

Assessment of Serum Nicotine Exposure from Modern Smoke-Free Tobacco Products

Protocol CSD0914

Prepared by

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1 SYNOPSIS

Title of Study:	Assessment of Serum Nicotine Exposure from Modern Smoke-Free Tobacco (MSFT) Products ¹
Objective of Study:	<p>The primary objective of this study is to:</p> <ul style="list-style-type: none">Determine uptake of nicotine in the blood over a 3-hour period following initiation of product use to clarify serum nicotine results found in previous studies.² <p>The secondary objectives of this study are to:</p> <ul style="list-style-type: none">Assess tobacco abstinence symptoms prior to and for 3 hours following initiation of product use after a 12-hour tobacco and nicotine abstinence period.Assess carboxyhemoglobin levels prior to and for 1 hour following initiation of product use.
Design of Study:	Fifteen generally healthy participants will abstain from tobacco/nicotine use for 12 hours prior to each of 5 weekly test sessions. One MSFT product or usual brand cigarette (UB) will be used by each participant per visit. Blood will be collected over the course of 3 hours just prior to and following product use to assess the serum nicotine concentrations and carboxyhemoglobin levels at various time points. Questionnaires will be administered to assess tobacco abstinence symptoms.
Main Criteria for Inclusion:	<ol style="list-style-type: none">Generally healthy males and females ages 21-55, inclusivePrimary tobacco use is smoking cigarettes and usual brand is in the FFLT categorySmokes 10-30 cigarettes per day and inhales the smokeNot postponing a decision to quit smoking to participate in the study
Main Criteria for Exclusion:	<ol style="list-style-type: none">Current oral lesionHistory of heart disease, kidney disease, lung disease, diabetes, uncontrolled hypertension, liver disease, neurological disorder, or psychiatric illnessCurrent diagnosis of peptic ulcer or irritable bowel syndrome

¹ Results from this study are not intended for product development purposes.

² Previous study results were potentially confounded by nicotine exposure from both cigarette smoking and MSFT product use.

Study Product:	<p>Participants will provide their own usual brand of FFLT cigarette at the first study visit. Other products to be tested include:</p> <ul style="list-style-type: none">• Tobacco Orbs, Fresh and Mellow• Tobacco Strips, Fresh• Tobacco Sticks, Mellow• Camel Snus, Frost and Mellow
Duration of Study:	<p>Planned Screening Duration: Approximately 14 days</p> <p>Number of Study Visits: 5 total, one per week for 5 weeks.</p> <p>Planned Enrollment Period: 8 weeks</p>
Criteria for Evaluation: Basic Safety	<p>Basic safety will be measured by oral exams, hemoglobin testing, and evaluation of adverse events.</p>
Criteria for Evaluation: Biomarker and Questionnaire Evaluations	<p>During the screening visit, participants will complete the Fagerström Test for Nicotine Dependence and the Mood and Physical Symptoms Scale (MPSS). Participants will keep product usage logs throughout the study. At each test session, timed blood samples will be collected to assess serum nicotine, cotinine and carboxyhemoglobin levels just prior to and for 3 hours following product use. Tobacco abstinence symptom questionnaires will be administered just prior to and for 3 hours following product use.</p>
Statistical Methods:	<p>Descriptive statistics will be computed for all endpoints.</p> <p>Two-sample t-tests will be used to compare differences in nicotine uptake from the current study to previous studies.</p> <p>For tobacco abstinence questionnaires and carboxyhemoglobin levels, one-way repeated measures analysis of variance will be used to assess changes over time for each of the products.</p> <p>Additional analyses may be performed if deemed necessary.</p>

2 INTRODUCTION

2.1 Background

R. J. Reynolds Tobacco Company (RJRT) is aggressively pursuing harm reduction efforts in its tobacco products portfolio. These efforts include the development and launch of modern smoke-free tobacco (MSFT) products, which currently include Camel Dissolvables and Camel Snus. Camel Dissolvables are tobacco products made primarily of finely milled tobacco that are designed to be completely consumed in the mouth without the need for spitting. Camel Orbs, Camel Strips, and Camel Sticks are examples of Camel Dissolvables. Camel Snus is a pouched, moist snuff product composed of pasteurized tobacco with a low sodium and moisture content. Similar to Camel Dissolvables, spitting is not required while using snus, allowing MSFT products to be used discreetly in places that do not allow smoking.

