- Template (or similar) language highlighted in TURQUOISE refers to specific procedures that are not common to ALL our studies (e.g., smoking topography). Include only when such procedure(s) utilized.
- **Red text** indicates substantive changes from previous CE short-term studies.
- *Language highlighted in YELLOW is changed from last CE review, mostly based on feedback from MDS and Legal, and needs final approval by CE (MDS note that some of this will also be a change from what MDS reviewed, based on interval Legal review.)*

A RANDOMIZED, CONTROLLED STUDY COMPARING THE SHORT TERM EXPOSURE TO SMOKE CONSTITUENTS OF *(investigational product)* TO *(reference product)* IN ADULT SMOKERS DURING CONTROLLED SMOKING

Philip Morris USA Study No.
MDS Pharma Services Project No.
MDS Pharma Services Protocol No.

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(Signature) (Date)

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e.g.	For example (Latin: *exempli gratia*)
i.e.	That is to say (Latin: id est)
Inc.	Incorporated
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNAL-glucuronides	[4-(methylnitrosamino)-1-(3-pyridyl) but-1-yl]-β-N-D-glucosiduronic acid and [4-(methylnitrosamino)-1-(3-pyridyl) but-1-yl]-β-O-D-glucosiduronic acid
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
CLIA-88	Clinical Laboratory Improvement Amendments of 1988
CO	Carbon monoxide
COHb	Carboxyhemoglobin

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1. INTRODUCTION

1.1 Smoking-Related Disease

In 2002, an estimated 45.8 million adults in the United States were current smokers.[1]

Scientific evidence of the harm to smokers from tobacco use has accumulated over decades. The medical and scientific consensus is that cigarette smoking causes lung cancer, heart disease, emphysema, and other serious diseases in smokers.[2] Smokers are far more likely to develop serious diseases, like lung cancer, than non-smokers (USDHHS 1989, Nelson et al. 1994).

1.2 Principles of Harm Reduction

Given the established relationship of smoking and various disease conditions, it would seem desirable to reduce the risk of these diseases by avoiding smoking altogether or stopping smoking as soon as possible. However smoking behavior is a complex psychological, sociological, and physical event that may be very difficult for some individuals to overcome. Fewer than 10% of smokers who attempt to quit each year succeed.[2] Although the goal for most smokers is to achieve a minimum risk of smoking-related diseases through smoking cessation, for some individuals a reduction in risk while continuing to smoke may be a possible alternative.

1.3 Biomarkers Related to Smoking

The number of chemical compounds to which a smoker is exposed when smoking a cigarette has been estimated as 4,800 (Green and Rodgman 1996). The rate and amount of exposure to these chemicals are complex functions of cigarette composition and design, rate of smoking, burn temperature, and many other factors (Baker 1999). The relative delivery of mainstream smoke constituents (e.g. nicotine and tar) of different cigarettes is typically determined in smoke generated by machines operated under defined smoking regimens [e.g. the Federal Trade Commission (FTC) method] (Federal Trade Commission 1997). Tobacco smoke uptake by smokers is not only influenced by cigarette composition and design, but also by many smoker dependent parameters [e.g. ventilation hole blocking (Baker and Lewis 1997), puffing patterns, and number of cigarettes smoked (Bridges et al. 1990, Hofer et al. 1991)]. Therefore, it is difficult to

establish dose-effect relationships for toxic smoke constituents in humans, as the dose/uptake is difficult to predict.

Studies suggest that machine-derived smoke composition data are not suitable quantitative measures of smoker exposure to smoke constituents (National Cancer Institute Monograph No.13, 2001). In this study, to determine the exposure of adult smokers to cigarette smoke, a direct evaluation of the levels of smoke constituents or their metabolites (i.e., biomarkers) in appropriate body fluids will be performed. Biomarkers can be organized into three categories as defined below.[2] Some biomarkers may overlap two or more of these categories.

Biomarker of exposure (BOE): A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose.

Biomarker of biologically effective dose (BED): The amount that a tobacco constituent or metabolite binds to or alters a macromolecule; estimates of the BED might be performed in surrogate tissue.

Biomarker of potential harm (BOPH): A measurement of an effect due to exposure; these include early biological effects, alterations in the morphology, structure, or function, and clinical symptoms consistent with harm; also includes "preclinical changes".

1.4 Rationale for Biomarkers Measured

The biomarkers in this study, listed below, were selected based on the following criteria (Benowitz 1999):

- Unique or nearly unique to tobacco smoke.
- Representative of particulate and gas/vapor phase tobacco smoke.
- Representative of health-relevant tobacco smoke constituents.
- Constituent metabolism understood.
- Concentration reflective of uptake of cigarette smoke constituent(s).
- Validated, sensitive and reliable analytical methods available.
- Sampling to acquire material for analysis only minimally invasive.

In addition, levels of the selected biomarkers have been shown to differ between adult smokers and non-smokers, and the majority change rapidly after smoking cessation.

Biomarkers of exposure:

- Nicotine and five of its metabolites (nicotine equivalents) in urine provide a measure of the amount of nicotine that enters the body.[3]

- NNK metabolites (i.e., NNAL and NNAL-glucuronides) in urine provides an estimate of NNK, a tobacco-specific nitrosamine [TSNA]).[4]

- 3-hydroxypropylmercapturic acid (3-HPMA) in urine provides an estimate of the allylic group found in acrolein (a toxic unsaturated aldehyde found in cigarette smoke).[5]

- 1,3-butadiene metabolite (i.e., monohydroxybutenyl mercapturic acid [MHBMA]) in urine provides a measure of exposure to 1,3-butadiene.

- *S*-Phenylmercapturic acid (*S*-PMA), a urinary metabolite derived from the conjugation of benzene epoxide with glutathione, provides a measure of exposure to benzene.

- Urinary metabolites of the noncarcinogenic polycyclic aromatic hydrocarbon (PAH) pyrene, total 1-hydroxypyrene (1-OHP), have been used as surrogate indicators of total PAH exposure. Total 1-OHP levels are generally higher in smokers' urine than in nonsmokers' urine.[6]

- Urine mutagenicity as measured by the Ames test is elevated in smokers.[7]

Biomarker of biologically effective dose:[2]

- Carboxyhemoglobin (COHb) is produced from the interaction of hemoglobin and carbon monoxide and results in decreased oxygen-carrying capacity of the blood. Carboxyhemoglobin is elevated in smokers.[8, 9]

1.5 Purpose of this Study

The use of a PREP may reduce the smoker's exposure to some of the chemical compounds to which the smoker is exposed when smoking in comparison to smoking a conventional cigarette. Philip Morris USA has developed *(PM USA: per Sec. 6.1, describe the investigational product, minimizing any confidential, proprietary details).*

The current study was designed to assess selected biomarkers in adult smokers smoking *(investigational product)* as a PREP under controlled smoking conditions (i.e., predetermined smoking times and an individually determined maximum number of cigarettes per day based on reported smoking history). Smoking behavior will be evaluated in the *(investigational product)* smoking group through the duration of the study.

In addition, smoking topography in all smokers will be measured twice daily to assess puffing behavior (number of puffs, puff volume, puff duration, inter-puff interval, and peak flow). Puffing profiles will be compared to quantify compensatory behavior to determine if the *(investigational product)* group alters puffing behavior when compared with *(reference product)* groups.

2. STUDY OBJECTIVES

2.1 Primary Objective

- To assess changes in *(list BOE, BED)* from Baseline to Days 6 and 7 in adult *(reference product)* smokers who have been randomly assigned to continue smoking *(reference product)*, to stop smoking or to switch to *(investigational product)*.

2.2 Secondary Objectives

- To assess in adult *(reference product)* smokers who have been randomly assigned to continue smoking *(reference product)*, to stop smoking or to switch to *(investigational product)*.
  - changes in *(list BOE, BED)* from baseline to Days 6 and 7.
  - changes in *(list BOE, BED)* from baseline to Days 1 through 5 and from baseline to Day 8.
  - changes in smoking behavior as evaluated by smoking topography and questionnaires.

