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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CLINICAL STUDY PROTOCOL

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SPONSOR AGREEMENT

I have read the following protocol and agree to the conduct of the study as described herein:

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Date

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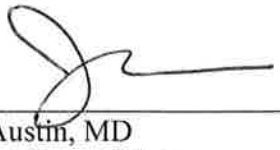
I have read the following protocol and agree to conduct the study as described herein:



02 MAR 2017

Jonathan Austin, MD
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Date

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SYNOPSIS

Altria Client Services LLC Protocol Number: ALCS-RA-16-11-EV

Protocol Title:

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

Number of Study Sites: One site in the United States in High Point, NC.

Study Purpose:

In Section VI (H) (b) of the Premarket Tobacco Product Applications (PMTAs) for Electronic Nicotine Delivery Systems,[11] the Food and Drug Administration recommends that applications "should provide data that adequately characterizes the likely impact of the new tobacco product on the health of both users and nonusers of tobacco products in order to support that marketing the new tobacco product would be appropriate for the protection of the public health."

The purpose of this study is to generate evidence for potential PMTAs for e-vapor products to support a conclusion that marketing the products would be appropriate for the protection of the public health. This study will characterize the levels of selected aerosol constituents in the exhaled breath of e-vapor users during 10 puffs of the candidate e-vapor products. This information will be used to model potential exposure to nonusers of the test products.

Study Products and Administration:

Test e-vapor products (EVPs):

- Product A: Product XL25F = Test EVP (currently marketed by Nu Mark LLC as MarkTen® XL Fusion [2.5% NBW]) [CVR2.6.8] Formula: 10321-75-1; Name: "Celine"; Cartridge Code: 751.
- Product B: Product XL40CB = Test EVP (currently marketed by Nu Mark LLC as MarkTen® XL Bold Classic [4.0% NBW]) [CVR2.6.8] Formula: 10381-44-B; Name: "Rosetta"; Cartridge Code: B44.
- Product C: Product XL35WM = Test EVP (currently marketed by Nu Mark LLC as MarkTen® XL Winter Mint [3.5% NBW]) [CVR2.6.8] Formula: 10353-34-C; Name: "Monica"; Cartridge Code: C34.
- Product D: Product XL40MB = Test EVP (currently marketed by Nu Mark LLC as MarkTen® XL Bold Menthol [4.0% NBW]) [CVR2.6.8] Formula: 10381-40-E; Name: "Spencer"; Cartridge Code: 40E.

SYNOPSIS (continued)

Study Objectives:

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.

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, average puff flow rate, average puff volume and average puff duration per cartridge and in total over the 12 hours during each 12 hours of ad libitum use
- To characterize responses to the Use Product Again Questionnaire after each 12 hour ad libitum use session

Study Design:

This is an open-label, 4-way crossover study designed to estimate the nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde, and acrolein levels in exhaled breath samples during use of four MarkTen® XL e-vapor products. The study will enroll approximately 32 adult e-vapor-using subjects. Each of the 32 subjects will provide two exhaled breath samples for all four test products. Subjects will make two visits to the site, one screening visit and one 4 day in-clinic visit to provide exhalation samples for four test products (one each day for 4 days). Subjects will also use their assigned test product ad libitum for 12 hours each day after the collection of exhaled breath samples. Subjects will be randomly assigned to a test product-use schedule at Visit 2, with one test product used per day.

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

SYNOPSIS (continued)

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 abstain from tobacco or nicotine use overnight, and on Day 1, subjects will be randomly assigned to a test product use schedule (1 test product per day for four days). 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 four sample collections: 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 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, over ~5 minutes (one puff every 30 seconds) collected in the respective sample collection containers:

- 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 "Rev 05 batteries with topography v2" batteries containing a topography recording chip ad libitum for the next 12 hours following the package insert instructions. These batteries have been validated to demonstrate no differences between the devices with or without the topography chip. Cartridges will be weighed before and after use. The "BVR2.3 Rev 05 battery with topography v2" 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.

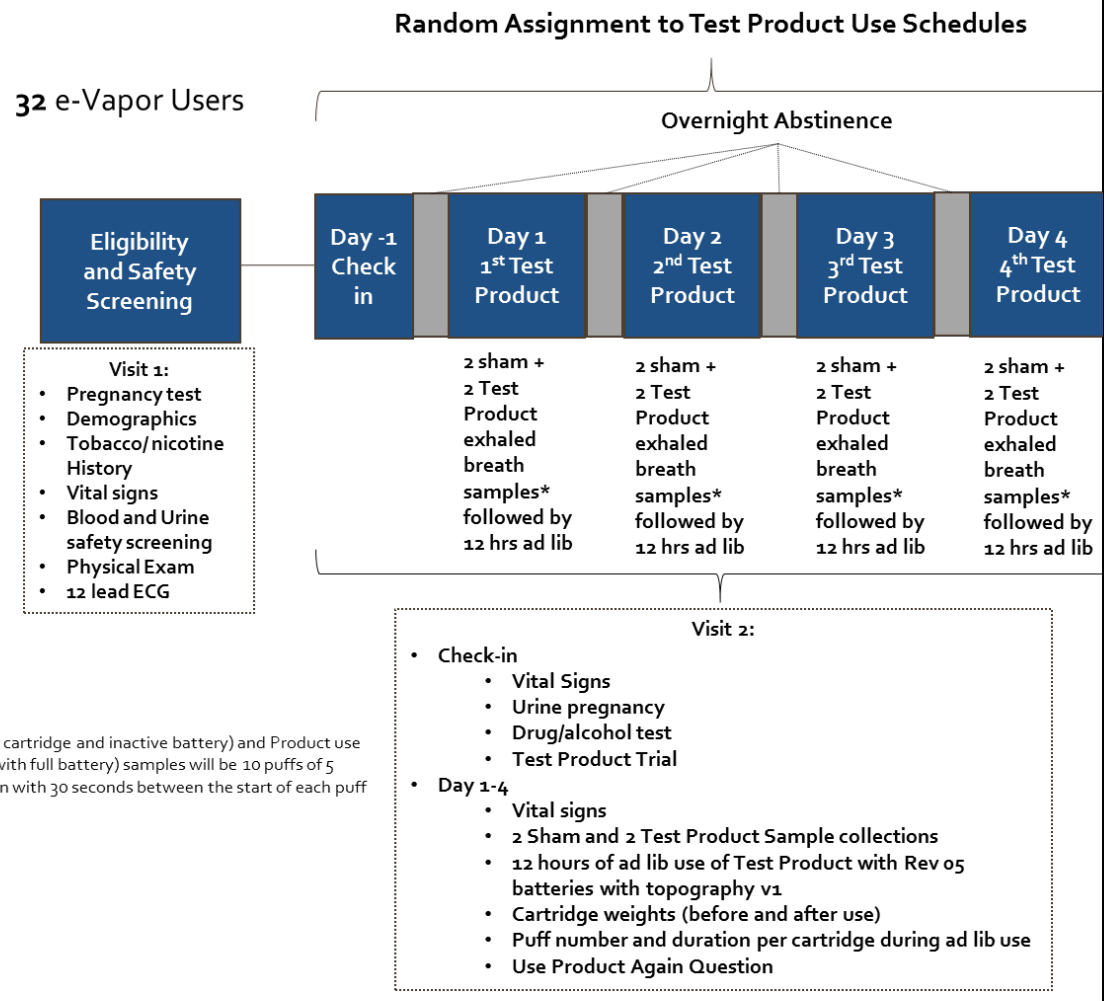
Upon completion of the ad libitum product use session on Day 4, subjects will undergo end-of-study assessments and be released from the site.

Overall study time and events are listed in Table 1.