Previous RJRT studies examined the use of MSFT products by human participants throughout a three-week product transition from exclusive, *ad libitum* use of usual brand (UB) cigarettes to dual use of one MSFT product concurrent with a 75-100% reduction goal in cigarette consumption. Separate studies were conducted with each MSFT product. Those studies evaluated several aspects of the tobacco transition, including product usage, biomarkers of tobacco exposure in blood and urine, and subjective responses to questionnaires. The studies involving Camel Strips and Camel Sticks included in-lab product use with the collection of blood samples just prior to and for 60 minutes following initiation of product use. The study in which participants used Camel Snus extended the collection of blood samples to 90 minutes following the initiation of product use. Serum nicotine concentrations were measured in the timed blood samples in an effort to understand the timing and level of nicotine uptake from this new class of products. Results have been obtained for serum nicotine levels following Strip and Stick use, but are still pending for the study employing Camel Snus.

Results from the analyses performed to date showed a steady decline in serum nicotine levels for 60 minutes following use of a single Strip or Stick in the lab setting. One probable reason for this observation was the protocol requirement for participants to abstain from tobacco use for only 30 minutes prior to their study visit. Since the typical half-life of nicotine in the blood is 30-120 minutes, only a portion of the nicotine present at the start of this abstinence would be cleared from the blood before participants used product during the test session. As a result, participants averaged serum nicotine concentrations of approximately 13ng/mL just prior to product use in the lab setting. The

clearance of this nicotine from the blood concurrent with nicotine uptake from Strip or Stick use still resulted in an overall decline in nicotine concentrations following MSFT use.

In an attempt to determine the serum nicotine contribution from in-lab MSFT use, the average rate of serum nicotine clearance across all participants was applied to each participant's starting nicotine concentration to estimate the clearance of the initial nicotine levels over time. Calculated values of remaining initial nicotine at each time point were subtracted from observed values to determine corrected concentrations that theoretically correspond to the nicotine absorbed specifically from Strip or Stick use. These corrections did indicate a serum nicotine increase resulting from Strip or Stick use; however, the validity of the half-life estimation on which the corrections are based is unknown.

Additionally, the time at which the maximum nicotine concentration occurred following product use could not be determined from the available data. The corrected nicotine concentrations still appeared to be rising 60 minutes following the initiation of Strip and Stick use in lab. Although we extended the time of blood collections following initiation of Snus use to 90 minutes, results are not yet available for comparison. To clarify the validity of the correction method described above and to determine the time at which peak nicotine concentrations occur following product use, a separate study employing a longer tobacco abstinence period and an extended period of timed blood sample collection is required.

2.2 Study Rationale

This study will clarify the accuracy of the serum nicotine "corrections" performed on the data obtained in previous local tobacco-use studies with MSFT. This will be accomplished by administering product to participants who have abstained from tobacco and nicotine use for 12 hours and collecting blood samples for nicotine analysis just prior to and for 3 hours following product use. Results will be compared to the subtraction method performed in previous studies to understand the utility of the method for estimating the nicotine contribution from a single product when background levels from previous product use are present. Results are not intended for product development use. In addition, this study will evaluate carboxyhemoglobin levels and the relief and recurrence of tobacco abstinence symptoms just prior to and for 3 hours following MSFT or UB use.

2.3 Safety Considerations

Details of participants' medical history will be discussed and recorded in a confidential setting at a screening visit. Prior to the administration of study product, participants will have an oral exam performed by a medical practitioner, will have hemoglobin levels tested to rule out anemia, and will have their health history reviewed by the study medical advisor to determine final eligibility. Trained, certified phlebotomists will perform all blood collections. All study staff have been trained in and will follow Occupational Safety and Health Administration (OSHA) safety guidelines pertaining to occupational exposure to blood-borne pathogens when handling biological samples and disposal of biohazardous materials.