3. SUMMARY OF STUDY DESIGN

3.1 Design

This research study will utilize a randomized, open-label, controlled, forced-switching, parallel design conducted at a single research center. Up to *(N)* self-affirmed *(reference product* or *tar range)* adult smokers with a daily cigarette consumption of 10 to 30 cigarettes/day will be initially enrolled (with neither gender constituting more than 60% of the total) into the Acclimation/Baseline determination phase (Days –3, –2, -1) to better ensure that at least *(N)* healthy, adult male and female smoking subjects are available for randomization into one of *(N)* parallel groups with *(N)* subjects in the *(reference product)* group, *(N)* subjects in the *(reference product)* group, *(N)* subjects in the *(investigational product)* group, and *(N)* subjects in the No-Smoking group.

Beginning on the morning of Day 1 and continuing through the end of Day 8, the subjects will participate in one of the following groups:

Group	Smoking (Days 1 through 8)	No. of Subjects
x	*(reference product)*	*(N)*
x	*(investigational product)*	*(N)*
x	No-Smoking	*(N)*

Members of each group will be randomized based on gender (male and female) and daily cigarette consumption (10-19 cigarettes/day and 20-30 cigarettes/day) on Day -3.

Smoking will only be permitted at designated smoking times throughout the study. Smoking will not be permitted from 2300 to 0700 during the study. Controlled smoking conditions (i.e., predetermined smoking times and an individually determined maximum number of cigarettes per day based on reported smoking history) were selected in order to reduce the variability of exposure and assure a range of different exposures in order to measure possible dose effects. Controlled smoking allows evaluation of the cigarette design and related smoking behavior excluding possible compensation by number of cigarettes smoked. For study integrity, all butts and smoked cigarettes (*include this term only* ***if*** *EHCSS* used) will be collected throughout the study. This will ensure that subjects do not have access to partially smoked cigarettes at any time during the study.

3.2 Clinical Procedures

Up to *(N)* subjects will be confined to the clinic on the evening of Day –4. At the time of check-in, the subjects will be required to give all of their cigarettes to the study staff. The subjects will be permitted to smoke *(reference product)* upon request to the study staff until 2300. On the morning of Day –3 beginning at 0700, the subjects will be monitored for cigarette consumption to determine their daily allotment of cigarettes for the remainder of the study. This Acclimation Day will be used to determine the maximum daily cigarette consumption permitted for each subject in the smoking groups during the study. Each smoking group will smoke according to a controlled regimen that is consistent with the total number of cigarettes they usually smoke in a day. All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff. During the Acclimation Day, each subject will be permitted to smoke up to 2 cigarettes more than the subject's usual daily maximum number according to the subject's smoking history obtained during subject recruitment and verified by the smoking history obtained at Screening. (Note: During the Acclimation Day, the maximum number of cigarettes permitted for subjects with a smoking history of 29 to 30 cigarettes/day will be 30 cigarettes/day.)

On subsequent study days, the maximum daily number of cigarettes for each subject in the smoking groups will be limited to the number actually smoked on the Acclimation Day. On each day, these subjects will be permitted to smoke up to their daily allotment of cigarettes at specified smoking opportunities offered at equal intervals between 0700 and 2300. On each day after the Acclimation Day, the total daily cigarettes smoked by each subject will be evenly divided over three periods during each day (0700 to 1219, 1220 to 1739, and 1740 to 2300). For example, a subject who smokes 15 cigarettes per day would be permitted to smoke 5 cigarettes per period. A subject who smokes 10 cigarettes per day would be permitted to smoke 3 cigarettes per period with one extra in the period of choice. All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff.

No subject in any group will be forced or required to smoke by the study staff at any time during the study, and a subject may quit smoking at any time.

Beginning on the morning of Day –2 and continuing through Day -1, Baseline determinations will be conducted. *(N)* subjects will be randomized

just prior to Day 1 into one of the *(N)* groups. Subjects will continue to smoke *(reference product)* at the level determined from the Acclimation Day through the end of Day -1. Beginning on Day 1, the subjects will participate within their randomization group for the remainder of the study. Each randomized group will be assigned to separate living quarters during the daily 0700 to 2300 smoking interval. No smoking will be allowed during meals. All cigarette smoking on Days -2, -1, 6, and 7 should occur indoors. All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff.

3.4 Urine Collection

Beginning on the morning of Day –2, all urine voided by members of each group will be collected in 24-hour intervals (approximately 0700 to 0700) until completion of the study, and the total weight of each 24-hour collection for each subject will be recorded. See Attachment A for complete details.

3.5 Biomarkers

Biomarkers of exposure:

- *(list)*

will be measured in 24-hour urine collections at Baseline and at selected days during the study (see Summary of Events Table).

Biomarker estimating biologically effective dose (BED):

- *(list)*

will be measured in blood samples at Baseline and at selected days during the study (see Summary of Events Table).

3.6 Smoking Topography *(This schedule is just an example: customize procedure to be study-specific as needed.)*

On Day –3, all subjects will be trained in the use of the Clinical Research Support System *Micro* (CReSSmicro™) portable measurement device manufactured by Plowshare® Technologies which will be used to gather smoking topography (*i.e.*, number of puffs, puff volume, puff duration, inter-puff interval, and peak flow) during the study. The subjects will

smoke one or more cigarettes using this device on Day –3 to assure proper training on this device.

All subjects will smoke the first cigarette of the day and the first cigarette after lunch using the Clinical Research Support System Micro (CReSSmicro™) portable measurement device at Baseline (Days –2 and -1). On Days 1 through 8, subjects in the smoking groups will smoke the first cigarette of the day and the first cigarette after lunch using the Plowshare device to gather smoking topography.

Each subject will be assigned one topography device for the study duration. No labels of any kind should be affixed to the device without the prior consent of Plowshare® Technologies.

Smoking profiles will be compared to determine whether or not adults will likely smoke the *(investigational product)* cigarettes in the same manner as they do *(reference product)* cigarettes.

3.7 Questionnaires

All subjects will complete the *Smoking History Questionnaire* at Screening. All subjects will complete the *Diet* and *Work Environment Questionnaires* at check-in (Day –4). All subjects will complete the *Fagerström Test for Nicotine Dependence*[27] at Screening. All subjects will complete the *Product Assessment Questionnaire* on Days -2 and -1. All subjects in the *(investigational product)* group will complete the *Product Assessment Questionnaire* on Days 1 through 8.

3.8 Clinical Examinations

The participants will be evaluated clinically with physical examinations, vital signs measurements, spirometry, and electrocardiograms.

During the study, blood samples collected for scheduled clinical laboratory tests and biomarkers will require approximately *(N)* mL of blood from each subject.

4. SUBJECT SELECTION

4.1 Source of Subjects

4.1.1 Adult subjects who are members of the community at large will be enrolled in this study.

4.1.2 Up to *(N)* subjects will be initially enrolled into the Acclimation period (with neither gender constituting more than 60% of the total) to better ensure that *(N)* subjects are available for randomization into 1 of *(N)* study groups.

4.2 Inclusion Criteria

4.2.1 Healthy smoking adult male and female subjects 21 to 65 years of age.

4.2.2 Smoking history of 10 to 30 cigarettes per day, for at least *(N)* months prior to Day -2, of manufactured *(non-menthol)* cigarettes with a tar content of *(N)* mg (FTC). Brief periods (i.e., up to 7 consecutive days) of non-smoking during the 3 months to 4 weeks prior to Day -2 (e.g., due to illness, trying to quit, participation in a study where smoking was prohibited) will be permitted at the discretion of the Investigator.

4.2.3 Smoked *(reference product)* as their exclusive brand (i.e., no other brands) without interruption (i.e., no brief periods of non-smoking) for at least 4 weeks prior to Day -2.

4.2.4 Female subjects who are heterosexually active and of childbearing potential (*i.e.*, not surgically sterile [bilateral tubal ligation, hysterectomy, or bilateral oophorectomy at least 6 months prior to Day -2] or at least 2 years naturally postmenopausal) must have been using one of the following forms of contraception and agree to continue using it through completion of the study:

- hormonal (*i.e.*, oral, transdermal patch, implant, or injection) consistently for at least 3 months prior to Day –2,
- double barrier (*i.e.*, condom with spermicide or diaphragm with spermicide) consistently for at least 2 weeks prior to Day –2,
- IUD for at least 3 months prior to Screening, or have a partner who has been vasectomized for at least 6 months prior to Day –2.

Female subjects of childbearing potential (*i.e.*, not surgically sterile [bilateral tubal ligation, hysterectomy, or bilateral oophorectomy at

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least 6 months prior to Day –2] or two years naturally postmenopausal) who are not currently engaging in heterosexual intercourse must agree to use one of the above methods of birth control, in the event that they have heterosexual intercourse during the course of the study.