SYNOPSIS (continued)

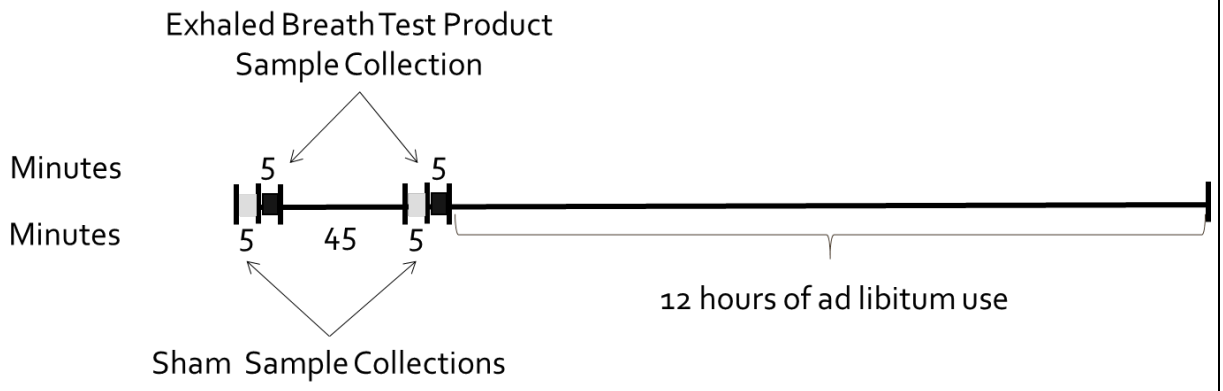
Study Diagram:

A: Overview



SYNOPSIS (continued)

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



Product Assignment Sequence and Randomization:

On Day 1 (Visit 2), subjects will be randomized to one of four product-use order sequences in a 1:1:1:1 ratio (n = ~8 subjects in each sequence: A B D C, B C A D, C D B A, D A C B). Subjects will also be randomized by sex.

Subject Population and Sample Size:

The overall study population will consist of approximately 32 adults (21 – 65 years of age) who use EVPs and who satisfy the criteria for enrollment.

Inclusion Criteria

Subject candidates must satisfy the following criteria before being enrolled in the study:

1. provide voluntary consent to participate, as documented by the signed institutional review board (IRB)-approved informed consent form (ICF) for the study;
2. be between the ages of 21 and 65 years, inclusive, at the time of screening (Visit 1);
3. be positive for tobacco use by urine cotinine measurement (≥ 500 ng/mL) at Visit 1 (screening);
4. have used nicotine-containing EVPs for the 3 months before Visit 1 (screening) and used nicotine-containing EVPs ("some days" or "every day") for the past 30 days and at least 4 out of the past 7 days before Visit 1 (screening) and at check-in for Visit 2;
5. have negative alcohol, amphetamines, opiates, cannabinoids, phencyclidine, and cocaine urine drug screening results (exhaled breath test for alcohol is also acceptable) at Visit 1 (screening) and at check-in Visit 2;
6. if female (*all* females), have a negative serum pregnancy test at Visit 1 (screening) and have a negative urine pregnancy test at check-in for Visit 2;
7. if female and heterosexually active and of childbearing potential (e.g., not surgically sterile [i.e., bilateral tubal ligation, hysterectomy, or bilateral oophorectomy] at least 6 months before Visit 1 [screening] or at least 2 years naturally postmenopausal [follicle stimulating hormone ≥40 IU/L at Visit 1 (screening)]), must be using one of the following forms of

SYNOPSIS (continued)

contraception and agree to continue using it through at least 30 days after the last study product use (if early terminated) or completion of the study:

- hormonal (e.g., oral, transdermal patch, implant, or injection) consistently for at least 3 months before Visit 1 (screening);
- double barrier (i.e., condom with spermicide or diaphragm with spermicide) consistently for at least 30 days before Visit 1 (screening);
- intrauterine device for at least 3 months before Visit 1 (screening);
- Essure® or similar nonsurgical sterilization procedure at least 3 months before Visit 1 (screening); or
- partner who has been vasectomized for at least 6 months (inclusive) before Visit 1 (screening);

8. if male and heterosexually active and capable of fathering a child (e.g., not vasectomized at least 6 months before Visit 1 [screening]), must be using a double barrier (i.e., condom with spermicide or diaphragm with spermicide) method of contraception from Check-in Visit 2 until at least 90 days after the last study product use (if early terminated) or completion of the study;
9. not plan to quit e-vapor use in the next 30 days;
10. be willing to use all assigned EVPs during the study; and
11. be willing and able to comply with the requirements of the study.

Exclusion Criteria

Subjects may be excluded from the study if the subject meets any of the criteria listed below at Visit 1 (screening) and Visit 2 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.

1. have a history or presence of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, urologic, diabetes, existing respiratory diseases (especially bronchospastic diseases and asthma), immunologic, psychiatric, cardiovascular disease, or any other condition(s) that, in the opinion of the investigator, would jeopardize the safety of the subject or impact the validity of the study results (Note: Chronic medical conditions controlled and on stable medications [over the past 3 months] may not be exclusionary per investigator discretion.);
2. have current evidence or any history of congestive heart failure;
3. have clinically significant abnormal findings on physical examination, vital signs, ECG, clinical laboratory results, or medical history, in the opinion of the investigator;
4. have systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg at Visit 1 (screening) or at check-in for Visit 2;
5. have estimated creatinine clearance (by Cockcroft-Gault equation) <80 mL/minute;
6. have liver enzymes (aspartate aminotransferase and alanine aminotransferase) ≥1.5 times the upper limit of normal at Visit 1 (screening);
7. have an acute illness (e.g., upper respiratory infection or viral infection) requiring treatment within 2 weeks before check-in at Visit 2;
8. have fever (>100.5°F) at Visit 1 (screening) or at check-in for Visit 2;

SYNOPSIS (continued)

9. have body mass index (BMI) greater than 40.0 kg/m^2 or less than 18.0 kg/m^2 at Visit 1 (screening);
10. have positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) at Visit 1 (screening);
11. have used prescription or over-the-counter bronchodilator medication (e.g., inhaled or oral β-agonists) within 12 months of Visit 1 (screening) and Visit 2;
12. have diabetes mellitus that is not controlled by diet or exercise alone, in the opinion of the investigator;
13. have used prescription antidiabetic medication or insulin therapy within 12 months of Visit 1 (screening) and Visit 2;
14. have used medication for depression or asthma within 12 months of Visit 1 (screening) and Visit 2;
15. have a history of drug or alcohol abuse within 12 months of Visit 1 (screening) and Visit 2;
16. have had allergic or other known adverse reactions to menthol, propylene glycol, or glycerol;
17. if female, be pregnant, nursing, or planning to become pregnant during the study;
18. have participated in a clinical study for an investigational drug, medical device, biologic, or for a tobacco product within 30 days before Visit 1 (screening) and Visit 2;
19. be a current or former employee of the tobacco industry or a first-degree relative (e.g., parent, spouse, sibling, child) of a current or former employee of the tobacco industry or a named party or class representative in litigation with any tobacco company;
20. have been involved in the development of the study design or conduct or be a first-degree relative (e.g., parent, spouse, sibling, child) of someone involved in the development of the study design or conduct;
21. be a current employee or personnel involved with the study at the site; or
22. have participated in two or more ALCS studies within the 12 months period before Visit 1 (screening) and check-in at Visit 2.

Study Duration:

The expected study duration from subject Visit 1 (screening) to the End of Study for each individual subject will be approximately 34 days.

Statistical Analysis:

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 provided for each analyte level (defined as the difference between the product sample value and the sham sample value), puff duration, number of puffs per cartridge, and for cartridge weight change by test product and use session (exhaled breath or ad libitum use). A linear mixed model for analysis of variance will be used on the analyte level in the exhaled breath samples, average puff duration, average puff flow rate, average puff volume, average puff number per cartridge, and cartridge weight changes. The model will include sequence, study product, and period as fixed effects and subject nested within-sequence

SYNOPSIS (continued)

as a random effect. Additional details of the statistical analysis will be provided in the statistical analysis plan.

