Characterization of Selected Aerosol Constituents Levels in the Exhaled Breath of Adult e-Vapor Users during Use of Four e-Vapor Products

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STATISTICAL ANALYSIS PLAN

Altria Client Services LLC

Protocol ALCS-RA-17-06-EV

Characterization of Selected Aerosol Constituents Levels in the Exhaled Breath of Adult E-vapor Users during Use of Four e-vapor Products

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Title: Characterization of Selected Aerosol Constituents Levels in the Exhaled Breath of Adult E-vapor Users during Use of Four e-vapor Products

Protocol: ALCS-RA-17-06-EV

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LIST OF ABBREVIATIONS

AE	Adverse event
AICC	Akaike Information Criterion - corrected
ALCS	Altria Client Services LLC
ANCOVA	Analysis of Covariance
APE	Alleged physical effect
BMI	Body mass index
ECG	Electrocardiogram
eCRF	Electronic case report form
ENDS	Electronic Nicotine Delivery Systems
EVP	e-vapor product
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	human immunodeficiency virus
HR	Heart rate
LLC	Limited Liability company
LOCF	Last Observation Carried Froward
LSMEANS	Least squares means
MAR	Missing At Random
MedDRA	Medical Directory of Regulatory Activities
MDL	Minimum Detection Limit
MI	Multiple Imputation
MMRM	Mixed Model Repeated Measures
OLS	Ordinary Least Squares
PEAE	Product -Emergent Adverse Event
PMTA	Premarket Tobacco Product Application
PT	Preferred term
REML	Restricted maximum likelihood
SAE	Serious adverse event
SAP	Statistical analysis plan
SCA	Sham-Corrected Amount
SOC	System Organ Class
SOC	System Organ Class
WHO DDE	World Health Organization Drug directory

1.0 STUDY INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

The primary objective of this study is to estimate the change in total amount of nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde, and acrolein levels in exhaled breath samples between sham (inactive battery and empty cartridge) and controlled test product use for four e-vapor products.

1.1.2 Secondary Objectives

The secondary objectives of this study are:

- To estimate e-liquid use by changes in cartridge weight
  - Before and after controlled use in exhaled breath sessions
  - Before and after use during 12 hours of ad libitum use (per cartridge and in total over the 12 hours)
- To estimate puff count, and average puff duration per cartridge and in total over the 12 hours of ad libitum use
- To characterize responses to the Use Product Again Questionnaire after each 12-hour ad libitum use session.

1.2 BACKGROUND

Innovative and novel tobacco-derived nicotine products like e-vapor products (EVPs) potentially offer reduced risk alternatives to adult consumers of conventional lit-end cigarettes.[1] The proven strategies of prevention and cessation can be complemented by making available tobacco products that have demonstrated lower risks than conventional cigarettes. There is overwhelming scientific evidence regarding a risk continuum in the range of tobacco products available currently in the market.

According to this body of evidence, combustible tobacco products like conventional cigarettes are the most risky, and non-combustible tobacco products like EVPs present

relatively lower risks. The Royal College of Physicians' new report, 'Nicotine without smoke: tobacco harm reduction,[2] has concluded that EVPs are significantly less risky than cigarettes and likely to be beneficial to public health. E-vapor products deliver nicotine in an aerosol that has a very different composition than a typical aerosol from conventional cigarettes. There are thousands of chemicals generated from the combustion of tobacco, many of which are carcinogenic.[3, 4] In contrast, far fewer chemicals are generated from heating an e-liquid consisting of carrier ingredients (usually propylene glycol and/or glycerol), nicotine, water, and flavors.[5] The likely reductions in exposure due to large differences in the chemical composition of aerosols between the cigarettes and EVPs presents harm reduction opportunities for adult smokers switching to EVPs. Nu Mark LLC (Nu Mark) is focused on responsibly developing and marketing innovative tobacco products, such as EVPs, that may reduce the risk of tobacco-related disease.

Altria Client Services LLC (ALCS) and Nu Mark LLC (Nu Mark) are undertaking various studies to evaluate the MarkTen® branded EVPs. These studies will provide scientific evidence to support a Premarket Tobacco Product Application (PMTA) to the Center for Tobacco Products at the Food and Drug Administration (FDA). This specific study will estimate the levels of selected constituents in the exhaled breath of adult EVP users during use of e-vapor products. Data from this study will be used to model potential exposure to non-users when MarkTen® XL e-vapor products are being used. This study is one component of a broader comprehensive framework that will provide the totality of evidence for the PMTA.