4.2.5 Voluntary consent to participate in this study documented on the signed ICF.

4.3 Exclusion Criteria

Subjects may be excluded from the study if there is evidence of any of the following criteria at Screening, at Acclimation/Baseline, or at any time during the study as appropriate. Exceptions may be permitted at the discretion of the Investigator in consultation with the Sponsor providing there would be no additional risk involved for the subject. Any exceptions will be documented.

4.3.1 History of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, urologic, pulmonary, immunologic, psychiatric, or cardiovascular disease, or any other condition that, in the opinion of the Investigator, would jeopardize the safety of the subject or impact the validity of the study results.

4.3.2 Clinically significant abnormal findings on the physical examination, medical history, or clinical laboratory results, in the opinion of the Investigator.

4.3.3 Use of nicotine-containing product other than manufactured cigarettes (*e.g.,* roll-your-own cigarettes, bidis, snuff, nicotine inhaler, pipe, cigar, chewing tobacco, nicotine patch, nicotine spray, nicotine lozenge, or nicotine gum) within 4 weeks prior to Day –2 or during study.

4.3.4 Self-reported puffers (*i.e.,* smokers who draw smoke from the cigarette into the mouth and throat but do not inhale).

4.3.5 History of drug or alcohol abuse within 12 months of Day –2.

4.3.6 Diabetes mellitus that is not controlled by diet/exercise alone, in the opinion of the Investigator.

4.3.7 Current evidence or any history of congestive heart failure.

4.3.8 Female subjects must not be pregnant, lactating, or intend to become pregnant from Screening through completion of study.

4.3.9 An acute illness (*e.g.*, upper respiratory infection, viral infection) requiring treatment within 2 weeks prior to Day –2.

4.3.10 Fever (>100.2°F) at Screening or at check-in (Day -4).

4.3.11 BMI greater than 40 kg/m^2 or less than 18 kg/m^2 at Screening.

4.3.12 FVC or FEV_1 <75% of predicted at Screening.

4.3.13 Clinically significant ECG abnormalities, in the opinion of the Investigator.

4.3.14 Hemoglobin < 12.0 grams/dL at Screening.

4.3.15 Carboxyhemoglobin < 1.5% at Screening or at check-in (Day -4).

4.3.16 Serum creatinine >1.3 mg/dL (females) or >1.5 mg/dL (males).

4.3.17 Liver enzymes ≥ 1.5 times the upper limit of normal.

4.3.18 Positive urine screen for alcohol or drugs of abuse at Screening or at check-in (Day -4).

4.3.19 Plasma donation within 7 days prior to Day –2.

4.3.20 Donation (standard donation amount or more) of blood or blood products (with the exception of plasma as noted in 4.3.19) or receipt of a whole blood or blood product transfusion within 8 weeks prior to Day –2.

4.3.21 Use of prescription anti-diabetic medication and/or insulin therapy within 12 months of Day -2.

4.3.22 Use of prescription or over-the-counter bronchodilator medication (*e.g.*, inhaled or oral β-agonists) within 12 months of Day –2.

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4.3.23 Use of antibiotic treatment within 2 weeks prior to Day –2.

4.3.24 Participation in a previous clinical study for an investigational drug, device, or biologic within 30 days prior to Day –2.

4.3.25 Subject or a first-degree relative (*i.e.*, parent, sibling, child) is a current or former employee of the tobacco industry or a named party or class representative in litigation with Philip Morris USA.

4.3.26 Subject or a first-degree relative (*i.e.*, parent, sibling, child) is a current employee of MDS Pharma Services.

4.3.27 History of sensitivity or allergy to caffeine. *(Include only if phenotyping w/ caffeine being performed in the study)*

4.4 Restrictions

4.4.1 No foods or beverages containing alcohol for 48 hours prior to check-in (Day –4).

4.4.2 No foods or beverages containing grapefruit for 7 days prior to check-in (Day –4) and during the study.

4.4.2 No strenuous exercise for 48 hours prior to check-in (Day –4) and during the study.

4.4.3 Subjects must have smoked *(reference product)* as their exclusive brand (i.e., no other cigarette brands) for 4 weeks prior to Day –2.

4.4.4 Smoking will only be permitted at designated smoking times throughout the study. No smoking will be permitted from 2300 to 0700 nor during meals during the study. All cigarette smoking on Days -2, -1, 6, and 7 should occur indoors.

4.5 Medications

Stable doses (*i.e.*, no dosage adjustments within 30 days prior to Day –2) of prescription or over-the-counter medications required to treat an Investigator-approved disease or condition (e.g., hypertension, seasonal rhinitis) are permitted. Hormonal contraceptives (*e.g.*, oral, transdermal patch, implant, injection) and hormonal replacement therapy are permitted.

Occasional use of over-the-counter analgesics (*e.g.*, acetaminophen, ibuprofen), antacids, H_2-antagonists, antihistamines, and nasal decongestants are permitted. Exceptions may be permitted at the discretion of the Investigator in consultation with the Sponsor, providing the medication in question would have no impact on the study. Any exceptions will be documented.

5. SCREENING

The Investigator or his/her designee must assess the following items during the interval Day -32 through Day -5.

5.1 Informed Consent

All prospective subjects will have the study explained by the Investigator or his/her designee.

All prospective subjects will be required to read, sign and date the study informed consent prior to any Screening/study procedures being performed. Written acknowledgment of the receipt of the full informed consent and the subject's freely tendered offer to participate will be obtained from each subject in the study and documented in the source documents. Each subject will receive a signed and dated copy of each informed consent form (ICF).

5.2 Medical History/Demographic Data

After the ICF is signed, medical history and demographic data, including name, sex, age (each subject must show proof of age with government-issued photo ID, e.g., driver's license), race, and tobacco use/history will be recorded for each subject.

5.3. Physical Examination

5.3.1 Height (in inches) and weight (in pounds) in indoor clothing with shoes off. Body mass index (BMI) will be calculated as weight (kg)/height (meters) squared.

5.3.3 Vital signs (respiratory rate, heart rate, blood pressure, oral temperature) in the sitting position after at least 10 minutes of rest and at least 15 minutes after the last cigarette smoked.

5.3.4 General observations and questioning by the Investigator or his/her designee (licensed physician).

5.3.5 12-Lead ECG.

5.3.7 Pulmonary function testing (FVC, FEV_1) in the standing position and according to the 1995 ATS Standardization of Spirometry Guideline.[y] Spirometry values for FVC and FEV_1 will be determined from at least three consistent efforts using KoKo Spirometry software. The best effort for each parameter will be recorded.

5.4 Clinical Laboratory Tests

All clinical laboratory tests will be conducted by a laboratory facility accredited by the Centers for Medicare and Medicaid Services (Clinical Laboratory Improvement Amendments of 1988 [CLIA-88]), MDS Pharma Services Clinical Laboratory, 621 Rose Street, Lincoln, Nebraska, 68502. Values for the laboratory parameters are to be within the normal ranges as determined by MDS Pharma Services Clinical Laboratory, Lincoln, NE. Subjects with Screening laboratory values outside the normal limits will be accepted into the study only after the Investigator or his designee (a physician) has determined that the abnormal values are "not clinically significant."

5.4.1 Hematology, consisting of hemoglobin, hematocrit, RBC, WBC with differential, and platelet count.

5.4.2 Serum chemistry, consisting of sodium, potassium, chloride, bicarbonate, ALT, AST, BUN, alkaline phosphatase, total bilirubin, glucose, creatinine, total protein, uric acid, and albumin.

5.4.3 Routine clinical urinalysis consisting of bilirubin, blood, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, and urobilinogen will be evaluated. Microscopic examination will be conducted if protein, leukocytes, nitrite and/or blood is detected. Microscopic analysis will include RBC, WBC, casts, and bacteria.

5.4.4 HIV antibody, hepatitis B surface antigen (HbsAg), and hepatitis C antibody (HCV) screens.

5.4.5 COHb drawn between 1500 and 1900 at the screening physical examination (repeated at check-in [Day –4]).

5.4.6 Serum pregnancy test for females (repeated at check-in [Day –4]).

5.4.7 Urine screen for ethanol, amphetamines, opiates, cannabinoids, and cocaine at Screening and check-in (Day –4)

6. MATERIALS

6.1 Study Smoking Supplies

All cigarettes will be supplied by the Sponsor. Production lot number and date of manufacturing (to be provided by Sponsor) will be documented in the study file.

