Day 6 for accountability. This will be documented in the appropriate log.

Immediately after use, all tobacco plugs of all used Menthol Tobacco Sticks will be separated from the filters and the tobacco plugs will be collected from Day 1 to Day 5, using dedicated vials for accountability and subsequent analysis of potential combustion occurrences (see also Section 7.8.2.2). This will be documented in appropriate log.

6.5.2.2 Ambulatory Period

THS 2.2 Menthol Arm:

Subjects must return any unused packs, empty packs and partially used packs of Menthol Tobacco Sticks and the THS 2.2 Menthol product components they have used to the site for accountability.

All tobacco plugs from Menthol Tobacco Sticks used after check-in at the investigational site until around 11:00 PM on Day 30 Visit, Day 60 Visit, and Day 90 Visit will be collected in dedicated vials for subsequent analysis of potential combustion occurrences.

mCC Arm:

No IP accountability will be done for mCC arm.

SA Arm:

No IP accountability will be done for SA arm.

6.5.3 Investigational Product Retention

The study site will destroy or return to the Sponsor any unused Menthol Tobacco Sticks and will return to the Sponsor the THS 2.2 Menthol product components upon study completion. Retention of THS 2.2 Menthol products will be documented.

Irrespective of the study arm at the time of Discharge from the clinic, the site staff will return to the subjects any remaining mCCs given to them on the day of Admission.

6.5.4 Compliance to Investigational Product

During the confinement period, compliance for all study arms will be ensured by strict dispensation of the products (stick by stick) and collection of used Menthol Tobacco Sticks/mCC butts will be documented in the appropriate log.

During the ambulatory period, subjects in the 3 study arms will capture, Day 6 to the time of Discharge on Day 90 Visit (Day 91), the number of product used (e.g., menthol and non-menthol CC, Menthol Tobacco Sticks, or any other tobacco /nicotine-containing products including NRT) on a daily basis in the product use electronic diary. The product use electronic diary will be supplied by Sponsor and distributed to the subjects by the study site personnel. The product use electronic diary will serve as a compliance tool in the 3 arms. On Day 6, the compliance to the product will be ensured using both the accountability log (from 06:30 AM to time of discharge) and the product use electronic diary. In case of discrepancy between the log and the electronic diary entries, the electronic diary will be considered as the primary source data.

In addition, in the SA arm, compliance will be chemically verified using an exhaled CO breath test during both the confinement and at the ambulatory visits. The cut-off point for the CO breath test value to distinguish mCC use versus SA use will be 10 ppm (Benowitz et al., 2002).

6.6 Restrictions

6.6.1 Smoking Restrictions and Restrictions to the Smoking Abstinence Arm

6.6.1.1 Confinement Period

To avoid smoke cross-contamination among the 3 study arms, subjects in the THS 2.2 Menthol and the mCC arm must smoke in separate rooms and the subjects allocated to the SA arm should not have access to the smoking rooms. Precautions should be taken to remove cues to smoking for subjects who are randomized to the SA arm.

In the THS 2.2 Menthol and SA arms, subjects will not be allowed to smoke any mCC or use any nicotine/tobacco-containing products (including NRT) from Day 1 (06:30 AM) until the time of Discharge on Day 6. In the mCC arm, subjects will not be allowed to use the THS 2.2 Menthol, any nicotine/tobacco containing products and other mCC than brought to the site by the subject. In the SA arm, intensive support including psychological support will be provided upon the request of the subject or of the Investigator/site staff.

Smoking will generally only be allowed during the designated smoking times, from 06:30 AM onwards to around 11:00 PM (for more details on time restrictions see Section 6.2). In general, the performance of scheduled procedures has priority over the wish of a subject to use the product. However, this is different at Day 5 due to the assessment of the nicotine profile. If the subject wants to use the product at Day 5 around the time of the blood draw, he/she should use the product first and the blood will be drawn after product use.

6.6.1.2 Ambulatory Period

Subjects in the THS 2.2 Menthol arm will be instructed to exclusively use THS 2.2 Menthol and subjects in the SA arm will be instructed to remain abstinent from smoking with or without NRT.

In the THS 2.2 Menthol and mCC arms, product use will be allowed during the Day 30 Visit, Day 60 Visit, and Day 90 Visit (for more details on time restrictions see Section 6.2).

Subjects in the SA arm may use NRT (no other medication supportive to smoking abstinence will be allowed) if considered necessary by the Investigator or if requested by the subject. NRT products will be used as per the product label, and may be purchased by subjects at a pharmacy. Subjects will be reimbursed. Intensive support including psychological support will be provided upon the request of the subject or of the Investigator/site staff.

6.6.2 Dietary Restrictions

6.6.2.1 Confinement Period

A standard diet will be designed by a dietician. For each meal, the caloric and fat content should be controlled in order to avoid a "high-fat" diet. The FDA guidance on food-effect studies for bioequivalency testing identifies a high-fat diet as a diet which maintains "approximately 50 percent of total caloric content of the meal and is high in calories (approximately 800 to 1000 calories) (FDA, 2002)."

In order to avoid any effect on assessment of BoExp, grilled or pan-fried meat, smoked pre-cooked meats (e.g., tuna, ham, corned beef, and meats), smoked bacon and sausage will not be permitted (Tiffany et al., 1991). In addition, to avoid any effect on the measurement of CYP1A2 activity, alcohol, broccoli, Brussels sprouts, cauliflower, grapefruit, and xanthine-containing foods and beverages (coffee, tea, chocolate, cocoa, mate, guarana, etc.) will be forbidden (Faber et al., 2004) except when the subject is asked to take caffeine tablet for CYP1A2 measurement. Consumption of quinine-containing drinks (e.g., tonic water) will not be allowed.

Subjects are not allowed to bring their own food or beverages to the investigational site. Meals will be served according to the schedules provided in Section 9. Additional light snack, fruits, and raw vegetables can be distributed to the subjects without restrictions at any time during confinement as long as they fulfill the above requirements. Consumption of water is allowed as desired. The same menu and meal schedule will be administered uniformly for all subjects in all study arms. In addition for the purpose of the Ames test planned on Day 0 and Day 5, the menus served on Day -1 and Day 4 will be identical.

Fasting state has to be observed for at least 10 hours prior to blood draws for:

1. Safety laboratory on screening, Day 0, Day 6.
2. Risk markers in serum/plasma/blood on Day 0 and Day 6.
3. Serum/plasma bio-banking samples for further analysis of BoExp and risk markers on Day 0, and Day 6.
4. Blood bio-banking for transcriptomics on Day 0 and Day 6.
5. Oxysterols in plasma on Day 0 and Day 6.

On Day 0 and Day 6, subjects must not have eaten at least 30 minutes prior to collection of buccal and nasal epithelial samples.

6.6.2.2 Ambulatory Period

The above dietary restrictions are not applicable for the ambulatory period. However, 1 day prior to the Day 90 Visit, and during the visit on site, subjects will be asked by the site staff to refrain from consuming grapefruit or grapefruit-containing products, or quinine-containing drinks (e.g., tonic water). Alcohol, broccoli, Brussels sprouts, cauliflower, chargrilled meat, xanthine-containing foods and beverages (e.g., coffee, tea, chocolate, cocoa, mate, guarana) will not be allowed on site during the Day 90 Visit.

A fasting state has to be observed for at least 10 hours prior to blood draws for:

1. Safety laboratory on Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91).
2. Risk factor assessments in serum/plasma/blood on Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91).
3. Serum/plasma bio-banking samples for further analysis of BoExp and risk markers at the Screening Visit, on Day 90 Visit (Day 91).
4. Blood bio-banking for transcriptomics on Day 90 Visit (Day 91).
5. Oxysterols in plasma on Day 90 Visit (Day 91).

On Day 90 Visit (Day 90), subjects must not have eaten at least 30 minutes prior to collection of nasal epithelial and buccal samples.

6.7 Concomitant Medication

No medication should be taken during the study from the screening to the EOS (time of discharge from Day 90 Visit (Day 91) plus 28 days safety follow-up period) without prior informing the Investigator. However, the Investigator is responsible for the medical care of the subjects during their participation in this study. Any decisions regarding the prescribing of medication will be made in the best interests of the subject.

In the SA arm, subjects may be provided with NRT if considered necessary by the Investigator or requested by the subject. The use of medication supportive for smoking abstinence other than NRT will not be allowed.

Concomitant use of NSAIDs and acetylsalicylic acid (including over-the counter products) is not allowed, as all of them could interfere with risk markers such as 11-DTX-B2. Acetaminophen will be allowed at a daily total dose of up to 3000 mg. Any medication (except medication containing estrogen) with an impact on the CYP1A2 and CYP2A6 metabolism (as prescription and over-the-counter products) as shown in Table 3 must be avoided.

If the use of a concomitant medication cannot be avoided for the subject's safety, it must be fully documented in the Source Document and transcribed into the eCRF (for details, see Section 7.4.7).

The drugs and substances shown in Table 3 are a selection of drugs considered to have an impact on CYP1A2 and/or CYP2A6 activity (Chang et al., 1999, Ingelman-Sundberg et al., 1999, Lacy et al., 2007). Prior to database close, concomitant medication will be assessed according to their potential impact on CYP1A2 and CYP2A6 activity and potential impact on the study results.

Concomitant medication will first be assessed at the Screening Visit. To be eligible for the study, any medication with impact on CYP1A2 and CYP2A6 metabolism must be discontinued at least 2 weeks prior to Admission to the clinic or for at least 5 half-lives (whichever is longer). They must not be used during the entire study until the Day 91 (completion of the study).

Medication containing estrogens (e.g. for contraception and for hormone replacement therapy), even though known to be CYP1A2 inhibitors, will be allowed in this study but must be documented on the eCRF.

Table 3. Examples of Medications with Effects on CYP1A2 and CYP2A6 Activity

Drug name	Substance Class
Rifampicin and fluoroquinolones including ciprofloxacin and ofloxacin,	Antibiotics
Fluvoxamine, fluoxetine, paroxetine, bupropion, duloxetine, amitriptyline, imipramine, sertraline, mirtazapine, citalopram, thioridazine	Antidepressant
Haloperidol, perphenazine, chlorpromazine, propoxyphene fluphenazine, clozapine, olanzapine	Neuroleptic
Phenobarbital, primidone, carbamazepine	Antiepileptic
Cholorquine, quinidine	Antirheumatic
Clotrimazole, terbinafine, fluconazole, ketoconazole, miconazole	Antimycotic
Erythromycin, ciprofloxacin, clarithromycin, norfloxacin	Antibiotic
Cimetidine, chlorpheniramine, diphenhydramine, ranitidine	H_2-receptor antagonist
Amiodarone, verapamil, mibefradil, mexiletin, propafenone, propranolol, lidocaine	Antiarrhythmic
Losartan, amlodipine, nifedipine,	Antihypertensive
Drospirenone, estrogens	Hormonal contraception, hormonal replacement therapy (estrogens)
Fluvastatin	Cholesterol-lowering agent
Theophylline	Antispasmodic pulmonological agent/Bronchodilator agent
Omeprazole, lansoprazole	Proton pump inhibitor
Interferon	Antiviral/Immunomodulating agent
Methoxsalen	Anti-psoriatic
Modafinil, diclofenac, rofecoxib	Analgesic
Insulin	Anti-diabetic

Drug name	Substance Class
Sildenafil	Erectile dysfunction
Quinine	Leg cramps
St. John's Wort	Over-the-counter (herbal remedy) antidepressant
Psoralen	Anti-psoriatic (substance class Furocoumarins)
Pilocapine	Cholinergic agonists (e.g., used for Glaucoma Therapy)

Data sources: Chang et al., 1999, Ingelman-Sundberg et al., 1999, Lacy et al., 2007. The list is not exhaustive.

7 STUDY PROCEDURES

Personnel performing study measurements or recordings must have the appropriate training fully documented. Quality control (QC) measures have to be in place. An overview of all study procedures is shown in the Schedule of Events (Appendix 1). In this section, only the expected/planned time points for the various measurements are described. As not all subjects can undergo a procedure at the same time, adequate time windows are given for each study procedure and each time point (Section 9). Site personnel will adhere to the site's Standard Operating Procedures (SOPs) for all activities. Appropriate medical advice will be provided to the subject in case of any medial findings requiring health care.

7.1 Informed Consent/Subject Information Sheet

Prior any study assessments is performed, the subject will be asked to provide his consent to participate to the study (ICF/subject information sheet for study participation) (Section 1.3).

In addition to the ICF/subject information sheet for study participation, the subject will be asked to provide his separate consent for two kinds of bio-banking (Section 1.3.2).

- ICF/subject information sheet to the additional bio-banking of serum/plasma/urine samples for further measurements of BoExp and risk markers.
- ICF/subject information sheet to the additional bio-banking of blood sample for further transcriptomics (pharmacogenomics) analysis/samples for nasal epithelial collection and samples from buccal collection.

The subject's participation in the study does not depend on their consent for all kind of bio-banking and will be separate to that for study participation. The three consents will be captured in the eCRF.

7.2 Information on the Risk of Smoking and Smoking Cessation Advice and Debriefing

Each subject will be given information on the risks of smoking and smoking cessation advice 6 times during the study: at the Screening Visit, at Admission (Day -2), at Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91). This will take the form of a brief interview according to the recommendations of the U.S. Public Health Service (U.S. Department of Health and Human Services, 2008). Details of the interview will be recorded in the Source Document File. Information on the risk of smoking and smoking cessation advice will be given to the subjects on an individual basis during a face-to-face meeting between the subject and the Investigator, and may additionally be given in a group session.

In addition, a debriefing of subjects will be done at each information on risk of smoking/ smoking cessation advice session to address any intended or unintended beliefs participants have about the candidate MRTP. The goal of the debriefing would be to help ensure that subjects exit the study with an accurate understanding of product risks, including an understanding that the candidate MRTP has not been demonstrated to be less harmful than mCC.

7.3 Support for the Smoking Abstinence Arm

All subjects in the SA arm will be closely monitored from Day 1 to Day 6 and during the ambulatory visits until time of discharge of the Day 90 Visit (Day 91) by the site staff for possible signs and symptoms of nicotine withdrawal. This includes clinical monitoring, e.g., vital signs, physical examination, and body weight when available. It will also involve close monitoring of the subject's behavior, mood, and any AEs. A psychologist may be contacted and will be available upon subject's request, or if considered necessary, upon the request of the Investigator or designee.

7.4 Clinical Assessments

Any clinically relevant medical condition detected during the Screening Visit has to be documented as a concomitant disease. This also applies to clinically relevant findings in laboratory values, vital signs, and ECGs detected during the Screening Visit. Any untoward medical occurrence in a subject detected during the study which was not present at the Screening Visit must be documented as an AE. Worsening of a pre-existing condition from the Screening Visit onwards will also be documented as an AE. If a clinically relevant finding is detected during the Screening period, the Investigator or designee needs to check if inclusion criterion No. 04 is still fulfilled.

7.4.1 Demographic Data

Demographic data (sex, date of birth/age, ethnicity, and race) will be recorded at the Screening Visit.

7.4.2 Identification of the Current Cigarette Brand

Identification of the current mCC brand(s) smoked by the subject will be done at the Screening Visit and at Day -2. At the Screening Visit, smokers will be asked to bring a pack of their current mCC brand(s) to the site. On Day -2, subjects will hand their mCC supply for the entire confinement period to the site staff. Site staff will document the brand name. A photograph of the front and the side of the cigarette pack supplied by the subject will be taken by the study site staff in addition to recording the brand name. These photographs will

be considered as Source Documentation. A copy of the photographs will be provided to the Sponsor electronically (as Digital Video Disc or Compact Disc).

7.4.3 Smoking History Assessment

Subjects will be asked about their smoking history. At Screening and on the Day of Admission (Day -2), this will include questions to evaluate whether the subject has smoked for at least the last 3 consecutive years, to determine the number of mCC smoked during the previous 4 weeks, and to check if the CCs smoked during the previous 4 weeks were mCCs. This self-reported mCC daily consumption will be used for eligibity. In addition, the subject will be asked if he/she has used nicotine-containing products other than commercially available mCC (either tobacco-based products or NRT), electronic cigarettes, or similar devices, within 4 weeks prior to assessment.

At Screening and on the day of Admission (Day -2), subjects will also be asked if they are ready to abstain from smoking for up to 91 days (as required in the study protocol inclusion criteria No. 06). Only subjects who are prepared and able to comply with this requirement will be considered for participation in the study.

7.4.4 Intention to Quit Smoking

Intention to quit smoking will be assessed at Screening, Day -2, Day 30 visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90) by the means of Prochaska ‘Stage of Change’ questionnaire see Section 7.8.3.8.

Only smokers who are in the precontemplation stage (who are not willing to quit within the next 6 months) will be enrolled in the study.

7.4.5 Demonstration and Trial of the THS 2.2 Menthol

All subjects will be shown a demonstration of the THS 2.2 Menthol product at the Screening Visit. On Day -2, as the last procedure of the eligibility assessments on that day, subjects will be offered a product test of the THS 2.2 Menthol (using of up to three THS Menthol Tobacco Sticks). In female subjects, the THS 2.2 Menthol test must only be done after pregnancy is excluded by a negative urine pregnancy test. Enrolment takes place after all requested inclusion and exclusion criteria have been satisfactorily met at Day-2. Only subjects who are willing and able to use the product can participate in the study (as required in the study protocol inclusion criteria No. 06). The product test will be the last assessment prior to enrolment.

7.4.6 Product Preference

In order to perform a complementary analysis on subjects' preference, the following question will be asked to the subject;

"Which product would you prefer to be randomized to:

- THS 2.2 Menthol.
- mCC.
- SA.
- no preference."

This question will be asked on Day -2 after enrolment in all subjects. If the subject would have preferred to be randomized to SA arm, then, the Prochaska 'Stage of Change' questionnaire will be re-administered If the subject says that he is planning to quit within 6 months, the subject will be discontinued from enrolment during the study. .

7.4.7 Medical History, Concomitant Disease, Previous and Ongoing Medications

Relevant medical history will be documented at the Screening Visit. Any concomitant disease will be documented at the Screening Visit. Medical history is defined as any condition that started prior to and ended prior to Screening. A concomitant disease is defined as any condition that started prior to the Screening Visit and is still ongoing at the Screening Visit.

Prior medication taken 4 weeks prior to Screening Visit and any concomitant medication needs to be documented. Any medication which was started prior to the Screening Visit and is still being taken by the subject will be considered a concomitant medication. Medication initiated after Screening is also referred to as concomitant medication. This applies to both prescription and over-the-counter products.

Records of any medication taken must include the drug name (preferably both generic and trade name), route of administration (e.g., oral, intravenous), total daily dose/unit (e.g. expressed in mg, mL, or IU), indication, the start and (if applicable), the stop date (day, month and year). Any therapy changes (including changes of regimen) during the study are to be documented. Any concomitant medication that is still being taken by the subject at the EOS will be recorded on the eCRF.

7.4.8 Physical Examination

A physical examination will be conducted at the Screening Visit, at Admission (Day -2), at Discharge from the clinic on Day 6, on Day 30 Visit (Day 30), Day 60 Visit (Day 60), and on Day 90 Visit (Day 91).

7.4.9 Body Height, Weight and Waist Circumference

Body weight will be recorded on the Screening Visit, on Admission (Day -2), on Discharge on Day 6, on Day 30 Visit (Day 30), Day 60 Visit (Day 60), and on Day 90 Visit (Day 91). Body height will be measured only at the Screening Visit. Waist circumference will be measured on Admission (Day -2) and on Day 90 Visit (Day 91). Appropriate medical advice will be provided to the subject in case of any medical findings requiring health care.

Body mass index (BMI) will be calculated from the body weight and height using the following formula:

$$BMI = \frac{\text{weight in kilograms}}{\text{height in meters}^2} \quad (\text{kg/m}^2)$$

Weight and waist circumference will also be analyzed as risk markers (Section 7.5.2).

7.4.10 Vital signs

Systolic and diastolic blood pressure, pulse rate and respiratory rate will be measured at the Screening Visit, at Admission (Day -2), in the morning of every day of the confinement period (i.e., Days -1 to 6), and at each ambulatory visit (Day 30 Visit on Day 30, Day 60 Visit on Day 60 and Day 90 Visit on Day 91). All measurements will be made after the subject has rested for at least 5 minutes in a supine position and at least 15 minutes after smoking/product use. For every measurement, it will be documented if the subject has smoked within 15 minutes prior to the measurement.

7.4.11 Other Clinical Assessments

7.4.11.1 Spirometry to Assess Full Lung Functions

The spirometry test will be performed in accordance with the 2005 guideline of the American Thoracic Society/European Respiratory Society Joint Task Force on the standardization of spirometry (ATS/ERS, 2005a). Spirometry predicted values will be standardized to the Third National Health and Nutrition Examination Survey (NHANES III) predicted set. All personnel performing full lung function testing should have the appropriate training and QC

measures should be put into place and be properly documented and filed at the pulmonary function laboratory (including the records of the calibration, if applicable). A certified respiratory therapist should perform the assessment and the results are being assessed by a pulmonologist. The subject will be at rest for at least 15 minutes prior to lung function testing. All lung function manoeuvres will be recorded with the subject in a sitting position throughout the study.

The full lung function tests will be performed prior to product use (mCC or THS 2.2 Menthol) on Day 0 (baseline values), Day 6, and Day 90 Visit (Day 91). Full lung function tests will include the recording of FEV_1, FVC, the ratio FEV_1 to FVC, and MEF 25-75 (using spirometry with bronchodilator), lung volumes (VC, TLC, and IC) using the helium dilution technique and the DLCO and KCO (using the single breath technique for CO). The assessments must be performed in the following sequence:

- Gas transfer (DLCO, KCO).
- Lung volume (VC, TLC, and IC).
- Spirometry with bronchodilator.

For full lung functions, the following must be performed:

1. The ambient temperature, barometric pressure and humidity must be measured and entered into the software of the instrument.
2. The instrument will be calibrated prior to every test.
3. The subject will wear nose clips.
4. The subject will be asked to place the mouthpiece in their mouth and breathe normally. They are then instructed to breathe in to total lung capacity (TLC) and exhale as hard and as fast as they possibly can until they reach forced residual volume (FRV). At this point they will be instructed to inhale as hard and as fast as possible until they are back to TLC.

Spirometry with and without bronchodilator

The following recommendations should be followed:

1. The instrument should be calibrated prior to the procedure.
2. The subject will be instructed to take a full inspiration away from the cardboard mouthpiece, seal his/her lips around the mouthpiece, making sure that his/her tongue does not occlude the mouthpiece, and perform an expiratory manoeuvre.
3. The subject will be instructed to do the following: breathe out as rapidly and forcefully into the mouthpiece as possible and continue until he/she feels his/her lungs are completely empty.

Spirometry without bronchodilator

Spirometry will be conducted without bronchodilator at screening only. Values for FEV_1, FVC, the ratio FEV_1 to FVC, and MEF 25/75 will be recorded.

The spirometry without bronchodilator must be done prior to the spirometry with bronchodilator and must be done at least 1 hour after smoking.