1.3 STUDY DESIGN

This is an open-label study designed to estimate the nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde, and acrolein levels in exhaled breath samples during use of 4 MarkTen® XL e-vapor products (Table 1). The study design is summarized in Figure 1.

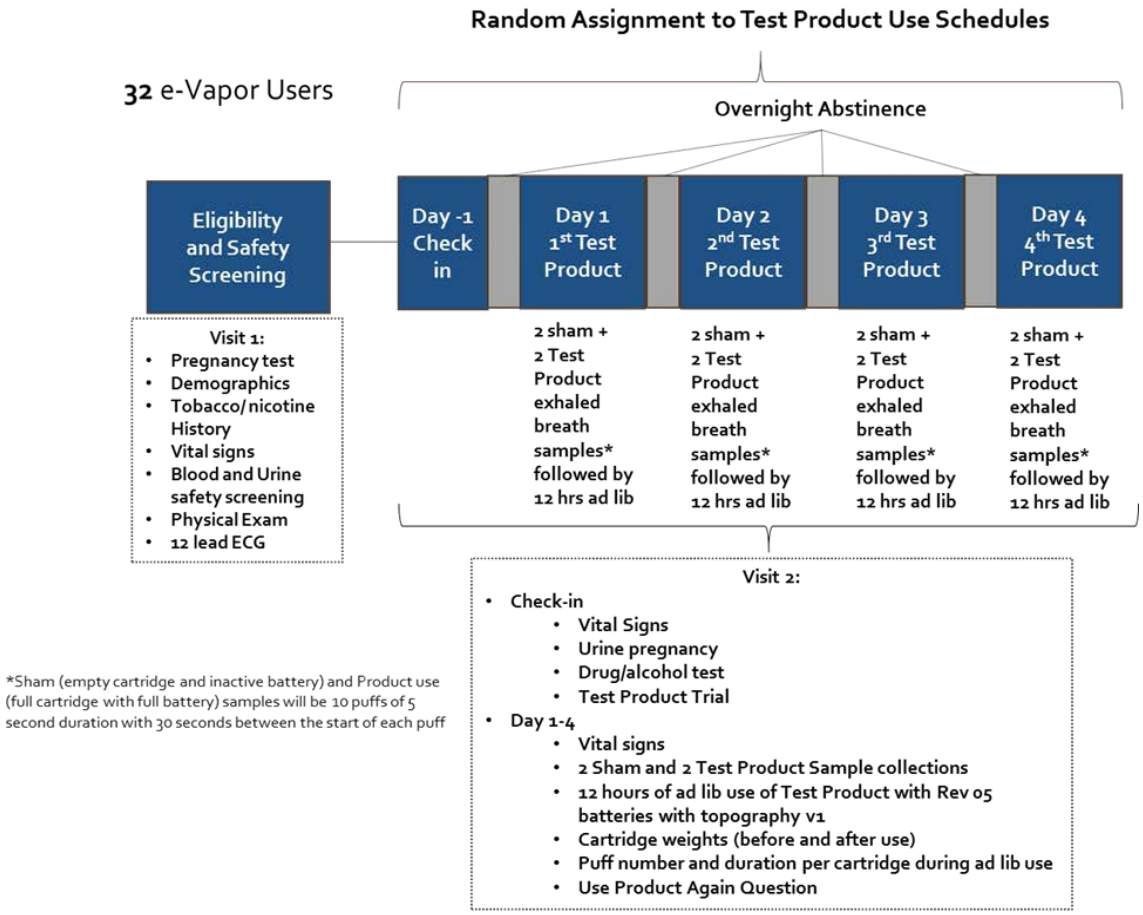
Table 1. Test Product Summary

Test Product	Test Product Nickname (letter)	Flavor	Percent Nicotine Content, by weight
XL25F	Celine (A)	Fusion	2.5%
XL40B	Rosetta (B)	Bold Classic	4.0%
XL35WM	Monica (C)	Winter Mint	3.5%
XL40B	Spenser (D)	Menthol	4.0%