3 STUDY OBJECTIVES

The primary objective of this study is to:

- Determine uptake of nicotine in the blood over a 3-hour period following initiation of product use to clarify serum nicotine results found in previous studies.

The secondary objectives of this study are to:

- Assess tobacco abstinence symptoms prior to and for 3 hours following initiation of product use after a 12-hour tobacco and nicotine abstinence.
- Assess carboxyhemoglobin levels prior to and for 1 hour following initiation of product use.

4 STUDY DESIGN AND RANDOMIZATION

This is a randomized, crossover, single-center study of 15 participants who smoke and are willing to abstain from all tobacco and nicotine use for 12 hours prior to test sessions. At the beginning of each test session, participants' expired CO levels will be measured and must register ≤ 10 ppm in order to continue with the test session that day. Participants will be willing to use one MSFT product or smoke one UB cigarette at each 3.5-hour study visit and to have blood samples collected and answer questionnaires over that period of time. All participants will provide and smoke their UB cigarette at Test Session 1. For Test Sessions 2, 3, 4, and 5, participants will be randomized to one of five groups (A, B, C, D, or E, n=3 for each group). By necessity, this study is unblinded due to the

very different visual appearances of the products. Products will be presented to groups, corresponding to days of the week, in the following orders:

Table 1. Product Presentation Order for Randomized Groups

Group	Test Session 2	Test Session 3	Test Session 4	Test Session 5
A	Strip	Snus	Orb	Stick
B	Orb	Strip	Stick	Snus
C	Strip	Snus	Orb	Stick
D	Stick	Orb	Snus	Strip
E	Snus	Stick	Strip	Orb

5 SUBJECT SELECTION

5.1 Screening Procedures

Screening procedures will be completed in 1 visit. Procedures will be performed per the study flow chart ([Table 2](#)).

5.2 Inclusion Criteria

Participants who meet the following criteria may be included in the study:

1. Males or females, ages 21-55, inclusive
2. Weighs at least 110 pounds
3. Is in generally good health
4. Smokes 10-30 cigarettes per day and inhales the smoke
5. Primary tobacco use is smoking cigarettes
6. Smokes FFLT combustible cigarettes, 85s or 100s, menthol or non-menthol
7. Is not postponing a decision to quit smoking to participate in study
8. Has prior experience using smokeless or dissolvable tobacco products, but does not currently use routinely
9. Has not given a whole blood donation in at least 8 weeks (56 days)

5.3 Exclusion Criteria

The following will exclude participants from the study:

1. Has current oral lesion(s), as determined by the medical advisor or designee
2. History of heart disease, kidney disease, asthma or any other lung disease, diabetes, uncontrolled hypertension, liver disease, neurological disease, or psychiatric illness
3. Hemoglobin below 12.5 g/dL
4. Positive for HIV, Hepatitis B, or Hepatitis C
5. Hemophilia or any other bleeding disorders
6. Clotting disorders with concomitant use of anticoagulants
7. Women who are pregnant, breastfeeding, intend to become pregnant during the course of the study, or have given birth in the last 12 months
8. BMI > 40
9. History of illegal IV drug use
10. Drinks more than 14 alcoholic beverages per week
11. Employed by a tobacco company, a sub-contractor of a tobacco company, or handles tobacco as part of their job
12. Current diagnosis of peptic ulcers or irritable bowel syndrome
13. Current use of nicotine replacement products or intention to use nicotine replacement products during the course of the study
14. Afternoon exhaled CO measurement of >15ppm at screening visit

5.4 Selection of Participants

Participants who meet all the inclusion criteria and none of the exclusion criteria are eligible to be randomized/enrolled into the study. Fifteen participants and up to 15 alternates will be included in the study. Fifteen alternates who meet all the inclusion and none of the exclusion criteria will be included through the end of the first test sessions. If a participant's expired CO levels are >10 ppm at the beginning of Test Session 1, that participant will be excused from the study and an alternate will be invited to participate. If that alternate's expired CO level is ≤10 ppm, the alternate will become a participant and will be included in the remainder of the study.

If a participant's expired CO levels are >10 ppm at any single test session after Test Session 1, the participant will be excused from that test session and asked to attend a make-up test session the week following Test Session 5. If a participant's expired CO

levels are >10 at any two test sessions after Test Session 1, that participant will be excused from the study.