Comparative Product:

(reference product) (PM USA to provide smoke machine yields as published on PM USA website for mg tar, mg nicotine, mg CO [FTC] *and COMPLETE product descriptive details*)

Investigational Product:

(investigational product) (PM USA to provide smoke machine yields for mg tar, mg nicotine, mg CO [FTC] *and COMPLETE product descriptive details but minimizing any confidential, proprietary product details*)

Lighters:

Gas lighters (blue flame type) will be provided and controlled by the clinical site. Subjects will be instructed to use these lighters exclusively for lighting *(reference product)* and (*investigational product)* cigarettes during the study.

All cigarette products will be stored in a locked, limited-access area in the clinic site pharmacy under temperature- and humidity-controlled indoor conditions. For 3 days prior to dispensing, cigarette products will be stored in a locked, limited-access humidor at pre-specified temperature and humidity. A daily allotted amount of cigarettes for each subject in the smoking groups will be transferred into the study unit in subject-specific,

bar-coded containers for use at the designated smoking times. Any necessary handwritten markings on cigarette containers or container labels should be written with a grease pencil only unless otherwise pre-approved by the PM USA Study Manager. When handling or dispensing cigarettes, site personnel should wear gloves.

6.2 Study Materials Accountability

The pharmacy staff at the site will coordinate shipping of all cigarettes for the study with the Sponsor. The pharmacy will document the date each shipment was received and recorded in the inventory records. The site pharmacy will document and reconcile the total number of cigarette packs shipped to the site, the total number of cigarette packs dispensed during a study, and the total number of unopened packs remaining at the end of a study's clinical conduct. The site pharmacy will pull one unopened carton of each study cigarette from the first shipment and store it at the site until PM USA reviews the final study report.

The daily allotment of study smoking materials for each subject will be maintained in a locked area on the study unit until required for use at the designated smoking times. Individual cigarette dispensing records will be maintained for each subject. The site study staff will document in the ClinQuick™ system each cigarette dispensed. At the end of each 24-h interval, the number of cigarettes dispensed by the study staff to each subject, as recorded in the ClinQuick™ system, will be considered the number of cigarettes smoked for that day (cpd). No other reconciliation will be done. The site pharmacy may then destroy any unused loose cigarettes for that 24-h interval according to the site's Standard Operating Procedure for drug destruction and complete a Certificate of Destruction.

After statistical database lock occurs and after monitoring of cigarette accountability is completed, the site will destroy any unused cigarettes (except for the one reserved carton) according to its Standard Operating Procedure for drug destruction and the PM USA Clinical Evaluation Clinical Process *"Destruction of unused study cigarettes"* and complete a Certificate of Destruction.

6.3 Subject Randomization

MDS Pharma Services will prepare the randomization schedule. A total of up to *(N)* subjects will be initially enrolled in the study (with neither gender constituting more than 60% of the total). Following the Acclimation Day

(Day –2) to determine each subject's maximum permitted daily cigarette consumption, *(N)* subjects will be randomly assigned to *(N)* groups with *(N)* subjects in Group x [(*reference product)* group), *(N)* subjects in Group x ((*investigational product)* group), and *(N)* subjects in Group x (No-Smoking group).

Subjects will be randomized into each group based on gender and cigarette consumption (10-19 cigarettes/day and 20-30 cigarettes/day) as determined from Acclimation Day (Day –2). Treatments will be randomized in blocks of *(N)* subjects, consisting of 1 subject for *(reference product)*, *(N)* subjects for (*investigational product)*, and *(N)* subject for No-Smoking group. To ensure that treatments are as balanced as possible within each allocation group (i.e., gender and cigarette consumption), randomization order will be numbered consecutively within each allocation group. Randomization order numbers will be associated with randomized treatment. For example, within gender, the subjects in the 10 to 19 cigarettes/day group will be numbered starting with the first randomization order number for that group. Subjects in the 20 to 30 cigarettes/day group will be numbered starting with the next available number following the last subject in the 10 to 19 cigarettes/day group and ending with the last number assigned in that allocation group (up to *(N)*).

7. STUDY CONDUCT

The following study conduct procedures will be observed during the study as appropriate.

7.1 Check-in Procedures (Day –4)

7.2.1 Brief written assessment (medical/medication history) to affirm that the exclusion criteria/restrictions have not been violated since the Screening visit.

7.2.2 Brief physical examination and vital signs (respiratory rate, heart rate, blood pressure, oral temperature) in the sitting position after at least 10 minutes of rest and at least 15 minutes after the last cigarette smoked.

7.2.3 Urine sample for urinalysis and testing for alcohol and drugs of abuse.

7.2.4 Blood sample collection for clinical laboratory analysis (hematology, clinical chemistry) including a serum pregnancy test for females.

7.2.5 Blood sample collection between 1500 and 1900 for COHb.

7.3 Subject Confinement

The subjects will be admitted to the study unit in the evening of Day –4. They will remain in the unit until completion of all the study events at the end of Day 8.

7.4 Meal Schedule/Dietary Considerations

A standardized diet (*i.e.*, low mutagenicity, low PAHs) designed by a dietitian to minimize possible confounding influences for biomarkers assessed will be used. The following will not be permitted: broiled or pan-fried meat, pre-cooked meats (*e.g.*, tuna, ham, corned beef, smoked lunchmeats), bacon, and sausage.[7] These specially-designed meals will be served throughout the study according to a meal plan at the following approximate times:

Breakfast:	0800
Lunch:	1200
Dinner:	1700
Evening snack:	2000 to 2100

Subjects may not smoke during meals.

One full serving of each meal will be labeled, frozen and then stored for possible future analysis by the Sponsor. These frozen meals may be discarded when the Sponsor authorizes release of the final Study Report.

The same menu and approximate meal schedule will be administered uniformly to all subjects in all groups. The meal menu will be the same on Days –2, 6, and 8 and on Days –1 and 7.

Hard candies and gum will be available from 0700 through 2300 daily beginning on Day –3.

Consumption of water will be permitted ad lib during the study.

Consumption of caffeinated beverages will be permitted with meals or snacks during the study

Consumption of alcoholic beverages will not be permitted during the study.

7.5 Smoking Considerations

Beginning at 0700 on Day -3, the subjects will be permitted to continue smoking *(reference product)* and will be monitored for cigarette consumption to determine their daily allotment of cigarettes for the remainder of the study. This Acclimation Day will be used to determine daily cigarette consumption permitted for each subject in the smoking groups during the study. Each subject will smoke according to a controlled regimen that is consistent with the total number of cigarettes they usually smoke in a day. During the Acclimation Day, each subject will be permitted to smoke up to 2 cigarettes more than the subject's usual daily maximum number according to the subject's smoking history obtained during subject recruitment and verified by the smoking history obtained at Screening. (Note: During the Acclimation Day, the maximum number of cigarettes permitted for subjects with a smoking history of 29 to 30 cigarettes/day will be 30 cigarettes/day.) All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff.

On subsequent study days, the maximum daily number of cigarettes for each subject in the smoking groups will be limited to the number actually smoked on the Acclimation Day. On each day, these subjects will be permitted to smoke up to their daily allotment of cigarettes at specified smoking opportunities offered at equal intervals (approximately every 32 minutes) between 0700 and 2300. On each day after the Acclimation Day, the total daily cigarettes smoked by each subject will be evenly divided over three periods during each day (0700 to 1219, 1220 to 1739, and 1740 to 2300). For example, a subject who smokes 15 cigarettes per day would be permitted to smoke 5 cigarettes per period. A subject who smokes 10 cigarettes per day would be permitted to smoke 3 cigarettes per period with one extra in the period of choice.

All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff.

No smoking will be allowed during meals. All cigarette smoking on Days -2, -1, 6, and 7 should occur indoors. All cigarettes smoked by each subject from 0700 through 2300 will be documented by the study staff.

A RANDOMIZED, CONTROLLED STUDY COMPARING THE SHORT TERM EXPOSURE TO SMOKE CONSTITUENTS OF *(investigational product)* TO *(reference product)* IN ADULT SMOKERS DURING CONTROLLED SMOKING

Philip Morris USA Study No.
MDS Pharma Services Project No.
MDS Pharma Services Protocol No. 466-06

Sponsor's Contact:

MDS Pharma Servis Program Director:

MDS Pharma Services Project Manager:

VERSION:

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No subject in any group will be forced or required to smoke by the study staff at any time during the study, and a subject may quit smoking at any time.