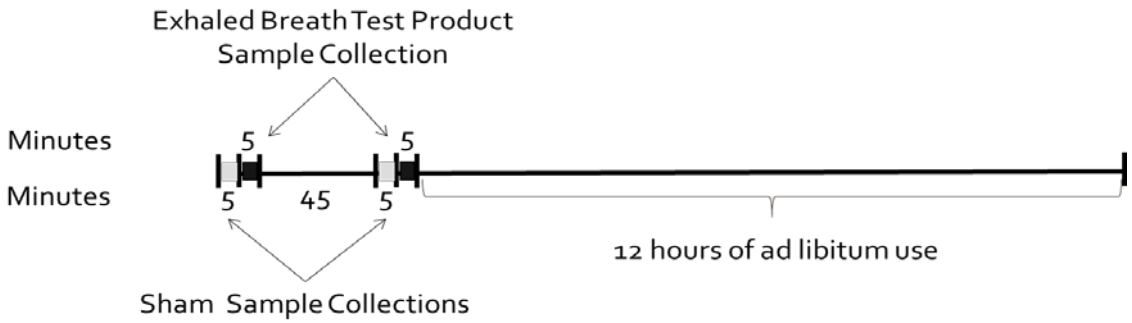
The study will enroll approximately 32 adult e-vapor-using subjects. Each of the 32 subjects will provide samples for all four test products within the framework of a 4-test product, 4-sequence crossover design. Subjects will make two visits to the site, one screening visit and one visit to provide exhalation samples for four products. Test product sequences will be randomly assigned to subjects at Visit 2 with one test product used per day, for 4 days. Following breath collections, each subject will also use their assigned test product ad libitum for 12 hours each day after the collection of exhaled breath samples. There will be a 10 hour and 55-minute overnight washout period between test product crossovers.

Figure 1. Study Diagram

A. Overall Diagram



B. Daily Test Product Use: Day 1 through Day 4



1.4 DEFINITION OF THE BASELINE ASSESSMENT

Data collected at the screening visit (e.g., vital signs; Visit 1), will be considered to be baseline values. For primary responses collected from a subject only during Visit 2 the sham concentrations of the each analyte, in a given period, will be considered the baseline (i.e., pre-test product use) concentration of each analyte for that subject, in that period. The sham analyte means will be used to compute test product post-use minus pre-use differences (= sham-corrected amount = SCA) of each of the 7 analyte concentrations of each subject, in each period.

1.5 SAMPLE SIZE

A sample size of 32 subjects was selected based primarily on practical considerations. A sample size of 32 will yield 8 complete replications of each sequence for the experiment, assuming there is no dropout or other loss of response data. No imputations are planned for lost data; however, subjects who dropout may be replaced at the discretion of the sponsor.

1.6 RANDOMIZATION

Each subject will be assigned to one of 4 test product sequences using a Williams design. The Williams design employs a Latin square, in which no test-product follows another specific test-product more than once in a given replication of the experiment (e.g., B will never follow A more than once in a 4x4 crossover design matrix). This property yields a balanced cross-over design wherein there is uniformity within both periods and sequences. In a balanced design, each of the test product types occurs the same number of times in each period and the number of subjects who receive test product type i in one period and test product type j in the next period is the same for all $i \neq j$ (Jones and Kenward 2003).

For this study, if the 4 test products are designated A, B, C, and D, the following 4x4 matrix (Table 2) represents one possible assignment of the 4 possible sequences to 4 different subjects. Thus the matrix represents one complete replication of the 4-period-4-sequence crossover experiment.

Table 2. Test Product Type Assignment in a Single Replication of a 4x4 Williams Crossover Design

Sequence	Test product type Assignment By Period			
	1	**2**	**3**	**4**
1	A	B	D	C
2	B	C	A	D
3	C	D	B	A
4	D	A	C	B

The randomizations will be done using a SAS macro program.[6]

1.7 STUDY PROCEDURES

At Visit 1 (screening), subjects (21–65 years of age) will complete vital sign assessment, serum pregnancy test (female subjects), urine drug/alcohol screen, the Demographics Questionnaire and Tobacco and Nicotine Product-Use History Questionnaire, blood hematology, clinical chemistry, urinalysis, 12 lead ECG and a physical exam.

Approximately 32 eligible adult subjects (no more than 60% of either sex) may enroll into the study. Subjects will self-report that they have been using EVPs "some days" or "every day" for the past 30 days and for at least 4 out of the past 7 days.