Spirometry with bronchodilator

At the Screening Visit, on Day 0, on Day 6, and on Day 90 Visit (Day 91), spirometry with bronchodilator will be performed and post-bronchodilator values for FEV_1, FVC, the ratio FEV_1 to FVC, and MEF 25/75 will be recorded.

The spirometry will be performed in all subjects 15-30 minutes post administration of around 400 μg salbutamol (usually equivalent to 2 -4 puffs assuming 90 μg/puff).

The results from $FEV_{1,}$ and the ratio FEV_1 to FVC at screening will be used for eligibity criteria.

Lung volume measurements (VC, TLC, and IC)

Lung volume measurements (VC, TLC, and IC) This procedure will be conducted as part of the full lung function tests on Day 0, Day 6, and Day 90 Visit (Day 91) and the following values will be recorded: VC, FRC, IC and TLC values.

The helium dilution technique will be used in accordance with the recommendations of the American Thoracic Society/European Respiratory Society Joint Task Force on the standardization of the measurement of lung volumes (ATS/ERS, 2005c). This technique is a closed-circuit system where a spirometer is filled with a mixture of helium and oxygen. The closed-circuit rolling seal spirometer will be filled to a starting volume of six liters with a mixture of containing helium, oxygen and balance room air. Oxygen will be set to 30% so that all test subjects will be comfortable; exact contents will be analysed The subject will be asked to seal their lips around the mouthpiece and breathe normally on the closed-circuit while the helium mixes and equilibrates. During this time carbon dioxide will be removed by a chemical absorber and oxygen will be automatically replaced. Once equlibration has occurred, the subject will be asked to perform one or more a vital capacity efforts to end the test.

Gas Transfer (DLCO and KCO)

This procedure will be conducted as part of the full lung function tests on Day 0, Day 6, and Day 90 Visit (Day 91) using the single breath technique according to the recommendations of the American Thoracic Society/European Respiratory Society Joint Task Force on the standardization of the single-breath determination of carbon monoxide uptake in the lung

ATS/ERS, 2005b. DLCO and KCO (DLCO to alveolar volume (VA) ratio) values will be recorded.

As recommended by the guideline of single breath technique, gas transfer values will be adjusted to COHb levels. To that purpose, blood sampling for COHb measurement will be taken prior to gas transfer assessment on Day 0, Day 6, and Day 90 Visit (Day 91).

The subject will be asked to seal their lips around the mouthpiece, with a nose clip on the nose. They will then be asked to breathe room air for a few tidal breaths.

The subject will then be instructed to:

1. To inhale as far as possible. This is estimated as at least 90 % of the subject's VC.
2. To hold their breath for 10 seconds without straining. The subject should relax against the shutter and be encouraged not to breath out or breath in against it, as this will alter the intrathoracic pressure and the pulmonary haemodynamics, resulting in either an increase (breathing in) or decrease (breathing out) in DLCO.
3. To blow as far as possible. On completion of the manoeuvre, the subject will be rested in a sitting position, before repeating the test a minimum of two times. The time between manoeuvres will be at least 4 minutes.

7.4.11.2 Electrocardiogram

An electrocardiogram (ECG) will be recorded at Screening, on Day 6, at Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 91). ECG testing will be performed as per the site's local practice. A standard 12-lead ECG will be recorded after the subject has rested for at least 5 minutes in a supine position.

The following parameters will be documented: heart rate, PR interval, QRS interval, QT interval, and QTc interval corrected by the ECG machine according to Bazett's formula and Federici's formula. Every ECG has to be assessed as normal, abnormal – clinically not relevant, or abnormal – clinically relevant. A diagnosis has to be provided on the eCRF for all ECGs assessed as abnormal – clinically relevant. All ECG print-outs will be interpreted by a qualified physician. Any print-outs of ECGs on thermo-sensitive paper must be photocopied and stapled together for inclusion in the Source Documents.

7.4.11.3 Chest X-ray

A chest X-ray (anterior-posterior and left lateral views) will be assessed during the Screening period to exclude subjects with relevant pulmonary diseases. Subjects will be referred to a radiology facility for this procedure. No new examination is required if the subject can present a chest X-ray with anterior-posterior and left lateral views at the Screening Visit which is not older than 6 months.

7.5 Biomarker Assessment

All bioanalytical assays and laboratory assessments except the assay to measure oxysterols will be carried out using validated methods (Sections 7.6 and 7.7). The bioanalytical methods used will be documented in the Bioanalytical Plans/Reports. A list of laboratories is provided in Appendix 2.

Precautions should be taken during blood sampling and processing to prevent the contamination of samples with environmental smoke.

7.5.1 Biomarker of Exposure

7.5.1.1 Exhaled CO and COHb

Carboxyhemoglobin measured in blood and exhaled CO will be investigated as a measure of exposure to CO in all three study arms. A CO breath test should be conducted in timely conjunction with the blood sampling for COHb, where applicable. In the SA arm, the CO breath test will serve as a verification of compliance during the confinement period and ambulatory visits (Section 6.5.4).

CO Breath Test:

Carbon monoxide in exhaled breath will be measured using the Smokerlyzer® device, such as the Micro 4 Smokerlyzer® device or similar in all 3 study arms.

During the confinement period on Days -1 to Day 5, the CO breath test will be conducted 4 times per day. The first assessment should be conducted within 15 minutes prior to the first product use. The other 3 assessments should be conducted as described in Section 9.

For subjects in the SA arm from Day 1 onwards, the first CO breath test will be done between 08:00 AM and 09:30 AM. The other 3 assessments should be conducted, as described in Section 9.

On Day -2 and Day 6, and during the ambulatory period on Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90), the CO breath tests will be conducted once, irrespective of time of product use.

Carboxyhemoglobin

Carboxyhemoglobin will be assessed on a daily basis from Day-2 until Day 6.

On Day -1, and from Day 1 to Day 4: one blood sample will be collected in the evening between 08:00 PM and 09:30 PM.

On Day 0, two blood samples will be collected: one blood sample will be collected prior to gas transfer assessment and prior to product use (the COHb levels measured will serve for adjustment of gas transfer values only), the second blood sample will be collected in the evening between 08:00 PM and 09:30 PM.

On Day 5: one blood sample will be collected within 15 minutes prior first product use. The 3 other blood samples will be collected as described in Section 9.

On Day 6: one blood sample will be collected prior to the gas transfer assessment and prior to product use (the COHb levels measured will serve for adjustment of gas transfer values only).

For subjects in the SA arm from Day 1 onwards, the first COHb will be done between 08:00 AM and 09:30 AM. The 3 other blood samples will be collected, as described in Section 9.

On Day 30 Visit (Day 30), and Day 60 Visit (Day 60): For all study arms, one blood sample will be collected during the visit, irrespective of the time of product use.

On Day 90 Visit: one blood sample will be collected on Day 90, irrespective of the time of product use. One blood sampling will be collected on Day 91 prior to gas transfer assessment, and product use (the COHb levels measured will serve for adjustment of gas transfer values only).

7.5.1.2 Plasma Nicotine and Cotinine

Nicotine and cotinine concentrations will be measured in plasma to evaluate the exposure to nicotine in all three study arms. No sampling for PK profile will be done for subjects in the SA arm (Day 5 to Day 6) and only one blood sampling will be done on each of Day 5 and Day 6.

- <u>On Day 0 to Day 4 (all study arms):</u>

  One blood sample per day will be taken in the evening between 08:00 PM and 09:30 PM.

- <u>Nicotine/Cotinine Pharmacokinetic (PK) Profile on Day 5 and Day 6 (THS 2.2 Menthol and mCC arms only):</u>

  In total, 9 blood samples will be drawn on Day 5. The first blood sample on Day 5 will be drawn within 15 minutes prior to the first product use. On Day 5, the start time of the first product use (T_0) will serve as reference for the time to peak concentration. An additional 8 blood samples will be drawn in 2 hour intervals after the start of product use. The last blood sample should be drawn no later than 11:00 PM, corresponding to the end of product use. At all time points, if the subject wants to use the product around the time of

the blood draw, he/she should use it first and the blood will be drawn after product use. Depending on the time of the first product use, it may be that fewer than 8 blood samples will be collected from a subject after T_0.

On Day 6, two blood samples will be drawn. The first one will be 20 hours after T_0 and the second blood sample will be 24 hours after T_0 (with T_0 being the start time of first product use at Day 5).

- On Day 5 and Day 6 (SA arm only):

  On Day 5, one blood sample will be drawn in the evening between 08:00 PM and 09:30 PM.

  On Day 6, one blood sample will be drawn between 08:00 AM-09:30 AM.

- On Day 30 Visit (Day 30), Day 60 Visit (Day 60), Day 90 Visit (Day 90) (all study arms):

  One blood sample will be drawn during these visits, irrespective of the time of product use.

7.5.1.3 Other Biomarkers of Exposure

BoExp will be measured as per the Schedule of Events (Appendix 1):

- In 24-hour urine collection samples during both confinement and ambulatory periods from Day 0 onwards to Day 5 and on Day 30 Visit, on Day 60 Visit , and on Day 90 Visit.
- In 4-hour urine fraction during both confinement and ambulatory periods collected on Day -1, and Day 90 Visit (Day 91).

The following BoExp will be measured:

- Selected primary BoExp: MHBMA, 3-HPMA, S-PMA, and Total NNAL.
- Selected secondary BoExp: Total 1-OHP, Total NNN, 4-ABP, 1-NA, 2-NA, o-tol, NEQ, CEMA, 3-hydroxybenzo(a)pyrene, HEMA, S-BMA and HMPMA.

For normalization of BoExp, creatinine will also be measured in the 24-hour urine and 4-hour urine samples.

7.5.2 Other assessments

7.5.2.1 Risk Markers

The following risk markers will be assessed in this study:

- Systolic and diastolic blood pressure, hs-CRP, fibrinogen, homocysteine, blood glucose, LDL, HDL, TG, TC, HbA1c, sICAM-1, WBC count, Apo A1, Apo B, 8-epi-PGF2α, 11-DTX-B2, platelet count, weight, and waist circumference.

The assessment of systolic and diastolic blood pressure, blood glucose, TG, TC, platelet count, weight, and waist circumference will not be repeated because they are part of the safety parameters or clinical evaluation.

Selected risk markers will be evaluated at the following time points in at least 10-hour of fasting state conditions for the assessments which require blood, serum, or plasma:

- Systolic and diastolic blood pressure: to be evaluated as risk markers on Day 0, Day 6, Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 91). The results from vital signs on Day 0, Day 6, Day 30 Visit, Day 60 Visit, and Day 90 Visit will be used.
- hs-CRP, homocysteine, blood glucose, LDL, HDL, TG, TC in serum: to be evaluated as risk markers on Day 0, at Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91). Samplings will be planned to measure hs-CRP, homocysteine, LDL, HDL at the mentioned timepoints. The results on blood glucose, TG, and TC from the safety laboratory panel on Day 0, Day 30 Visit, Day 60 Visit, and Day 90 Visit will be used.
- Fibrinogen in plasma: to be evaluated as risk markers on Day 0, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91). Samplings will be planned to measure fibrinogen at the mentioned timepoints.
- HbA1c in serum: to be evaluated as risk markers at Day 0, and Day 90 Visit (Day 91). Samplings will be planned to measure HbA1c at the mentioned timepoints.
- Apo A1 and Apo B in serum on Day 0, and Day 90 Visit. Samplings will be planned to measure Apo A1 and Apo B at the mentioned timepoints.
- sICAM-1 in serum: to be evaluated as risk markers on Day 0, Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61) , and Day 90 Visit (Day 91). Samplings will be planned to measure sICAM-1 at the mentioned timepoints.
- 8-epi-PGF2α and 11-DTX-B2 in urine: to be evaluated as risk markers in 24-hour urine on Day 0, Day 5, on Day 30 Visit, Day 60 Visit, and Day 90 Visit and in 4-hour urine fraction in Day 90 Visit. Samplings will be planned to measure 8-epi-PGF2α and 11-

DTX-B2 at the mentioned time points. The results will be normalized to creatinine and express as concentration adjusted for creatinine.

- WBC and platelet count in whole blood: to be assessed as risk markers on Day 0, Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91). The results from the safety laboratory panel will be used.
- Waist circumference and weight: evaluated on Day -2 and on Day 90 Visit (Day 91) (as evaluated as part of the clinical examination, see Section 7.4.9).

7.5.2.2 CYP1A2 activity test

CYP1A2 activity will be assessed in plasma by measuring PX and CAF concentrations and calculating the PX/CAF molar metabolic ratio (Faber et al., 2004) on Day 0, Day 5, and Day 90 Visit (Day 90). Samples to measure PX and CAF will be drawn approximately 6 hours (±15 minutes) after the intake of one "vivarin" caffeine tablet (around 200 mg caffeine) with 240 mL ±10 mL water (Faber et al., 2004).

The exact time of intake of the caffeine tablet in the morning and of the time of blood sampling 6 hours [±15 minutes] later must be recorded.

7.5.2.3 CYP2A6 activity test

CYP2A6 activity will be measured in plasma on Day 0, Day 6, and Day 90 Visit (Day 91), using the molar metabolic ratio of *trans*-3'-hydroxycotinine to cotinine (Jacob et al., 2011). Blood sampling for CYP2A6 has to be done prior to product use. CYP2A6 activity drives the metabolism of nicotine to cotinine and subsequent metabolites.

7.5.2.4 Ames Mutagenicity Test

Urine mutagenicity, a biomarker for measuring mutagen load, will be measured on Day 0, Day 5 and Day 90 Visit in 24-hour urine. The urinary determination of each sample will be done in one bacterial strain (*S. typhimurium* strain YG1024), using S9 metabolic activation and 4 doses for each of the urine extracts.

7.5.2.5 Oxysterols

Oxysterols including 6α-hydroxy-5α-cholestane, 7α-hydroxycholesterol, 5α,6α-epoxycholestanol, 7-ketocholesterol, 7β-hydroxycholesterol, 5β,6β-epoxycholestanol, 24(R)-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol in addition to total cholesterol will be measured in plasma on Day 0, Day 6 and Day 90 Visit (Day 91) using a non-validated method. Based on the outcome of the study, it will be determined if the assay

will be further validated. The samples need to be taken in at least 10 hours of fasting condition.

7.6 Laboratory Assessments

A list of laboratories is provided in Appendix 2.

7.6.1 Clinical Chemistry, Hematology, and Urine analysis for the Safety Panel

Hematology, clinical chemistry and urine analysis for the safety panel will be measured at Screening, Day 0, Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and at Day 90 Visit (Day 91). Blood samples will be taken after at least 10 hours of fasting (Section 6.6.2). The urine test will be performed semi-quantitatively as a urine test. Parameters to be measured are listed in Table 4.

Table 4. Clinical Laboratory Parameters for Safety Panel

Hematology	Clinical Chemistry	Urine analysis

• Hematocrit
• Hemoglobin
• Mean corpuscular hemoglobin (MCH)
• Mean corpuscular hemoglobin concentration (MCHC)
• Mean corpuscular volume (MCV)
• Platelet count
• Red blood cell (RBC) count
• White blood cell (WBC) count
• Differential WBC count:
  • Neutrophils
  • Basophils
  • Eosinophils
  • Lymphocytes
  • Monocytes
	• Albumin
	• Total protein
	• Alkaline phosphatase (AP)
	• Alanine aminotransferase (ALT)
	• Aspartate aminotransferase (AST)
	• Blood urea nitrogen (BUN)
	• Creatinine
	• Gamma-glutamyl transferase (GGT)
	• Fasting Glucose
	• Lactate dehydrogenase (LDH)
	• Potassium
	• Sodium
	• Total bilirubin
	• Direct bilirubin
	• Total cholesterol
	• Triglycerides
		• pH
		• Bilirubin
		• Glucose
		• Nitrite
		• Red blood cell traces
		• Protein
		• Specific gravity

7.6.2 Serology

A test for Hepatitis B surface antigen, Hepatitis C virus, and human immunodeficiency virus (anti-HIV1/2) will be done at Screening.

7.6.3 Urine Drug Screen

A urine drug screen will be performed at the study site at the Screening Visit and on the day of Admission (Day -2). The urine will be screened for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates.

7.6.4 Urine Cotinine Screening

A urine cotinine test will be performed at Screening in order to confirm the subject's smoking status.The test must detect cotinine with a cotinine threshold of ≥200 ng/mL.

7.6.5 Alcohol Test

Subjects will have a urine or breath alcohol test at the Screening Visit and at Admission to the clinic (Day -2).

7.6.6 Urine Pregnancy Testing

All female subjects will undergo pregnancy testing at the Screening Visit, at Admission (Day -2), at Day 6, at Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 91). Female subjects with a positive pregnancy test at the Screening Visit or at Day -2 cannot be enrolled and will be considered a screening failure. The product test at Admission must be done only in female subjects with a negative urine pregnancy test. In any case of a positive urine pregnancy test, the Investigator or designee will inform the subject about the risks associated with smoking during pregnancy.

The post-menopause is formally defined as the time after which a woman has experienced 12 consecutive months of amenorrhea (lack of menstruation) without a period. If a woman claims she is post-menopausal, but has had her menses within 12 months, a follicle stimulating hormone test must be performed and must be within acceptable limits.

All pregnancies detected during the study must be reported and handled as described in Section 8.5.

7.7 Sampling Handling and Storage

All blood samples are to be tested at a central laboratory with the exception of COHb blood samples, and the safety laboratory panel at screening only which will be tested at a local laboratory (Appendix 2). The samples for measurement of oxysterols will to be tested at PMI R&D Laboratory, Neuchâtel, Switzerland. The urine test for the safety laboratory, urine pregnancy tests, urine drug screen, and urine cotinine tests will be done by personnel at the study sites.

Detailed procedures for sample collection and handling of samples are described in a separate Sample Handling Manual (SHM). Safety laboratory samples will be destroyed as per the laboratory's standard procedures. All other samples (except bio-banking samples) will be destroyed after the clinical study report (CSR) has been finalized. The facility/-ies at which the samples are stored will be informed in writing by the Sponsor when destruction of the samples will be allowed.

The bioanalytical lab(s) are listed in Appendix 2.

7.7.1 Blood samples

Venous blood samples will be collected by qualified and trained site personnel. Subjects should be in a seated position during blood collection. The maximal total volume of blood drawn for each subject will be around 310 mL, which includes 40 mL for safety and repeated analysis, 30 mL of blood for long term storage of the bio-banking samples for further analysis of BoExp and risk markers (only if additional consents are given) and 15 mL for long-term storage bio-banking samples for further analysis of transcriptomics (only if additional consents are given) (Section 7.7.3).

7.7.2 Urine samples

Spot urine samples will be used for the urine drug screen, urine cotinine screen, urine pregnancy test, and safety urinalysis.

<u>24-hour urine collection during confinement period</u>

Subjects will empty their bladders shortly before 06:30 AM on the study day indicated in the Schedule of Events (Appendix 1) and discard the urine. The collection period will start at 06:30 AM ± 30 minutes and will end on the following day at 06:29 AM ± 30 minutes. Shortly before 06:29 AM, after nearly 24-hours of urine collection, subjects will empty their bladder again and this urine will be used as the final portion of the 24-hour urine sample.

At time of Discharge on Day 6, subjects will empty their bladder shortly before 06:29 AM. This will be the last urine portion for the 24-hour urine for the Day 5 dot mark in the Schedule of Events.

<u>24-hour urine collection during ambulatory period</u>

Twenty four-hour urine will be collected on Day 30 Visit, Day 60 visit, and Day 90 visit. Subject will be asked to come at site in the morning on Day 30, Day 60 and Day 90 and will remain overnight until respectively Day 31, Day 61, and Day 91. Subject will be asked to empty his bladder on Day 30, Day 60, Day 90 shortly before 09:00 AM (this urine will be discarded). Then collection period will start at 09:00 AM ± 30 minutes and will end on the following day. After nearly 24-hours of collection at 08:59 AM ± 30 minutes, subject will empty his bladder again and this urine will be used as the final portion of the 24-hour urine sample.

<u>24-hour urine collection during both confinement and ambulatory periods</u>

During the sampling period, all urine passed must be collected and put into the sampling bottle, with the exception of about 10 mL for the spot urine tests (described above). No urine must be passed into the toilet. The volume of 24-hour urine, the start and the end time of urine collection will be recorded by the study site staff. For assessment of urine BoExp,

creatinine for normalization of urine BoExp, 8-epi-PGF2α and 11-DTX-B2, sample bio-banking and urine mutagenicity, aliquots from the 24-hour urine collection will be taken. In the Schedule of Events for the 24-hour urine collection (Appendix 1), the dot corresponds to the day on which the 24-hour urine collection period starts. For example, for NEQ measured at Day 5 in the 24-hour urine collection that starts on Day 5 and ends later on Day 6.

4-hour urine collection on Day -1 and Day 90 Visit (Day 91)

Four-hour urine fraction will be collected for each subject on Day -1 and on Day 90 Visit (Day 91). On Day -1, each subject will empty his/her bladder shortly before 10:00 AM, and the volume of urine collected will be discarded. The collection will start at 10:00 AM ± 30 minutes (empty bladder) and will end 4 hours later at 02:00 PM ± 30 minutes. On Day 90 Visit (Day 91), the 4-hour fraction will start immediately after the end of 24-hour urine collection (the start of 4-hour fraction will correspond to the end of 24-hour collection).

The volume of the 4-hour urine fraction, the start and end time of urine collection have to be recorded by the study staff.

7.7.3 Bio-banking Long Term Storage

The facility at which the samples are stored will follow their procedures for destruction of banked samples if a subject withdraws their consent for coded sample bio-banking.

7.7.3.1 Bio-banking Long Term Storage of Blood or Urine

If a subject gives consent for sample bio-banking for further analysis of BoExp/risk markers, additional samples of urine from the 24-hour collection and serum/plasma will be collected as follows:

- Samples will be collected from the 24-hour urine collections that started on Day 0 and Day 5 and Day 90 Visit (Day 90) (10 tubes of 10 mL each per time point).
- Serum/plasma will be collected on Day 0, Day 6, Day 90 Visit (Day 91) (30 mL of blood in total with 2 tubes of 5 mL of blood draw per timepoint: from one tube two X 1 mL of serum and plasma will be collected and stored).

If a subject gives consent for sample bio-banking of whole blood for further transcriptomics analysis, blood will be collected as follows:

- Blood will be collected on Day 0, Day 6, and Day 90 Visit (Day 91) (15 mL in total with 5 mL per timepoint. The 5 mL will be split into two tubes of 2.5 mL each).

The blood samples for transcriptomics and the data related to these samples will be anonymized and will be the subject of a separate report. Anonymized data and samples are initially single or double coded where the link between the subjects' identifiers and the unique code(s) is subsequently deleted. This is applicable for the blood bio-banking for transcriptomics only.

The samples intended for sample bio-banking will be kept frozen; separate from the other samples collected, and will be shipped to a central storage facility according to the SHM. After the final CSR is signed, samples of plasma/serum/blood will be stored for a maximum of 5 years and samples of urine will be stored for a maximum of 2 years. The blood bio-banking for transcriptomics will be stored for a maximum of 5 years.