6 STUDY PROCEDURES

6.1 Schedule of Study Procedures

Table 2. Study Flow Chart

Study Procedures	Screening	Test Sessions					Final Oral Exam
		1	2	3	4	5	
Informed Consent	X						
Medical History (including concomitant medications)	X						
Height	X						
Weight	X						
Demographics	X						
Administer FTND	X						
Oral Exam	X						X
Hemoglobin testing	X						
Dispense single-unit trial pack and guidelines for use	X						
Schedule date and time of test sessions	X						
Distribute weekly product usage log	X	X	X	X	X		
Measure expired CO	X	X	X	X	X	X	
Schedule date and time of final oral exam					X		
Assess Adverse Events		X	X	X	X	X	X
Record time last cigarette was extinguished		X	X	X	X	X	
Collect product usage log		X	X	X	X	X	
Participants provide 1 UB cigarette to study staff		X					
Collect in-lab cigarette butt for YIU analysis		X					
Dispense single unit MSFT product for timed use during test session			X	X	X	X	
Collect snus pouch following in-lab use for SAU analysis ^a			X	X	X	X	
Collect timed blood samples relative to initiation of product use via hep-loc IV catheter for nicotine, cotinine, and COHb analysis ^b		X	X	X	X	X	
Measure COHb levels in whole blood samples		X	X	X	X	X	
Administer Mood and Physical Symptoms Scale ^c	X	X	X	X	X	X	
Dispense 6 units of the MSFT product to be presented for use at next test session and guidelines for use		X	X	X	X		
Collect unused MSFT products			X	X	X	X	

NOTES:

^a Used snus pouches will only be collected during the test session at which participants are scheduled to use snus in the lab according to the randomization schedule.

^b For nicotine and cotinine analysis, blood samples will be drawn at -2, 0, 3, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 minutes with respect to initiation of in-lab product use. For carboxyhemoglobin measurements, blood samples will be drawn at -2, 30, and 60 minutes.

^c The Mood and Physical Symptoms Scale will be administered ≤ 10 minutes prior to and at 5, 15, 30, 45, 60, 90, 120, 150, and 180 minutes following initiation of in-lab product use.

6.2 Study Materials and Administration

Each participant's study materials will be determined by a randomization schedule and the study protocol. For each dispensation/collection of study product, the date and amount dispensed/returned will be recorded in the source documents. Compliance with the protocol will be confirmed by oral query and assessment of completed product log sheets. Use of all products in the lab will be timed to determine duration of use.

UB Cigarettes

At screening, participants will provide study staff with one pack of their usual brand of cigarettes. Study staff will photocopy the front and return the pack to the participant. At Test Session 1, participants will provide the study staff with one usual brand (UB) cigarette that staff will dispense to the participant to smoke in the lab.

MSFT Products

The MSFT products to be tested in this study include Camel Snus (Frost and Mellow), Camel Orbs (Fresh and Mellow), Camel Strips (Fresh), and Camel Sticks (Mellow). All products are currently available in the marketplace. Following completion of the screening oral exam and fulfillment of all inclusion and no exclusion criteria, participants will receive a trial pack containing one of each product and variety to sample during the week prior to Test Session 1. At the end of Test Sessions 1-4, participants will receive a 6-unit supply of the product to which they are randomized for use during their next test session. Participants will also receive instructions for use and will be asked to use 1 unit per day according to instructions to become accustomed to using each product prior to in-lab use. During Test Sessions 2-5, participants will use 1 unit of the MSFT product that was provided to them for use over the previous week. In the case of Camel Snus and Camel Orbs, participants will be asked to choose one variety to take home with them. They will be presented with the same variety in the lab during their test session.

6.3 Product Use Instructions

Each product will have accompanying instructions for in-lab use. For each product, participants will not be permitted to eat or drink until the product has completely dissolved or has been removed in the case of snus. Participants will also be asked not to spit during or after use of each product. Use of all products in the lab will be timed to determine duration of use.