At Visit 2, upon arrival at the site, vital signs, urine pregnancy test (female subjects), and urine drug/alcohol screen will be conducted. After check in, subjects will engage in a brief product trial with each e vapor product following an assigned schedule (i.e., ad libitum use for 10 minutes) to get accustomed to using the products. Trials of each e-vapor product will be separated by approximately 30 minutes (from the end of each product trial). Subjects who react negatively (i.e., unwilling to use and/or cannot tolerate the product [e.g., experience adverse events (AEs) that will prevent them from continuing to use the product as judged by the investigator]) to any of the Nu Mark e-vapor products during the product trial will not continue in the study. Subjects will be given a facilitated " Use the Product Again Questionnaire" after the product trials. After all assessments are complete on Day –1, subjects will be randomly assigned to a test product use schedule (1 Test Product per day for four days). Subjects will abstain from tobacco or nicotine use overnight. On Day 1, Subjects will complete an

exhaled breath test product use session with their assigned test product. An exhaled breath test product use session will consist of the following: one sample with an empty cartridge and inactive battery [sham condition] and one sample using the assigned test product with all exhaled breath collected in one type of trapping container, followed by at least 45 minutes of rest, then the sham sample and same test product sample collection with a second type of trapping container. Each sample will consist of all the exhaled breath occurring during 10 puffs, each with 5 second puff duration (+/- 1 second), over approximately 5 minutes (one puff approximately every 30 seconds) collected in the respective sample collection container:

- Trapping Container 1: captures nicotine, glycerin, propylene glycol, and menthol
- Trapping Container 2: captures formaldehyde, acetaldehyde, and acrolein
- Test product cartridges will be weighed before and after each collection.

Upon completion of the exhaled breath product use session, the subjects will be allowed to use new cartridges with freshly charged "BVR2.3 Rev 05 batteries with topography v1" batteries ad libitum for the next 12 hours following the package insert instructions. These batteries have been verified to perform within acceptance criteria of batteries without topography chip. Cartridges will be weighed before and after use. The "BVR2.3 Rev 05 battery with topography v1" will record all puffs and duration of puff during the 12 hours of ad libitum use. After the 12 hours of ad libitum use, subjects will complete the Use the Product Again Questionnaire.

After overnight tobacco and nicotine abstinence, the subjects will have vital signs recorded and repeat the exhaled breath test product use session and 12-hour ad libitum use session with their assigned test product on days 2 through 4 (1 test product per day). After each 12-hour ad libitum use session, subjects will complete the Use the Product Again Questionnaire.

Upon completion of the ad libitum product use session on Day 4, subjects will undergo end-of-study assessments and be released from the site.

The 4 test products are described in Section 1.3. The Schedule of Assessments (SOA) is provided in Table 3.

Table 3. Schedule of Assessments

EVENT	Visit 1 (Day –30 to Day –1)	Visit 2 (Day –1 Check-in)	Visit 2 (Day 1 through Day	End of Study
Age verification	X			
Informed consent	X			
Medical history	X			
Review of inclusion and exclusion criteria	X	X		
Demographics (including questionnaire)	X			
Tobacco and Nicotine Product-Use History Questionnaire	X			
Vital signs[1]	X	X	X	
Urine cotinine measurement	X			
HIV, HBsAg, anti-HCV screening	X			
Body weight, body height, and body mass index	X			
Clinical chemistry, hematology, and urinalysis	X			
Urine drug and alcohol screening[2]	X	X		
Serum pregnancy test (all females only)	X			
Urine pregnancy test (all females only)		X		
FSH test (post-menopausal females only)	X			
Review of concomitant medications	X	X	X	X
Quit Assist™ Website Referral	X			X
Review of adverse events	X	X	X	X
Electrocardiogram	X			
Physical examination	X			X[3]
Check-in procedures		X		
Confinement		X	X	
Product trial		X		
Willing To Use Questionnaire		X		
Weigh cartridges before and after use (both exhaled breath product use session and ad libitum use session) record batteries used with each cartridge			X	
Exhaled breath product use session[4]			X	
12 hour ad libitum use with "BVR2.3 Rev 05 battery with topography v2" for recording puff parameters. The batteries used with each cartridge will be documented.			X	
Use the Product Again Question (after each 12 hour ad libitum use session)			X	
Discharge from clinic				X

HIV = human immunodeficiency virus; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus.

1. Vital signs (respiratory rate, pulse rate, blood pressure, oral temperature), will be measured in the sitting position after at least 5 minutes of rest at least 15 minutes from last tobacco use.
2. Exhaled breath test for alcohol is also acceptable.
3. Brief physical examination (symptom driven).
4. A product use session will consist of the following: one sample with an empty cartridge and inactive battery [sham condition] and one sample using the assigned test product with all exhaled breath collected in one type of trapping container, followed by at least 45 minutes of rest, then the sham sample and same test product sample collection with a second type of trapping container. Each sample will consist of **all** the exhaled breath occurring during 10 puffs, each with 5 second puff duration (+/- 1 second), over approximately 5 minutes (one puff approximately every 30 seconds) collected in the respective sample collection containers

2.0 STUDY POPULATIONS

2.1 DEFINITIONS OF POPULATIONS FOR ANALYSIS

2.1.1 Exhaled Breath Population

The Exhaled Breath Population (referred in the Protocol as "the Exhaled Breath Population") will include all subjects who used at least one test product and from whom at least 1 exhaled breath was collected.