7.7.3.2 Bio-Banking Long-term Storage from Nasal Epithelial Collection/ Buccal Collection.

If a subject gives consent for sample bio-banking to collect and store samples, samples from nasal epithelial and from buccal collection will be collected on Day 0, Day 6, and Day 90 Visit (Day 90) according to respective procedures Appendix 7 and Appendix 8. Site personnel will be trained to follow the procedures.

For nasal epithelial collection the staff needs to investigate if the subject has presented any allergy to lidocaine if the subject wants to have the nostril numbed prior to starting the procedure.

The samples and the all the related data will be anonymized and will be the subject of a separate report. Anonymized data and samples are initially single or double coded where the link between the subjects' identifiers and the unique code(s) is subsequently deleted. The nasal epithelial and buccal samples will be stored for a maximum of 5 years.

7.8 Other Study Procedures

7.8.1 Product Use Electronic Diary

A product use electronic diary will be used for the documentation of used Menthol Tobacco Sticks, smoked CCs (menthol and non-menthol), used NRTs product, or the use of other nicotine/tobacco containing products. All subjects (including those subjects randomized to the SA arm) must complete this diary on a daily basis from the time of Discharge on Day 6 until the time of Discharge on Day 90 Visit (Day 91). Subjects will be trained by site staff in the use of this electronic diary during the confinement period at the time the diary is delivered to the subject.

7.8.2 Human Smoking Topography Assessment

Human smoking topography involves the measurement of each smoker's unique way of smoking mCCs or using Menthol Tobacco Sticks using the HST SODIM® portable device. The HST SODIM® device, model SPA/M (SODIM® Instrumentation, Fleury les Aubrais, France) is a device which is used to measure smoking topography (Appendix 4). It consists of a special sample holder (containing a constriction in the middle) which is placed between the smoker's mouth and the filter of the mCC or Menthol Tobacco Stick being smoked/used. The holder is connected by two narrow tubes to a portable data logger/recording system (Appendix 4 for a description of the device).

At Day 0, the HST SODIM® device has to be used for all mCC smoked for all subjects. On Day 1 and Day 4 of the confinement period, the HST SODIM® device has to be used for every smoking event for all subjects in the mCC and THS 2.2 Menthol arms. On Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90) of the ambulatory period, HST assessment will start between 08:30 and 09:30 AM with 4 hours recording. Smoking topography with the HST SODIM® device will **not** be done in subjects smoking mCC that are incompatible with the HST SODIM® device (e.g., slim mCC). For subjects in the SA arm, no HST assessments will be performed.

For each subject, one HST SODIM® device will be assigned at Day -1, which will be used by that subject on all HST assessment days (in the case of malfunction, the device will be exchanged). HST SODIM® devices will be assigned to all subjects smoking mCCs which are compatible with the HST SODIM® device at each cohort according to the screening number.

The Sponsor will provide training on the use of the HST SODIM® device to the study site staff. The study site staff will, in turn, provide training to the subjects. All HST SODIM® devices will be returned to the Sponsor after completion of the study.

7.8.2.1 Human Smoking Topography Parameters

The HST SODIM® device measures and records the flow and other per-puff parameters listed in Table 5, the per cigarette parameters shown in

Table 6 will be derived (representing average values or totals per cigarette).

Prior to calculation of the per cigarette parameters, the Sponsor HST group will validate the data and discard any invalid data. Only valid data for per-cigarette parameters a will be part of the study database and will be analyzed.

Table 5. Human Smoking Topography – Per-Puff Parameters

Description	Variable	Unit
Puff number	Ni	
Puff volume	Vi	mL
Puff duration	Di	S
Average flow [Vi/Di]	Qmi	mL/s
Peak flow	Qci	mL/s
Inter puff interval	Ii	s
Sum of Ii and Di	DFi	s
Work [INT Pmi*FinalFlow*dt]	Wi	mJ
Average pressure drop	Pmi	mmWG
Peak pressure drop	Pci	mmWG
Average resistance [Pmi/Qmi]	Rmi	mmWG/mL/s
Peak resistance [Pci/Qci]	Rci	mmWG/mL/s

Table 6. Human Smoking Topography – Per-Cigarette Parameters

Description	Variable	Formula	Unit
Total number of puffs	NPC	∑ Ni	
Total puff volume	TVOL	∑ Vi	mL
Average puff volume	AvgVi	∑ Vi / NPC, i=1 ... NPC	mL
Average puff duration	AvgDi	∑ Di / NPC, i=1 ... NPC	s
Total puff duration	TDi	∑ Di	s
Average flow	AvgQmi	∑ Qmi / NPC, i=1 … NPC	mL/s
Peak flow	AvgQci	∑ Qci / NPC, i=1 … NPC	mL/s
Total inter puff interval	TIi	∑ Ii	s
Average inter puff interval	AvgIi	∑ Qci / NPC, i=1 … NPC	s
Total smoking duration	TDFi	∑ DFi	s
Total Work	TWi	∑Wi	mJ
Average Work	AvgWi	∑Wi / NPC, i=1 … NPC	mJ
Average pressure drop	AvgPmi	∑Pmi / NPC, i=1 … NPC	mmWg
Average Peak pressure drop	AvgPci	∑Pci / NPC, i=1 … NPC	mmWg
Smoking Intensity	SMINT	TVOL/TDFi	mL/s

Description	Variable	Formula	Unit
Puffing Time Index	PTI	(100*TDi)/TDFi	%
Puff Frequency	PFeq	NPC/(TDFi/60)	Min-1

7.8.2.2 Visual Inspection of the Tobacco Plugs

All THS tobacco plugs collected during the study will be sent to the Sponsor for subsequent visual inspection to determine whether combustion occurred during product use (Section 9).

7.8.3 Questionnaires

The subject questionnaires and the VAS used in this study will be entered by the subject directly in an electronic patient reported outcomes (ePROs) device or on paper copy. All subject reported outcome data will be provided in English or Spanish and instructions will be provided in the subject's local language. The questionnaires and the VAS will be reviewed for completeness by the study site staff and subjects will be requested to complete any missing information.

Symptoms or worsening of symptoms documented on any of the questionnaires or the VAS do not need to be documented as additional AEs because the questionnaires and the VAS will be analyzed as part of the final report. However, it is at the discretion of the Investigator to decide whether to document such symptoms as additional AEs. The main source for AE collection will be the face-to-face interview between the subject and study site staff, using open, non-directive questions (Section 8.2).

7.8.3.1 *Fagerström Test for Nicotine Dependence (revised version)*

Potential nicotine dependence will be assessed via a questionnaire at Screening and on Day 90 Visit (Day 90) using the Fagerström Test for Nicotine Dependence (FTND) in its revised version (Fagerström et al., 2012).

The questionnaire consists of six questions which will be answered by the subject himself/herself. The scores obtained on the test permit the classification of nicotine dependence into three levels: Mild (0-3 points), moderate (4-6 points), and severe (7-10 points) (Fagerström et al., 2012).

7.8.3.2 *Assessment of Cough*

Subjects will be asked to assess the respiratory symptom 'cough' on a VAS, on three Likert scales, and with an open question on a daily basis during the confinement period (from Day 0 to Day 6), and at every visit on Day 30 Visit (Day 31), Day 60 Visit (Day 61) and Day 90 Visit (Day 91). From Day 0 to Day 6 only, assessment of cough must be done prior to start of

product use/smoking and no later than 10:00 AM. On Day 31 and Day 61, and Day 91, assessment of cough will be conducted irrespective of the time of product use but no later than 10:00 AM.

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Subjects will be asked if they have experienced a regular need to cough, e.g., whether they have coughed several times in the previous 24 hours prior to assessment. If the answer is 'yes', subjects will be asked to complete a VAS, 3 Likert scales, and to answer the open question.

On the VAS, subjects will assess how bothersome their cough was during the previous 24 hours. The VAS ranges from 'not bothering me at all' to 'extremely bothersome.'

Furthermore, subjects will assess the intensity and frequency of cough and the amount of sputum production during the previous 24 hours on Likert scales.

The intensity of cough will be assessed on a 5-point Likert scale ranging from 1 to 5, with 1 = very mild – 2 = mild – 3 = moderate – 4 = severe – 5 = very severe.

The frequency of cough will be assessed on a 5-point Likert scale ranging from 1 to 5, with 1 = rarely – 2 = sometimes – 3 = fairly often – 4 = often – 5 = almost always.

The amount of sputum production will be assessed on a 4-point Likert scale ranging from 0 to 3, with 0 = no sputum – 1 = a moderate amount of sputum – 2 = a larger amount of sputum – 3 = a very large amount of sputum.

Finally, subjects will be asked to share any other important observations with the staff about their coughing.

7.8.3.3 *Modified Cigarette Evaluation Questionnaire*

Product evaluation will be assessed using the Modified Cigarette Evaluation Questionnaire (MCEQ) (Cappelleri et al., 2007). The MCEQ assesses the degree to which subjects experience the reinforcing effects of smoking, by measuring:

- Smoking satisfaction (satisfying, tastes good, enjoys smoking).
- Psychological rewards (calms down, more awake, less irritable, helps concentrate, reduces hunger).
- Aversion (dizziness, nauseous).
- Enjoyment of respiratory tract sensations (single-item assessment).
- Craving reduction (single-item assessment).

The MCEQ will be completed by subjects during the confinement period on a daily basis from Day -1 to Day 5 and on every ambulatory visits on Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90). On Day -1 and Day 0, all subjects will complete the questionnaire. From Day 1 onwards, only subjects who are randomized to the THS 2.2 Menthol and mCC arms will complete this questionnaire. The subjects will complete the questionnaire by themselves.

7.8.3.4 *Questionnaire of Smoking Urges (QSU-brief)*

To assess the urge-to-smoke, all subjects will be asked to fill-in a 10-item brief version of the QSU (Cox et al., 2001). The QSU-brief is a self-reported questionnaire with 10 items to be rated on a 7-point scale, ranging from 1 (strongly disagree) to 7 (strongly agree). Higher scores in this questionnaire indicate a higher urge to smoke.

The QSU-brief will be completed by the subject himself/herself on a daily basis from Day -1 to Day 5, and on every visit during the ambulatory period, i.e., Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90).

7.8.3.5 *Minnesota Nicotine Withdrawal Scale (revised version)*

The MNWS revised version is a valid and reliable scale that has been used previously to examine signs and symptoms of withdrawal from cigarette smoking (Hughes et al., 1986, Hughes et al., 2008). It consists of 2 scales: a 'self-report scale' and an 'observer scale.'

For the purpose of this study, only the self-reporting scale will be used and filled-in by the subject. Furthermore, the subject's weight will not be recorded for the purpose of the MNWS. At the end of the assessment of the questionnaire, the subject's pulse rate will be recorded.

Subjects will be asked to rate the items for the previous 24 hours on a scale ranging from 0 to 4 (where 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe).

The MNWS (revised version) will be completed on a daily basis from Day 0 to Day 6, and at every visit during the ambulatory period, i.e., Day 30 Visit (Day 31), Day 60 Visit (Day 61) and Day 90 Visit (Day 91). From Day 0 to Day 6 only, MNWS questionnaire must be asked prior to start of product use/smoking and no later than 10:00 AM. On Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91), assessment of MNWS will be conducted irrespective of the time of product use but no later than 10:00 AM.

HST Questionnaire

A specific questionnaire, used for exploratory purposes has been developed to evaluate the impact of the utilization of the HST SODIM® device on smoker's smoking/inhalation experience in terms of ritual disruption.

This is a questionnaire with 5 items to be rated on a 5-point scale and open questions. Subjects will be asked by the Investigator to complete the HST questionnaire on:

- Day 0 for all subjects smoking mCC compatible with the HST SODIM® device (i.e., non-slim mCC)
- Day 4, Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90) for all subjects in the THS 2.2 Menthol and mCC arms (except mCC which are not compatible with the HST SODIM® device).

7.8.3.6 *Socio-Economic Status*

As part of the characterization of the study population it is important to measure variables that have been shown to be related to nicotine dependence and product reinforcing value. Based on prior tobacco research these factors include age, gender, ethnicity, tobacco use history, educational as well as socio-economic status.

Socio-economic status (SES) information is recorded in similar manner in the clinical program, in behavioral research and will be eventually assessed in postmarked studies once the product is commercialized. In order to predict and evaluate the effect of alternative, potentially less harmful tobacco product use might have in adult smokers the socio-economic status constitutes an important demographic characteristic. SES data will be reported across the randomized clinical studies and will be collected in observational pre-market and post-market studies. At screening the subjects will be informed in detail about the exams and evaluations planned during the study, and similarly notified about the SES assessment which will be done on Day 4 once they provided informed consent and were enrolled into the study.

On Day 4, subjects will fill a questionnaire, which will allow the Sponsor to determine subject's Socio-Economic Status (SES). Subjects will be asked a series of questions related to their education, occupational status, size and annual income of their household. These data will be used to create a measure for SES that categorizes subjects into low, moderate and high SES (King et al., 2011). The method used to create SES tertiles will be described in the SAP. If the subject does not want to answer the questionnaire, he will not be withdrawn from the study.

7.8.3.7 **Current and Past Smoking Behavior**

Subjects will be assessed for their current and past smoking behavior at baseline on Day -1 and on Day 5 by the means of 2 questionnaires. The questionnaires will be asked with the following sequence:

- A standard questionnaire (Behavioral Risk Factor Surveillance System Questionnaire 2011) (CDC, 2011a) which is validated.

- A Smoking Questionnaire (SQ) to be validated. Immediately after the administration of the SQ, some supplemental questions will be collected on the completion of the SQ questionnaire.

The Behavioral Risk Factor Surveillance and the SQ are self-administrated to be answered by subjects. The supplemental questions will be asked by the site.

The standard questionnaire is the questionnaire used in Behavioral Risk Factor Surveillance System Questionnaire 2011 (CDC, 2011a), Section 7 on tobacco use. The standard questionnaire consists of 5 questions. All 5 questions are close-ended questions and the responses are exhaustive and mutually exclusive.

- Question 1 collects information on the 100 cigarettes criterion.
- Question 2 allows for a broad classification of current cigarette smoking behavior (daily, occasional, non).
- Question 3 captures quitting experience during past 12 months.
- Question 4 records past smoking duration.
- Question 5 provides information regarding other tobacco use e.g. smokeless tobacco.

The SQ focuses on self-reported current and past cigarette smoking behavior. The SQ consists of 8 questions. The first 3 questions are close-ended questions and the responses are exhaustive and mutually exclusive. The last 5 questions refer to the individual smoking history and are answered to the degree applicable.

- Question 1 allows for a broad classification of current cigarette smoking behavior (daily, occasional, ex, non).
- Questions 2 and 3 collect information on the 100 cigarettes criterion and on ever smoking (defined as smoking at least one cigarette per day).
- Question 4 captures age of initiation and Questions 5 and 6 capture the current as well as total quitting duration, respectively.
- The brand of cigarettes predominantly smoked in the last 12 months of smoking is captured in Question 7.
- Question 8 captures the tobacco smoking history. Daily numbers of manufactured cigarettes, as well as hand-rolled cigarettes, cigars, and pipes consumed, separately for seven time periods.

Finally, the supplemental questions consist of 8 questions. This supplemental question 1 is related to the time which the subject spends to complete the SQ. Question 2-7 are close-

ended questions and to be answered yes or no. Question 8 is open-end allowing for addition comments provided by subjects.

The daily mCC consumption reported in these questionnaires will not be used for eligibility. The data from these questionnaires will be described as part of the CSR and further analysis will be the subject of a separate report.

7.8.3.8 *Prochaska 'Stage of Change' Questionnaire: Intention to Quit Smoking*

The Prochaska 'Stage of Change' questionnaire will be used to assess the smokers' mental state for the intention to quit (7.4.4 and Appendix 5 for staging algorithm; DiClemente et al., 1991 and Velicer et al, 1995). There are 5 stages of change describing smokers and former smokers: 1. Precontemplation, 2 Contemplation. 3. Preparation, 4. Action and 5. Maintenance. In the *precontemplation* stage, the individual does not recognize smoking as a problem.

In the *contemplation* stage, the individual is gathering information about smoking, such as contacting a health care provided, or tobacco quit line for information on the effects of smoking or cessation classes. During this stage, the stress and inconvenience of quitting smoking is greater than the immediate and possible long-term health effects from continued smoking. In the *preparation stage*, intention and behavior begin to come together and the subject is preparing to enter the action stage in the next 30 days. It is necessary for the subject to recognize the benefits of not smoking, before a subject can enter the *action* stage and as a result, change their smoking behavior. After six months of not smoking, the individual reaches the *maintenance* stage when different skills may be needed to prevent relapse from those employed in the initial behavior change.

The Prochaska 'Stage of Change' questionnaire will be performed at Screening, Admission (Day -2), Day 30 Visit (Day 30), Day 60 Visit (Day 60) and at Day 90 Visit (Day 90). The Prochaska 'Stage of Change' questionnaire will be asked to the subject prior to product trial.

8 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Events

The FDA MRTP guideline (FDA, 2012a) specifies this definition towards tobacco products, as follows:

An AE is any health-related event associated with the use of a tobacco product in humans, which is adverse or unfavorable, whether or not it is considered tobacco-product related.

8.1.2 Serious Adverse Events

A Serious Adverse Event (SAE) is defined as, but not limited to, any untoward medical occurrence that:

- Results in death.
- Is life-threatening.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

Any pre-planned hospitalizations that are known at the time of signing the ICF will not be recorded as SAE; however they will be recorded as AEs only. Any AE that occurs during this pre-planned hospitalization will be considered according to the above definitions.

8.2 Assessment of Adverse Events

The Investigator or designee is responsible for obtaining, assessing, and documenting all AEs during the study.

8.2.1 Collection of Information

Adverse event information will be collected from the time of signature of the ICF/Subject Information Sheet onwards until EOS either by the Investigator or designee via spontaneous reporting or by the use of consistent, open, non-directive questions from study site staff (e.g., "Have you had any health problems since the previous visit/How are you feeling since you were last asked?"). At the discretion of the Investigator or designee, the collection of AE information may also be triggered from the review of the subject questionnaires and the VAS. However, during the confinement period and during the ambulatory visits, the main source for AE collection will be face-to-face interview(s) with the subject.

Information recorded will include: verbatim description of the AE, start and stop dates and times, seriousness, severity (intensity), action taken (e.g., whether or not the AE led to the subject's withdrawal from the study), and outcome (e.g., resolved, withdrawal due to AE).

For each AE, the intensity will be graded on a 3-point intensity scale (mild, moderate, severe) using the definitions provided in Section 8.2.5.

Any exacerbation/worsening or increased frequency of an AE or pre-existing condition shall be evaluated and recorded.

Correct medical terminology/concepts are preferred when recording AE terms, and abbreviations must be avoided. Wherever possible, a diagnosis is to be used to describe an AE as a diagnosed medical condition rather than individual signs and symptoms (e.g., record 'pneumonia' rather than 'fever', 'cough', 'pulmonary infiltrate' or 'septicemia' rather than 'fever' and 'hypotension' following blood sample).

Any AE that meets the serious criteria must be recorded both on the AE report form of the eCRF and on a separate SAE report form (Section 8.3).

8.2.2 Period of Collection

From the time of signature of the ICF/Subject Information Sheet onwards until EOS, all AEs (includes SAEs) will be collected by the study site staff as described below.

8.2.3 Screening Period

All existing health conditions identified during the Screening period will be recorded as concomitant disease and the subject's eligibility for admission to the study will be reviewed. Any AEs which occur during the Screening period will be captured by the study site staff and assessed by the Investigator or designee in order to establish relationship or relatedness in respect to study procedures. Only the study procedures-related AEs will be reported in the CSR and in accordance with respective regulatory guidelines.

8.2.4 Admission Day until End of the Study

From Admission onwards until Day of Discharge, all AEs will be actively collected by the study site staff.

Any new, clinically relevant, abnormal finding or worsening of a pre-existing condition/concomitant disease detected during the study including the safety follow-up period will be documented as an AE and/or SAE as described in the Safety Management Plan.

During the safety follow-up period new AEs and/or SAEs will be recorded after spontaneous reporting by the subject. Serious AEs will be reported by the Investigator as described in this document and the Safety Management Plan. Any ongoing AEs/SAEs during the safety follow-up period will be actively followed up by the site until they have been resolved, stabilized (i.e., no worsening of condition), or an acceptable explanation has been found.

At the end of the safety follow-up period all ongoing AEs/SAEs will be followed up by the Investigator or designee on behalf of the Sponsor (Section 8.3) until they have resolved, stabilized (i.e., no worsening of condition), or an acceptable explanation has been found.

8.2.5 Intensity of Adverse Event

For each AE, the intensity will be graded by the Investigator on a 3-point intensity scale (mild, moderate, severe) using the following definitions:

Mild: The AE is easily tolerated and does not interfere with daily activity.

Moderate: The AE interferes with daily activity, but the subject is still able to function.

Severe: The AE is incapacitating and requires medical intervention.

According to CIOMS (Council for International Organizations of Medical Sciences) VI Working group, changes in severity (Intensity) and maximum intensity of adverse events must be documented.

8.2.6 Relationship to Investigational Product and Relationship to Study Procedures

It is difficult to establish a firm method to distinguish an adverse reaction (that is AE that is causally related to the IP) from a clinical adverse event that is temporally associated to the use of an IP.

In general, all AEs and/or SAEs will be assessed by the Investigator or designee as either ‘related’ or ‘not related’ to IP as described below. In addition to the assessment of the relationship of the clinical event to the IP, the Investigator or designee shall document a potential relationship of the clinical event to any particular study procedure.

Not related: The temporal relationship of the clinical event to IP administration makes a causal relationship unlikely, or, concomitant medication, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.

Related: The temporal relationship of the clinical event to study IP administration makes a causal relationship possible, and concomitant medication, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

8.2.7 Expectedness

An AE will be regarded as 'unexpected' if its nature or severity is not consistent with information already known about the IP, and is not listed in the current IB. The IB provides further detail on signs or symptoms that might be expected with the use of the IP, including information relating to device malfunction or misuse.

8.3 Reporting and Follow-Up of Serious Adverse Events

Any SAEs reported or observed during the study after signature of the ICF/Subject Information Sheet until the end of the safety follow-up period (i.e., up to 28 days after study Discharge on Day 90 Visit) whether or not attributable to the IP, to any other medication or to any study procedures, or any SAE related to the product and spontaneously reported after the safety follow-up must be reported by the Investigator or other study site staff **within 24 hours after first awareness by any party involved in the study** to [redacted] and to the Sponsor.

An SAE report form must be faxed or e-mailed as an attachment to:

[redacted]	**Fax number:**	[redacted]
	Toll-free fax:	[redacted]
	E-mail:	[redacted]
	Address:	[redacted]
Sponsor Contact: [redacted], MD,	**Phone:**	+41 [redacted]
	Mobile:	+41 [redacted]

Medical Safety Officer	**E-mail:**	[redacted]
	Address:	Philip Morris Products S.A. R&D Innovation Cube Quai Jeanrenaud 5 2000 Neuchâtel Switzerland

The Investigator is responsible for local reporting (e.g., to the IRB) of SAEs that occur during the study, according to local regulations.

Any SAE will be reported to the Center for Tobacco Products Office of Science within 15 business days after the report is received by the Sponsor.