Descriptive statistics will be calculated for the safety parameters. No formal statistical tests are planned for safety data.

Table 1. Summary of Time and Events

EVENT	Visit 1 (Day –30 to Day –1) Screening	Visit 2 (Day 1 Check-in)	Visit 2 (Day 1 through Day 4)	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		
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 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, over 5 minutes (one puff every 30 seconds) collected in the respective sample collection containers

LIST OF ABBREVIATIONS

AE	adverse event
ALCS	Altria Client Services LLC
APE	alleged physical effect
BMI	body mass index
CFR	Code of Federal Regulations
CLIA-88	Clinical Laboratory Improvement Amendments of 1988
CRF	case report form
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
ICF	informed consent form
IRB	institutional review board
MN	micronucleus
NBW	nicotine by weight
OECD	Organization for Economic Cooperation and Development
PK	pharmacokinetic
PMTA	Premarket Tobacco Product Application
SAE	serious adverse event
SAP	statistical analysis plan

1.0 Introduction and Study Rationale

1.1 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 are undertaking various studies to evaluate the EVPs including the MarkTen® branded EVPs. These studies will provide the scientific evidence in support of a Premarket Tobacco Product Application (PMTA) submission to the Center of Tobacco Products at the Food and Drug Administration (FDA). Before issuing a marketing order, the FDA must determine, based on the contents of the PMTA and other data available to the FDA, that marketing the new product that is the subject of the PMTA would be appropriate for the protection of the public health. The FDA's Draft PMTA Guidance for Electronic Nicotine Delivery Systems[6] (ENDS; or in other words EVPs) (Draft ENDS PMTA Guidance) suggests that applicants include, in addition to other information, results from studies addressing

topography and use patterns, an assessment of abuse liability, and results from human studies assessing biomarkers and health risks to users and non-users of the products.

This 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.

The following sections summarize the preclinical studies on the products proposed for testing in this study as well as human studies conducted on earlier versions of MarkTen® branded products.

1.2 Product Information

1.2.1 Preclinical Testing and Toxicological Assessment

Altria Client Services LLC implements an EVP toxicological assessment ("Product Stewardship") program based on the concept that, while the use of any tobacco product has an inherent risk, ingredients and components used in Nu Mark's products and any interaction of e-vapor device and e-liquid to form aerosols should not increase the inherent risk. The toxicological assessment is based on the totality of all of the available scientific evidence and, when appropriate, considers the harm reduction potential of EVPs for cigarette smokers.

Nu Mark uses the highest quality ingredients available, food-grade generally recognized as safe flavors and United States Pharmacopeia-grade aerosol formers (propylene glycol and glycerol) and nicotine. The use levels are determined based on estimated consumer usages, material-specific scientific data in the literature, and applicable regulatory information.

Toxicological data are already available for many individual ingredients commonly used in consumer products. However, there is no toxicological data on specific product formulations. Therefore, ALCS uses a weight-of-evidence approach in assessing e-vapor ingredients and products considering the following information:

- Individual material evaluation and risk assessment

- Chemistry analyses of formulations and product aerosols
- Flavor mixtures (prototype formulations): in vivo inhalation study in rats
- Product-specific formulation: in vitro (genotoxicity and cytotoxity) testing

The approach is consistent with the FDA's recommendations in the Draft ENDS PMTA Guidance (May 2016), for "a full assessment of the toxicological profile associated with the new product, which includes in vitro and in vivo toxicological studies." In the assessment of genotoxicity potential, the FDA refers to International Council for Harmonization (ICH) S2(R1) guidance and Organization for Economic Cooperation and Development (OECD) protocols.[7,8,9]

Altria Client Services LLC has performed the following battery of in vitro tests and if needed, in vivo genotoxicity test for the relevant e-liquid formulations can be performed:

1. In vitro bacterial gene mutation test (Ames assay; OECD471);
2. In vitro mammalian cytotoxicity test (Neutral Red Uptake [NRU] assay; OECD129);
3. In vitro mammalian genotoxicity test (micronucleus [MN] assay; OECD487); and
4. In vivo mammalian genotoxicity test (MN assay; OECD474 and Comet assay; OECD489) (if needed).

The test products included in this study are outlined in Table 2.

Table 2. Test Products Used in This Study

Non-menthol Test Products
Product A: Product XL25F = Test e-vapor product (currently marketed by Nu Mark LLC as MarkTen® XL Fusion [2.5% NBW]) [CVR2.6.8] Formula: 10321-75-1; Name: "Celine"; Label: 751.
Product B: Product XL40CB = Test e-vapor product (currently marketed by Nu Mark LLC as MarkTen® XL Bold Classic [4.0% NBW]) [CVR2.6.8] Formula: 10381-44-B; Name: "Rosetta"; Label: B44.
Menthol Test Products
Product C: Product XL35WM = Test e-vapor product (currently marketed by Nu Mark LLC as MarkTen® XL Winter Mint [3.5% NBW]) [CVR2.6.8] Formula: 10353-34-C; Name: "Monica"; Label: C34.
Product D: Product XL40MB = Test e-vapor product (currently marketed by Nu Mark LLC as MarkTen® XL Bold Menthol [4.0% NBW]) [CVR2.6.8] Formula: 10381-40-E; Name: "Spencer"; Label: 40E.

NBW = nicotine by weight.

A brief summary of in vitro assay results for the e-vapor formulas included in this study is provided below.

- Product XL35WM and Product XL40MB: These 3 formulations were negative in all 3 in vitro (Ames, NRU, and MN) assays.

- Product XL25F and Product XL40CB: Both formulations were negative in Ames and Neutral Red Uptake assays. In MN assay, Product XL25F formulation was positive and Product XL40CB formulation was equivocal (neither clearly positive nor negative as defined in the OECD487, i.e., inconclusive). While none of the e-liquid ingredients in the formulations are known mutagens or carcinogens, mixtures of e-liquid could potentially trigger genotoxic signals in cellular systems. For pharmaceutical drug candidates, OECD guidelines recommend in vivo genotoxicity assays to further investigate genotoxicity potential detected by an in vitro system (OECD474 and 489). Accordingly, ALCS is conducting in vivo genotoxicity testing of Product XL25F and Product XL40CB, using a conservative approach that evaluates two independent endpoints (in vivo MN and in vivo Comet responses) based on the combined OECD474 and 489. Preliminary results for Product XL25F are negative at both in vivo endpoints. Results for Product XL40CB are pending.

As outlined above, these testing results need to be considered in the context of all available evidence. In addition to in vitro assays, flavor mixture formulations were tested as aerosols via in vivo 90-day inhalation exposures in rats. While not product-specific, these investigations allow holistic evaluations of respiratory and

systemic responses in rodents inhaling aerosols of e-vapor flavor mixtures, similar to the human mode of exposure. The results from this testing did not show a meaningful difference in biological endpoints between the vehicle only and the vehicle with flavor study groups. In addition, Werley et al (2016) demonstrated that 90-day inhalation exposures from an example EVP (MarkTen® CVR1.3) did not show meaningful difference in biological endpoints between the vehicle only and the vehicle with flavor study groups.[10]

In summary, based on the totality of evidence including the individual material assessment, literature review, and collective in vitro and in vivo testing, we believe there is no concern for use of the test Products in this clinical study. All four formulations being used in this study are currently available in the commercial market (since 8 August 2016).

1.2.2 Human Studies on Previous Nu Mark E-Vapor Products

ALCS has conducted five in-clinic human studies and two ambulatory studies. These studies are summarized in the following three subsections.