2.1.2 Per-Protocol Population

The per-protocol (PP) population will include all subjects who sufficiently comply with the protocol and who do not have any major protocol deviations (Section 2.3).

2.1.3 Safety Population

The safety analysis set will include all subjects who used at least 1 of the study products.

2.1 PROTOCOL DEVIATIONS

Major protocol deviations will include non-compliance with inclusion criteria 3, 4 or 5 and/or exclusion criteria 7, 11, 19, and 20. (All inclusion and exclusion criteria can be seen in Sections 4.1 and 4.2 of the protocol.) major protocol deviation designations will be reviewed and updated (as necessary) shortly after the completion of the study. Decisions as to what to do about subjects who had major protocol deviations will be made at that time.

All protocol deviations will be documented and included in an appendix listing.

3.0 ENDPOINTS AND COVARIATES

3.1 PRIMARY ENDPOINT

The primary endpoints for the study are each subject's sham-corrected amount of nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde and acrolein in exhaled breath from 10 puffs in each period for each of the 4 e-vapor products.

3.2 SECONDARY ENDPOINTS

Secondary endpoints will include:

Cartridge weight changes (before and after exhaled breath sessions), cartridge weight changes per cartridge and in total during 12 hours of ad libitum use, puff count, and average puff duration per cartridge and in total by subject during 12 hours of ad libitum use, and responses to Use Product Again Questionnaire.

3.3 SAFETY ENDPOINTS

The safety endpoints of the study are:

- Vital signs (pulse rate, diastolic and systolic blood pressure)
- Adverse events

4.0 STATISTICAL ANALYSIS SPECIFICATIONS

4.1 GENERAL

Unless otherwise stated, all data will be listed by subject, study product, and study day (and time point as necessary) and summarized by test product and study day (and time point as necessary). Tabular summaries (by test product and visit) of continuous endpoints will include standard descriptive statistics (number of observations, mean, median, SD, CV%, and range). Categorical data will be summarized as counts and percentages of numbers of subjects in each category (e.g., male and female genders). For categorical measurements (e.g., adverse experience severity) collected at separate visits, the denominator of the percentages of each category will be the number of subjects with a nonmissing value at the visit. For all other categorical measurements, except where otherwise noted, the denominator of percentages will be the number of subjects with nonmissing response data for the test product group.

For all analyses, the baseline response and covariate values will be defined per Section 1.4 of this statistical analysis plan. The electronic case report forms will be used to capture study results and data.

All tables and listings will be produced by using SAS®, Version 9.4.

4.2 METHODS FOR HANDLING DROPOUTS AND MISSING DATA

The primary and some secondary responses will be analyzed in a mixed effects ANOVA/ANCOVA framework wherein subjects and dependencies among multiple measures of the same endpoint, taken from the same subject (one in each of 4 periods), will be modeled as random effects. One advantage of such models is that the model estimates will be unbiased as long as any data that may be missing, are missing at random (MAR).[7, 8] This method of dealing with missing data has been demonstrated by Food and Drug Administration statisticians and others to be superior to LOCF.[9, 10]

Missing safety and demographic data will not be imputed.

4.3 SPECIFICATIONS FOR DISPLAYS

Displays will be produced by using the Courier New 8-point font. Headers will also be in Courier New 8-point font.

All displays are intended to be printed as landscape on 8.5 × 11-in paper. The top and bottom margins will be 0.50 in, and the left and right margins will be 0.75 in.

Relative to the number of digits after the decimal in the original data, summary statistics will have the following number of digits after the decimal: mean, median, and percentiles - one more digit; standard deviation and standard error - two more digits; minimum, maximum, and range - same number of digits. Summary statistics will not exceed four digits after the decimal. Some laboratory parameters or other data may require deviation from this rule.

Percentages will be displayed with one digit after the decimal.

4.4 INTERIM ANALYSES

No interim analyses are planned for this study.

5.0 SUBJECT ENROLLMENT AND DISPOSITION

Subject enrollment, inclusion in analysis populations, number of subjects discontinuing before study completion, termination status, and the reason for premature discontinuation will be summarized by sequence with frequencies and, where appropriate, percentages of enrolled subjects. Data will also be provided in an appendix listing by subject.