Any additional/follow-up information that becomes available after the initial SAE report form has been completed will be forwarded to [redacted] and the Sponsor within 24 hours after first awareness by any person at the site using a follow-up to the existing SAE report form.

The follow-up SAE report form must include the minimum information required as described in the safety management plan for form completion and only changed/new information needs to be specified. Information provided in the follow-up SAE report form supersedes any information that was initially reported.

All SAEs will be followed up by the Investigator or designee and/or [redacted] until their resolution or until the Investigator considers the event to be stabilized (i.e., no worsening of condition), or an acceptable explanation has been found (e.g., a chronic condition).

The SAE report form to be used in this study is provided as a separate document and included in the study master file. All SAEs will be recorded, in addition to the SAE report form.

8.4 Reporting of Other Events Critical to Safety Evaluations

8.4.1 Abnormal Results of Laboratory Tests

Any clinical safety laboratory test result that is outside of the normal reference range will be reviewed by the Investigator or designee and assessed for clinical relevance. If the abnormal laboratory results was detected after screening, this should be recorded as AE.

The grading scheme shown in (reference to the Common Terminology Criteria for Adverse Events and Common Toxicity Criteria [CTCAE] version 4.03) will be used by the Investigator or designee to assess abnormal laboratory AEs as follows:

- All Grade 1 abnormal laboratory values will be evaluated by the Investigator with respect to baseline value and clinical relevance. If considered to be clinically relevant the Investigator or designee must report it as an AE. All Grade 2 and higher abnormal laboratory values must be reported as or linked to an AE/concomitant disease.
- If a subject has Grade 2 and higher abnormal laboratory values at Screening it is at the discretion of the Investigator or designee to enroll the subject or not. This decision must be documented in the source documentation and captured in the eCRF.
- If there is any worsening in grade from Grade 2 and above during the study the Investigator or designee must report this worsening as an AE.
- Where there is no grading available, the abnormal laboratory value will be evaluated by the Investigator or designee and assessed for clinical relevance. If considered to be clinically relevant, the Investigator will report it as an AE.
- Any other abnormal clinical laboratory result (including those that are not part of the core safety assessments) can, at the discretion of the Investigator or designee, be reviewed and assessed. Even if they do not meet the criteria of the CTCAE grading scheme (please see above), the Investigator or designee may consider them to be of clinical relevance and, if they are, must report them as AEs.
- In general, laboratory values will be recorded as ‘increased <lab parameter>’ or ‘decreased <lab parameter>’ to ensure consistency of recording/coding.

All other information (e.g., relationship to IP, intensity, seriousness, outcome) will be assessed as for other AEs.

8.5 Reporting and Follow-Up of Pregnancies

For pregnancies detected after signature of the ICF/subject information sheet and prior to first THS 2.2 Menthol use, the subject will be considered as a screening failure and removed from the study. No Pregnancy Form will be filled, however the diagnosed pregnancy must be captured in the Screen Failure eCRF.

Any pregnancy diagnosed after first exposure to the IP and potentially associated to exposure to the IP, including pregnancies spontaneously reported to the Investigator or designee after the EOS must be reported by the Investigator or designee and followed-up until the pregnancy outcome is reached. Potentially associated with exposure to the IP is defined as the conception date being calculated as after first exposure and before the last exposure to the IP.

The Investigator or designee will complete a Pregnancy Form (provided as a separate document) for all pregnancies diagnosed (including positive urine pregnancy tests).

The procedure to report a pregnancy and provide any additional/follow-up information to [redacted] and the Sponsor must be followed in the same manner and within the same timelines as described for an SAE (Section 8.3). In addition, each pregnancy has to be reported as a non-serious AE. No invasive study procedures, including drawing of blood must be done in such subjects after the discovery of pregnancy.

[redacted] will follow up pregnancies only if they were detected after first product use (i.e., after THS 2.2 Menthol product test on Admission Day). If pregnancies are to be followed up, they will be followed up until an outcome is reached (e.g. normal delivery, spontaneous abortion, or voluntary termination). Any pregnancy complication, adverse pregnancy outcome or maternal complications will be recorded.

The Investigator is responsible for informing the IRB of any pregnancy that occurs during the study and its outcome, according to local regulations.

8.6 Adverse Events Leading to Withdrawal

Subjects who are withdrawn from the study because of an AE will undergo the safety procedures (see Section 9.5), as described for the day of Discharge, as soon as possible and will enter the period of safety follow-up. The Investigator or designee and/or [redacted] will follow up these AEs until they have resolved, stabilized (i.e., no worsening of condition), or an acceptable explanation has been found.

8.7 Investigational Device Misuse

Any occurrences of THS Tobacco Stick Holder or THS Charger misuse (use not in accordance with its label and instruction) by a subject will be documented by the Investigator or his/her designated staff using a Device Issue Log.

Investigational device misuse may result in use-related hazards.

Use-related hazards are derived from the US Food and Drug Administration Medical Device Use-Safety Guidance (FDA, 2012c):

- Hazards caused specifically by how a device is used
- Unanticipated use scenarios (e.g., modification of charging unit, applying any chemicals, using CCs, mechanical damage of the unit, etc.) that result in hazards must be documented and reported by the Investigator.

8.8 Investigational Device Malfunction

Any occurrences of malfunction of the THS Tobacco Stick Holder or THS Charger will be documented by the Investigator or his/her designated staff using a Device Issue Log.

Furthermore, any malfunctions of the THS Tobacco Stick Holder or THS Charger that lead to an AE/SAE will follow the same processes as described above.

9 STUDY ACTIVITIES

A detailed schedule of assessment can be found in Appendix 1. The time points shown are to be considered at the time of assessment for the first subject. As not all subjects can be treated at the same time, a short time window will be implemented for subsequent subjects. Measurements not conducted at the exact time point, but conducted within the given time window (if applicable) do not constitute a protocol deviation but an accepted variability for the given time point.

In general, if no start time for the procedures is provided, then the procedure can be performed at any time during the day.

9.1 Screening Visit

The Screening Visit will be performed within 4 weeks (Day -30 to Day -3) prior to Admission (Day -2). Subjects will attend the investigational site in at least 10-hour fasting state for clinical laboratory to be assessed. First, the ICF along with study information should be given to the subject. When/if the ICF is signed, the other screening procedures can be performed in the order deemed most practical. While it is recommended to complete as much screening procedures as possible in one day, it is permissible to complete those over more than one day. Smoking is allowed during screening.

Table 7 shows the procedures that will be performed at the Screening Visit:

Table 7. Time Schedule – Screening Visit

Time	Blood sample	Procedures	Additional information
Start of procedure		**Screening**	
		Informed consent for study participation and two additional consents for bio-banking	
		Information on the risk of smoking/smoking cessation advice and debriefing	
		Demographic data collected	
		Identification of current mCC brand	
		Medical history/concomitant disease	
		Prior medication (within 4 weeks prior to the Screening Visit) and concomitant medication	
		Smoking history	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Screening**	
		Intention to quit smoking within the next 6 months (Prochaska 'Stage of Change' questionnaire)	
		Readiness comply to study protocol (e.g., readiness to abstain from smoking for up to 91 days)	
		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
	√	Clinical laboratory parameters (hematology, clinical chemistry)	To be done after at least 10 hours of fasting
		Urine safety analysis	
		Urine pregnancy test (all females only)	
	√	HIV, hepatitis B and C	
		Physical examination, and height, weight, calculation of BMI	
		THS 2.2 Menthol product demonstration	
		Chest X-ray (if not performed within 6 months prior to Screening Visit)	
		Urine drug screen	
		Urine cotinine screening test	
		Alcohol urine or breath test screen	
		Fagerström Test for Nicotine Dependence (FTND)	
		Spirometry first without and then with short-acting bronchodilator (recording of FEV1, FVC, FEV_1/FVC, and MEF 25-75 only)	Has to be done at least 1 hour after smoking At rest for at least 15 minutes prior to lung function testing In sitting position
		ECG	At least 5 minutes in supine position prior to recording

Time	Blood sample	Procedures	Additional information
Start of procedure		**Screening**	
		AE/SAE recording	If the screening visit is performed on two separate days, the AE/SAE questions will be asked again
		Inclusion/exclusion criteria	

Abbreviations: AE = adverse event; BMI = body mass index; mCC = menthol conventional cigarette(s); ECG = electrocardiogram; HIV = human immunodeficiency virus; SAE = serious adverse event; THS = tobacco heating system.

The sequence of assessments/events is given just for illustrative purposes. The sequence will be at the discretion of the site after signature of the ICF.

9.2 Confinement Period (Days -2 to 6)

9.2.1 Admission (Day -2)

The procedures of Day -2 can be performed in order deemed most practical, except the product test which will be the last assessment prior to enrolment after all eligibility criteria have been met and will be done in female only if the pregnancy test is confirmed negative

Table 8 shows the procedures that will be performed at Admission (Day -2):

Table 8. Time Schedule – Day -2

Time	Blood sample	Procedures	Additional information
Start of procedure		**Admission**	
		AE/SAE recording; Concomitant medication, prior medication	All day
Time of admission-06:30 PM		Urine pregnancy test (females only)	
Time of admission-06:30 PM		Information on the risk of smoking/smoking cessation advice and debriefing	
Time of admission-06:30 PM		Smoking history	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Admission**	
Time of admission-06:30 PM		Readiness to comply to study protocol (e.g readiness to abstain from smoking for up to 91 days)	
Time of admission-06:30 PM		Urine drug screen	
Time of admission-06:30 PM		Urine cotinine screening test	
Time of admission-06:30 PM		Alcohol urine or breath test screen	
Time of admission-06:30 PM		CO breath test	Irrespective of the time of product use
Time of admission-06:30 PM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
Time of admission-06:30 PM		Physical examination, and weight, waist circumference, and BMI	Weight and waist circumference to be evaluated also as risk markers
Time of admission-06:30 PM		Identification of current mCC brand	
Time of admission-06:30 PM		Intention to quit smoking in the next 6 months or less (Prochaska 'Stage of Change' questionnaire)	Has to be done prior to product trial
Time of admission-06:30 PM		Product test of THS 2.2 Menthol and subsequently readiness to use THS 2.2. Menthol	Must be done only after the pregnancy test is confirmed negative. The product test is the last assessment prior to enrolment.
Time of admission-06:30 PM		Inclusion/exclusion criteria	
Time of admission-06:30 PM		Enrolment	After all inclusion and exclusion criteria have been satisfactorily met
		Preference product question	Has to be done after enrolment
Before 09:00 PM		Dinner	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Admission**	
Around 11:00 PM		End of smoking period	

Abbreviations: AE = adverse event; BMI = body mass index; mCC = menthol conventional cigarette(s); CO = carbon monoxide; SAE = serious adverse event; THS = tobacco heating system.

9.2.2 Baseline Period (Day-1 06:30 AM to Day 1 06:29 AM)

Table 9 and Table 10 shows the assessments that will be performed at baseline (Day -1 and Day 0, respectively):

Table 9. Time Schedule – Day -1

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day -1**	
		AE/SAE questioning; Concomitant medication	All day
		CO breath test	Within 15 minutes before first mCC.
06:30 AM		Beginning of smoking	
10:00 AM +/-30 min		Start collection of 4-hour urine to Day -1 sampling bottle	Subject must empty his bladder shortly prior to 10:00 AM. This urine will be discarded prior to starting collection. The 4-hour fraction will be collected over a period of 4 hours ± 30 minutes
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
12:00 PM-01:30 PM		CO breath test	
02:00 PM +/-30 min		End of 4-hour urine collection and sampling to urine samples to be taken from the 4-hour urine after the final portion of 4-hour urine fraction has been added to Day -1 sampling bottle.	BoExp (primary, secondary and NEQ), creatinine, Risk markers: 11-DTX-B2, 8 epi-PGF2α.
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM		CO breath test	
Afternoon		Snacks	
Evening until 09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	

08:00 PM-11:00 PM	MCEQ questionnaire QSU-brief questionnaire Assessment of past and current Smoking behavior : • Behavioral Risk Factor Questionnaire • Smoking Questionnaire and supplemental data	See Section 7.8.3 for all questionnaires. Behavorial Risk factor questionnaire has to be asked prior to smoking questionnaire. Supplemental questions will be asked immediately after the smoking questionnaire
Around 11:00 PM	End of smoking	
06:30 AM-11:15 PM	Collection of all smoked mCC butts	For accountability

Abbreviations: AE = adverse event; COHb = carboxyhemoglobin; mCC = menthol conventional cigarette(s); CO = carbon monoxide; MCEQ = Modified Cigarette Evaluation Questionnaire; QSU = Questionnaire of Smoking Urges; SAE = serious adverse event.

Table 10. Time Schedule – Day 0

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day 0**	
		AE/SAE questioning; Concomitant medication	All Day
		Randomization	At any time of the day after eligibility criteria have been met. Subjects are not to be informed of their assigned arm until Day 1
06:30 AM+/-30min		Start collection of 24-hour urine to Day 0 sampling bottle	Subject must empty his bladder shortly prior to 06:30 AM. This urine will be discarded prior to starting collection.
	√	CYP2A6 activity in plasma	Has to be done prior to smoking
		CO breath test	Within 15 minutes before first mCC.
	√	COHb in blood	Has to be done prior to smoking and prior to gas transfer assessment

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day 0**	
		Full lung function (single breath test for CO first, then helium dilution technique, and finally spirometry with bronchodilator,)	Has to be done prior to smokingSubject at rest for at least 15 minutes prior to lung function testing In sitting position
		MNWS questionnaire	Has to be done prior to smoking but no later than 10:00 AM
		Assessment of cough	Has to be done prior to smoking but no later than 10:00 AM
06:30 AM to 11:00 PM		HST	HST SODIM® device has to be done for all product uses, if compatible mCCs are smoked
06:30 AM		Beginning of smoking	
	√	Clinical laboratory parameters*(hematology, clinical chemistry) and risk markers (hs-CRP, fibrinogen, homocysteine, LDL, HDL, HbA1c, sICAM-1, Apo A1, Apo B)	Has to be done after at least 10 hours of fasting. * Blood glucose, TG, TC, WBC, platelet count from safety will be also evaluated as risk markers
	√	Blood sampling for bio-banking for transcriptomics	If consent is obtained Has to be done after at least 10 hours of fasting
	√	Bio-banking for BoExp/risk markers in serum/plasma (if consent is obtained)	If consent is obtained Has to be done after at least 10 hours of fasting
	√	Oxysterols	Has to be done after at least 10 hours of fasting
		Urine safety analysis	
Before 11:00 AM		Breakfast	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day 0**	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement Systolic and diastolic blood pressure to be evaluated also as risk marker
10:00 AM-11:30 AM		A caffeine tablet containing approximately 200 mg of caffeine ± 240 mL ±10 mL of water	The time of the tablet intake must be recorded
12:00 PM:01:30 PM		CO breath test	
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM	√	CYP1A2 activity in plasma	6 hours ± 15 minutes after intake of caffeine tablet
04:00 PM-05:30 PM		CO breath test	
In the afternoon before 09:00 PM		Nasal Epithelial Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Prior to starting the procedure, the subject will be asked to blow his nose
In the afternoon before 09:00 PM		Buccal Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Subject will be asked to rinse his mouth with a minimum of 20 mL of water prior to sample collection
Afternoon		Snacks	
Before 09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-09:30 PM	√	Nicotine, cotinine in plasma	
08:00 PM-11:00 PM		HST questionnaire	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day 0**	
08:00 PM-11:00 PM		MCEQ questionnaire (all subjects) QSU-brief questionnaire (all subjects)	
Around 11:00 PM		End of smoking	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Baseline Day 0**	
06:30 AM-11:15 PM		Collection of all mCC butts	For accountability

Abbreviations: AE = adverse event; Apo: Apolipoprotein; BoExp = biomarkers of exposure; BP = blood pressure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; CYP = cytochrome P450 enzyme; HbA1c = hemoglobin A1c; hs-CRP = high-sensitive C-reactive protein; HDL = high density lipoprotein; HST = human smoking topography; LDL = low density lipoprotein; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; sICAM-1 = soluble inter-cellular adhesion molecule; TC = total cholesterol; TG = triglycerides; WBC = white blood cell count.

9.2.3 Exposure Period in Confinement (Day 1 06:30 AM to time of Discharge on Day 6)

The tables in this Section show the procedures that will be performed during the confinement period (Day 1 to time of Discharge on Day 6). Table 11 shows the procedures that will be performed on Day 1:

Table 11. Time Schedule – Day 1

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 1**	
		AE/SAE questioning; Concomitant medication	All day
		Support for smoking abstinence (SA arm only), if needed	All day
Before 06:30 AM		Subjects are informed of their assigned study arm	
06:29 AM+/-30min		Urine sample to be taken from the 24-hour urine after final portion of urine has been added to Day 0 sampling bottle.	BoExp (primary, secondary and NEQ), creatinine, and Ames mutagenicity in 24-hour urine. Risk markers: 11-DTX-B2, 8 epi-PGF2α. Bio-banking for BoExp/risk markers (if additional consent is obtained) Must be prior to first product use of Day 1

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 1**	
06:30 AM+/-30min		Start collection to Day 1 sampling bottle 24-hour urine	
		Assessment of cough (VAS)	To be done prior to product use, but no later than 10:00 AM
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM
		CO breath test	Within 15 minutes before first product use (for THS 2.2 Menthol and mCC arms) or between 08:00 AM and 09:30 AM (SA arm)
06:30 AM-11:00 PM		HST	In the THS 2.2 Menthol and mCC arms, it should be done for all smoking events with the HST SODIM® device, if compatible mCCs are smoked
06:30 AM		Beginning of product use	
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
12:00 PM-01:30 PM		CO breath test	
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM		CO breath test	
Afternoon		Snacks	
In the evening before 09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-09:30 PM	√	Nicotine, cotinine in plasma	
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire	
Around 11:00 PM		End of product use	
6:30 AM-11:15 PM		Collection of used mCC butts	For accountability
6:30 AM-11:15 PM		Collection of all tobacco plugs from Menthol Tobacco Sticks	For accountability and further analysis

Abbreviations: 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; HST = human smoking topography; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; THS = tobacco heating system.

Table 12 shows the assessments that will be performed on Day 2 of the confinement period:

Table 12. Time Schedule – Day 2

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 2**	
		AE/SAE questioning; Concomitant medication	All day
		Support during smoking abstinence (SA arm only), if needed	All day
6:29 AM+/-30min		Urine samples to be taken from the 24-hour urine after the final portion of urine has been added to Day 1 sampling bottle.	BoExp (primary, secondary, and NEQ) and creatinine
6:30 AM+/-30min		Start collection to Day 2 sampling bottle 24-hour urine	
		Assessment of cough	To be done prior to product use, but no later than 10:00 AM
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM
		CO breath test	Within 15 minutes before first product use (for THS 2.2 Menthol and mCC arms) or between 08:00 AM and 09:30 AM (SA arm)
6:30 AM		Beginning of product use	
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
12:00 PM-01:30 PM		CO breath test	
Before 02:30 PM		Lunch	
04:00 PM-05.30 PM		CO breath test	
Afternoon		Snacks	
06:30 PM-09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-09:30 PM	√	Nicotine, cotinine in plasma	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 2**	
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire	
Around 11:00 PM		End of product use	
6:30 AM-11:15 PM		Collection of used mCC butts	For accountability
6:30 AM-11:15 PM		Collection of all tobacco plugs from Menthol Tobacco Sticks	For accountability and further analysis

Abbreviations: AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; THS = tobacco heating system.

Table 13 shows the assessments that will be performed on Day 3 of the confinement period:

Table 13. Time Schedule – Day 3

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 3**	
		AE/SAE questioning; Concomitant medication	All day
		Support during smoking abstinence (SA arm only), if needed	All day
6:29 AM+/-30min		Urine samples to be taken from the 24-hour urine after the final portion of urine has been added to Day 2 sampling bottle	BoExp (primary, secondary, and NEQ) and creatinine
6:30 AM+/-30min		Start collection to Day 3 sampling bottle 24-hour urine	
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM
		Assessment of cough	To be done prior to product use, but no later than 10:00 AM

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 3**	
		CO breath test	Within 15 minutes before first product use (for THS 2.2 Menthol and mCC arms) or between 08:00 AM and 09:30 AM (SA arm)
6:30 AM		Beginning of product use	
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
12:00 PM-01:30 PM		CO breath test	
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM		CO breath test	
Afternoon		Snacks	
In the evening before 09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-09:30 PM	√	Nicotine, cotinine in plasma	
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire	
Around 11:00 PM		End of product use	
6:30 AM-11:15 PM		Collection of used mCC butts	For accountability
6:30 AM-11:15 PM		Collection of all tobacco plugs from Menthol Tobacco Sticks	For accountability and further analysis

Abbreviations: AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; THS = tobacco heating system.

Table 14 shows the assessments that will be performed on Day 4 of the confinement period:

Table 14. Time Schedule – Day 4

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 4**	
		AE/SAE questioning; Concomitant medication	All day
		Support during smoking abstinence (SA arm only), if needed	All day
		Socio-economic status questionnaire	At any time of the day
6:29 AM+/-30min		Urine sample to be taken from 24-hour urine after final portion of urine has been added to Day 3 sampling bottle.	BoExp (primary, secondary, and NEQ) and creatinine
6:30 AM+/-30min		Start collection to Day 4 sampling bottle 24-hour urine	
		Assessment of cough	To be done prior to product use, but no later than 10:00 AM.
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM.
		CO breath test	Within 15 minutes before first product use (for THS 2.2 Menthol and mCC arms) or at 08:00 AM ±-09:30 AM (SA arm)
06:30 AM-11:00 PM		HST	In the THS 2.2 Menthol and mCC arms, it should be done for all smoking events with the HST SODIM® device, if compatible mCCs are smoked
6:30 AM		Beginning of product use	
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
12:00 PM-01:30 PM		CO breath test	
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM		CO breath test	
Afternoon		Snacks	
In the evening before 09:00 PM		Dinner	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 4**	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-09:30 PM	√	Nicotine, cotinine in plasma	
08:00 PM-09:30 PM		HST questionnaire for all subjects in the THS 2.2 Menthol and mCC arms	If compatible mCC are smoked
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire	
Around 11:00 PM		End of product use	
6:30 AM-11:15 PM		Collection of used mCC butts	For accountability
6:30 AM-11:15 PM		Collection of tobacco plugs from all Menthol Tobacco Sticks butts	For accountability and further analysis

Abbreviations: AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; HST = human smoking topography; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; THS = tobacco heating system

Table 15 shows the assessments that will be performed on Day 5 of the confinement period:

Table 15. Time Schedule – Day 5

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 5**	
		AE/SAE questioning; Concomitant medication	All day
		Support for smoking abstinence (SA arm only), if needed	All day
6:29 AM+/-30min		Urine samples to be taken from 24-hour after final portion of urine has been added to Day 4 sampling bottle.	BoExp (primary, secondary, and NEQ) and creatinine
6:30 AM+/-30min		Start collection to Day 5 sampling bottle 24-hour urine	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 5**	
		Assessment of cough	To be done prior to product use, but no later than 10:00 AM.
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM.
		CO breath test	Within 15 minutes before first product use (for THS 2.2 Menthol and mCC arms) or between 08:00 AM and 09:30 AM (SA arm)
	√	COHb in blood	Within 15 minutes before first product use for THS 2.2 Menthol and mCC arms) or between 08:00 AM and 09:30 AM (SA arm)
	√	Nicotine, cotinine in plasma	For THS 2.2 Menthol and mCC arms: within 15 minutes before first product use (T_0): then additional blood samples at 2 hour intervals from T_0 until 11:00 PM. Each sample has a time window of +5 minutes For SA arm: only one blood sampling between 08:00 PM-09:30 PM
6:30 AM		Beginning of product use	
Before 11:00 AM		Breakfast	
Before 11:30 AM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement
10:00 AM-11:30 AM		A caffeine tablet containing around 200 mg of caffeine ± 240 mL ±10 mL of water.	The time of caffeine tablet intake should must be recorded
12:00 PM-01:30 PM		CO breath test	
12:00 PM-01:30 PM	√	COHb in blood	
Before 02:30 PM		Lunch	
04:00 PM-05:30 PM		CO breath test	
04:00 PM-05:30 PM	√	COHb in blood	
04:00 PM-05:30 PM	√	CYP1A2 activity in plasma	6 hours ±15 minutes after intake of the caffeine tablet
04:30 PM-05:00 PM		Snacks	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 5**	
In the evening before 09:00 PM		Dinner	
08:00 PM-09:30 PM		CO breath test	
08:00 PM-09:30 PM	√	COHb in blood	
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire Assessment of past and curent smoking behavior : - Behavioral Risk Factor Questionnaire - Smoking Questionnaire - Supplemental questions	See Section 7.8.3 for all questionnaires
Around 11:00 PM		End of product use	
6:30 AM-11:15 PM		Collection of used mCC butts	For accountability
6:30 AM-11:15 PM		Collection of tobacco plugs from Menthol Tobacco Sticks	For accountability and further analysis

Abbreviations: AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; CYP = cytochrome P450 enzyme; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence SAE = serious adverse event; THS = tobacco heating system.