1.2.2.1 In-clinic Pharmacokinetic Studies

Altria Client Services LLC sponsored 3 pharmacokinetic (PK) studies of EVPs since 2012. The first PK study (CEL-LIQ-01-12) included 2 prototype EVPs (CVR1.2 NS [PM 10305-54-B] non-menthol and CVR1.2 MS [PM 10305-57-A] menthol flavored) containing 2.0% tobacco-derived NBW. The purpose of this PK study was to characterize the nicotine plasma PK profile from single and multiple (1 use [10 inhalations] each hour over 12 hours) uses of the prototypes. This randomized, single-blind, 4-period crossover PK study was conducted in 27 (14 males, mean age 36.3 years) healthy adult cigarette smokers. Subjects were instructed to use the prototype EVPs by taking ten 5-second puffs over a 5-minute period, repeating the exposure every hour for 12 hours. Six subjects (25%) experienced 11 mild adverse events (AEs) on the day they were using the CVR1.2 NS product, and 7 subjects (29%) reported 13 mild AEs on the day of CVR1.2 MS product use. The most common AE was throat irritation (4 subjects), followed by dyspepsia (2 subjects) and nausea (2 subjects).

The second PK study (CEL-LIQ-01-13) included 2 prototype e-cigarette products (CVR1.3 NB3 [10305-126-NB3%] containing 3.0% tobacco-derived NBW and CVR1.3 NB5 [10305-126-NB5%] containing 5.0% tobacco-derived NBW). Twelve adult smokers (6 males) participated in the study. For each product, the subjects took ten 5-second inhalations within a 5-minute period, and repeated the product use episode at 1-hour intervals for a total of 12 product use episodes. One subject reported 6 AEs (including dry lip, nausea, vomiting, and dizziness) following the use of the CVR1.3 NB3 product, and 3 subjects experienced 15 AEs (including nausea, vomiting, feeling hot, burning sensation, dizziness, nervousness, and hiccups) following the use of the CVR1.3 NB5 product. One subject discontinued due to mild AEs of nausea, vomiting, and nervousness during the CVR1.3 NB5 use period.

The third PK study (ALCS-E45-01-14) included 4 prototype EVPs containing 2.5% to 4.5% tobacco-derived NBW. The purpose of the PK study was to characterize the nicotine plasma PK profile from single and multiple (1 use each hour over 10 hours) uses of the prototypes. Adult subjects were instructed to use the prototype EVPs by taking 10 puffs over a 10-minute period, repeating the exposure every hour for 10 hours. Five (21%) of the 24 randomized subjects experienced 14 mild AEs that were considered likely related to study product. The most common AE (11 events in 2 subjects) was throat irritation, followed by 1 AE each of dry throat, headache, and dizziness.

1.2.2.2 In-clinic Topography Studies

Altria Client Services LLC has also sponsored 2 puff topography studies. The first study (CRT-LIQ-01-13) evaluated puffing topography measurements in subjects using a prototype EVP, CVR1.3 (PM-10305-87A). Thirteen (6 males) adult cigarette smokers and 10 (4 males) adult e-vapor users were enrolled in this study. Subjects were provided 2 cartridges (2.0% tobacco-derived NBW) for ad libitum use over a 7-hour period. Twelve (6 males) of the 13 cigarette smokers returned to the study site for additional puff measurements using 4 prototypes (2.0% tobacco-derived NBW). The subjects were instructed to take ten 5-second puffs over a 5-minute period. The subjects repeated this puff regimen 6 times a day (3 times per prototype) on 2 separate days. One reported AE of headache (mild) was considered possibly related to study product.

The second puff topography study (ALCS-M10-05-14) evaluated puffing topography measurements in 3 different groups of tobacco product users: adult smokers, adult smokers and e-vapor users (dual users), and adult exclusive e-vapor users. During the study, subjects used CVR1.5 EVPs (2.5% tobacco-derived NBW in both Menthol and Classic flavors) ad libitum over 8 consecutive hours. Ninety-one generally healthy adult subjects (48 males) were enrolled, and 89 subjects completed the study. On average, subjects used approximately 2.9 CVR1.5 e-vapor cartridges over the 8 hours, and no AEs were reported.

1.2.2.3 Ambulatory Studies

In addition, ALCS sponsored an ambulatory vaping exposure study (COV-M10-01-14) in 2014. One hundred forty-eight (70 males) healthy adult smokers were enrolled in the randomized control study. One hundred and three (48 males) subjects were allowed to smoke their own brand conventional cigarettes and use an e-vapor CVR1.5 (1.5% tobacco-derived NBW, either Classic or Menthol flavored) product ad libitum and 45 (22 males) subjects smoked their conventional cigarettes ad libitum without using EVPs for 4 weeks. Of the 103 subjects assigned to the e-vapor group, 34 reported 44 AEs, and 9 of the 45 subjects assigned to the smoking group reported 13 AEs. Out of the 44 AE reports from the subjects assigned to the CVR1.5 e-vapor group, 4 mild AEs were considered to be possibly related to the Test Products by the investigators (2 upper respiratory tract infection, 1 throat irritation, and 1 headache).

Altria Client Services LLC also sponsored an ambulatory tobacco product use study (ALCS-M10-02-14). Two hundred twenty-six (104 males) adult cigarette smokers were randomized into 5 groups investigating the use of cigarettes with the use of CVR1.5 EVPs (1.5% tobacco-derived NBW in Classic [C15] or Menthol [M15] flavor and 2.5% tobacco-derived NBW in Classic [C25] or Menthol [M25] flavor) for 3 weeks. Two groups were instructed to use their own brand cigarettes and CVR1.5 EVPs (n=51, C15/M15; n=49, C25/M25) ad libitum and 2 groups were instructed to use their own brand cigarettes ad libitum and at least 1 CVR1.5 e-vapor cartridge per day (n=52, C15/M15; n=49, C25/M25). The control group (n=25) continued to smoke their own brand cigarettes ad libitum. A total of 26 subjects (11.5% of enrolled subjects) reported 37 post-randomization AEs (1 post-randomization serious AE [SAE] occurred in the control group and was not related to Test Product). In the 201 subjects randomized to CVR1.5 EVP use groups, 15 mild or moderate AEs from 9 subjects (4% of CVR1.5 e-vapor use groups) were considered as unlikely, possibly,

or likely related to the Test Products by the investigators (no definitely related AEs were reported). Of these 15 unlikely, possibly, or likely related AEs, the only AE reported by more than 1 subject across all of the CVR1.5 use groups was mild oral discomfort (2 subjects; 1% of the CVR1.5 e-vapor use groups).

1.2.3 Consumer Response Center Data

Adverse events (AE) related to the use of Nu Mark's e-vapor products (MarkTen®) have been spontaneously reported by consumers to the ALCS Call Center, "Consumer Response Center", since August 2013. While ALCS continuously monitors and evaluates these AEs, no investigation is conducted to verify these health-related complaints associated with the use of MarkTen® e-vapor products (approximately 59.6 million cartridges sold). As of December 31, 2016, there were 645 consumers who reported 1,206 AEs using the MedDRA Preferred Terms (PT). The top ten PTs were: thermal burns (11.19%), burns second degree (9.12%), throat irritation (5.22%), cough (5.06%), oral discomfort (4.89%), nausea (4.31%), headache (4.06%), burns first degree (3.98%), electric shock (2.74%), and dizziness (2.24%). There were four consumers who reported AEs that met the Common Terminology Criteria for Adverse Events v4.0 criteria for "Severe". The "Severe" PTs were: eye swelling, swelling face, swollen tongue, facial paralysis, cough, haematemesis, upper respiratory tract infection, gastrointestinal injury, aphonia, and thermal burn, with two consumers being hospitalized. The majority (79.44%) of the AEs reported to the Consumer Response Center were classified as "Mild".

Based on the information presented, it is anticipated that the four EVPs in this study will be well tolerated.