Camel Orbs

Participants will be asked to use one Orb to completion by placing the Orb between their cheek and gum and occasionally moving to a different location in the mouth during use.

Camel Strips

Participants will be asked to use one Strip to completion by placing the Strip on the top of the tongue/roof of mouth or by folding and placing between the lip and gum.

Camel Sticks

Participants will be asked to use ½ of a Stick to completion by placing the Stick portion between their cheek and gum and occasionally moving to a different location in the mouth during use.

Camel Snus

Participants will be asked to place one pouch between either upper or lower lip and gum and to leave in place for a minimum of 15 and a maximum of 30 minutes. Occasional movement of the pouch will be suggested, but not required.

6.4 Diet, Fluid, Tobacco, and Activity Restriction

Participants will be required to abstain from all tobacco and nicotine use for 12 hours prior to each test session. To facilitate tobacco and nicotine abstinence, participants will be asked to end use of tobacco/nicotine at 8:30 p.m. the evening prior to their test session and will be asked to report to the test facility at 8:30 the next morning. At the start of each visit, participants will be asked what time they finished using their last nicotine-containing product. Smoking abstinence will be confirmed by measuring the amount of carbon monoxide in participants' expired breath at the start of each study visit.

Participants must register an expired CO level of ≤ 10 ppm at the start of each test session to participate further that day.

Participants will be asked not to eat, drink, or spit during product use in the lab. Other than those requirements, there are no dietary, fluid, or activity controls throughout this study with the exception of excessive alcohol consumption, which will be grounds for dismissal. Excessive alcohol consumption consists of more than 14 drinks per week (one drink = 1 ounce hard liquor [1 mixed drink], one 6-ounce glass of wine, or one 12-ounce beer). No restriction on activity is required.

6.5 Concomitant Medications

Any medication taken by a participant during the course of the study and the reason for its use will be recorded in the source documents.

6.6 Test Session Eligibility

Expired CO measurements will determine whether a participant is eligible to complete each test session. Participants must register ≤ 10 ppm CO to participate further in each test session. Refer to [Section 5.4](#) “Selection of Participants” for additional details.

6.7 Sample Collection and Processing

Blood samples will be collected via an indwelling IV catheter inserted in the antecubital region of the forearm once the expired CO eligibility has been met at each test session. Blood samples for serum nicotine/cotinine analysis will be collected at -2, 0, 3, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 minutes with respect to initiation of product use. Blood samples will be collected to measure carboxyhemoglobin levels at -2, 30, and 60 minutes with respect to initiation of product use. The total volume of blood to be drawn in each test session is approximately 88mL. For the entire study, a total of approximately 440mL will be drawn, which is just under one pint of blood.

Samples to be analyzed for nicotine and cotinine will be collected in serum separator tubes, allowed to sit for at least 30 minutes at room temperature and will be centrifuged at 3000 rpm for 20 minutes at 8°C. Serum will be aliquoted, frozen at -70°C, and shipped to a contract lab for analysis.

Samples to be analyzed for carboxyhemoglobin saturation will be collected in tubes containing EDTA. Whole blood will be analyzed within 15 minutes of sample collection using a CO-oximeter.

The cigarette filter remaining after UB smoking in the lab at Test Session 1 will be collected for yield-in-use analysis of nicotine. Butts will be processed by clipping off a 1-cm portion from the mouth end. Each 1-cm tip will be stored in a separate amber glass jar, frozen at -70°C and shipped to a contract lab for analysis.

The snus pouch used by each participant in the lab will be collected and analyzed for the amount of remaining nicotine. Each pouch will be placed in a separate amber jar, frozen at -70°C, and shipped to a contract lab for analysis.

6.8 Timing of Questionnaire Assessments

The Fagerström Test for Nicotine Dependence will be administered at the screening visit. The Mood and Physical Symptoms Scale (MPSS), a questionnaire evaluating tobacco abstinence symptoms, will be administered at the screening visit, and in each test session ≤ 10 minutes prior to the start of product use and at 5, 15, 30, 45, 60, 90, 120, 150, and 180 minutes following initiation of product use.

7 ADVERSE EXPERIENCES

Any untoward medical occurrence experienced by a participant from the time of providing informed consent until completion of the final oral exam, whether or not considered related to the use of study product by the Medical Advisor, will be recorded as an adverse experience (AE).