Table 16 shows the assessments that will be performed on Day 6, prior to the time of Discharge from the confinement period:

Table 16. Time Schedule – Day 6

Time	Blood sample	Procedure	Additional information
Start of procedure		**Exposure period procedures**	
		AE/SAE recording; Concomitant medication	All day until discharge

		Support for smoking abstinence (SA arm only), if needed	All day until discharge
	√	Nicotine, cotinine in plasma	In THS 2.2 Menthol and mCC arms, 20 and 24 hours blood sampling after T0 of Day 5. Each sample has a time window of ±5 minutes In SA arm, one blood sample will be drawn at between 08:00 AM and 09:30 AM
6:29 AM+/-30min		Urine samples to be taken from the 24-hour urine after final portion of urine has been added to Day 5 sampling bottle	BoExp (primary, secondary, and NEQ) and creatinine, and Ames mutagenicity Risk markers: 11-DTX-B2, 8 epi-PGF2α Bio-banking for BoExp/risk markers if consent is obtained
	√	CYP2A6 activity in plasma	Has to be done prior to product use
		Assessment of cough	To be done prior to product use, but no later than 10:00 AM
		MNWS questionnaire	To be done prior to product use, but no later than 10:00 AM
	√	COHb in blood	Has to be done prior to gas transfer assessment for all study arms and prior to product use in the THS 2.2 Menthol and mCC arms
		Full lung function (single breath test for CO first, then helium dilution technique, and finally spirometry with bronchodilator)	Has to be done prior to product use in the THS 2.2 Menthol and mCC arms Subject at rest for at least 15 minutes prior to lung function testing In sitting position
6:30 AM		Beginning of product use	
	√	Clinical laboratory parameters (hematology, clinical chemistry) *, risk markers (sICAM-1)	Has to be done after at least 10 hours of fasting. *Platelet count, and WBC to be evaluated also as risk markers
	√	Bio-banking for BoExp/ risk markers in serum/plasma (if consent is obtained)	Has to be done after at least 10 hours of fasting
	√	Bio-banking for transcriptomics (if consent is obtained)	Has to be done after at least 10 hours of fasting

	√	Oxysterols	Has to be done after at least 10 hours of fasting
Prior to discharge of Day 6		Breakfast	
Prior to discharge of Day 6		Physical examination, and weight and calculated BMI	
Prior to discharge of Day 6		CO breath test	Irrespective of the time of product use
Prior to discharge of Day 6		Urine safety analysis	
Prior to discharge of Day 6		Urine pregnancy test (all females only)	
Prior to discharge of Day 6		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after product use prior to measurement. Systolic and diastolic blood pressure to be assessed as risk markers
Prior to discharge of Day 6		ECG	At least 5 minutes in supine position prior to recording
		Nasal Epithelial Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Prior to starting the procedure, the subject will be asked to blow his nose
Prior to discharge of Day 6		Buccal Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Subject will be asked to rinse his mouth with a minimum of 20 mL of water prior to sample collection
Prior to discharge of Day 6		Information on risk of smoking/smoking cessation advice and debriefing	
Prior to discharge of Day 6		Distribution of product use electronic diary	To be completed by the subject every day from time of Discharge on Day 6 until next visit. All Menthol Tobacco Sticks/mCC and any tobacco/nicotine containing products have to be recorded.
		Collection of all used Menthol Tobacco Sticks and smoked mCC butts	For accountability

		Time of Discharge from confinement-/beginning of the ambulatory exposure period	

Abbreviations: 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event; BMI = body mass index; BoExp = biomarkers of exposure; BP = blood pressure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; CYP = cytochrome P450 enzyme; ECG = electrocardiogram; MNWS = Minnesota Nicotine /Withdrawal Scale; PK = pharmacokinetics; SA = smoking abstinence; SAE = serious adverse event; sICAM-1 = soluble inter-cellular adhesion molecule; WBC = white blood cell count.

9.3 Ambulatory Period (from time of Discharge on Day 6 to Day 90 Visit)

For Day 30 Visit (Day 30 and Day 31), Day 60 Visit (Day 60 and Day 61), and Day 90 Visit (Day 90 and Day 91), a time window of +/- 5 days will be allowed with respect to Day 6, Day 30 Visit and Day 60 Visit respectively. The time of opening and end of the visit are given as an estimate.

Table 17 shows the assessments that will be performed on Day 30 Visit and Day 60 Visit of the ambulatory period:

Table 17. Time Schedule – Day 30 Visit and Day 60 Visit (from the time of check-in in the morning prior to 08:30 AM to check-out of the subject on the day after)

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 30 and Day 60**	
In the morning prior to 08:30 AM		Opening of the Day 30 Visit and Day 60 Visit	No product restriction prior to the opening of the visit Product use will be allowed on site in the THS 2.2 Menthol and mCC arms from the time of check-in in the morning to around 11:00 PM. The use of THS 2.2 menthol in smokers allocated to mCC and SA arms will be forbidden
		Support for smoking abstinence (SA arm only) if needed	At any time of the day
		AE/SAE questioning; Concomitant medication	At any time during the day

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 30 and Day 60**	
08:30 AM-09:30 AM		Start of HST recording for 4 hours	In the THS 2.2 Menthol and mCC arms, the HST SODIM® device has to be used for all smoking events, if compatible mCCs are smoked HST recording will be done only on the subject's assigned product
09:00 AM ± 30 min		Start of collection of 24-hour urine fraction on the defined Visit	The subject must empty his bladder and discard the urine prior to starting urine collection BoExp (primary, secondary, and NEQ), creatinine Risk markers: 11-DTX-B2 and 8-epi-PGF2α
Before 11:00 AM		Breakfast	
10:00 AM-11:30 AM		CO breath test	Irrespective of the time of product use
10.00 AM-11:30 AM	√	COHb in blood	Irrespective of the time of product use
10.00 AM-11:30 AM	√	Nicotine, cotinine in plasma	One blood sample will be drawn, irrespective of the time of product use
12:30 PM-01:30 PM		End of collection of 4 –hour HST recording	The HST should be recorded over a period of 4 hours ± 15 minutes.
Before 02.30 PM		Lunch	
02:30 PM-07:00 PM		ECG	At least 5 minutes in supine position prior to recording
02:30 PM-07:00 PM		Urine Pregnancy test	
02:30 PM-07:00 PM		Physical examination, including weight calculated BMI	
02:30 PM-07:00 PM		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after product use prior to measurement At any time during the day Systolic and diastolic blood pressure to be evaluated also as risk markers
08:00 PM-09:30 PM		HST questionnaire (THS 2.2 Menthol and mCC arms only)	If compatible mCC are smoked

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 30 and Day 60**	
08:00 PM-11:00 PM		MCEQ questionnaire (THS 2.2 Menthol and mCC arms only), QSU-brief questionnaire Intention to quit smoking in the next 6 months or less (Prochaska 'Stage of Change' questionnaire)	
In the evening before 09:00 PM		Dinner	
Around 11:00 PM		End of product use	
08:00 AM-11:15 PM		Collection of empty/partially used Menthol Tobacco Stick	At any time of the day For accountability Partially used packs will be returned to the subject
08:00 AM-11:15 PM		Collection of tobacco plugs from each used Menthol Tobacco Sticks	For further analysis

Abbreviations: 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; INR = international normalized ratio;HDL: high density lipoprotein; LDL: low density lipoprotein; hs CRP: high sensitivity C-reactive Protein; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SAE = serious adverse event; TG = triglycerides; s-ICAM-1 soluble inter-cellular adhesion molecule; TC: total cholesterol; THS = tobacco heating system; WBC = white blood cell count.

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 31 and Day 61**	
		Support for smoking abstinence (SA arm only) if needed	At any time of the day
		AE/SAE questioning; Concomitant medication	At any time during the day

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 31 and Day 61**	
	√	Clinical laboratory parameters* (hematology, clinical chemistry), and risk markers (s-ICAM-1, hs-CRP, fibrinogen, homocysteine, LDL, HDL)	Sample to be collected after at least 10 hours of fasting. *Blood glucose, TG, TC, WBC, and platelet count from safety lab will be also evaluated as risk markers
06:30 AM		Beginning of product use	
08:59 AM ± 30 min		End of urine collection and urine sample to be taken from the 24-hour urine after final portion of urine has been added to Day 30 or Day 60 sampling bottles	BoExp (primary, secondary, and NEQ), creatinine Risk markers: 11-DTX-B2 and 8-epi-PGF2α
Before 10:00 AM		Assessment of Cough, and MNWS questionnaire	Irrespective of the time of product use but no later than 10:00 AM
Before 10:00 AM		Breakfast	
Prior to check-out of the given visit		Urine safety analysis	
Prior to check-out of the given visit		Information on the risk of smoking/smoking cessation advice and debriefing	
Check-out		End of the Visit	

Abbreviations: 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event; BoExp = biomarkers of exposure; CO = carbon monoxide; COHb = carboxyhemoglobin; HDL: high density lipoprotein; ; hs CRP: high sensity lipoprotein; HST = human smoking topography; LDL: low density lipoprotein; mCC = menthol conventional cigarette(s); MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine Withdrawal Scale; SAE = serious adverse event; s-ICAM-1: soluble inter-cellular Molecule 1; TC: total cholesterol; TG = triglycerides; THS = tobacco heating system; QSU = Questionnaire of Smoking Urges; WBC = white blood cell count.

The procedures for the last visit which will be conducted on Day 90 Visit +/- 5 days of the study are shown in Table 18.

The time of opening and end of the visit are given as an estimate.

Table 18. Time Schedule – Day 90 Visit (from the time of check-in in the morning prior to 08:30 AM of Day 90 to time of discharge of Day 91)

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 90**	
In the morning		Opening of the Visit	No product restriction prior to the opening of the visit. Product use will be allowed on site in the THS 2.2 Menthol and mCC arms from the time of check-in in the morning to around 11:00 PM The use of THS 2.2 menthol in smokers allocated to mCC and SA arms will be forbidden
		AE/SAE questioning; Concomitant medication	At any time during the day
		Support for smoking abstinence (SA arm only), if needed	At any time of the day
08:30 AM-09:30 AM		Start of HST recording for 4-hour	In the THS 2.2 Menthol and mCC arms, the HST SODIM® device has to be used for all smoking events if compatible mCCs are smoked HST recording will be done only on the subject's assigned product
09:00 ± 30 min		Start of collection of 24-hour urine fraction	The subject must empty his bladder and discard the urine prior to starting urine collection
Before 11:00AM		Breakfast with a caffeine tablet containing approximately 200 mg of caffeine ± 240 mL ±10 mL of water	The time of caffeine tablet intake must be recorded
10:00 AM-12:30 PM		CO breath test	Irrespective of the time of product use
10:00 AM-12:30 PM	√	COHb in blood	Irrespective of the time of product use

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 90**	
10:00 AM-12:30 PM	√	Nicotine, cotinine in plasma	One blood sample will be drawn, irrespective of the time of product use
12:30 PM-01:30 PM		End of collection of HST recording	The HST should be recorded over a period of 4 hours ± 15 minutes.
Before 02:30 PM		Lunch	
In the afternoon	√	CYP1A2 activity in plasma	6 hours ±15 minutes after intake of the caffeine tablet
In the afternoon before 09:00 PM		Nasal Epithelial Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Prior to starting the procedure, the subject will be asked to blow his nose
In the afternoon before 09:00 PM		Buccal Collection	Only if consent is obtained Subject must not have eaten at least 30 minutes prior to collection Subject will be asked to rinse his mouth with a minimum of 20 mL of water prior to sample collection
08:00 PM-09.30 PM		HST questionnaire (THS 2.2 Menthol and mCC arms only)	If compatible mCC are smoked
08:00 PM-11:00 PM		Fagerström Test for Nicotine Dependence (FTND)	
08:00 PM-11:00 PM		MCEQ (THS 2.2 Menthol and mCC arms, only)	
08:00 PM-11:00 PM		QSU-brief questionnaire, Intention to quit smoking in the next 6 months or less (Prochaska 'Stage of Change' questionnaire)	
In the evening before 09:00 PM		Dinner	
Around 11:00 PM		End of product use	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 90**	
08:00 AM-11:15 PM		Collection of empty/partially used menthol THS tobacco stick and mCC packs	At any time of the day For accountability Partially used mCC packs will be returned to the subject
08:00 AM-11:15 PM		Collection of tobacco plugs from each used Menthol Tobacco Sticks	All day For further analysis

Abbreviations: 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event;; BMI = body mass index; BoExp = biomarkers of exposure; mCC = conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; CYP = cytochrome P450 enzyme; ECG = electrocardiogram; HbA1c = hemoglobin A1c; hs-CRP = high-sensitive C-reactive protein; HDL = high density lipoprotein; HST = human smoking topography; LDL = low density lipoprotein; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SAE = serious adverse event; sICAM-1 = soluble inter-cellular adhesion molecule; TG = triglycerides; THS = tobacco heating system; WBC = white blood cell count.

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 91**	
		Support for smoking abstinence (SA arm only), if needed	At any time of the day
		AE/SAE questioning; Concomitant medication	At any time during the day
	√	CYP2A6 activity in plasma	Has to be done prior to product use
	√	COHb	Has to be done prior to gas transfer assessment

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 91**	
		Full lung function (single breath test for CO first, then helium dilution technique, and finally spirometry with bronchodilator,)	Has to be done prior to product use in the THS 2.2 Menthol and mCC arms Subject at rest for at least 15 minutes prior to lung function testing In sitting position At rest for at least 15 minutes prior to lung function testing In sitting position
		Beginning of product use	
	√	Clinical laboratory parameters* (hematology, clinical chemistry), and risk markers (s-ICAM-1, hs-CRP, fibrinogen, homocysteine, LDL, HDL, TG, HbA1c, Apo A1, Apo B)	Sample to be collected after at least 10 hours of fasting. *Blood glucose, TG, TC, WBC, and platelet count from safety to be evaluated also as risk markers
	√	Oxysterols	Sample to be collected after at least 10 hours of fasting
	√	Bio-banking for transcriptomics	Sample to be collected after at least 10 hours of fasting If consent is obtained
	√	Bio-banking for BoExp/risk markers in serum/plasma	Sample to be collected after at least 10 hours of fasting If consent is obtained
Before 10:00AM		Assessment of Cough, and MNWS questionnaire	Irrespective of the time of product use but no later than 10:00 AM
Before 11:00 AM		Breakfast	
08:59 ± 30 min		End of 24-hour urine collection and urine sample to be taken from the 24-hour urine after final portion of urine has been added to Day 90 sampling bottle	BoExp (primary, secondary, and NEQ), creatinine, Ames mutagenicity Urine bio-banking if consent is obtained Risk Markers: 11-DTX-B2 and 8-epi-PGF2α.

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 91**	
		Start collection of 4-hour urine to Day 91 sampling bottle	4-hour urine collection will start immediately after the end of collection of 24-hour urine The 4.hour fraction will be collected over a 4-hour period ± 30 minutes
Before 02:30 PM		Lunch	
		End of 4-hour urine collection and sampling of urine samples to be taken from the 4-hour urine after the final portion of 4-hour urine fraction has been added to Day 91 sampling bottle.	BoExp (primary, secondary and NEQ), creatinine, 4-hour urine fraction. Risk markers: 11-DTX-B2, 8 epi-PGF2α.
In the afternoon prior to discharge of Day 91		Physical examination, including weight calculated BMI, and waist circumference	Weight and waist circumference to be evaluated also as risk markers
In the afternoon prior to discharge of Day 91		Vital signs (blood pressure, pulse rate, respiratory rate)	At least 5 minutes in supine position and at least 15 minutes after smoking prior to measurement At any time during the day Systolic and diastolic blood pressure to be evaluated also as risk markers
In the afternoon prior to discharge of Day 91		ECG	At least 5 minutes in supine position prior to recording
In the afternoon prior to discharge of Day 91		Urine safety analysis and pregnancy test	
From 06:30 to time of discharge of Day 91		Collection of empty/partially used menthol THS tobacco stick	At any time of the day Partially used packs will be returned to the subject after accountability is performed
In the afternoon prior to discharge of Day 91		Return of electronic diaries by the subject to the site staff	
In the afternoon prior to discharge of Day 91		Information on the risk of smoking/smoking cessation advice and debriefing	

Time	Blood sample	Procedures	Additional information
Start of procedure		**Day 91**	
		Time of Discharge On Day 90 Visit	

Abbreviations: 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event;; Apo. Apolipoprotein; BMI = body mass index; BoExp = biomarkers of exposure; mCC = conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; CYP = cytochrome P450 enzyme; ECG = electrocardiogram; HbA1c = hemoglobin A1c; hs-CRP = high-sensitive C-reactive protein; HDL = high density lipoprotein; HST = human smoking topography; LDL = low density lipoprotein; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine /Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SAE = serious adverse event; sICAM-1 = soluble inter-cellular adhesion molecule; TG = triglycerides; THS = tobacco heating system; WBC = white blood cell count.

* Platelet count, WBC, blood glucose, triglycerides, and total cholesterol from safety will also be assessed as risk markers

9.4 Safety Follow-up Period

All subjects participating in the product trial on Day -2 and are not enrolled into the study will enter a 28-day safety follow-up period.

After subjects have completed the Day 91 safety assessments (or if they are prematurely withdrawn from the study), they will enter a 28-day safety follow-up period.

During the 28-day safety follow-up period, there will be spontaneous reporting by the subject of new AEs and new SAEs. Any ongoing AEs/SAEs will be actively followed-up by the site.

Any AEs or SAEs that are ongoing at the end of the 28-day safety follow-up period will be handled as described in Section 8.

9.5 Early Termination Procedures

The following safety assessments as described on Day 6 will be performed as early termination procedures (Section 9.2.3):

- AE/SAE recording.
- Clinical laboratory parameters (hematology, clinical chemistry, and urine safety analysis).
- Physical examination.
- Full lung function.
- Urine pregnancy.
- Vital signs.

- ECG, and
- Information on the risk of smoking/smoking cessation advice, and debriefing.

10 CONTROL AND QUALITY ASSURANCE

10.1 Monitoring

The CRO Clinical Research Associate ("Monitor") will be responsible for the monitoring of the study. Monitoring will be performed according to CRO's SOPs and as per the agreed monitoring plan with the Sponsor.

The Investigator shall permit the Monitor to review study data as frequently as considered necessary to ensure that data are being recorded in an adequate manner and that protocol adherence is satisfactory.

The Investigator shall access medical records for the Monitor in order that entries in the eCRFs may be verified. The Investigator, as part of their responsibilities, is expected to ensure that the study adheres to GCP requirements.

An Investigator's meeting will be held prior to the site initiation visit. During this meeting, the general training of the study procedures and specific training on selected procedures will be done and documented.

Subsequent to the Investigator's meeting, and before the first subject is screened into the study, site initiation visit will be conducted by the Monitor and, if necessary, with Sponsor or its authorized representative. The purpose of the site initiation visit is described in the monitoring plan.

During the study, the Monitor will have regular contact with the study site, including interim monitoring visits. The purpose of these visits is described in the monitoring plan.

Communication by telephone, mail, and e-mail may be used as needed to supplement site visits. The Investigator and study personnel will cooperate with the Monitor, provide all appropriate documentation, and will be available to discuss the study.

The Monitor and the Sponsor's personnel will be available between visits should the Investigator or other staff at the sites need information and advice.

Site visits will be made at regular intervals during the study. The frequency of the monitoring visits will be defined in the monitoring plan agreed with the Sponsor.

The Investigator, or a designated member of the Investigator's staff, must be available during the monitoring visit to review the data and resolve any queries, and to allow direct access to the subject's records for source data verification.

10.2 Training of Staff

A formal meeting (Investigator's meeting) will be conducted prior to site initiation. During this meeting, the Sponsor or its authorized representative will discuss the requirements of the clinical study protocol and related documents and will also provide training in the relevant systems and other study-specific procedures. The activities of this meeting will be described in the monitoring plan.

Further to the Investigator meeting, the Investigator or designee will ensure that appropriate training relevant to the study is provided to all staff involved in the study, and that any new information relevant to the performance of this study is forwarded to the staff involved in a timely manner. The Investigator or designee will maintain a record of all individuals involved in the study.

10.3 Audits and Inspections

Good Clinical Practice regulations require that there are independent inspections of clinical program activities. Such inspections may be performed at any time before, during and/or after the study.

Authorized representatives of the Sponsor, regulatory agencies and/or an IRB may perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, ICH/GCP guidelines, and any applicable regulatory requirements. The Investigator or designee will contact the Sponsor or the authorized representative immediately if contacted by a regulatory agency about an inspection at their site.

The Investigator and study staff are responsible for maintaining a comprehensive and accurate filing system of all study-related documentation that will be suitable for inspection at any time by the Sponsor, its authorized representative, and/or regulatory agencies. In signing this protocol, the Investigator or designee understands and agrees to provide access to the necessary documentation and files.

11 DATA MANAGEMENT ACTIVITIES

All Data Management Activities will be described in detail in the Data Management Plan (DMP) and documents specified therein.