6.0 BASELINE EVALUATIONS

This section addresses data that were collected only at the screening visit.

6.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographic and baseline characteristic data will be collected and will include age (based on date of informed consent), sex, ethnic group, race, height, weight, and BMI. Age will be calculated as an integer in years (rounded down) as the difference between the subject's date of informed consent and the date of birth. All demographic and baseline characteristic data available from the eCRF will be summarized and listed.

6.2 MEDICAL HISTORY

All medical and surgical history data, as well as current medical conditions will be summarized by category of medical condition and listed.

6.3 TOBACCO/NICOTINE PRODUCT-USE HISTORY

Subjects will be required to report tobacco product and nicotine product-use histories to satisfy study inclusion and exclusion criteria. The following characteristics of the subject's usual brand will be documented: brand, brand style, and flavor. The number of uses per day (single number, not a range) will also be listed and summarized (pooled over all brands) (n, median, mean, standard deviation, CV% and range).

6.4 USE TEST-PRODUCTS AGAIN SCORES

"Use the Product Again" VAS raw scores which can range from 0 to 100 mm (measured from left to right on the VAS), will be included in subject listings, but no summary statistics will be computed from them. The raw scores will be converted to a binary, "unwilling" vs. "willing" to use, response as follows:

$$\text{VAS} < 50 \text{ mm} \approx \text{unwilling to use again VAS} \geq 50 \text{ mm} \approx \text{willing to use again.}$$

An additional 3-level ordinal response will be defined as follows:

VAS < 50 mm ≈ unwilling to use again VAS = 50 mm ≈ don't care

VAS > 50 mm ≈ willing to use again.

Summary statistics [n (%)] will be computed for each level of the binary and 3-level endpoint, by test-product and by sequence. Summary statistics (n, minimum, maximum, median, mean, and standard deviation) of raw VAS scores will be computed by test-product, sequence and by binary "willing to use" category.

6.5 CLINCAL LABORATORY PARAMETERS

Clinical chemistry, hematology, urinalysis, urine cotinine, and viral assay data will be collected from each subject at the screening visit; however, there are no planned follow-up clinical lab assessments. Screening lab data will be listed for all subjects with flags for abnormal values and will be summarized. Clinical laboratory parameters will be summarized by parameter within panels (clinical chemistry, hematology, urinalysis, etc.), per Section 4.1.

6.6 ELECTROCARDIOGRAM DATA

12-lead Electrocardiogram (ECGs) will be performed at visit 2. Each subject's ECG will be scored as "normal", "not being clinically significantly abnormal, or "clinically significantly abnormal". The QT interval, QTc, PR, QRS duration and heart rate also will be recorded.

If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

$QTcF = QT / (RR)^{1/3}$ where RR = 60/HR (if not provided)

The average of the triplicate readings collected from each subject will be calculated for each ECG parameter. Summary statistics (n, mean, median, SD, min and max) will be calculated and tabulated for each ECG parameter and by sequence and test product.

7.0 EVALUATION OF RESPONSE PARAMETERS

7.1 ANALYSIS OF PRIMARY RESPONSE ENDPOINTS

Sham and test product-derived sample amount of each analyte as well as sham-corrected amount (SCA) of each analyte will be summarized, by test product, per Section 4.1. Sham-corrected analyte amounts will be computed by subtracting corresponding sham response values from test product response values.

Mixed effects ANCOVA models will be fit to separately to the following sham-corrected amount of 7 analytes that will be measured in exhaled breath repeatedly in each subject over the 4 days of Visit 2:

1. Nicotine
2. Glycerin
3. Propylene glycol
4. Menthol
5. Formaldehyde
6. Acetaldehyde
7. Acrolein

The Minimum Detection Limit (MDL) of each analyte is provided in Table 4.

Table 4. Limits of Detection for Exhaled Breath Analytes

Analyte	Nominal MDL (µg)
Propylene glycol	5
Vegetable glycerin	5
Nicotine	0.5
Menthol	0.5
Formaldehyde	0.2
Acetaldehyde	0.2
Acrolein	0.2

Analyte concentrations that are below the Minimum Detection Limit (MDL) will be set to the MDL value for analyses.