7.1 Definition and Grading Intensity of Adverse Experiences

An adverse experience is defined as any untoward medical occurrence experienced by a study participant, whether or not considered related to use of study product by the Medical Advisor.

AEs are all:

- unfavorable changes in general health status;
- subjective or objective signs/symptoms;
- concomitant diseases or accidents;
- clinically relevant adverse changes in laboratory parameters observed in a participant in the course of a clinical study.

All adverse experiences, whether volunteered, elicited, or noted on physical examination, will be recorded throughout the study.

The severity of AEs will be categorized as follows:

- **MILD** = experience is minor and does not cause significant discomfort to subject or change in activities of daily living; participant is aware of symptoms, but symptoms are easily tolerated;
- **MODERATE** = experience is an inconvenience or concern to the subject and causes interference with activities of daily living, but the participant is able to continue with activities of daily living;
- **SEVERE** = experience significantly interferes with activities of daily living, and the participant is incapacitated and/or unable to continue with activities of daily living.

7.2 Criteria for Determining Relationship to Study Product

The Medical Advisor will make a determination of the relationship of the adverse experience to the study material using a 4-category system (not related, possible, probable, or definite) according to the following guidelines:

- **NOT RELATED** = an adverse experience that does not follow a reasonable temporal sequence from use of the study product and that can be reasonably explained by other factors, including underlying disease, complications, concomitant medications, or concurrent treatment;
- **POSSIBLE** = an adverse experience that follows a reasonable temporal sequence from the use of the study product (including the course following discontinued use of the study product) and that can not be excluded as being possibly caused by the study product (e.g., existence of similar reports attributed to the suspected study product and/or its analogues; reactions attributable to the pharmacological effect of the study material), although other factors such as underlying disease, complications, concomitant medications, or concurrent treatment are presumable;
- **PROBABLE** = an adverse experience that follows a reasonable temporal sequence from use of the study product (including the course following discontinued use of the study product) and that can be excluded as being possibly caused by other factors, such as underlying disease, complications, concomitant medications, or concurrent treatment;
- **DEFINITE** = an adverse experience that follows a reasonable temporal sequence from use of the study product (including the course following discontinued use of the study material), follows a

known or hypothesized cause-effect relationship, and (if appropriate) satisfies the following:

- positive results obtained in study product sensitivity tests;
- toxic level of the study product present in blood or other body fluids.

8 STATISTICAL ANALYSES

Statistical analyses will include descriptive statistics for the following serum nicotine endpoints: area under the time-versus-concentration curve (AUC), AUC “rise”, and peak “rise.” In addition, descriptive statistics will be provided for questionnaire responses, carboxyhemoglobin levels, snus-after-use analysis (amount of nicotine remaining, amount of nicotine extracted, and percent extraction), and yield-in-use analysis of usual brand cigarettes. An independent sample t-test will be used to compare the nicotine endpoints from this study to those of previous studies in an effort to verify the mathematical modeling previously applied. A one-way, repeated measures analysis of variance will be used to assess changes in carboxyhemoglobin levels and nicotine craving scores for each of the products. Nonparametric versions of the aforementioned tests will be substituted if the data sufficiently deviate from a Gaussian (normal) distribution.

9 ADMINISTRATIVE ASPECTS

9.1 Informed Consent

Written informed consent for the study will be obtained from all participants before protocol-specific procedures are carried out. The investigator or designee will explain the purpose of the study and the nature of the test product. The potential participants will be informed that participation is voluntary and that they can withdraw from the study at any time. The informed consent process shall be documented by the use of a written informed consent form (ICF) approved by the HRRC and will be signed by the participant prior to protocol-specific procedures being performed. The participant will be given a copy of the signed ICF.

9.2 Records and Data

Signed informed consent and medical history forms will be locked in a safe only accessible by study staff when not in use. All other data will identify participants by a unique participant ID# and a study-specific number. Data will be stored in computer files and, in some cases, as paper source documents. Following study completion, all source documents and records will be maintained in secure data storage indefinitely by RJRT.