11.1 Data Capture

11.1.1 Case Report Forms and Study Records

With the exception of subject-reported outcome data, all results from the clinical assessments will be recorded in the Source Documents by the Investigator or their authorized designee and then captured in the eCRFs at the study site. The subject questionnaires and the VAS will be entered by the subject directly in an electronic Patient Reported Outcomes (ePRO) device or on paper copy. Trained study personnel will be responsible for capturing the data from the observations, tests, and assessments specified in the protocol in the Source Documents and then transferring the data into the eCRF according to the eCRF Completion Guidelines.

The Investigator has ultimate responsibility for the collection and reporting of all data related to the clinical study and ensuring that the data are accurate, authentic/original, legible, timely (contemporaneous), enduring, and available when required. The eCRF must be electronically signed by the Investigator to attest that the data contained in the eCRF are true and accurate. Any corrections made to source documents and/or eCRFs must be clearly recorded, without obscuring the original values and be accompanied by the date of change, reason for change, and identification of the person making the change. The eCRF for each subject will be checked against the source documents at the study site by the Monitor. Instances of missing or unclear data will be discussed with the Investigator for resolution. For the ePRO diary, all subject reported outcome data will be provided in English and instructions will be provided in the subject's local language. An eCRF will be generated for all subjects that sign informed consent.

11.1.2 Protocol Deviations

All protocol deviations will be entered into the Clinical Trial Management System (CTMS) or other approved format.

Information from the source documents will represent the primary source of protocol deviations. Information following site monitoring and other manual reviews will be documented in the site visit reports, follow-up letters, audit documentation, or other manual review and will be recorded and tracked in the CTMS or other approved format. Telecommunications and other verbal communications regarding deviations will be considered and handled as important communication, documented and tracked as protocol deviations, as necessary.

Individual entries for protocol deviations that are recorded in the CTMS, or other approved format, following site monitoring and other manual reviews will be reviewed against the individual data points in the eCRF database but will not be formally reconciled with the eCRF database (e.g., their description or occurrence date). The overall procedure for managing protocol deviations is described in the SOPs and/or agreed upon procedure of the CRO Data Management Team. All deviations will be reviewed periodically, as determined at study start, to identify trends to improve monitoring and/or potential impact on the statistical analysis.

11.2 Data Handling

All study data will be managed by the Data Management Team at the CRO. The overall procedures for quality assurance of clinical study data are described in the SOPs of the CRO Data Management Team. The Data Management Team at the CRO will prepare a DMP, to be reviewed and approved by the Sponsor, prior to the start of data entry. This document will describe, in detail, the Data Management-related procedures and processes.

All data of all subjects enrolled and screening failures who experience an AE during the study (from time of informed consent to end of the safety follow-up period) will be captured in the Source Documents and all AEs will entered in the study database.

All data collected during the study is property of the Sponsor irrespective of the location of the database and the Data Management CRO.

11.2.1 Data Validation

The data will be validated as defined in the DMP and Data Validation Specifications. Discrepancies will be generated electronically as necessary.

Data queries will be raised for discrepant or missing data. All changes to data will be captured in the database with a comprehensive audit trail.

11.2.2 Coding

Adverse events, medical/surgical history, and prior/concomitant medication will be classified according to the terminology of the latest version of the following dictionaries:

Medical history:	Medical Dictionary for Regulatory Activities (MedDRA®)
Adverse events:	MedDRA®
Medications:	WHO Drug Dictionary Enhanced and Anatomical Therapeutic and Chemical classification system

THS device issues and malfunction will be classified according to C54451/Medical Device Problem Codes, FDA, CDRH (FDA, 2012b).

11.2.3 Database Lock

When all outstanding Data Management issues have been resolved and all validation, quality review and cleaning activities are complete, the database or selected data is/are declared soft locked. Access to change data in the soft-locked database or to change selected data at this time is limited.

After data review by the Sponsor, resolution of all raised queries and QC of the changed data the database or selected data upon Sponsor approval as applicable is/are declared locked.

Any changes to the database after that time can only be made by written agreement between the Sponsor and the Data Management and Statistical Team at the CRO. Any of those changes must be formally documented.

After study completion, the study database will be transferred to the Sponsor in the format specified in the DMP in Clinical Data Interchange Standards Consortium Study Data Tabulation Model (SDTM) Data Structure Specifications.

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

Full details of the statistical analysis will be given in a Statistical Analysis Plan (SAP). Any changes to the planned statistical methods will be documented in the Clinical Study Report. The statistical evaluation will be performed using SAS®, version 9.2 or later.

12.1.1 Stratification Criteria

For the primary analysis of the BoExp, the following stratification criteria will be used:

1. Sex (male; female).
2. Average daily CC consumption over the last 4 weeks as reported during the Screening.

12.1.2 Definitions for Statistical Data Analysis

Baseline:

In general, baseline will be the last available time-point prior to Day 1, 06:30 AM.

THS 2.2 Menthol users:

Subjects switching from mCC to THS 2.2 Menthol whose product use pattern categorization is primarily THS 2.2 Menthol or predominantly THS 2.2 Menthol over a defined period will be considered as THS 2.2 Menthol users during that period. See Section 12.3 for further details on the product use.

Dual use:

Subjects switching from mCC to THS 2.2 Menthol whose product use pattern categorization is dual mostly THS 2.2 Menthol, dual balanced or dual mostly CC over a defined period will be considered as dual users. See Section 12.3 for further details on the product use.

CC users:

Subjects switching from mCC to THS 2.2 Menthol or subjects continuing to use mCC whose product use pattern categorization is primarily CC or predominantly CC over a defined period will be considered as CC users during that period. See Section 12.3 for further details on the product use.

Smoking abstinence:

Smoking abstinence during a defined period is defined as using no tobacco-containing product as reported by the subject on the electronic diary.

Non-compliance to randomization arm:

- Confinement:
  Use of any nicotine or tobacco–containing product other than the assigned product.
  Exhaled CO breath test >10 ppm for subjects from the SA arm
- Ambulatory:
  Non-compliance in the THS 2.2 Menthol and SA arms will be defined in reference to the time period
  -]Day 6-Day 30 Visit]
  -]Day 30 Visit-Day 60 Visit]
  -]Day 60 Visit-Day 90 Visit]

  as the use of more than 2 CC during a single day within a time period or the use of on average more than 0.5 CC per day over the exposure study.

12.1.3 Descriptive Statistics

All data will be presented in listings, ordered by product arm and subject, unless otherwise specified.

Descriptive statistics for continuous variables (number of subjects [n], number and percent of subjects with data, mean, standard deviation [SD], median, first and third quartiles, minimum and maximum for continuous data, and the n and absolute and relative [%] frequency for categorical data) will be presented by product arm and overall at each time point, where applicable.

For BoExp, the geometric mean and coefficient of variation (CV) will be presented in addition to the mean and SD.

Analyses over time will be descriptive statistics of parameters at each assessment timepoint.

12.1.4 Handling of Missing Values and of Values Outside the Detection Limits

Missing values for the BoExp will be imputed using the last observation carried forward (LOCF) approach.

Values below the lower limit of quantification (LLOQ) will be imputed using 0.5 x LLOQ. For values above the upper limit of quantification (ULOQ), the ULOQ will be used for calculation and reporting in summary tables. The number of values below LLOQ or above ULOQ will be presented in each summary table.

For questionnaire data total scores and domain or subscale scores may use a certain degree of imputation by averaging across individual item scores.

Further details will be provided in the SAP.

12.1.5 Significance Level for Inferential Analysis

Unless stated otherwise, all statistical tests will be two-sided and conducted at the 5% level, and all quoted confidence intervals will be two-sided 95% confidence intervals.

The primary endpoints will be tested using a multiple testing procedure to preserve the overall alpha level by simultaneously testing the endpoints using a closed procedure with each test performed at an one-sided alpha level of 2.5%. This implies that statistical significance is required for all primary endpoints in order to be able to make confirmatory claims about any of the endpoints.

Further details will be provided in the SAP.

12.2 Determination of Sample Size and Power Consideration

The following discussion addresses the ability to demonstrate on Day 5 a reduction of at least 50% on four selected primary BoExp and on Day 90 on a fifth primary BoExp in smokers switching from mCC to THS 2.2 Menthol as compared to smokers continuing to smoke mCC.

Table 19 describes the expected coefficients of variation (CV) and mean ratios (MR) between THS 2.2 Menthol and the two control arms in COHb, 3-HPMA, MHBMA, and S-PMA on Day 5 based on data from a controlled, randomized, open-label, 3-arm parallel single-center confinement study to investigate exposure to selected smoke constituents in smokers switching from CCs to smoking article (SMAR) cigarettes for 5 days, the YVD-CS01-EU study (ClinicalTrials.gov: ID: NCT00812279) sponsored by PMI. The mean ratios and coefficients of variations for SMAR/CC are expected to be the same as THS 2.2 Menthol/mCC.

Table 19. Expected Mean Ratios and Coefficients of Variation for THS 2.2 Menthol/mCC

	THS 2.2 Menthol /mCC Mean Ratio (CV)	THS 2.2 Menthol/SA Mean Ratio (CV)
COHb	0.40 (0.32)	2.10 (0.20)
3-HPMA	0.30 (0.50)	1.70 (0.33)
MHBMA	0.15 (0.70)	1.00 (0.35)
S-PMA	0.20 (0.70)	1.15 (0.42)

Abbreviations: 3-HPMA = 3-hydroxypropylmercapturic acid; mCC = menthol conventional cigarettes; COHb = carboxyhemoglobin; CV = coefficients of variation; MHBMA = monohydroxybutenyl mercapturic acid; MR = mean ratios; S-PMA = S-phenylmercapturic acid; SA = smoking abstinence; THS 2.2 = Tobacco Heating System 2.2.

Table 20 describes the expected coefficients of variation (CV) and mean ratios (MR) between THS 2.2 Menthol and the mCC control arm in Total NNAL on Day 90 based on data from a Philip Morris USA-sponsored randomized, controlled, switching, open-label, parallel-group, single-center study in 90 male and female adult smokers evaluated six biomarkers of tobacco smoke exposure over a 12-week period (Frost-Pineda et al., 2008). The mean ratios and coefficients of variations for EHCJLI /CC are expected to be the same as THS 2.2 Menthol/mCC.

Table 20. Expected Mean Ratios and Coefficients of Variation for THS 2.2 Menthol/mCC

	THS 2.2 Menthol /mCC MR (CV)
Total NNAL	0.30 (0.60)

Abbreviations: Total NNAL = total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; MR = mean ratio; THS 2.2 = Tobacco Heating System 2.2; mCC = menthol conventional cigarettes; CV = coefficient of variation.

Table 21 describes the expected coefficients of variation (CV) and mean ratios (MR) between THS 2.2 Menthol and the mCC control arm in COHb, 3-HPMA, MHBMA and S-PMA based on data from a single-center, open-label, randomized, controlled, 2-arm parallel group study to evaluate the exposure to selected smoke constituents in smoking, apparently healthy subjects switching from conventional cigarettes to THS 2.1 compared to subjects continuing to smoke CC for 5 days, the ZRHX-EX-01 study (ClinicalTrials.gov: ID: NCT01780714) sponsored by PMI. The mean ratios and coefficients of variations for THS 2.1/CC are expected to be the same as THS 2.2 Menthol/mCC.

Table 21. Expected Mean Ratios and coefficients of Variation for THS 2.2 Menthol/mCC

	THS 2.2 Menthol /mCC MR (CV)
COHb	0.44 (0.14)
3-HPMA	0.28 (0.20)
MHBMA	0.11 (0.47)
S-PMA	0.07 (0.50)

Abbreviations: 3-HPMA = 3-hydroxypropylmercapturic acid; mCC = menthol conventional cigarettes; COHb = carboxyhemoglobin; CV = coefficient of variation; MHBMA = monohydroxybutenyl mercapturic acid; MR = mean ratio; S-PMA = S-phenylmercapturic acid; THS 2.2 = Tobacco Heating System 2.2.

Based on these two sets of assumptions on the mean ratios and coefficients of variations for the four primary BoExp on Day 5, the power to demonstrate a reduction was computed.

The comparison will be run on the per protocol population as defined in Section 12.4.2.

Subjects are expected not to have any reason for exclusion from the per protocol population in the confinement period for THS 2.2 Menthol arm and in ambulatory and confinement condition for the mCC arm.

50% of the subjects are expected to be excluded from the per protocol population in the THS 2.2 Menthol arm in the ambulatory (mostly due to lack of compliance to the randomized product). Thus the expected populations on which the reductions will be assessed as described in Table 22.

Table 22. Sample Size to Assess the Reductions

	Sample size to assess the reduction (THS 2.2 Menthol:/mCC)
COHb	80:40
3-HPMA	80:40
MHBMA	80:40
S-PMA	80:40
Total NNAL	40:40

Abbreviations: 3-HPMA = 3-hydroxypropylmercapturic acid; Total NNAL = total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol mCC = menthol conventional cigarettes; COHb = carboxyhemoglobin; MHBMA = monohydroxybutenyl mercapturic acid; S-PMA = S-phenylmercapturic acid; THS 2.2 = Tobacco Heating System 2.2.

Table 23 describes the expected power to demonstrate a reduction on 5 primary BoExp in smokers switching from CC to THS 2.2 Menthol as compared to those continuing to smoke mCC, using one-sided test with 2.5% type I error probability using the assumptions from YVD-CS01-EU and ZRHX-EX-01 given a sample size of 160 smokers (~80 in THS 2.2, ~40 in CC, and ~40 in the SA arm). Therefore subjects will be enrolled into the study until there are 160 smokers in the FAS population.

Table 23. Expected power (YVD-CS01-EU and ZRHX-EX-01 studies assumptions)

	Reduction					
Assumptions	50%	51%	52%	53%	54%	55%
YVD-CS01-EU	94%	88%	81%	70%	56%	38%
ZRHX-EX-01	98%	97%	92%	76%	41%	6%

Power considerations related to secondary endpoints of biological changes:

The sample size is sufficient to obtain 95% CIs for the ratio between (geometrical) mean levels of primary BoExp in THS 2.2 Menthol and SA with upper and lower limits deviating not more than 18% from the point estimates, with an 80% overall probability of achieving the desired precision of estimating the true mean.

This study has 80% power using a one-sided test with 2.5% type I error probability:

- To detect a 0.631 [mL/min/kg] (29%) difference between THS 2.2 Menthol and mCC in CYP1A2 activity, as measured by the caffeine clearance, assuming a SD of 0.564. Effect size and variability are derived from data obtained in the YVD-CS01-EU study sponsored by PMI.
- To detect a 24.71 [ng/g creatinine] (20%) difference between THS 2.2 Menthol and mCC in 11-DTX-B2, assuming a SD of 43.78, as reported by Saareks et al., 2001. The anticipated effect size of THS 2.2 Menthol is assumed to be about 90% of the effect of smoking cessation reported in the paper by Saareks et al., 2001.

12.3 Product use

The FDA Draft Guidance on Modified Risk Tobacco Product Applications Draft (Section VI-A-2) (FDA, 2012a), requires that an MRTP Application should contain scientific evidence about the effect the product may have on tobacco use behavior among current tobacco users including consideration of areas such as the expected rates of use of the tobacco product by current tobacco users and the use of the tobacco product in conjunction with other tobacco products.

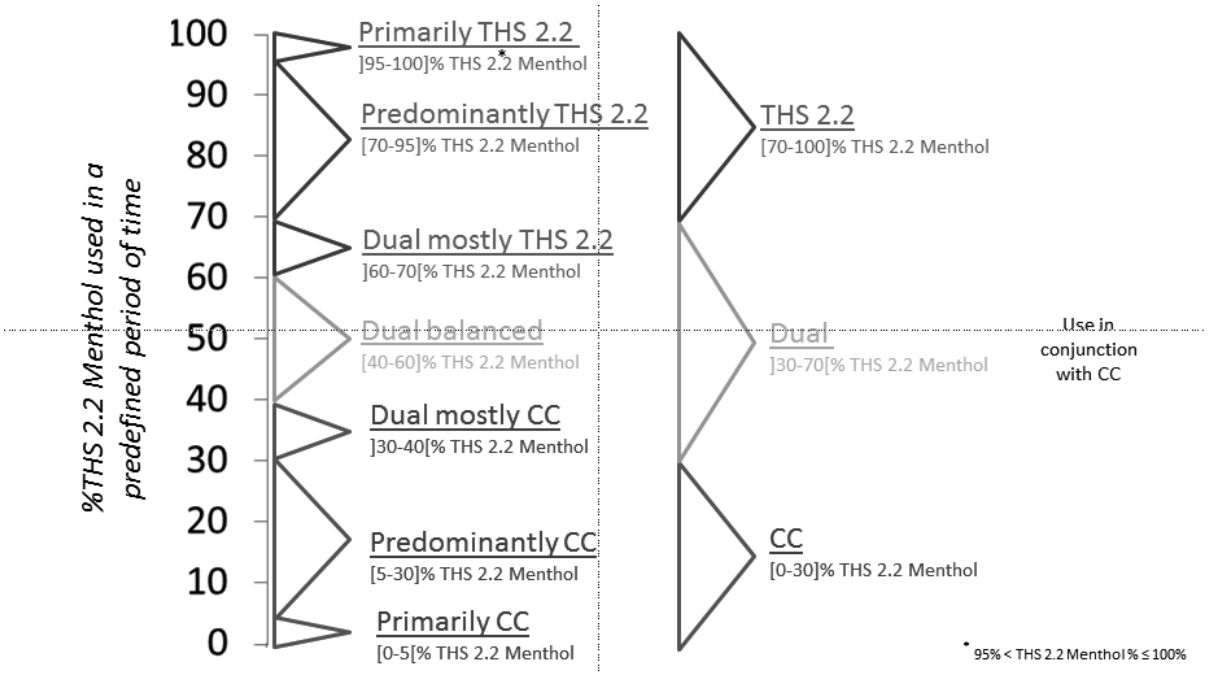
Although subjects are being requested to use solely the product allocated to their respective study arm, it is considered that during the ambulatory period not all subjects randomized to the THS 2.2 Menthol arm might be exclusively using THS 2.2 Menthol at all times during the study. Subjects may concomitantly use THS 2.2 Menthol and CC (dual-use). To assess dual use of THS 2.2 Menthol and CC, PMI has defined dual-use with regards to using THS 2.2 Menthol in the following way:

Dual-use will be calculated as percentage based on their reported THS 2.2 Menthol Tobacco Sticks consumption and the number of conventional cigarettes smoked (menthol and non-menthol) by study period:

- On Day 30 Visit, using the reported number of CC (menthol and non-menthol) and/or Menthol Tobacco Sticks consumption since time of Discharge of Day 6,
- On Day 60 Visit, using the reported number of CC (menthol and non-menthol)and/or Menthol Tobacco Sticks consumption after Day 30 Visit and
- On Day 90 Visit, using the reported number of CC (menthol and non-menthol)and/or Menthol Tobacco Sticks consumption after Day 60 Visit.

Figure 2 presents a detailed overview on the definition of the product use categories.

Figure 2. Product Use Pattern Categorization



The more granular categorization scheme will be used for the definition of the per-protocol population and for the description of the product use patterns observed in the study whereas the less granular scheme will be used for the presentation of other study endpoints (e.g. safety endpoints) to better understand the impact of product.

As the calculation of the sample size was based on the primary objective (reduction of Biomarkers levels in THS 2.2 Menthol relative to mCC), we assumed 50% of the subjects in the THS 2.2 Menthol arm would be using THS 2.2 Menthol exclusively and therefore will be included in the per-protocol population on Day 90.

In order to further optimize the assessment of BoExp and increase the comparability of the levels of biomarkers between THS 2.2 Menthol and SA the confounding effects of the use of any other tobacco or nicotine containing product (other than the assigned product) need to be controlled for. Therefore a subject will be considered abstinent based on the following categorization:

- "Abstinence": 100% abstinence from tobacco or nicotine containing product use other than the assigned product

- Predominantly abstinent: not more than 0.5 uses of any tobacco or nicotine containing product (other than the assigned product) per day on average and no more than two uses on a single day occur.
- "Not abstinent": more than 0.5 uses of any tobacco or nicotine containing product (other than the assigned product) per day on average or more than two uses on a single day occur.

The purpose of the defined threshold was to reduce the confounding effects of the use of any other tobacco or nicotine containing product (other than the assigned product). The results of a study PMI conducted in 2011 (PIPA_CEMA_01 study) to evaluate the capacity of the urinary biomarker CEMA to detect smoking of small amounts of CC use (down to 2 CCs) per day was used to help us identify this threshold. The results of this study show that CEMA used as a urinary biomarker of exposure to acrylonitrile is able to discriminate between non-smokers and smokers who have smoked only 2 CCs and 4 CCs with a maximum level of sensitivity and specificity at an optimal threshold of the 2.56 µg/L (CEMA Cmax in non-smokers) for CEMA and 3.76 µg/g for CEMA concentration adjusted for creatinine. For non-smokers CEMA and CEMA concentration adjusted for creatinine were greater than the LLOQ on only one occasion.

12.4 Analysis Population

The per-protocol (PP) population will be the primary analysis for BoExp, and risk markers. The FAS will be the primary analysis set for compliance to randomization arm, exposure and questionnaires. Exposure and questionnaires will be described by randomization arm and according to the product use (exclusive THS 2.2 Menthol, dual-use of THS 2.2 Menthol and mCC, mCC exclusive, SA) groups.

A sensitivity analysis will be run on the compliant population for the for BoExp and risk markers.

The primary population for the assessment of safety will be the safety population. Safety will be summarized presented by randomization arm and according to product use (exclusive THS 2.2 Menthol, dual-use of THS 2.2 Menthol, Menthol and mCC, mCC exclusive, SA).

12.4.1 Full Analysis Set

The FAS consists of all the randomized subjects who had at least one post-randomization product use experience, if randomized to THS 2.2 Menthol or mCC, have at least one valid non-safety assessment (THS 2.2 Menthol, mCC, SA arms)..

12.4.2 Per Protocol Population

The PP population is a subset of FAS and includes all randomized subjects who

- Have had compliance to their randomized arm (see Section 12.1.2 for the definition of non-compliance to randomized arm).
- Have not been misrandomized, and
- Have no major protocol deviation (to be further described in the SAP).

12.4.3 Safety Population

The safety population consists of all the subjects who had at least one exposure to THS 2.2 Menthol (product test at Admission Day). Subjects in the safety population will be analyzed according to actual exposure.

12.4.4 Compliant Population

The compliant population will be a subset of the Per Protocol Population for subjects from the THS 2.2 Menthol arm who are exclusive THS 2.2 Menthol users or exclusive mCC users, as defined in Section 12.3or for subjects from the mCC arm.

12.5 Primary Analysis

12.5.1 Primary Endpoint Analysis Variables

The primary endpoints are:

- COHb on Day 5
- MHBMA on Day 5
- 3-HPMA on Day 5
- S-PMA on Day 5
- Total NNAL on Day 90.

See Section 3.4.1.

Evaluation criterion:

The study will be considered successful, if the study demonstrates a 50% reduction or more for all five primary BoExp in the THS 2.2 Menthol arm compared to the mCC arm, using a one-sided test with 2.5% type I error probability.

12.5.2 Baseline Comparability

Not applicable.

12.5.3 Descriptive Analysis

Primary endpoints will be summarized as described in Section 12.1.3 on the FAS.