On Days –2 and -1, each subject will continue to smoke *(reference product)* up to the maximum daily number determined on the Acclimation Day. Beginning at 0700 on Day 1, subjects will participate within their randomization group for the remainder of the study. Subjects randomized to the No-Smoking group will not be permitted to smoke after 2300 on Day -1.

Subjects will use only the blue flame gas lighters provided to light cigarettes. The lighting of a cigarette will be recorded as its start time.

Throughout the study, all smoked cigarettes and butts will be collected for study integrity and to ensure that subjects do not have access to partially smoked cigarettes at any time during the study. The return of a smoked cigarette or butt will be recorded as its stop time.

7.6 Baseline Investigations

From 0700 on Day –2 through 2300 on Day -1, Baseline investigations will be conducted on all subjects.

7.6 Smoking/No-Smoking Groups

Beginning at 0700 on Day 1, the subjects will participate within their randomization group for the remainder of the study.

The groups used in this study are:

Group	Smoking (Days 1 through 8)	No. of Subjects
x	*(reference product)*	*(N)*
x	*(investigational product)*	*(N)*
x	No-Smoking	*(N)*

7.7 Smoking Topography *sample language only*

Each subject will be assigned one topography device for the study duration. No labels of any kind should be affixed to the device without the prior consent of Plowshare® Technologies. Devices will be date/time-synchronized with the ClinQuick system prior to dispensing to subjects.

On Day –3, all subjects will be trained in the use of the Clinical Research Support System *Micro* (CReSSmicro™) portable measurement device manufactured by Plowshare® Technologies which will be used to gather smoking topography (*i.e.*, number of puffs, puff volume, puff duration, inter-puff interval, and peak flow) during the study. The subjects will smoke one or more cigarettes using this device on Day –3 to assure proper training. The interval between inserting the cigarette into the topography device ("new cig" time recorded on the device) and lighting the cigarette (recorded in ClinQuick as the cigarette start time) should be less than 10 minutes.

All subjects will smoke the first cigarette of the day and the first cigarette after lunch using the Clinical Research Support System Micro (CReSSmicro™) portable measurement device at Baseline (Days –2 and -1). On Days 1 through 8, subjects in the smoking groups will smoke the first cigarette of the day and the first cigarette after lunch using the CReSSmicro™ device to gather smoking topography.

7.8 Blood Sampling for Biomarkers

The blood sampling procedure should not interrupt a cigarette being smoked by the subject.

7.8.1 Carboxyhemoglobin

A 5 mL blood sample for COHb analysis will be drawn in a sodium heparin (green top) vacutainer tube at 1900 ± 30 minutes on Days –2 and -1 (Baseline), 1, 2, 3, 4, 5, 6, 7, and 8 (*[N]* mL of blood total).

Immediately following collection the blood sample will be gently inverted to mix. The whole blood samples will be properly labeled and stored at ambient temperature until shipped for analysis

The subjects will have approximately *(N)* mL of blood collected during the study for biomarker analysis and approximately *(N)* mL of blood for the Screening, check-in (Day –4), and Day 8 clinical laboratory evaluations

including a serum pregnancy test and carboxyhemoglobin at Screening and check-in (Day –4).

7.9 Urine Collection for Biomarkers/Analyte

All urine voided by each subject will be collected from approximately 0700 to 0700 on Days –2 through 8 and refrigerated at 2 to 8°C. The total weight of each 24-h collection will be recorded for each subject. The site study staff will flag 24-h collections < 500 g as suspect and query the subject as to its completeness (*i.e.*, full 24 hours), document the subject's response, and review with the subject proper collection of complete 24-h urine (see Attachment A).

The following urine biomarkers and analyte will be analyzed in each group as outlined below:
This is an EXAMPLE; actual will be study-specific.

All Groups		
Urine Biomarker/Analyte	**Number of Aliquots/ Aliquot Size Required**	**Study Day(s)**
A1. Nicotine equivalents	2 aliquots of 5 mL each	-2 through 8
A3. Total 1-OHP	2 aliquots of 5 mL each	-2, -1, 6, 7
A4. 1,3-butadiene metabolite (MHBMA)	2 aliquots of 5 mL each	-2, -1, 6, 7
A5. 3-HPMA	2 aliquots of 5 mL each	-2, -1, 6, 7
A6. NNAL and NNAL-glucuronides	4 aliquots of 5 mL each	-2, -1, 6, 7
A7. Mutagenicity	1 aliquot of 500 mL	-1, 7
A8. S-PMA	2 aliquots of 5 mL each	-2, -1, 6, 7
A2. Creatinine	2 aliquots of 5 mL	-2 through 8

Detailed container, processing, storage, and shipping information is located in Attachment X. *which will be study-specific*

Following the removal of the necessary urine for the urine biomarker aliquots, two 100 mL aliquots (2 x 100 mL) from each 24-hour collection interval or pooled 24-hour collection interval will be stored in a HDPE container in a freezer set at or below –20°C until shipped for analysis.

7.15 Safety Assessments

7.15.1 Brief physical examination with vital signs (respiratory rate, heart rate, blood pressure, oral temperature) in the sitting position after at least 10 minutes of rest and at least 15 minutes after the last cigarette smoked at check-in (Day –4). Complete physical examination with vital signs (respiratory rate, heart rate, blood pressure, oral temperature) in the sitting position after at least 10 minutes of rest and at least 15 minutes after the last cigarette smoked on Day 8.

7.15.2 Body weight (in pounds) in indoor clothing with shoes off in the morning of Days -2 and 8 before breakfast.

5.3.6 12-Lead ECG at Screening and on Day 8.

5.3.7 Pulmonary function testing (FVC, FEV_1) at Screening and on Day 8.

7.15.3 Clinical laboratory analysis (hematology and clinical chemistry) on Day –4 (check-in) and on the morning of Day 8.

7.15.4 Clinical laboratory analysis (urinalysis) on Day –4 (check-in) and on the morning of Day 9 after closing out the 24h urine collection for Day 8.

7.15.4 Serum pregnancy test at check-in (Day -4) (female subjects). A subject with a positive pregnancy test will be removed from the study, referred to a smoking cessation clinic if she requests, and advised to seek prenatal care and counseling from her primary care provider.

7.15.5 Urine screen for alcohol and drugs of abuse (*i.e.*, amphetamines, opiates, cannabinoids, cocaine).

7.15.6 Brief written assessment (medical/medication history) of the subject to affirm that the eligibility criteria/restrictions have not been violated at check-in (Day –4).

7.15.7 The subjects will be instructed to inform the study physician and/or nurses of any adverse events that may occur at any time during the study.

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7.16 Questionnaires

The following study questionnaires will be administered throughout the study as noted below:

The *Fagerström Test for Nicotine Dependence*[27] (Attachment x) at Screening to all subjects.

The *Smoking History Questionnaire* (Attachment x) at Screening to all subjects.

The *Diet Questionnaire* and *Occupation and Work Environment Questionnaire* (Attachments XX) at check-in (Day –4) to all subjects.

The *Product Assessment Questionnaire* (Attachment x) between 1500 and 1800 on Days -2 and -1 to all subjects and on Days 1 through 8 to the *(investigational product)* group.

8. ADVERSE EVENTS

8.1 Adverse Events

The following is the definition for an **adverse event (AE)**:

Any unfavorable and unintended sign (including an abnormal laboratory finding[a]), symptom, or disease[b] temporally associated with the use of an investigational product[c], **whether or not** related to the investigational product.[999, 1000]

[a] For this study, a laboratory adverse event is defined as an abnormal laboratory finding that is determined by the Investigator to be clinically significant for that subject.

[b] This includes a newly developed, worsened preexisting, recurring intermittent or intercurrent illness, injury, or condition.

[c] "Investigational product" includes the reference product which all subjects are allowed to smoke during the check-in and baseline phases, from initial confinement until randomization, as it is administered as part of the study.

All adverse events occurring during this clinical trial after the subject has signed the ICF document must be recorded in the CRF, including the date and time of onset and outcome of each event. Events captured between Screening and check-

in occur prior to investigational product usage and will be documented as medical history and **not** adverse events.

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an adverse event. **Planned** surgery permitted by the clinical study protocol and the condition(s) leading to this surgery are not adverse events.

No causal relationship with the study products or with the clinical study itself is implied by the use of the term "adverse event."