1.2.4 Product Warnings

The adult subjects will be informed that the EVPs used in this research bear the following warning:

> **WARNING:** This product is not a smoking-cessation product and has not been tested as such. This product is intended for use by persons of legal age or older, and not by children, women who are pregnant or breastfeeding, or persons with or at risk of heart disease, high blood pressure, diabetes, or taking medicine for depression or asthma. Nicotine is addictive and habit forming, and it is very toxic by inhalation, in contact with the skin, or if swallowed. Nicotine can increase your heart rate and blood pressure and cause dizziness, nausea, and stomach pain. Inhalation of this product may aggravate existing respiratory conditions. Ingestion of the nonvaporized concentrated ingredients in the cartridges can be poisonous.

For products sold in the State of California, the following warning will be present at points of sale:

> CA Proposition 65 **WARNING**: This product can expose you to chemicals including glycidol, which is known to the State of California to cause cancer, and nicotine, which is known to the State of California to cause birth defects or other reproductive harm. For more information, go to www.P65Warnings.ca.gov.

Glycidol is detected in the aerosols generated from ALCS's products. International Agency for Research on Cancer has classified glycidol as "probably carcinogenic to humans (Group 2A)" (http://monographs.iarc.fr/ENG/Classification/latest_classif.php). ALCS's scientific test data, however, show that the anticipated human exposures are substantially below the established regulatory limits (https://www.osha.gov/dts/chemicalsampling/data/CH_243700.html).

In summary, as stated in section 1.2.1, based on the totality of evidence including the individual material assessment, literature review, and collective in vitro and in vivo testing, we believe there is no concern for use of the test products in this clinical study. All four formulations being used in this study are currently available in the commercial market (since 8 August 2016).

1.3 Study Purpose

In Section VI(H)(b) of the PMTAs for ENDS,[11] the FDA recommends that applications "should provide data that adequately characterizes the likely impact of the new tobacco product on the health of both users and nonusers of tobacco products in order to support that marketing the new tobacco product would be appropriate for the protection of the public health."

The purpose of this study is to generate evidence for potential PMTAs for e-vapor products to support a conclusion that marketing the products would be appropriate for the protection of the public health. The study will characterize the levels of selected aerosol constituents in the exhaled breath of e vapor users during 10 puffs of the candidate e-vapor Products. This information will be used to model potential exposure to nonusers of the test products.

2.0 Study Objectives

2.1 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.

2.2 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, average puff flow rate, average puff volume and average puff duration per cartridge and in total over the 12 hours during each 12 hours of ad libitum use
- To characterize responses to the Use Product Again Questionnaire after each 12-hour ad libitum use session

3.0 Summary of Study Design

3.1 Design

This is an open-label, 4-way crossover study designed to estimate the nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde, and acrolein levels in exhaled breath samples during use of four MarkTen® XL e-vapor products. The study will enroll approximately 32 adult e-vapor-using subjects. Each of the 32 subjects will provide two exhaled breath samples for all four test products. Subjects will make two visits to the site, one screening visit and one 4 day in-clinic visit to provide exhalation samples for four test products (one each day for 4 days). Subjects will also use their assigned test product ad libitum for 12 hours each day after the collection of exhaled breath samples. Subjects will be randomly assigned to a test product-use schedule at Visit 2, with one test product used per day.

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, a 12 lead ECG and complete a physical exam.

Approximately 32 eligible subjects (no more than 60% of either sex) may enroll into the study. Subjects will self-report that they have been using e-vapor products (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 abstain from tobacco or nicotine use overnight, and on Day 1, subjects will be randomly assigned to a test product use schedule (1 Test Product per day for four

days). 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 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, over 5 minutes (one puff every 30 seconds) collected in the respective sample collection containers:

- 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 v2" 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 v2" 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.

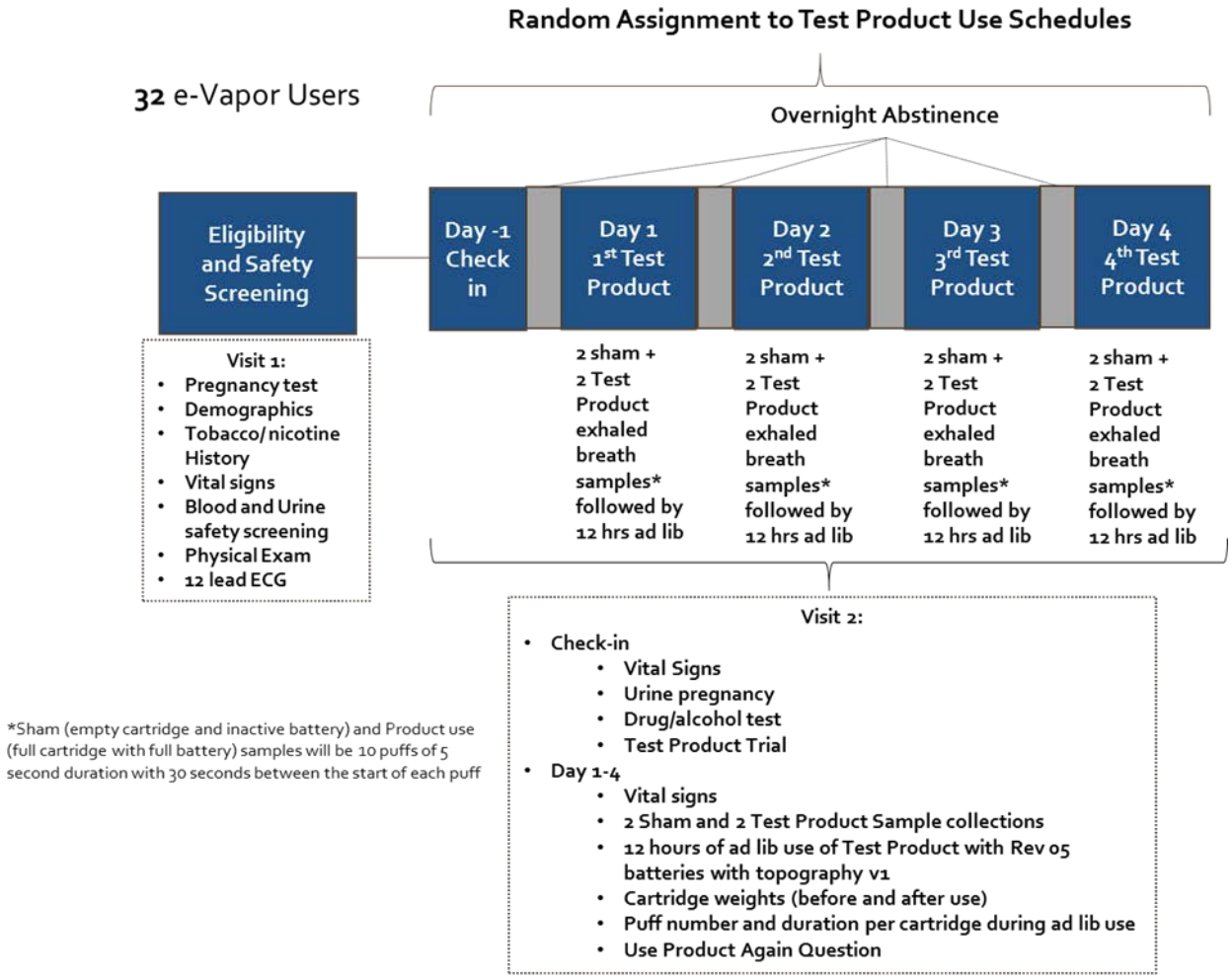
After overnight tobacco and nicotine abstinence, the subjects will have vital signs recorded and repeat the exhaled breath test product use session and 12-hour ad libitum use session with their assigned test product on days 2 through 4 (1 test product per day). After each 12-hour ad libitum use session,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