Two types of sampling containers were used for collection of the analytes from each subject's exhaled breath (Table 5). Sampling container 1 with one tube and one pad was used for capturing nicotine, glycerin, propylene glycol, and menthol while Sampling container 2 with one tube and 2 pads was used for capturing formaldehyde, acetaldehyde, and acrolein. Exhaled breath samples were collected in 2 independent sampling sessions, separated by 45 minutes, within each period (Figure 1B). Sampling container 1 was used to trap exhaled breath in the first sampling session, while sampling container 2 was used in the second sampling session.

There are two subsamples (1 tube + 1 pad) of each analyte, in each sampling container 1 and three subsamples (1 tube + 2 pads) of each analyte in sampling container 2. Enthalpy Analytical Company will provide data sets with the tube and pad analyte amount from each of the two types of Sample container, for each subject. Pad and tube MDL values of the same analyte will be different from one another. For each sampler, the final amounts for a given analyte will be computed in one of three ways:

1. When both the amounts of analyte in pad(s) and tube are above MDL, the exhaled breath sample (EBS) value will be the sum of the pad and tube values
2. When the amounts of analyte in pad(s) and tube are both below MDL; EBS value will be the sum of the two MDL values. However, since the MDL is volume dependent, the sum will be considered as below MDL (i.e. <0.5).
3. When the amount of one analyte is MDL, but the other is above MDL. EBS will be the sum of the MDL value and the above BLLOQ value.

Descriptive statistics (number of observations, number of missing, mean, SD, median, minimum, maximum, the first quartile, the third quartile, 95% confidence interval, and coefficient of variation) will be tabulated for each of these analytes by test product. Frequency (n and %) of MDL values will be summarized for each product. If the percentage of MDL values for an analyte is more than 50% in any product group, the analyte will not be analyzed with a mixed model.

Each mixed model will be fit to the data by restricted maximum likelihoods. Covariance structures to account for dependencies among repeated measures, within subjects, will be selected for each model using AIC criteria.[11] Four candidate covariance structures will be considered: compound symmetry, 1st order autoregressive, 1st order autoregressive with a random subject effect, and unstructured. Each model will include period-specific sham values (as a covariate), sequence, study product, and period as fixed effects; subjects nested within-sequence will be modeled as random effects. Standard residual analyses will be used to examine validity of assumptions for the fixed effect distributions. Response variables will be appropriately transformed (e.g., log, reciprocal, or square root) if the residuals indicate distributional assumptions don't hold. The models will be refit to the transformed data and residual analyses again be performed verify that the transformations corrected the problems.

Mean SCA response with (95% confidence limits), for each test product, will be estimated using SAS Least square means methods in each model. Estimates will be back-transformed to the original scale as necessary. Analyte LSmean estimates (with 95% CIs) for each test product will be tabulated for each model. As an example, the SAS Proc Mixed code to fit the Mixed effects ANCOVA model [with an AR(1) covariance structure] is provided below:

```
ods graphics on;
proc mixed data=evap_data method=REML;
   class seq test_product period subject;
   model Analyte = sham § seq period test_product/residual  DDFM=KR;
   Random subject (seq); †
   Repeated /type=AR(1) subject=subject(seq); ‡
  lsmeans test_product/cl alpha=0.05;
  ods output lsmeans=mndat;
title1 'Fit a 4x4 crossover design mixed effects ANCOVA';
title2 'with a baseline(sham) covariate adjustment'; run;
```

§ amount (µg) of exhaled analyte from sham is entered in the model as a "baseline" covariate

† explicit inclusion of the subject in a random statement will depend upon the selected covariance structure (Little et al. 2000).

‡ AR1 requires a Random subjects statement, but the UN structure does not.

7.2 ANALYSIS OF SECONDARY RESPONSE ENDPOINTS

Descriptive Statistics (n, mean, SD, Qtr1, median, Qtr3 and CV) will be provided, by product, for the following measurements:

- Exhaled breath sessions
  - Cartridge weight change by sampling session and overall
- 12 hour ad-libitum use session
  - Cartridge weight change by cartridge and overall (Template provided)
  - Total cartridge weight change (Template provided)
  - Number of cartridges used
  - Puff count by cartridge and overall
  - Total puff count
  - Average puff duration by cartridge and overall
  - Total puff duration by cartridge and overall
  - Average puff duration of the total puffs
  - Total puff duration
- Complete cartridge use over 12-hour ad-libitum use session
  - Puff count per complete cartridge (Template provided)
  - Average puff duration per complete cartridge
  - Total puff duration per complete cartridge
  - Cartridge weight change per complete cartridge

Definition of complete cartridge use: If a subject replaced a cartridge with a new cartridge, the previous cartridge is considered a complete cartridge. If subject only used one cartridge for the day, none would be counted as complete cartridge.