The Investigator will review each event and rate each reported sign or symptom on a 3-point severity scale. The following definitions for **rating severity**[999] will be used:

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

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

Severe: The adverse event is incapacitating and requires medical intervention. *Note: This is not the same as "serious," which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.*

Each AE will also be assessed by the Investigator for **relationship to study "treatment" (causality)** using the following grades of certainty[1001, 1002] (the strength of a causal association may be revised as more information becomes available):

Not related:

Clearly and definitely due to extraneous cause (e.g., disease, environment)

Unlikely:

a. Does not follow a probable temporal (i.e., time) sequence from use of study "treatment."
b. Does not follow a known pattern of response to the study "treatment."

VERSION:

c. Could plausibly have been produced by the subject's clinical state/underlying disease or other drugs or chemicals the subject received.
d. Does not reappear or worsen when the study "treatment" is re-administered.

Possible:

a. Follows a reasonable temporal (i.e., time) sequence from use of study "treatment"
b. Follows a known pattern of response to the study "treatment."
c. Could also have been produced by the subject's clinical state/concurrent disease or other drugs or chemicals the subject received.

Likely:

a. Follows a reasonable temporal (i.e., time) sequence from use of study "treatment."
b. Follows a known pattern of response to the study "treatment."
c. Could not readily have been produced by the subject's clinical state/concurrent disease or other drugs or chemicals.
d. Follows a clinically reasonable response on withdrawal (dechallenge), *i.e.*, disappears or decreases when the study "treatment" is stopped or reduced.
e. Rechallenge information is **not** required to fulfill this definition.

Definitely:

a. Follows a reasonable temporal (i.e., time) sequence from use of study "treatment"
b. Follows a known pattern of response to the study "treatment."
c. Cannot be explained by the subject's clinical state/ concurrent disease or other drugs or chemicals.
d. Follows a clinically reasonable response on withdrawal (dechallenge), *i.e.*, disappears or decreases when the study "treatment" is stopped or reduced.
e. Recurs with re-exposure to study "treatment" (rechallenge). *NOTE: Re-exposure of the subject is NOT required, but the "certainly/definitely related"*

VERSION:

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category may only be used when recurrence is observed.

8.2 Serious Adverse Events

The following is the definition for a **serious adverse event (SAE):**

A serious adverse event is any adverse study experience occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse study experience[a]
- in-patient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity[b]
- a congenital anomaly/birth defect.[1000]

[a] "Life-threatening" means that the subject was at immediate risk of death at the time of the SAE; it does not refer to a SAE that hypothetically might have caused death if it were more severe.

[b] "Persistent or significant disability/incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

Important medical events that may or may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse study experience when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. An example is allergic bronchospasm requiring intensive treatment in an emergency room or at home.

All serious adverse events, **whether or not** considered study-related, must be reported by telephone and by fax to the Sponsor within 24h of the site's learning of the SAE or, at the latest, on the following workday. The Sponsor's representative to contact about this study is:

xxxxx
Study Manager
Clinical Evaluation
Philip Morris USA Research Center
615 Maury Street
Richmond, VA 23224
Phone: (804) 274-xxxx

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Cellular: (804) xxx-xxxx
FAX: (804) 274-xxxx

The Investigator must also inform the IRB, in compliance with GCP reporting guidelines, **and the site monitor of an SAE, whether or not** considered study-related. The Investigator will submit a written report (for this study, a MedWatch form [FDA 3500A] will be used) electronically within 15 working days to the Sponsor, **whether or not** the SAE is considered study-related. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the study "treatment." Information not available at the time of the initial report (e.g., end date, laboratory values) must be documented on a follow-up SAE form.

8.3 AE/SAE Follow-Up

Each adverse event, whether serious or non-serious, will be followed to resolution regardless of whether the subject is still participating in the study. Where appropriate, medical tests and examinations will be performed to document resolution of the adverse event. Outcome may be classified as resolved, improved, unchanged, worse, fatal, or unknown (lost to follow-up).

8.4 Pregnancy

Pregnancy occurring during a study in a female study subject will be documented* in a note to file and as a protocol deviation in the clinical conduct study report (CCSR) to the IRB. Pregnancy itself is not a serious adverse event. The Investigator or designee will discontinue the pregnant subject from the study, advise her to discontinue smoking, refer her to a smoking cessation clinic if she requests, and advise her to seek prenatal care and counseling from her primary care provider. Advice given will be documented in the subject's source document.

The site clinical staff will request the pregnant subject to notify the site of the outcome of the pregnancy (i.e., birth, loss, or termination). To help ensure this, the site clinical staff will follow up with the subject until the end of pregnancy, if in compliance with the site's SOP and with the subject's consent. This request and the subject's response will be documented in the subject's source document.

* A positive urine pregnancy test will be confirmed by a serum pregnancy test, (preferably done at the investigative site if the subject is willing) or by the subject's primary care provider (with documentation provided to the investigative site).

9. REMOVAL OF SUBJECTS FROM STUDY, EARLY TERMINATION

Subjects will be advised that they are free to withdraw from the study at any time. Subjects who elect to quit smoking during the study period and choose to continue in the study will remain in their assigned group and will finish the study according to that group's schedule. If they withdraw from the study they will receive a pro-rated stipend. The Investigator may remove a subject if he feels this action is in the best interest of the subject. At the discretion of the Investigator, and in consultation with the Sponsor, a subject may be removed for failure to adhere to the requirements of the protocol. In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's source documents.

If a subject terminates early from the study and has received investigational product provided by the Sponsor,* all[@] of the safety data normally required at the end of the study should, if possible, be obtained (see "Early Termination" procedures in the Time and Event Schedule). Subjects with adverse events will be followed until the event has resolved. Appropriate supportive and/or definitive therapy will be administered as required.

Neither subjects withdrawing from the study nor those removed by the Investigator after Day –2 will be replaced. Subjects withdrawing or removed from this study cannot re-enter.

Subjects who check-in but are not randomized because of excess number of subjects or because of failure to fulfill ongoing eligibility criteria will be considered "non-randomized subjects". Early termination procedures will not apply to these non-randomized subjects.

* "Investigational product" includes the reference product which all subjects smoke during the check-in and baseline phases, from initial confinement until randomization, as it is administered as part of the study.

[@] Repeating the check-in safety tests may be waived at the Investigator's discretion for subjects who terminate between check-in and randomization. The Investigator should record in the subject's source documents the reason for waiving the repeat safety tests.

10. ANALYTICAL METHODOLOGY

The following is an example; this section will be study-specific.

10.1 Clinical Laboratory

37 TABLE OF CONTENTS
38 *WILL NEED TO BE RENUMBERED, RECONFIGURED TO MATCH TEXT*
39

VERSION:

3063094335

Values for the clinical laboratory parameters will be determined by MDS Pharma Services Clinical Laboratory, Lincoln, Nebraska, which is an accredited laboratory facility, or other accredited clinical laboratory facilities accredited by the Centers for Medicare and Medicaid Services (Clinical Laboratory Improvement Amendments of 1988 [CLIA-88]). Hematology, clinical chemistry, and urinalysis will be analyzed using standard clinical laboratory procedures.

COHb will be analyzed spectrophotometrically.

Urine creatinine will be analyzed by rate alkaline picrate (Jaffe).

10.2 Analytical Laboratory

Biomarkers as noted below will be analyzed using validated analytical methods with appropriate quality controls according to the *FDA Guidance for Industry: Bioanalytical Method Validation* (May, 2001) and in accordance with FDA Good Laboratory Practice regulations (21 CFR Part 58):

Urine nicotine and five major nicotine metabolites (nicotine, cotinine, *trans*-3'-hydroxycotinine, nicotine-*N*-glucuronide, cotinine-*N*-glucuronide, and *trans*-3'-hydroxycotinine-*O*-glucuronide) will be reported individually and summed as nicotine equivalents, analyzed by LC-MS/MS.

NNAL (total and free) will be analyzed by LC-MS/MS.

3-HPMA will be analyzed by LC-MS/MS.

Urine *S*-PMA will be analyzed by LC-MS/MS.

Total 1-OHP will be analyzed by LC-MS/MS.

MHBMA will be analyzed by LC-MS/MS.

Mutagenicity will be evaluated from concentrated urine using the Ames test.

11. STATISTICAL METHODS

The following is an example; complete, study-specific details can be found in the study's Statistical Analysis Plan.