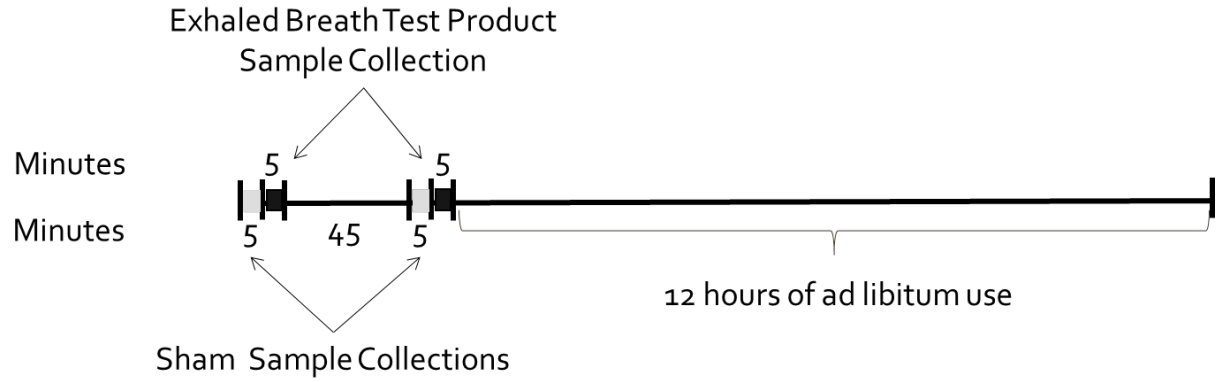
Overall study time and events are listed in Table 1. Overall design of the study is shown in Figure 1.

Figure 1. Study Diagram

A. Overall Diagram



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



3.2 Endpoints

The following study endpoints will be assessed:

- Primary endpoint: total amount of nicotine, glycerin, propylene glycol, menthol, formaldehyde, acetaldehyde and acrolein in exhaled breath for four e-vapor products (sham value corrected).
- Secondary endpoints: 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, average puff flow rate, average puff volume and average puff duration per cartridge and in total by subject during 12 hours of ad libitum use, responses to Use Product Again Questionnaire.

4.0 Subject Selection and Discontinuation

4.1 Inclusion Criteria

Subject candidates must satisfy the following criteria before being enrolled in the study:

1. provide voluntary consent to participate, as documented by the signed institutional review board (IRB)–approved informed consent form (ICF) for the study;
2. be between the ages of 21 and 65 years, inclusive, at the time of screening (Visit 1);
3. be positive for tobacco use by urine cotinine measurement (≥ 500 ng/mL) at Visit 1 (screening);
4. have used nicotine-containing EVPs for the 3 months before Visit 1 (screening) and use of nicotine-containing EVPs (“some days” or “every day”) for the past 30 days and at least 4 out of the past 7 days before Visit 1 (screening) and at check-in for Visit 2;
5. have negative alcohol, amphetamines, opiates, cannabinoids, phencyclidine, and cocaine urine drug screening results (exhaled breath test for alcohol is also acceptable) at Visit 1 (screening) and at check-in for Visit 2;

6. if female (*all* females), have a negative serum pregnancy test at Visit 1 (screening) and have a negative urine pregnancy test at check-in for Visit 2;

7. if female and heterosexually active and of childbearing potential (e.g., not surgically sterile [i.e., bilateral tubal ligation, hysterectomy, or bilateral oophorectomy] at least 6 months before Visit 1 [screening] or at least 2 years naturally postmenopausal [follicle-stimulating hormone ≥40 IU/L at Visit 1 (screening)]), must be using one of the following forms of contraception and agree to continue using it through at least 30 days after the last study product use (if early terminated) or completion of the study:

    - hormonal (e.g., oral, transdermal patch, implant, or injection) consistently for at least 3 months before Visit 1 (screening);
    - double barrier (i.e., condom with spermicide or diaphragm with spermicide) consistently for at least 30 days before Visit 1 (screening);
    - intrauterine device for at least 3 months before Visit 1 (screening);
    - Essure® or similar nonsurgical sterilization procedure at least 3 months before Visit 1 (screening); or
    - partner who has been vasectomized for at least 6 months (inclusive) before Visit 1 (screening);

8. if male and heterosexually active and capable of fathering a child (e.g., not vasectomized at least 6 months before Visit 1 [screening]), must be using a double barrier (i.e., condom with spermicide or diaphragm with spermicide) method of contraception from check-in at Visit 2 until at least 90 days after the last study product use (if early terminated) or completion of the study;

9. not plan to quit e-vapor use in the next 30 days;

10. be willing to use all assigned EVPs during the study; and

11. be willing and able to comply with the requirements of the study.

4.2 Exclusion Criteria

Subjects may be excluded from the study if the subject meets any of the criteria listed below at Visit 1 (screening) and Visit 2 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.

1. have a history or presence of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, urologic, diabetes, existing respiratory diseases (especially bronchospastic diseases and asthma), immunologic, psychiatric, cardiovascular disease, or any other condition(s) that, in the opinion of the investigator, would jeopardize the safety of the subject or impact the validity of the study results (Note: Chronic medical conditions controlled and on stable medications [over the past 3 months] may not be exclusionary per investigator discretion);

2. have current evidence or any history of congestive heart failure;

3. have clinically significant abnormal findings on physical examination, vital signs, ECG, clinical laboratory results, or medical history, in the opinion of the investigator;

4. have systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg at Visit 1 (screening) or at check-in for Visit 2;

5. have estimated creatinine clearance (by Cockcroft-Gault equation) <80 mL/minute;

6. have liver enzymes (aspartate aminotransferase and alanine aminotransferase) ≥1.5 times the upper limit of normal at Visit 1 (screening);

7. have an acute illness (e.g., upper respiratory infection or viral infection) requiring treatment within 2 weeks before check-in at Visit 2;

8. have fever (>100.5°F) at Visit 1 (screening) or at check-in for Visit 2;

9. have body mass index (BMI) greater than 40.0 kg/m^2 or less than 18.0 kg/m^2 at Visit 1 (screening);

10. have positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) at Visit 1 (screening);

11. have used prescription or over-the-counter bronchodilator medication (e.g., inhaled or oral β-agonists) within 12 months of Visit 1 (screening) and Visit 2;

12. have diabetes mellitus that is not controlled by diet/exercise alone, in the opinion of the investigator;

13. have used prescription antidiabetic medication or insulin therapy within 12 months of Visit 1 (screening) and Visit 2;

14. have used medication for depression or asthma within 12 months of Visit 1 (screening) and Visit 2;

15. have a history of drug or alcohol abuse within 12 months of Visit 1 (screening) and Visit 2;

16. have had allergic or other known adverse reactions to menthol, propylene glycol, or glycerol;

17. if female, be pregnant, nursing, or planning to become pregnant during the study;

18. have participated in a clinical study for an investigational drug, medical device, biologic, or for a tobacco product within 30 days before Visit 1 (screening) and Visit 2;

19. be a current or former employee of the tobacco industry or a first-degree relative (e.g., parent, spouse, sibling, child) of a current or former employee of the tobacco industry or a named party or class representative in litigation with any tobacco company;

20. have been involved in the development of the study design/conduct or be a first-degree relative (e.g., parent, spouse, sibling, child) of someone involved in the development of the study design/conduct;

21. be a current employee or personnel involved with the study at the site; or

22. have participated in two or more ALCS studies within the past 12-month period before Visit 1 (screening) and check in at Visit 2.

4.3 Subject Discontinuation

Subjects will be advised that they are free to withdraw from the study at any time and for any reason.

Investigators are urged to enroll only those eligible subjects who are likely to complete the entire study and who are willing to comply with the requirements of the protocol. Subjects withdrawn or removed from this study cannot re-enter.

Subjects will be instructed during the informed consent process that all data collected up to the point of withdrawal are intended for analysis. Subjects will also be instructed that they may notify the investigator if they choose at any time to withdraw consent for analysis of these data.

4.4 Subject Discontinuation Criteria

Subject participation in this study may be discontinued for any of the following reasons:

- withdrawal of informed consent;
- any clinical AE, laboratory abnormality, or other medical condition (including pregnancy) that indicates to the investigator that continued participation is not in the best interest of the subject;
- non-compliance with study procedures;
- termination of the study by the sponsor, United States (US) FDA, or other regulatory authorities; or
- lost to follow-up.