Mixed models will be applied to the following measurements:

- Total puff count over the 12-hour ad-libitum use session

- Total puff duration over the 12-hour ad-lib use session
- Average puff duration of the total puffs over the 12-hour ad-lib use session
- Total cartridge weight change over the 12-hour ad-lib use session
- Cartridge weight change calculated in each exhaled breath sampling session

Each model will include fixed effect terms for product, period and sequence. Subjects within sequence will be modeled as random effects. Models will be fit with SAS Proc Mixed, using the procedures described in Section 7.1.

7.3 EVALUATION OF SAFETY PARAMETERS

All safety analyses will be performed on the safety analysis set. Safety data, presented by test product, will be summarized on an "as-treated" basis using descriptive statistics (e.g., n, mean, standard deviation, median, minimum, maximum for continuous variables; n [%] for categorical variables). Safety variables will include test product use-emergent adverse events (PEAEs), and vital signs.

7.4 ADVERSE EVENTS

All AE data for the safety population will be coded (to the lowest-level term) by using the Medical Dictionary for Regulatory Activities (MedDRA, Version 17.1). AEs will be listed in by-subject data listings, including verbatim term (investigator terms from the case report form), preferred term, test product, severity, and relationship to test product. Summary tables of frequency counts of AEs will be provided by primary system organ class (SOC), preferred term, and test product. Frequency counts of AEs will also be summarized by severity and relationship to test product and overall.

A PEAE is defined as an adverse event that:

- Emerges during exposure to a test product, having been absent previously, or:
- Worsens in severity during sessions within visits 2, when the adverse event is continuous in duration.

The incidence (%) will be determined by calculating the number of subjects with at least 1 event and the percentage of subjects with PEAEs by preferred term. The

incidence of PEAEs will be reported as the number (percentage) of subjects with PEAEs by SOC and preferred term. A subject will be counted only once within a SOC and preferred term, even if the subject experiences more than one PEAE within a specific SOC and preferred term. A missing or unknown value for the assessment will be considered worst. The number (percentage) of subjects with PEAEs will also be summarized by maximum severity (mild, moderate, or severe). If a given subject had more than one PEAE mapped to the same PT, then that PEAE will be represented according to the corresponding maximal level of severity.

The number (percentage) of subjects with PEAEs will also be summarized by relationship to test product. If a given subject had more than one PEAE mapped to the same PT, then that PEAE will be represented according to the strongest relationship to study product. Test product-related PEAEs include those adverse events considered by the investigator to be "definitely", "possibly", "probably", "unlikely", or "not" related to test product usage. The number (percentage) of subjects with test product-related PEAEs will also be summarized by maximum severity (mild, moderate, or severe).

The number (percentage) of subjects with serious adverse events (SAEs) will be summarized by MedDRA, SOC, and preferred term for each test product group. A subject data listing of all SAEs will be provided.

7.5 PRIOR AND CONCOMITANT MEDICATIONS

Prior and current medication information is collected at the screening assessment. Concomitant medication data are collected from baseline (Visit 2, Day 1) through the follow-up assessment. Prior medications will be listed by subject. A separate listing of concomitant medications will be made, also by sequence and subject. For both listings, start and stop dates (and times for the 4 to 12-hour assessments) of each medication will be included.

All investigator terms for medications recorded in the eCRF will be coded to an 11-digit code using the World Health Organization Drug Dictionary WHO DDE Sept 2015 version.

If a medication has an end date and end time that occur before the product use date and time, that medication will be considered a prior medication. If a medication has a start

date and start time that occur before the product use date and time but an end date and end time that occur after the product use date and time, that medication will be considered prior and concomitant. If a medication has a start date, start time, end date, and end time that occur after the product use date and time, that medication will be considered concomitant. Should a missing start date, start time, end date, or end time lead to ambiguity in whether a medication is prior or concomitant, the medication will be considered concomitant.

7.6 VITAL SIGNS AND OTHER PHYSICAL EXAMINATIONS

Vital signs will be collected during screening (Visit 1) and at Visits 2, before subjects receive their first test product. Subjects' screening visit will be considered their baseline assessment. Vital sign measurements include pulse rate, respiratory rate, oral body temperature, and seated blood pressure (systolic and diastolic). These measurements will be listed by visit and subject. Vital sign values and change from baseline values will be computed for each vital sign and summarized by visit.

Incidence of potentially clinically significant results in vital signs will be provided from the AE summary tables, if applicable.

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