11.1 General

SAS (SAS Institute, Version 8.1 or above) will be the software package for data presentation and summarization, including data listings, tables, graphs and statistical analyses. The data listings will include all data for the study.

Descriptive statistics will be provided for variables of subject demographics, biomarkers, smoking topography, smoking questionnaire, clinical outcome and safety, with sample size, mean, standard deviation, minimum, median and maximum for continuous variables and frequency tables for categorical variables. Additional descriptive statistics including CV% and percentiles may also be provided for continuous variables. Descriptive statistics for biomarkers will be based on four data types: original measurements and original measurements adjusted by number of cigarettes/day; percent change from baseline and percent change from baseline adjusted by number of cigarettes/day.

Shapiro-Wilk test statistics will be used to test for normality of primary and secondary biomarker variables. Baseline comparability between the treatment groups in demographic characteristics and primary biomarkers of exposure will be assessed by statistical analysis. One-way analysis of variance (ANOVA) will be used for continuous variables (e.g., age, BMI, nicotine equivalents) if the normality assumption is not markedly violated, otherwise, the Kruskal-Wallis test will be used. For categorical variables (e.g., gender and smoking intensity), exact tests will be used.

The intent-to-treat (ITT) subject population for data analysis is defined as all subjects who are randomized into the "treatment" groups and have valid evaluations at baseline and post-baseline. Data from subjects who terminate early or quit smoking during the study will be included up to the time study "treatment" stops. Missing data from scheduled assessments will be treated as missing. The statistical significance level will be 5% for two-sided testing.

No interim analysis is planned.

11.2 Statistical Analysis

Complete, study-specific details can be found in the study's Statistical Analysis Plan.

Primary Variable Analysis

Secondary Variable Analysis

Smoking Topography Analysis *(If performed)*

Questionnaire Analysis

Safety Analysis

Safety variables assessed in the study include adverse events, concomitant medications, physical examinations, vital signs, ECG, clinical chemistry, hematology, and urinalysis. All safety data will be listed in full for each subject in an Appendix. Abnormal laboratory values will be flagged with "L" for values below the lower limit of the Investigator's clinical laboratory's normal range, and "H" for values above the upper limit of the Investigator's clinical laboratory's normal range.

Adverse events will be coded using the MedDRA dictionary. Adverse events will be compared descriptively among the *(N)* "treatment" groups.

If a subject becomes pregnant during the study, all the pregnant subject's data from the visit at which the pregnancy was confirmed will be excluded from all analyses.

Concomitant medications will be coded using WHO Drug.

11.3 Sample Size Estimation

(study-specific)

12. DATA MANAGEMENT

Data management activities will be carried out by MDS Pharma Services according to their internally established SOPs. A data management plan and data capture forms (e.g., CRFs) will be prepared by MDS and provided to the Sponsor for review.

All the data generated during the course of the study will be captured electronically or entered in ClinQuick™, entered from source documents into Entrypoint® Plus (EP-Plus), or captured by data or device specific software for that procedure.

ClinQuick™, a fully integrated study management and data capture system, will be used for all data that can be captured electronically via barcode or electronic acquisition (*e.g.*, vital signs, meal times, blood draw times). Other data will be entered into ClinQuick™ by remote data entry (*e.g.*, adverse events, concomitant medications) or via double data entry by two different associates using an automated verification system within ClinQuick™ (*e.g.*, medical history, physical examinations, ECGs, inclusion/exclusion criteria).

Data from some source documents (*e.g.*, questionnaires) may be entered into EP-Plus. Any EP-Plus data will be entered into two separate files by two different data entry associates. These two files will be uploaded and compared to each other using the SAS® COMPARE procedure. Any discrepancies will be resolved and adjustments will be made to the data as necessary. This process will be repeated until all data values are an exact match between the two data files. This data is to be considered "clean" data.

All ClinQuick™ and EP-Plus clinical data will undergo a 100% quality control check prior to clinical database lock.

Edit checks will be performed as a validation routine using SAS® to check for errors and discrepancies such as missing data, data inconsistencies, and inappropriate data ranges. Corrections will be made as necessary to the EP-Plus and/or ClinQuick™ data prior to statistical database lock. Any changes to the data following database lock will be documented.

All 24h urine collections will be evaluated for completeness (i.e., full 24h) using a standardized bioplausibility algorithm based on 24h urine creatinine excretion. Collections determined to be biologically implausible will be excluded from analyses of 24h biomarker amount excreted but will be included in creatinine-adjusted analyses.

Topography data will be downloaded at each visit from the *CReSS Micro* devices to a dedicated computer at the investigative site. These files will be converted into a SAS dataset.

Any bioanalytical and/or clinical data received by the MDS statistical team from a source other than MDS' internal Data Management team procedure (*e.g.*, KoKo

Spirometry, Plowshare® Technologies topography) will be in electronic format and considered final audited data. This final audited data will be converted into SAS® datasets and stored in one singular study directory. Listings will be generated from these SAS® datasets and QC'ed against the final audited data originally received by the MDS statistical team. Data collected from specific software for a specific procedure

Case Report Forms will be printed off directly from the SAS® data files. Each CRF will be reviewed and signed by the Principal Investigator.

Upon completion of the study, the clinical data will be transferred to the Sponsor in SAS® format with supporting SAS® documentation (*e.g.*, SAS® programs, formats) according to the specifications of the Sponsor. Subject names, initials, date of birth (except year), and other personal identifiers will be removed from this data transfer file; any such information removed will be documented at the time of transfer.

13. MONITORING THE STUDY

The responsible Philip Morris USA monitor or designee will contact and visit the Investigator as necessary, and he/she will be allowed, upon request, to inspect the various records of the study (*e.g.*, ICFs, CRFs) in a manner consistent with Good Clinical Practice and all other applicable state and federal law.

It will be the monitor's or designee's responsibility to inspect the CRFs at regular intervals throughout the study to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The monitor will verify that each subject has consented in writing to direct access to study records as well as to the study procedures. Where the terms of the Informed Consent, Good Clinical Practice, and all other applicable state and federal law permit, the monitor should have access to laboratory test reports and other subject records needed to verify the entries on the CRF. The Investigator (or his/her designee) agrees to co-operate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

14. REPORTING FOR THE STUDY

14.1 Case Report Forms

Standard MDS Pharma Services Case Report Forms will be used. MDS Pharma Services will assure complete and accurate entries on the forms.

14.2 Study Report

A study report written consistent with ICH guidelines will be provided by MDS Pharma Services to the Sponsor. The report will include a description of the clinical conduct of the study, analytical methods and results, and the statistical analysis described in the statistical methodology section of the protocol.

At the time the draft study report is completed, the MDS Quality Assurance unit will audit the report against the SAS® data and the raw data. At the completion of the audit, a QA report will be issued internally allowing any finding to be addressed. Once all QA findings are addressed and resolved, the draft report will be issued to the Sponsor.

15. GENERAL

15.1 Confidentiality

All members of the Investigator's staff have signed confidentiality agreements with MDS Pharma Services. MDS Pharma Services will regard all information provided to the Investigator dealing with the study and information obtained during the course of the study as confidential.

Subject names, initials, date of birth (except year), and other personal identifiers will not be supplied to the Sponsor. Any such information that appears on any study document must be obliterated before a copy of the document is supplied to the Sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed during the consenting process that representatives of the Sponsor, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence and in accordance with local data protection laws.

The Investigator will maintain a personal subject identification list (i.e., subject numbers with the corresponding subject names) to enable records to be identified.

15.2 Responsibility of the Investigator

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the Declaration of Helsinki (revised version of Washington, 2002) and in a manner consistent with the ICH Guideline for Good Clinical Practice and the applicable section of the U.S. Code of Federal Regulations governing Protection of Human Subjects (21 CFR 50), Institutional Review Boards (21 CFR 56), and Responsibilities of Sponsors and Investigators (21 CFR 312 Subpart D).

The Investigator should ensure that all persons assisting with the study are adequately informed about the protocol, and amendments to the protocol, the study "treatments," and their study-related duties and functions.

The Investigator will maintain a list, including signatures, of subinvestigators and other appropriately qualified persons to whom significant study-related duties are delegated.

15.3 Procedure for Amendments to Protocol

No deviations from this protocol will be permitted, except in a medical emergency, without the approval of the Sponsor. The Investigator and the Sponsor will discuss any amendment to this study. If agreement is reached concerning the need for modification, this agreement will be made in a formal amendment to the protocol.