12.5.4 Confirmatory Analysis

The hypothesis to be tested is that the geometric mean level of the BoExp for THS 2.2 Menthol is lower relative to mCC.

For primary BoExp, the hypothesis will be tested on Day 5 for monohydroxylbutenyl-mercapturic acid (MHBMA), 3-hydroxypropylmercapturic acid (3-HPMA), S-phenylmercapturic acid (S-PMA), and carboxyhemoglobin (COHb), and on Day 90 Visit for Total NNAL.

The transformed BoExp data on a natural log scale will be analyzed by means of a generalized linear model using product arm as covariate adjusting for the following baseline information: sex, average cigarette consumption over the previous 4 weeks, and baseline value of endpoint. The test will be declared significant if the contrast THS 2.2 Menthol vs mCC is significant. Estimates of differences between groups will be back-transformed to provide relative effects.

Assumptions of the analysis of variance model will be tested. Markedly non-lognormally distributed BoExp data will be transformed or analyzed by appropriate non-parametric methods.

12.6 Secondary Analysis

12.6.1 Secondary Endpoint Analysis Variables

See Section 3.4.2.

More details on derivation rules will be given in the SAP.

12.6.2 Baseline Comparability

Not applicable.

12.6.3 Descriptive Analysis

In general, secondary endpoints will be summarized as described in Section 12.1.3 on the FAS.

12.6.4 Inferential Analysis

For the secondary BoExp and selected risk markers, the hypothesis to be tested is the same as described in Section 12.5.4. This will be tested for secondary BoExp on Day 5, and if significant, on Day 90.

12.6.5 Safety Analysis

In general, all safety data will be listed and tabulated on the safety population by study arm, using the approach described in Section 12.1.3. Safety variables collected during exposure periods will also be reported by product exposure.

Adverse event data will serve as the primary assessment of safety. Other safety variables monitored in this study include: respiratory symptoms (cough assessment VAS and Likert scales); vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate); electrocardiogram (ECG) data; clinical chemistry, hematology, concomitant medications, and urine analysis safety panel; physical examination.

The number and percentage of subjects with AEs and SAEs will be tabulated by system organ class (SOC) and preferred term (PT). Summaries will also be presented for AEs leading to withdrawal, AEs leading to death, AEs by relatedness to product exposure, AEs by severity, and laboratory AEs. Tabulations will be performed for both the number of subjects experiencing an event and the number of events.

The number and percentage of subjects with clinical findings by sequence for laboratory parameters will be summarized for the safety population. Shift tables showing change from baseline of clinical findings will be provided for: ECGs, physical examinations and laboratory parameters (both shifts in normal ranges and toxicity grades). Descriptive statistics will be summarized by visit and change from baseline for laboratory parameters, ECG, respiratory symptoms, and vital signs.

12.7 Exploratory Analysis

12.7.1 Exploratory Endpoint Analysis Variables

See Section 3.4.3.

12.7.2 Descriptive Analysis

In general, exploratory endpoints will be summarized as described in Section 12.1.3 on the FAS.

12.8 Demographics and Baseline Characteristics

Demographic and other baseline characteristics will be reported for FAS and safety population. Summary statistics will be provided by exposure group and stratified by sex and by cigarette consumption. Formal statistical analysis will not be performed on baseline demographic data.

12.9 Interim Analysis

There are no planned interim analyses.

12.10 Preference Analysis

At admission, subjects will be asked for their preferred product (THS 2.2 Menthol, mCC, SA, or no preference). A sensitivity analysis will be run for the exposure according to the subjects' preferred product.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Investigators and Study Administrative Structure

13.1.1 Investigator

Principal Investigator Covance Dallas Site:	Dr. William Lewis [redacted] Phone: [redacted]
Principal Investigator Covance Daytona Beach Site	Dr. Frank Farmer [redacted] Phone: [redacted]

13.1.2 Sponsor

Sponsor:	Philip Morris Products S.A. Quai Jeanrenaud 5. 2000 Neuchâtel. Switzerland. Phone: + 41 (58) 242 2111 Fax: + 41 (58) 242 2811
[redacted], PhD Manager Clinical Science	Phone: +41 [redacted] Mobile: +41 [redacted] E-mail: [redacted]@pmi.com
[redacted] MEng, MSc Staff Scientist (Expert Statistician)	Phone: +41 [redacted] Mobile: +41 [redacted] E-mail: [redacted]@pmi.com
[redacted] MD Medical Safety Officer	Phone: +41 [redacted] Mobile: +41 [redacted]

	E-mail: [redacted]@pmi.com
[redacted], PhD Clinical Study Manager	Phone: +41 [redacted] Mobile: +41 [redacted] E-mail: [redacted]@pmi.com

13.1.3 Other Responsibilities

All duties and responsibilities transferred to [redacted] by PMI will be defined in the agreement signed between the two parties.

[redacted]

[redacted]

Any SAEs or pregnancies will be handled by:

[redacted]

Details of the laboratories conducting the clinical safety laboratory services, biopharmaceutical analyses and the analyses of BoExp are shown in Appendix 2.

13.2 Subject Confidentiality

All information obtained during the conduct of the study with respect to the subjects' state of health will be regarded as confidential. A statement to this effect will be written in the information provided to the subject. An agreement to disclose any such information will be obtained from the subject in writing and signed by the subject, in compliance with all local and national data protection and privacy legislation.

The anonymity of subjects participating in this study will be maintained. Subjects will be identifiable by the Sponsor (or Sponsor's authorized representative) on eCRFs and other documents by their subject (or randomization) number/code, sex, and date of birth, but not by name, initial, or any other details relating to identifiable person (e.g., address, social security number, medical chart number, etc.). The assignment of a subject number/code for subject identification will be based on the appropriate data protection rules.

The blood samples for transcriptomics and the data related to these samples will be anonymized. Anonymized data and samples are initially single or double coded where the link between the subjects' identifiers and the unique code(s) is subsequently deleted. This is applicable for the blood bio-banking for transcriptomics only.

Any documents that allow full identification of the subject (e.g., the subject's signed Study Information Sheet and ICF) must be maintained in confidence by the Investigator. If any document relating to this study shows a subject's name or any other details relating to an identifiable person (e.g., address, social security number, medical chart number, etc.), the name or other identifiable details must be obscured before a copy of that document is supplied to the Sponsor or the Sponsor's authorized representative.

13.3 Access to Source Documentation

Subjects will be informed that, during the course of the clinical study, the Sponsor, any authorized representatives of the Sponsor, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and ensure that all personal information made available for inspection is handled in the strictest confidence and in accordance with national and local data protection and privacy legislation.

The Investigator and all study site staff involved with the study must permit direct access to source data/documents for study related monitoring, audits, IRB review, and regulatory inspection(s).

13.4 Record Retention

All records of data, source data and Source Documents (original records or certified copies), in any form (including, but not limited to, written, electronic, magnetic, optical records and

scans, X-rays, and ECGs) that describe or record the methods, conduct, and/or results of the study, the factors affecting the study, and the actions taken will be maintained by the Investigator/study site for the study, as required by ICH GCP and any other applicable local or national regulations. For X-rays, at least the radiologist's assessment is required as source documentation. If the actual image is available, it can be stored on CD as well.

Essential study documents/records, which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, are described in Section 8 of the ICH Tripartite Guideline for Good Clinical Practice (ICH Guideline for Good Clinical Practice E6 (R1), July 1996).

Essential documents must be retained by the Investigator for a minimum of:

- At least 15 years after completion or discontinuation of the study, or
- At least 2 years depending on, for example, the circumstances
- After formal discontinuation of clinical development of the IP.

These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor.

Examples of essential records/documents include, but are not limited to:

- Signed informed consent documents for all subjects and Master ICF/Subject Information Sheet.
- Subject identification code list, Screening Log, and Enrolment Log (if applicable).
- Record of all communications between the Investigator and the IRB, composition of the IRB.
- Record of all communications/contact between the Investigator, Sponsor, and its authorized representatives.
- List of sub-Investigators and other appropriately qualified persons to whom the Investigator has delegated significant study-related duties, together with their roles in the study, curricula vitae, and their signatures.
- Investigator Logs.
- eCRFs, study specific questionnaires (and associated data/scoring), subject diaries.
- AE reports and details of follow-up investigations, details of concomitant medication.
- All other Source Documents (e.g., chest X-rays, ECGs, consultation reports, physical examination, laboratory records) or any electronically captured study source data.
- Clinical laboratory reports, laboratory normal ranges.

- Original medical/hospital records, if applicable (the medical files of study subjects must be retained in accordance with local legislation and in accordance with the maximum period of time permitted by the hospital or study site).
- Record of any body fluids or tissue samples collected and retained.
- Device Issue Log, IP Accountability Logs, dispensing records.
- Information regarding subjects' discontinuation and any follow-up.

It is the responsibility of the Sponsor to inform the Investigator/study site as to when these documents no longer need to be retained.

The Investigator/study site must take measures to prevent accidental or premature destruction of these documents.

If an Investigator wishes to assign the study records to another party or move them to another location, the Sponsor must be notified in advance.

The Investigator must obtain written approval from the Sponsor before destruction of any records. Normally, these records will be held in the Investigator's archives. If an Investigator is unable to meet this obligation, they must ask the Sponsor for permission to make alternative arrangements. Details of these arrangements must be documented.

The Sponsor or Sponsor's authorized representative will maintain documentation relating to the study as long as the IP is on the market, and/or for 15 years after the CSR has been finalized.

13.5 Clinical Study Report

The Sponsor must ensure that a CSR for this study is prepared regardless of whether the study is completed or prematurely terminated.

The CSR will be written based on standards of the ICH Guideline for the Structure and Content of Clinical Study Reports. In certain circumstances, an abbreviated CSR may be acceptable. Submission of the CSR to the IRB will be complied with as requested by local requirements.

The results of the additional variables for analysis will be presented in reports separate from the study CSR.

13.6 Financial Disclosure

Investigators are required to provide financial disclosure information to the Sponsor. In addition, the Investigators must provide to the Sponsor a commitment to promptly update

this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

13.7 Publication and Disclosure Policy

This document contains information that is confidential and proprietary to the Sponsor. This information is being provided solely for the purpose of evaluation and/or conducting this clinical study for the Sponsor. Disclosure of the content of this document is allowed only to study personnel, IRB, or duly authorized representatives of regulatory agencies for this purpose under the condition that confidentiality is maintained. The contents of this document may not be used in any other clinical study, disclosed to any other person or entity without the prior written permission of the Sponsor. The foregoing shall not apply to disclosure required by any regulations; however, prompt notice will be given to the Sponsor prior to any such disclosure.

The Sponsor plans to disclose details of the study protocol on a web-based, publicly available, clinical trial register database (e.g., ClinicalTrials.gov).

14 REFERENCE LIST

Ashley et al., 2008

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Appendix 1 Schedule of Events

Table A1 Study Assessments (separate table [Table A2] shown for 24 hour urine collections)

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
Informed consent for study participation and two informed consents for bio-banking	●																
Admission/Discharge		●								●						●	
Information on the risk of smoking/smoking cessation advice and debriefing	●	●								●		●		●		●	
Monitoring/Intensive support for SA arm					●	●	●	●	●	●	●	●	●	●	●	●	
Inclusion/exclusion criteria	●	●															
Enrolment		●															
Randomization				●													
Demographics, medical history	●																

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
Concomitant diseases	●	●															
Socio-economic questionnaire								●									
Vital signs [a]	●	●	●	●	●	●	●	●	●	●	●		●			●	
Physical examination	●	●								●	●		●			●	
Body height and weight [b]	●	●								●	●		●			●	
Waist circumference [c]		●														●	
Spirometry [d]	●			●						●						●	
Prior/concomitant medication	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
B/U: Hematology, clinical chemistry, urine analysis [e]	●			●						●		●		●		●	
Electrocardiogram	●									●	●		●			●	
Chest X-ray [f]	●																
B: HIV, hepatitis B and C	●																
Urine cotinine Screening test	●																

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
U: Urine drug screen	●	●															
U: Pregnancy test	●	●								●	●		●			●	
Alcohol urine or breath test	●	●															
FTND	●														●		
Smoking history	●	●															
Intention to quit smoking in the next 6 months (prochaska 'Stage of Change' questionnaire)	●	●									●		●		●		
Readiness to comply with study protocol: to abstain from smoking for up to 91 days	●	●															
Identification of mCC	●	●															
THS 2.2 Menthol demonstration	●																
THS 2.2 Menthol product test and readiness to use the product [g]		●															

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
Question on product Preference		●															
Collection of mCC butts for accountability			●	●	●	●	●	●	●	●							
Collection tobacco plugs of used Menthol Tobacco Sticks for further analysis					●	●	●	●	●		●		●		●		
Collection of empty/partially used Menthol Tobacco Stick packs for accountability											●		●		●		
CO breath test [h]		●	●	●	●	●	●	●	●	●	●		●		●		
B: BoExp in blood: COHb [i]			●	●	●	●	●	●	●	●	●		●		●	●	
B: BoExp to nicotine in plasma: nicotine, cotinine [j]				●	●	●	●	●	●	●	●		●		●		
U: Ames Mutagenicity (see Table A2)				●					●						●		
U:all urinary BoExp in 24-hour urine (primary and secondary, and BoExp to				●	●	●	●	●	●		●		●		●		

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
nicotine) (see Table A2)																	
U:all urinary BoExp in 4-hour fraction (primary and secondary, and BoExp to nicotine) (see Table A3)			●													●	
B:Risk markers: hs-CRP, fibrinogen, homocysteine, LDL, HDL [k]				●								●		●		●	
B: Risk Marker: sICAM-1 [l]				●						●		●		●		●	
B: Risk Marker: HbA1c, Apo A1, and Apo B [m]				●												●	
U: Risk markers in 24-hour urine: 8-epi-PGF2α and 11-DTX-B2 (see Table A2) [n]				●					●		●		●		●		
U: Risk markers in 4-hour fraction: 8-epi-PGF2α and 11-DTX-B2 (see Table A3)			●													●	
One caffeine tablet (200 mg caffeine)				●					●						●		
B: CYP1A2 activity				●					●						●		

	Screening	Confinement Period								Ambulatory Period							Safety Follow-up [z]
											Day 30 Visit ± 5 days		Day 60 Visit ± 5 days		Day 90 Visit ± 5 days		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
B: CYP2A6 activity				●						●						●	
B: Oxysterols				●						●						●	
Product use diary [o]										●	●	●	●	●	●	●	
QSU-brief questionnaire [p]			●	●	●	●	●	●	●		●		●		●		
MNWS (revised version) [q]				●	●	●	●	●	●	●		●		●		●	
MCEQ (modified version; THS 2.2 Menthol and mCC arms) [r]			●	●	●	●	●	●	●		●		●		●		
HST (THS 2.2 Menthol and mCC arms) [s]				●	●			●			●		●		●		
HST questionnaire				●				●			●		●		●		
Assessment of cough [t]				●	●	●	●	●	●	●		●		●		●	
Risk Factor Surveillance System Questionnaire			●						●								
Smoking Questionnaire			●						●								
Supplemental questions [u]			●						●								
AE/SAE recording	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

	Screening	**Confinement Period**								**Ambulatory Period**							**Safety Follow-up [z]**
											Day 30 Visit ± 5 days		**Day 60 Visit ± 5 days**		**Day 90 Visit ± 5 days**		
Study Day	**-30 to -3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6 [y]**	**30**	**31**	**60**	**61**	**90**	**91**	**91 to 119**
B: Bio-banking for BoExp and risk markers [vw]				●						●						●	
U: Bio.banking for BoExp and risk markers [v]				●					●						●		
B: Bio-banking for transcriptomics [vw]				●						●						●	
Nasal Epithelial collection [x]				●						●					●		
Buccal Collection [x]				●						●					●		

Abbreviations: 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; AE = adverse event; Apo: Apolipoprotein; B = blood sample required; BMI = body mass index; BoExp = biomarkers of exposure; mCC = menthol conventional cigarette(s); CO = carbon monoxide; COHb = carboxyhemoglobin; CYP = cytochrome P450 enzyme; FTND = Fagerström Test for Nicotine Dependence; HbA1c = hemoglobin A1c; HDL = high density lipoprotein; HST = human smoking topography; HIV = human immunodeficiency virus; hs-CRP = high-sensitive C-reactive protein; HST = human smoking topography; LDL = low density lipoprotein; MCEQ = Modified Cigarette Evaluation Questionnaire; MNWS = Minnesota Nicotine Withdrawal Scale; QSU = Questionnaire of Smoking Urges; SA = smoking abstinence; SAE = serious adverse event; sICAM-1 = soluble inter-cellular adhesion molecule; THS = tobacco heating system; U = urine sample required; WBC = white blood cell count; TC: total cholesterol, TG: triglycerides.

a: Systolic and diastolic blood pressure, pulse rate, and respiratory rate (systolic and diastolic blood pressure will also be analyzed as risk markers on Day 0, Day 6, Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 91)).

b: Including height (only at Screening). Weight will be evaluated also as risk marker on Day -2 Visit on D 90 Visit (Day 91).

c: Waist circumference will be evaluated also as risk markers on Day -2 and Day 90 Visit (Day 91).

d: Spirometry without bronchodilator will be performed at screening only and must be done prior to spirometry with bronchodilator.

Spirometry with bronchodilator will be done at Screening, Day 0, Day 6, and Day 90 Visit (Day 91). At screening, spirometry with bronchodilator will be done at least 1 hour after smoking. On Day 0, Day 6, and Day 90 Visit, spirometry with bronchodilator will be performed prior to product use (mCC or THS 2.2 Menthol).

Spiromety using helium technique and spirometry using the single breath technique for CO will be performed Day 0, Day 6, and Day 90 Visit (Day 91) prior to product use.

e: WBC count, platelet count, from the safety laboratory panel to be evaluated also as risk markers on Day 0, Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61) and Day 90 Visit (Day 91). Blood glucose, TG, and TC from the safety laboratory panel to be evaluated as risk markers on Day 0, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91).

f: Pre-study chest X-ray (with anterior-posterior and left lateral views) may be used, if performed within 6 months prior to Screening.

g: THS 2.2 Menthol product test to be conducted as the last procedure of eligibility check at Day -2 (and after urine pregnancy test has been confirmed negative in female subjects to exclude pregnancy).

h: CO breath test; Days -1 to Day 5: the test will be conducted four times per day. On Day -1, the first test should be conducted within 15 minutes prior to the first product use. on Day 5, the first test should be conducted within 15 minutes prior to the first product use (for subjects in the THS Menthol 2.2 and mCC arms) and at between 08:00 AM and 09:30 AM for subjects in the SA arm. The other three tests should be conducted as described in Section 9. Day -2, Day 6, Day 30 Visit (Day 30), Day 60 Visit (Day 60) and Day 90 Visit (Day 90): once during the visit, irrespective of the time of product use.

i: COHb; Assessments should be done in conjunction with CO breath tests, where applicable.

Day -1 and from Day 1 to Day 4: one blood sample in the evening around 08:00 PM.

On Day 0, two blood samples will be collected: one blood sample will be collected prior to gas transfer assessment and prior to product use (the COHb levels measured will serve for adjustment of gas transfer values only), the second blood sample will be collected in the evening between 08:00 PM and 09:30 PM.

Day 5: one blood sample within 15 minutes prior to first product use (for subjects in THS 2.2 Menthol and mCC arms) and between 08:00 AM and 09:30 AM for subjects in the SA arm. The three other blood samplings will be conducted as described in Section 9.

On Day 6: one blood sample will be collected prior to the gas transfer assessment and prior to product use (the COHb levels measured will serve for adjustment of gas transfer values only).

Day 30 Visit (Day 30), Day 60 Visit (Day 60): one blood sample to be collected during the visit, irrespective of the time of product use.

On Day 90 Visit: one blood sample will be collected on Day 90, irrespective of the time of product use. One blood sampling will be collected on Day 91 prior to gas transfer assessment, and product use (the COHb levels measured will serve for adjustment of gas transfer values only).

j: Nicotine/cotinine; Day 0 to Day 4 (all study arms): one blood sample between 08:00 PM and 09:30 PM.

Day 5 and Day 6 (THS 2.2 Menthol and mCC arms): one sample within 15 minutes prior to the first product use; eight blood samples after the start of product use (T0), each at 2 hour intervals. On Day 6, two blood samples will be drawn. The first sample will be 20 hours after T0 and the second blood sample will be 24 hours after T0 (with T0 being the time of the first product use on Day 5).

Day 5 and Day 6 (SA arm): one blood sample in the evening between 08:00 PM and 09:30 PM on Day 5 and one blood sampling between 08:00 AM and 09:30 AM on Day 6.

Day 30 Visit (Day 30), Day 60 Visit (Day 60), Day 90 Visit (Day 90) (all study arms): one blood sample to be drawn during the visit, irrespective of the time of product use.

k:To be evaluated also as risk markers on Day 0, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91).

l: . To be evaluated also as risk markers on Day 0, Day 6, Day 30 Visit (Day 31), Day 60 Visit (Day 61), and Day 90 Visit (Day 91).

m: To be evaluated also as risk markers on Day 0, and Day 90 Visit (Day 91).

n: To be evaluated also as risk markers on Day 0, Day 5, Day 30 Visit, Day 60 Visit, and Day 90 Visit.

o: Daily during ambulatory period only (from time of Discharge on Day 6 to time of discharge of Day 91). Use of any tobacco/nicotine containing products will be captured in the e-diary.

o: QSU-brief: Daily, from Day -1 to Day 5 and at every visit during the ambulatory period, i.e. Day 30 Visit (Day 30), Day 60 Visit (Day 60) and Day 90 Visit (Day 90) .

q: MNWS daily from Day 0 to Day 6 prior product use, but no later than 10:00 AM and at every visit during the ambulatory period, i.e. Day 30 Visit (Day 31), Day 60 Visit (Day 61) and Day 90 Visit (Day 91) no later than 10:00 AM irrespective of the time of product use.

.

r: MCEQ: Day -1 to Day 5 on a daily basis, and on Day 30 Visit (Day 30), Day 60 Visit (Day 60) and Day 90 Visit (Day 90) in Day -1 and Day 0 on all subjects, from Day 1 in THS 2.2 Menthol and mCC arms.

s: On Day 0, HST assessment will be done in all subjects smoking mCC compatible with the HST SODIM® device. On Day 1, Day 4, Day 30 Visit (Day 30) , and Day 60 Visit (Day 60) and Day 90 Visit (Day 90), HST will be done in all subjects in the THS 2.2 Menthol and mCC arms. On Day 1 and Day 4, full day HST recording will be done. On Day 30 Visit (Day 30), Day 60 Visit (Day 60), and Day 90 Visit (Day 90), 4 hours of recording will be done. Smoking topography with the HST SODIM® device will not be done in subjects smoking mCC that are incompatible with the HST SODIM® device (e.g. slim mCC). No HST assessments will be done in subjects in the SA arm.

t: Cough questionnaire to be done daily from Day 0 to Day 6 prior product use but no later than 10: 00 AM and on Day 30 Visit (Day 31) Day 60 Visit (Day 61) and Day 90 visit (Day 91) no later than 10:00 AM, irrespective of product use.

u: Subjects will be assessed for their current and past smoking behavior at baseline on Day -1 and on Day 5 by the means of 2 questionnaires. The questionnaires will be asked with the following sequence:

1-a standard questionnaire (Behavioral Risk Factor Surveillance System Questionnaire 2011) (CDC, 2011a) which is validated

2 a Smoking Questionnaire (SQ) to be validated. Immediately after the administration of the SQ, some supplemental questions will be collected on the completion of the SQ questionnaire. This supplemental question 1 is related to the time which the subject spends to complete the SQ and should be completed by site.