Protocol deviations should not lead to subject withdrawal unless they indicate a significant risk to the subject's safety or jeopardize the scientific integrity of the study.

A subject withdrawn from the study due to any AE or clinically significant abnormal laboratory test values will be evaluated by the investigator or other monitoring physician and will be treated and/or followed up until the symptoms or values return to

normal or acceptable levels, or until the subject is lost to follow-up, as appropriate in the opinion of the investigator.

4.5 Discontinuation Procedures

Subjects who are initially confirmed as eligible during screening but are deemed ineligible before enrollment (randomization) will be considered a "screen failure."

If premature withdrawal from the study occurs for any reason, the investigator must determine the primary reason and record this information in the case report form (CRF). Subjects withdrawn or dismissed by the investigator after enrollment (Day 1) will undergo End of Study procedures as feasible.

4.6 Replacement Subjects

Subjects withdrawn from the study may be replaced at the discretion of the sponsor.

4.7 Concomitant Medications

Any concomitant medications taken from 30 days before Visit 1 (screening) through the end of study (or upon early termination) will be recorded.

Use of prescription or over-the-counter medications/supplements required to treat a non-exclusionary disease or condition are permitted at the discretion of the investigator, provided the medication in question would have no effect on interpretation of analyte values. In-clinic use of over-the-counter medications (e.g., acetaminophen, ibuprofen) is permitted at the discretion of the investigator as needed by subjects, provided the medication in question would have no effect on interpretation of analyte values.

5.0 Study Conduct

5.1 Procedures by Visit/Day

5.1.1 Visit 1 (Screening)

The following screening procedures will be completed within 30 days of subject enrollment:

- Verify age and identity with government-issued identification
- Explain study procedures and obtain signature on the informed consent form
- Review inclusion and exclusion criteria
- Document demographics (Demographics Questionnaire)
- Document tobacco/nicotine product-use history (Tobacco/Nicotine Product Use-History Questionnaire)
- Record medical history
- Measure vital signs
- Measure height, weight, and BMI
- Collect blood sample for clinical chemistry (at least 8 hours fasting), hematology (at least 8 hours fasting), serology, and serum pregnancy (all females only) tests
- Follicle-stimulating hormone test for postmenopausal females only
- Collect urine sample for urinalysis, drug and alcohol screening, and cotinine screening (exhaled breath test for alcohol is also acceptable)
- Review concomitant medications and AEs
- Electrocardiogram
- Physical examination
- Provide tobacco cessation information (Quit Assist™ website referral)

5.1.2 Visit 2: Day -1 Check-in

Subjects will return to the clinic for their second visit on Day -1, and the following procedures will be completed:

- Review inclusion and exclusion criteria
- Measure vital signs
- Collect urine sample for urine drug and alcohol screening (exhaled breath test for alcohol is also acceptable), urine pregnancy test (all females only)
- Product trial
    1. Subjects will use the products in an assigned order (A, B, C, D)

2. Subject will engage in brief product trial with each e-vapor product (i.e., ad libitum use for 10 minutes).
3. Trials of each e-vapor product will be separated by approximately 30 minutes (from the end of each product trial).

- Review concomitant medications and AEs
- Remind subjects of study restrictions

5.1.3 Visit 2: Day 1 through Day 4

Subjects will abstain from tobacco or nicotine use overnight and repeat the following procedures on Days 1, 2, 3 and 4:

- Measure vital signs
- Subjects will be randomly assigned to a product use schedule (section 5.3)
- Exhaled breath product-use session (1 test product per day for 4 days per randomization schedule)

1. 10 sham puffs (empty cartridge and inactive battery)
   - 5-second puff duration, one puff every 30 seconds for 5 minutes (Note: All exhaled breath will be captured after the first puff to ~60 seconds after the last puff in Trapping Container 1 with a nose clip in place.) All puff durations will be documented.
   - Subjects will be instructed to inhale as they normally use EVPs and not to only mouth puff.
   - Subjects will also be instructed to make every attempt to exhale directly into the collection device and minimize leakage of the exhaled vapor in the ambient air.
2. 10 puffs with assigned test product with fully charged standard battery (BVR2.3)
   - 5-second puff duration, one puff every 30 seconds for 5 minutes (Note: All exhaled breath will be captured after the first puff to ~60 seconds after the last puff in Trapping Container 1 with a nose clip in place.) All puff durations will be documented.
   - Subjects will be instructed to inhale the vapor as they normally use EVPs and not to only mouth puff.
   - Subjects will also be instructed to make every attempt to exhale directly into the collection device and minimize leakage of the exhaled vapor in the ambient air.
3. At least 45 minutes without any tobacco or nicotine use

4. Cartridge (only test product cartridges) will be weighed within 90 minutes prior to the first 10 puffs. The cartridge will be weighed again after the first exhaled breath measurement and before the second exhaled breath measurement.

5. 10 sham puffs (empty cartridge and inactive battery)
   - 5-second puff duration, one puff every 30 seconds (Note: All exhaled breath will be captured after the first puff to ~60 seconds after the last puff in Trapping Container 2 with a nose clip in place.) All puff durations will be documented.
   - Subjects will be instructed to inhale as they normally use EVPs and not to only mouth puff.
   - Subjects will also be instructed to make every attempt to exhale directly into the collection device and minimize leakage of the exhaled vapor in the ambient air.

6. 10 puffs with the test product
   - 5-second puff duration, one puff every 30 seconds (Note: All exhaled breath will be captured after the first puff to ~60 seconds after the last puff in Trapping Container 2 with a nose clip in place.) All puff durations will be documented.
   - Subjects will be instructed to inhale the vapor as they normally use EVPs and not to only mouth puff.
   - Subjects will also be instructed to make every attempt to exhale directly into the collection device and minimize leakage of the exhaled vapor in the ambient air.
   - Weigh cartridge (test product only) again (within 90 minutes)

- Subject will be allowed to use new cartridges with fully charged (with in 24hrs) "BVR2.3 Rev 05 batteries with topography v2" batteries ad libitum for the next 12 hours

- Measure weight of cartridges before and after ad libitum use (within 90 minutes)

- Record all cartridges used with each "BVR2.3 Rev 05 battery with topography v2". All start and stop times with each cartridge and "BVR2.3 Rev 05 battery with topography v2" will be documented.

- Document likelihood of subject using product again (Use the Product Again Questionnaire) after each 12-hour ad libitum use session

- Review concomitant medications and AEs

5.1.3.1 End of Study (or Early Termination)

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 following end-of-study procedures will be completed:

- Conduct brief physical examination (symptom driven)
- Review concomitant medications and AEs
- Provide tobacco-cessation information (Quit Assist™ website referral)

5.2 Study Procedures

5.2.1 Informed Consent

All prospective subjects will have the study explained by the investigator or their designee.

All prospective subjects will be required to read, sign, and date the ICF before any screening or study procedures are performed. Written acknowledgment of the receipt of 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 the ICF.

5.2.2 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.

Characteristics of the subject's usual e-vapor products will be documented in the Tobacco and Nicotine Use History Questionnaire.

5.2.3 Medical History and Demographics

Medical history and demographic data, including sex, age (each subject must show proof of age with government-issued identification [e.g., driver's license], which will

be photocopied as source documentation), race, and ethnicity, for each subject will be recorded.

5.2.4 Questionnaires

Self-administered questionnaires will be completed at various times throughout the study (see Table 1) with trained site staff present for assistance. The questionnaires to be used in this study are as follows:

- Demographics Questionnaire, Visit 1 (screening)
- Tobacco and Nicotine Product-Use History Questionnaire, Visit 1 (screening)
- Use the Product Again Questionnaire, Visit 2: Day 1 through Day 4

5.2.5 Physical Examination

All physical examinations will be conducted by the investigator or their designee (licensed physician). A general physical examination including observations and questioning by the investigator/ or the investigator's designee (licensed physician), will be completed at Visit 1 (screening). A symptom-driven physical examination will be performed at End-of-Study or Early Termination and at other times as necessary to evaluate AEs or other subject complaints. All findings will be documented.