All revisions and/or amendments to the protocol must be approved in writing by the MDS Pharma Services Institutional Review Board.

15.4 Institutional Review Board

This protocol, ICFs, and any amendments to the protocol will be reviewed and approved in writing by the MDS Pharma Services Institutional Review Board prior to commencement of the study. The study will not be initiated without the approval from the MDS Pharma Services Institutional Review Board. The MDS Pharma Services Institutional Review Board operations are in compliance with Part 56 of Title 21 of the Code of Federal Regulations. Notice that the protocol and any amendments to the protocol and ICF have been reviewed and approved by the MDS Pharma Services Institutional Review Board will be in the final study report.

15.5 Termination of Study

The Sponsor reserves the right to discontinue this study for administrative reasons at any time. The Investigator, in collaboration with the Sponsor, reserves the right to discontinue the study for safety reasons at any time.

15.6 Study Record Retention

Investigator-specific essential documents and all primary data, or copies thereof (*e.g.*, CRFs, laboratory records, data sheets, correspondence, photographs, computer records), which are a result of the original observations and activities of the study and are necessary for the reconstruction and evaluation of any study report will be retained in the investigative site's archives for a **minimum** of 20 years after the completion or termination of the study. It is the responsibility of the Sponsor to inform the Investigator as to when these documents no longer need to be retained. The study report and final database will be retained in the MDS Pharma Services' archives for a **minimum** of 20 years after the completion or termination of the study and will be available for inspection at any time by the Sponsor. At completion of the study (*i.e.*, at issuance of final study report), the final database will be transferred to the Sponsor. Subject names, initials, date of birth (except year), and other personal identifiers will be removed from this data transfer file; any such information removed will be documented at the time of transfer.

16. REFERENCES *Need to be study-specific. These (from K6/01/03) are left in b/c several reference the "boiler-plate" sections of this template protocol and should be retained or updated as/when appropriate.*

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VERSION:

3063094344

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VERSION:

3063094345

40
41

SUMMARY OF EVENTS

EVENT	Day -32 to Day -5	Day -4	Day -3	Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	End of Study	Early Termination
	Screen	Chk-In	Acclim	Baseline											
Informed Consent	X														
Check-in Procedures		X													
Confinement		X	X	X	X	X	X	X	X	X	X	X	X		
Randomization					X										
Discharge from clinic														X	X
Medical History	X	X[1]													
Review of Inclusion/Exclusion Criteria	X	X													
Physical Examination	X	X[2]											X		X*
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X		X*
Body Weight and Height	X			X[3]									X[3]		X[3]*
BMI	X														
ECG	X												X		X*
Pulmonary Function Tests (FEV_1, FVC)	X												X		X*
HIV, Hepatitis B and C serology	X														
Clinical Chemistry	X	X											X		X*
Hematology	X	X											X		X*
Urinalysis	X	X												X	X*
Urine Drug Screen	X	X													
Urine Alcohol Screen		X													
Pregnancy Test (females)	X	X													
Standardized Diet (low mutagen and PAH)		X	X	X	X	X	X	X	X	X	X	X	X		
Review of Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Review of Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X		X
Questionnaires															
Fagerström Test for Nicotine Dependence	X														
Smoking History Questionnaire	X														
Diet Questionnaire		X													
Occupation and Work Environment Questionnaire		X													
Product Assessment Questionnaire[4]				X	X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]		

42

VERSION:

EVENT *(continued)*	Day -32 to Day -5	Day -4	Day -3	Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	End of Study	Early Termination
	Screen	Chk-In	Acclim	Baseline											
Blood Biomarkers															
Carboxyhemoglobin	X[6]	X[6]		X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]		
24-hour Urine Biomarkers/Analyte															
24-Hour Urine Collection (~ 0700 to 0700)				X	X	X	X	X	X	X	X	X	X		
Nicotine Equivalents				X	X	X	X	X	X	X	X	X	X		
Total and free NNAL				X	X						X	X			
3-hydroxypropylmercapturic acid (3-HPMA)				X	X						X	X			
S-phenylmercapturic acid (*S*-PMA)				X	X						X	X			
Monohydroxybutenyl mercapturic acid (MHBMA)				X	X						X	X			
Total 1-hydroxypyrene (1-OHP)				X	X						X	X			
Mutagenicity[8]					X							X			
Creatinine				X	X	X	X	X	X	X	X	X	X		
Urine Storage (2 x 100 mL aliquots)				X	X	X	X	X	X	X	X	X	X		
Smoking Topography															
Smoking topography			X[10]	X	X	X	X	X	X	X	X	X	X		
Smoked Cigarette /Butt Collection															
Collection for Study Integrity		X	X	X	X	X	X	X	X	X	X	X	X		

EXPLANATION OF SUPERSCRIPTS

[1] Brief medical/medication history.
[2] Brief physical examination.
[3] Body weight only and in morning before breakfast.
[4] Between 1500 and 1800.
[5] *(Investigational product)* group only.
[6] Between 1500 and 1900.
[7] At 1900 ± 30 minutes.
[8] Ames assay using YG1024 with S9.
[9] The first cigarette of the day and the first cigarette after lunch for all smoking subjects on Days –2 through 8.
[10] For training purposes only, data collected will not be used for study analysis.
* The Investigator may waive the safety testing for a subject who terminates after confinement begins but before randomization; a waiver reason shall be recorded in the source documents.

VERSION:

LIST OF ABBREVIATIONS

(Protocol writer to include all protocol-specific abbreviations here. This is just an example and a specific protocol may require additions or deletions from what is in this list.)

≅	Approximately
±	Plus or minus
3-HPMA	3-hydroxypropylmercapturic acid
1-OHP	1-Hydroxypyrene
µg	Microgram(s)
µm	Micrometer(s)
AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BED	Biologically effective dose
BUN	Blood urea nitrogen
°C	Degrees Celsius
CFR	Code of Federal Regulations
CLIA-88	Clinical Laboratory Improvement Amendments of 1988
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EHCSS	Electrically Heated Cigarette Smoking System
ELISA	Enzyme-linked immunosorbent assay
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in the first second
FTC	Federal Trade Commission
FVC	Forced vital capacity
GC	Gas chromatography
GCP	Good Clinical Practice
Gluc	Glucuronide
H_2 antagonist	H_2 histamine receptor antagonist
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus

VERSION:

3063094348

65

HDL	High density lipoprotein
HDPE	High density polyethylene
HDPP	High density polypropylene
HIV	Human immunodeficiency virus
hr(s)	Hour(s)
IARC	International Agency for Research on Cancer
ICF	Informed consent form
ICH	International Conference on Harmonization
ID	Identification
IRB	Institutional Review Board
IUD	Intrauterine device
K_3EDTA	Tripotassium ethylenediaminetetraacetate
L	Liter(s)
LC	Liquid chromatography
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
mg	Milligram(s)
ML	Milliliter(s)
MS	Mass spectrometry
NaCl	Sodium chloride
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
PAH	Polycyclic aromatic hydrocarbon
PREP	Potential reduced-exposure products
RBC	Red blood cell
RCF	Relative centrifugal force
RPM	Revolutions per minute
SAE	Serious adverse event
SOP(s)	Standard operating procedure(s)
S-PMA	*S*-phenylmercapturic acid
TSNA	Tobacco-specific nitrosamine
USA	United States of America
WBC	White blood cell
HDL	High density lipoprotein
HDPE	High density polyethylene
HDPP	High density polypropylene
HIV	Human immunodeficiency virus
hr(s)	Hour(s)
IARC	International Agency for Research on Cancer
ICF	Informed consent form

VERSION:

3063094349

ICH	International Conference on Harmonization
ID	Identification
IRB	Institutional Review Board
IUD	Intrauterine device
K3EDTA	Tripotassium ethylenediaminetetraacetate
L	Liter(s)
LC	Liquid chromatography
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
mg	Milligram(s)
ML	Milliliter(s)
MS	Mass spectrometry
NaCl	Sodium chloride
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
PAH	Polycyclic aromatic hydrocarbon
PREP	Potential reduced-exposure products
RBC	Red blood cell
RCF	Relative centrifugal force
RPM	Revolutions per minute
SAE	Serious adverse event
SOP(s)	Standard operating procedure(s)
S-PMA	S-phenylmercapturic acid
TSNA	Tobacco-specific nitrosamine
USA	United States of America
WBC	White blood cell