The Behavioral risk factor surveillance and the SQ are self-administrated to be answered by subjects. The supplemental questions will be asked by the site.

v: Samples will only be taken if additional consent for bio-banking is given by the subject.

w: Has to be done in at least 10 hours of fasting condition.

x:Subject must not have eaten 30 minutes prior to the start of procedures.

y: All examinations listed at the Day of Discharge (Day 6) will be conducted in subjects whose participation in the study is prematurely terminated and for all subjects who have tried the product on admission

z: Spontaneous reporting of new AEs/SAEs by the subject and active follow-up of ongoing AEs/SAEs by the site.

Table A2 Schedule for 24-hour Urine Collection Assessments

	Baseline Period 24-hour urine	**Confinement Exposure Period 24-hour urine**					**Ambulatory Exposure Period 24-hour-urine**		
	Day 0 to Day 1	**Day 1 to Day 2**	**Day 2 to Day 3**	**Day 3 to Day 4**	**Day 4 to Day 5**	**Day 5 to Day 6**	**Day 30 to Day 31**	**Day 60 to Day 61**	**Day 90 to Day 91**
BoExp in urine [a]	●	●	●	●	●	●	●	●	●
Creatinine	●	●	●	●	●	●	●	●	●
11-DTX-B2, 8-epi-PGF2 α	●					●	●	●	●
Ames test	●					●			●
Bio-banking [b]	●					●			●

a: MHBMA, 3-HPMA, S-PMA, Total NNAL, 1-OHP, total NNN, 4-ABP, 1-NA, 2-NA, o-tol, CEMA, HEMA, 3-hydroxybenzo(a)pyrene, HMPMA, S-BMA, NEQ.

b: Samples will only be taken if additional consent for the relevant sample bio-banking is given by the subject

Abbreviations: 1-NA = 1-aminonaphthalene; 2-NA = 2-aminonaphthalene; 1-OHP = 1-hydroxypyrene; 3-HPMA = 3-hydroxypropylmercapturic acid; 4-ABP = 4-aminobiphenyl; 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; BoExp = biomarker(s) of exposure; CEMA = 2-cyanoethylmercapturic acid; HEMA = 2-hydroxyethyl mercapturic acid; HMPMA = 3-hydroxy-1-methylpropyl-mercapturic acid; NEQ = nicotine equivalents; Total NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; total NNN = N-nitrosonornicotine; MHBMA = monohydroxybutenyl mercapturic acid; S-BMA = S-benzylmercapturic acid; S-PMA = S-phenylmercapturic acid.

Table A3 Schedule for 4-hour Urine Collection Assessments

	Baseline Period 4-hour urine fraction	**Ambulatory Period 4-hour urine fraction**
	Day -1	**Day 90 Visit (Day 91)**
BoExp in urine [a]	●	●
Creatinine	●	●
11-DTX-B2 and 8-epi-PGF2 α	●	●

a: MHBMA, 3-HPMA, S-PMA, Total NNAL, 1-OHP, total NNN, 4-ABP, 1-NA, 2-NA, o-tol, CEMA, HEMA, 3-hydroxybenzo(a)pyrene, HMPMA, S-BMA, NEQ.

Abbreviations: 1-NA = 1-aminonaphthalene; 2-NA = 2-aminonaphthalene; 1-OHP = 1-hydroxypyrene; 3-HPMA = 3-hydroxypropylmercapturic acid; 4-ABP = 4-aminobiphenyl; 8-epi-PGF2α = 8-epi-prostaglandine F2α; 11-DTX-B2 = 11-dehydro-thromboxane B2; BoExp = biomarker(s) of exposure; CEMA = 2-cyanoethylmercapturic acid; HEMA = 2-hydroxyethyl mercapturic acid; HMPMA = 3-hydroxy-1-methylpropyl-mercapturic acid; NEQ = nicotine equivalents; Total NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; total NNN = N-nitrosonornicotine; MHBMA = monohydroxybutenyl mercapturic acid; S-BMA = S-benzylmercapturic acid; S-PMA = S-phenylmercapturic acid.

Appendix 2 Participating Laboratories

[redacted]

[redacted]

[redacted]

More details will be found in the study laboratory manuals.

For bio-banking of samples:

[redacted]

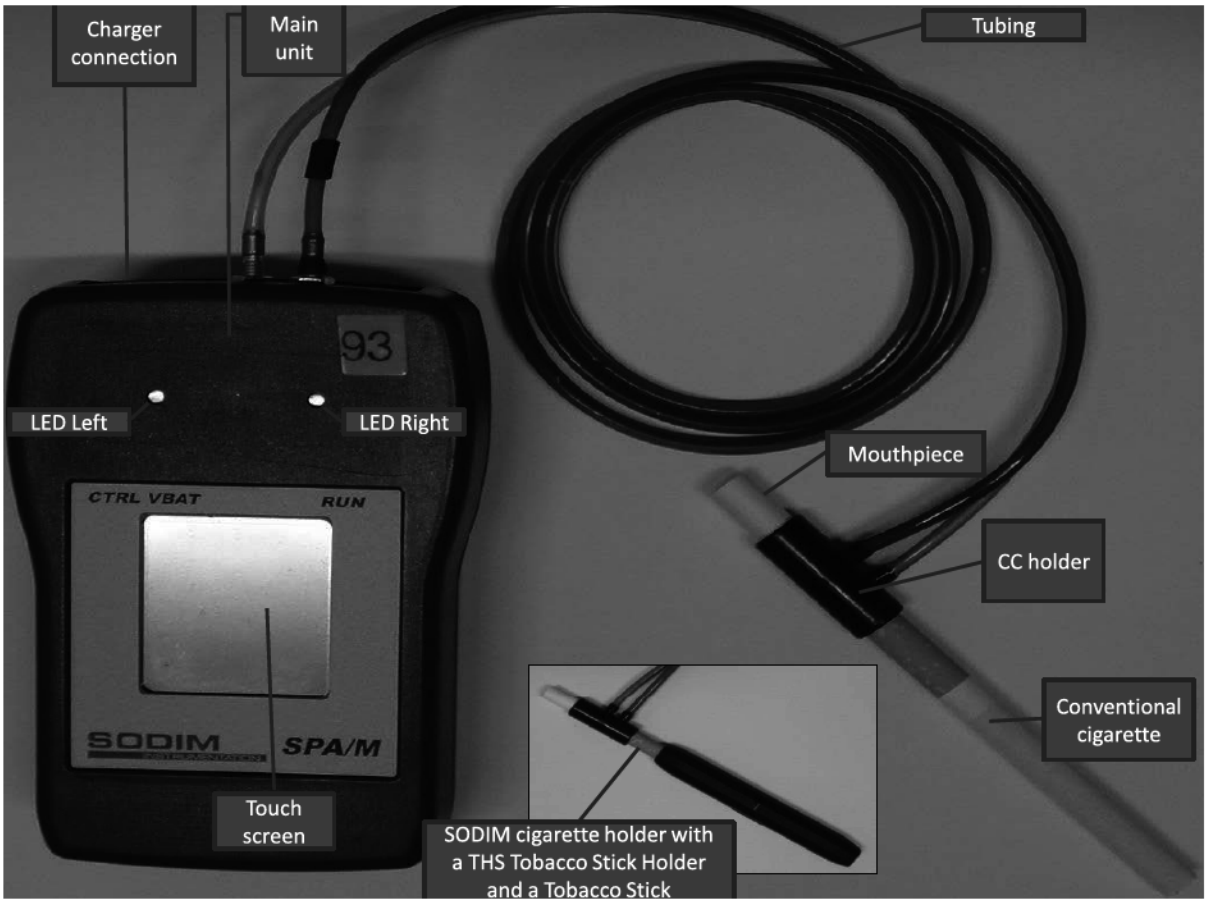
R&D PMI Laboratory
Quai Jeanrenaud, 5
2000, Neuchâtel
Switzerland

Appendix 3 Investigational Product and Instructions for Use

The product user guide will be provided as a separate document.

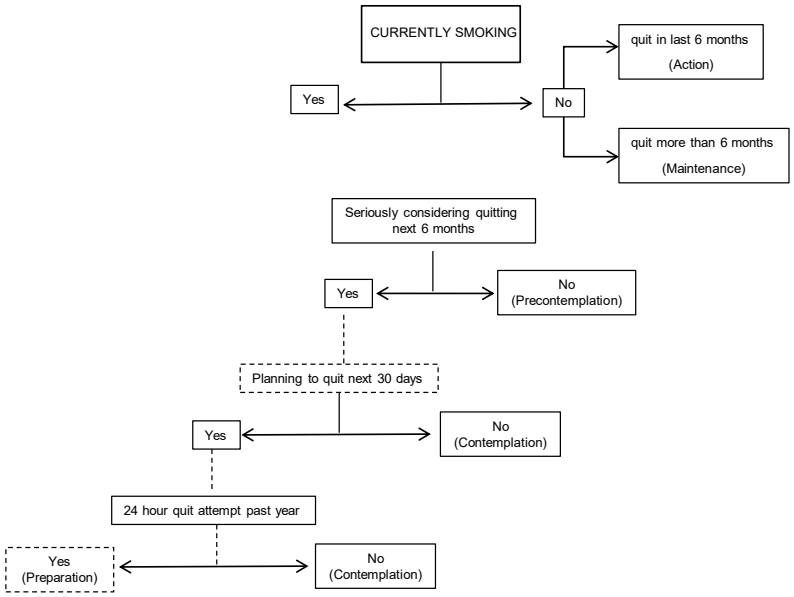
Appendix 4 HST SODIM® Device Description

An image of the HST SODIM® device used for assessing Human Smoking Topography (HST) is provided below. Full instructions for use of the device will be provided to the staff before study start.



Appendix 5 Prochaska 'Stage of Change' Questionnaire

The Prochaska questionnaire is structured as follows:



The questionnaire to be asked, is as follows, with the scoring sheet not to be read aloud:

Smoking algorithm for the Classification of the stages of change for smoking cessation

Assessment

1. Are you currently a smoker?

A) Yes, I currently smoke

B) No, I quit within the last 6 months

C) No, I quit more than 6 months ago

D) No, I have never smoked

Smokers only

2. In the last year, how many times have you quit smoking for at least 24 hours? ___

3. Are you seriously thinking of quitting smoking?

A) Yes, within the next 30 days

B) Yes, within the next 6 months

C) No, not thinking of quitting

Scoring Sheet (do not read to subject):

1. Are you currently a smoker?

A) Yes, I currently smoke

B) No, I quit within the last 6 months (ACTION STAGE)

C) No, I quit more than 6 months ago (MAINTENANCE STAGE)

D) No, I have never smoked (NONSMOKER)

Smokers only

2. In the last year, how many times have you quit smoking for at least 24 hours? ---

3. Are you seriously thinking of quitting smoking?

A) Yes, within the next 30 days (PREPARATION STAGE if they have one 24-hour quit attempt in the past year, if there was no quit attempt in the past year, then CONTEMPLATION STAGE)

B) Yes, within the next 6 months (CONTEMPLATION)

C) No, not thinking of quitting (PRECONTEMPLATION)

Appendix 6 Abnormal Laboratory Values

ABNORMAL LABORATORY VALUES RATING: SERUM CHEMISTRY PARAMETERS

Serum Chemistry*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Sodium – Hyponatremia (mmol/L) ** (1)	<LLN - 130	-	<130 - 120
Sodium – Hypernatremia (mmol/L) ** (1)	>ULN - 150	>150 - 155	>155 - 160; hospitalization indicated
Potassium – Hyperkalemia (mmol/L)** (1)	>ULN - 5.5	>5.5 - 6.0	>6.0 -7.0; hospitalization indicated
Potassium – Hypokalemia (mmol/L) ** (1)	<LLN - 3.0	<LLN - 3.0; symptomatic; intervention indicated	<3.0 - 2.5; hospitalization indicated
Glucose – Hypoglycemia ** (1) (mg/dL) (mmol/L)	<LLN – 55; <LLN – 3.0	<55 – 40; <3.0 – 2.2	<40 – 30; <2.2 – 1.7
Glucose – Hyperglycemia: ** (1) Fasting (mg/dL) (mmol/L) Non-fasting (mg/dL) (mmol/L)	 >ULN-160; >ULN-8.9 - -	 >160-250; >8.9-13.9 - -	 - - >250-500; >13.9-27.8; hospitalization indicated
Blood Urea Nitrogen (BUN) (mg/dL) (2)	23 – 26	27 – 31	> 31
Creatinine increased** (1)	>1 – 1.5 x baseline; >ULN – 1.5 x ULN	>1.5 – 3.0 x baseline; >1.5 – 3.0 x ULN	>3.0 x baseline; >3.0 – 6.0 x ULN
Albumin – Hypoalbuminemia** (1) (g/dL) (g/L)	<LLN – 3; <LLN - 30	<3 – 2; <30 - 20	<2; <20
Total Protein – Hypoproteinemia (2) (g/dL)	5.5 – 6.0	5.0 – 5.4	< 5.0
Alkaline phosphatase increased** (1)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN
ALT / AST increased** (1)	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 – 20.0 x ULN
Gamma-glutamyl transferase (GGT) increased (1)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN
Blood bilirubin increased ** (1)	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 ULN
Cholesterol high** (1) (mg/dL)	>ULN - 300; >ULN - 7.75	>300-400; >7.75-10.34	>400-500; >10.34-12.92

(mmol/L)			
Triglycerides - Hypertriglyceridemia [1]			
(mg/dL)	150 – 300;	>300 – 500;	>500 – 1000;
(mmol/L)	1.71 – 3.42	>3.42 – 5.70	>5.70 – 11.40

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GGT = gamma-glutamyl transferase; LLN = lower limit of the normal range; ULN = upper limit of the normal range.

Data Sources:

(1) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, U.S. Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research - Guidance for Industry.

(2) Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, U.S. Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research - Guidance for Industry.

* Those parameters that are not listed do not have grading categories in either the CTCAE or the FDA guidance documents and will therefore be reviewed by the Investigator and only reported as an AE if considered to be clinically relevant.

** Where parameters in this table are listed in both the CTCAE and the FDA guidance documents, and each document has different values within each grading category, the grading in CTCAE guidance document predominates.

ABNORMAL LABORATORY VALUES RATING: HEMATOLOGY PARAMETERS

Hematology*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin (Female) – (g/dL) [(1)]	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4
change from baseline value – (g/dL) [(1)]	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0
Hemoglobin (Male) – (g/dL) [(1)]	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4
change from baseline value – (g/dL) [(1)]	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0
Hemoglobin increase – (g/dL) [(2)]	Increase in >0 – 2 above ULN or above baseline if baseline is above ULN	Increase in >2 – 4 above ULN or above baseline if baseline is above ULN	Increase in >4 above ULN or above baseline if baseline is above ULN
WBC Increase – (cell/mm^3) [(1)]	10,800 – 15,000	15,001 – 20,000	20,001 – 25,000
WBC Decrease - (cell/mm^3) [(2)]**	<LLN – 3000; <LLN – 3.0 x 10^{-9}/l	<3000 - 2000; <3.0 – 2.0 x 10^{-9}/l	<2000 - 1000; <2.0 – 1.0 x 10^{-9}/l
Lymphocytes Increase - (cell/mm^3) [(2)]	-	>4,000 – 20,000	>20,000
Lymphocytes Decrease - (cell/mm^3) [(2)]**	<LLN – 800; <LLN – 0.8 x 10^{-9}/l	<800 - 500; <0.8 – 0.5 x 10^{-9}/l	<500 - 200; <0.5 – 0.2 x 10^{-9}/l
Neutrophils Decrease - (cell/mm^3) [(2)]**	<LLN – 1500; <LLN – 1.5 x 10^{-9}/l	<1500 - 1000; <1.5 – 1.0 x 10^{-9}/l	<1000 - 500; <1.0 – 0.5 x 10^{-9}/l
Eosinophils - (cell/mm^3) [(1)]	650 – 1500	1501 - 5000	>5000
Platelets Decrease - (cell/mm^3) [(2)]**	<LLN – 75,000; <LLN – 75.0 x 10^{-9}/l	<75,000 – 50,000; <75.0 – 50.0 x 10^{-9}/l	<50,000 – 25,000; <50.0 – 25.0 x 10^{-9}/l

Abbreviations: LLN = lower limit of the normal range; ULN = upper limit of the normal range; WBC = white blood cell.

Data Source:

(1) Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, U.S. Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research - Guidance for Industry.

(2) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

* Those parameters that are not listed do not have grading categories in either the CTCAE or the FDA guidance documents and will therefore be reviewed by the Investigator and only reported as an AE if considered to be clinically relevant.

** Where parameters in this table are listed in both the CTCAE and the FDA guidance documents, and each document has different values within each grading category, the grading in CTCAE guidance document predominates.

ABNORMAL LABORATORY VALUES RATING: URINALYSIS PARAMETERS

Urine*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Protein** (1)	1+ proteinuria; urinary protein <1.0 g/24 hours	2+ proteinuria; urinary protein 1.0-3.4 g/24 hours	Urinary protein ≥3.5 g/24 hours
Glucose (2)	Trace	1+	2+
Blood – Hematuria ** (1)	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; urinary catheter or bladder irrigation indicated; limiting instrumental ADL	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective endoscopic, radiologic or operative intervention indicated; limiting self-care ADL

Abbreviations: ADL = activities of daily living; IV = intravenous.

Data Source:

(1) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

(2) Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, U.S. Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research - Guidance for Industry.

* Those parameters that are not listed do not have grading categories in either the CTCAE or the FDA guidance documents and will therefore be reviewed by the Investigator and only reported as an AE if considered to be clinically relevant.

** Where parameters in this table are listed in both the CTCAE and the FDA guidance documents, and each document has different values within each grading category, the grading in CTCAE guidance document predominates.

Appendix 7 Procedure for Nasal Epithelial Collection

Materials to prepare ahead before sample collection:

- 2 Cyto-Pak soft brushes (Item #CP-5B, Medical Packaging Corporation)
- 2 of 2-mL round bottom microfuge tube with cap, sterile (Fisher Scientific Catalog # 02-681-375), containing: 1mL RNAprotect Cell Reagent (Qiagen Catalog #76526). These tubes are labeled “a” and “b” for first and second sampling, respectively
- Nasal speculum (Bionix # 9877, Bionix Medical Technologies, Toledo, OH)
- 1% Lidocaine Solution may be administered at participant request using a syringe with removable needle, which would be removed prior to use.
- RNaseZap (Ambion Catalog # AM9780; or AM9782) to decontaminate equipment and working area before handling the biological material.
- Tube rack
- Tube labels
- Wire cutter
- 70% isopropyl alcohol wipes (Fisher Scientific Catalog # 19-015-744)
- Liquid nitrogen or a quick access to -80^{o}C storage

Protocol for the preparation of the nasal collection:

1. Ask subject to blow his/her nose
2. Place the two of 2-mL round bottom microfuge tubes filled with 1 mL RNAprotect Cell Reagent in the tube.
3. Treat the wire cutter with few sprays of RNaseZap, wipe the cutter with paper towels, then wipe the cutter with 2 isopropyl alcohol wipes
4. If the subject wishes to have the nostril numbed for the procedure, use a 1% Lidocaine Solution.

   A. Draw 1 mL of Lidocaine into a syringe with a removable needle. Remove the needle once completed.

   B. Have the participant hold a paper towel under his/her nose.

   C. Have the participant verbally and rapidly repeat the letter "K" over and over again. Explain that this helps prevent the Lidocaine from getting into the throat, which can feel/taste unpleasant.

D. While the participant is saying the letter "K", quickly administer the Lidocaine into the nostril against the outside of the nose, which is opposite the septum.

Protocol of the nasal collection:

1. Use the nasal speculum to widen the left nostril then locate the inferior turbinate (a pen light may help to visualize the area
   1.1. While the speculum still widening the nostril, insert the Cyto-Pak soft brush into the nostril just past the inferior turbinate and press against the outside of the nostril (opposite from the septum) while being rotated. This step should take approximately 3 seconds
   1.2. Remove the Cyto-Pak soft brush from the nostril and immediately place the brush head in the 2-mL round bottom microfuge tube filled with 1 mL RNAprotect Cell Reagent (tube “a” for the first collection)
   1.3. Cut off the shaft of the brush with the cleaned wire cutter
   1.4. Repeat steps 1.1.-1.3. with the second Cyto-Pak soft brush (in the same nostril) for the second collection that will be collected in tube “b”
2. Vortex sample to ensure that all collected cells come into contact with the RNAprotect Cell Reagent
3. Store samples at -80^{o}C

Note: All samples should not undergo any freeze-thaw cycle prior to shipping.

Appendix 8 Procedure for Buccal Collection

Material required:

- Cytobrush Plush®GT Sterile (Cooper Surgical # C0112)
- 2 of 2-mL round bottom microfuge tube with cap, sterile (Fisher Scientific Catalog # 02-681-375), containing 1 mL RNALater (Qiagen Catalog # 76104). These tubes are labeled "a" and "b" for left and right cheek, respectively
- Tube rack
- Tube labels
- RNaseZap (Ambion Catalog # AM9780; or AM9782) to decontaminate equipment and working area before handling the biological material.
- 2 pair of scissors (autoclaved to sterilize then subsequently wiped with RNaseZap, see detailed protocol below as water vapor in most autoclaves contains RNases)
- 70% isopropyl alcohol wipes
- Liquid nitrogen or a quick access to -80°C storage

Protocol for the preparation of the buccal collection:

1. Ask subject to rinse their mouths with a minimum of 20 mL of water before the sample collection
2. Treat the scissors with few sprays of RNaseZap®, wipe the cutter with paper towels, then wipe the cutter with 2 isopropyl alcohol wipes
3. Place the two of 2-mL round bottom microfuge tubes ("a" and "b" filled with RNALater® reagent) in the tube rack.

Protocol of the buccal collection (two samples will be collected from each subject, one from each side of the inner cheek):

1. Collect buccal sample using sterile Cytobrush Plush®GT Sterile by insert the brush into the mouth
2. Applied sufficient lateral pressure to make contact with the buccal mucosa and bend the plastic shaft of the brush slightly (left cheek first, then right cheek)
3. Spin the brush in place, for 10 seconds in a single direction, while applying consistent pressure to the mucosa
4. Withdraw the brush from the cheek

5. Immediately place the brush head in the 2-mL round bottom microfuge tube filled with 1 mL RNALater reagent (tube “a” first, then tube “b”)
6. Cut off the shaft of the brush with the sterile scissor
7. Repeat steps 1-6 with the second Cytobrush Plush®GT Sterile (for the other side of the cheek)
8. Vortex sample to ensure that all collected cells come into contact with the RNALater reagent

Store samples at -80°C until shipment.

Note: All samples should not undergo any freeze-thaw cycle prior to shipping.