5.2.6 Height, Weight, and Body Mass Index

Height (cm) and weight (kg) will be measured with subjects wearing indoor clothing with shoes off. BMI will be calculated as weight (kg)/height (m^2).

5.2.7 Electrocardiogram

A 12-lead ECG will be performed after subjects have rested in a supine position for at least 5 minutes and at least 15 minutes after last tobacco or nicotine product use. The ECG will be documented as normal, having a not clinically significant abnormality, or having a clinically significant abnormality. In addition, ventricular rate, PR interval, QRS duration, and QT interval (corrected using Fridericia's formula and uncorrected) will be noted on the CRF.

5.2.8 Clinical Laboratory Tests

The clinical laboratory tests listed below and performed at screening and other time points as applicable (see Table 1) will be conducted by a local laboratory accredited by the Centers for Medicare and Medicaid Services (Clinical Laboratory Improvement Amendments of 1988 [CLIA-88]). Analyte values must fall within laboratory normal ranges or be deemed "not clinically significant" in the opinion of the investigator or the investigator's designee (licensed physician). Parameters to be evaluated are as follows:

- Clinical chemistry (at least 8 hours fasting): albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate or total carbon dioxide, blood urea nitrogen, chloride, creatinine (creatinine clearance will be calculated using Cockcroft-Gault formula at screening only), glucose, potassium, sodium, total bilirubin, total protein, and uric acid.
- Hematology: hematocrit, hemoglobin, platelet count, red blood cell count, and white blood cell count with differential.
- Urinalysis: bilirubin, blood, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, and urobilinogen. (A microscopic examination will be conducted if protein, leukocyte esterase, nitrite, or blood are detected. Microscopic analysis will include red blood cells, white blood cells, casts, and bacteria.)
- Serology: HIV, HBsAg, and HCV antibody screenings.
- Urine cotinine: a positive test will be required (≥ 500 ng/mL) for participation in the study.
- Pregnancy: serum or urine human chorionic gonadotropin (all females only) as appropriate (Section 5.2.9).
- Follicle-stimulating hormone: postmenopausal females only.
- Urine drug screening: amphetamines, phencyclidine, opiates, cannabinoids, and cocaine (Section 5.2.10).
- Alcohol screening: ethanol breath test or urine screening (Section 5.2.11).

5.2.9 Pregnancy Test

Urine or serum pregnancy tests will be completed by the clinical site staff at Visit 1 (serum) and check-in Visit 2 (urine). A positive test result will eliminate subjects from further participation in the study.

5.2.10 Urine Drug Screen

Urine drug screens will be completed by the clinical site staff for drugs of abuse (amphetamines, phencyclidine, opiates, cannabinoids, and cocaine) at Screening and Check-in for each visit. Positive test results (for any reason) will eliminate subjects from further participation in the study.

5.2.11 Alcohol Urine or Breath Test

A urine or breath alcohol test will be performed at Screening and Check-in for each visit. A positive test result will eliminate subjects from further participation in the study.

5.2.12 Vital Signs

Vital signs (respiratory rate, pulse rate, blood pressure, and oral temperature) will be assessed with subjects in the sitting position after at least 5 minutes of rest and at least 15 minutes after last tobacco use.

5.2.13 Analytical Methodology

5.2.13.1 Clinical Laboratory

Values for clinical laboratory parameters will be determined by clinical laboratory facilities accredited by the Centers for Medicare and Medicaid Services (CLIA-88). Hematology, clinical chemistry, and urinalysis will employ standard clinical laboratory procedures.

5.2.13.2 Exhaled Breath Collection

Exhaled breath condensate samples will be collected using the special exhaled breath sample-collection system provided by Enthalpy Analytical. A fresh Single-Subject Longitudinal Sampling Kit will be used for each sample.

The mouth piece on the collection tubes will be replaced between replicated measurements. The hand-held collection portion of the device may be reused between subjects, as long as the mouth piece has been replaced.

Exhaled breath condensate samples will be collected on a particulate filter located immediately beyond the mouth piece and followed by a cryogenically cooled trap. After sample collection is completed, the special exhaled breath sample-collection system will be transported to the on-site laboratory for processing.

The particulate filter will consist of a 44-mm glass fiber pad housed in a plastic filter holder. The filter will be removed from the holder and used for measurement of propylene glycol, glycerin, menthol and nicotine. The liquid in the cryogenically cooled trap will be removed from the special exhaled breath sample-collection system and analyzed for propylene glycol, glycerin, and nicotine. A separate sample will be collected for measurement of aldehydes in exhaled breath samples. The particulate filter will consist of a 44-mm glass fiber pad housed in a plastic filter holder treated with 2,4-dinitrophenylhydrazine and analyzed for aldehydes. The liquid in the cryogenically cooled trap will be removed from the special exhaled-breath sample-collection system, treated on-site with 2,4-dinitrophenylhydrazine, and analyzed for aldehydes.

5.2.14 Analysis of Exhaled Breath Samples

The following constituents will be measured in the exhaled breath samples:

- Trapping Container 1: propylene glycol, glycerin, nicotine, menthol
- Trapping Container 2: formaldehyde, acetaldehyde, acrolein

The actual time and type of sample collection will be documented.

Samples will be shipped to the following:

Enthalpy Analytical, Inc.
800-1 Capitola Dr.
Durham, NC 27713
Phone: 919-850-4392
Fax: 919-850-9012
www.enthalpy.com

5.2.15 12 hour ad libitum Product Use Session

The "BVR2.3 REV05 w/ topography v2 battery" will be used during the 12-hour ad libitum topography session. All devices will be fully charged, cleared, and time reset prior to dispensing (within 24hrs, specific USB adaptor and connection to a computer is required). The subjects will use a "BVR2.3 REV05 w/ topography v2 battery" for all EVP use during the 12 hours. The use of the "BVR2.3 REV05 w/ topography v2 battery" is transparent to the user and collects the date, time, duration of each puff, puff flow rate, as well as the battery voltage during each puff. All "BVR2.3 REV05 w/ topography v2 batteries" data will be downloaded via the topography battery specific USB connector by the site staff and transferred to Cato Research for analysis.

- The subjects will be instructed, according to product insert, that "when the battery needs to be recharged, the LED tip will blink rapidly and repeatedly" and "when the cartridge is almost empty, you will notice a reduction in flavor and vapor." The subjects will be instructed to inform the site staff when either event occurs.

- When the battery needs to be recharged, the site staff will replace the depleted "BVR2.3 REV05 w/ topography v2 battery" with a freshly charged (within 24hrs) "BVR2.3 REV05 w/ topography v2 battery" using the same cartridge.

- When the cartridge is almost empty the site staff will replace with a freshly charged (within 24hrs) "BVR2.3 REV05 w/ topography v2 battery" and new cartridge.

- The site staff will document each "BVR2.3 REV05 w/ topography v2 battery" used with each cartridge and time of changing the "BVR2.3 REV05 w/ topography v2 battery" or "BVR2.3 REV05 w/ topography v2 battery" and cartridge.

5.2.16 Tobacco-Cessation Information

The investigator or designee at Screening and at the End-of-Study or upon Early Termination will advise all adult tobacco users that to reduce the health effects of using tobacco products, the best thing to do is to quit. The investigator or their designee will also refer all adult tobacco users to the Quit Assist™ website, which contains citations to a number of third-party information sources, including websites, telephone resources, and other organizations with additional information.

5.3 Subject Randomization

On Day 1 (Visit 2), subjects (no more than 60% of either sex) will be randomized to one of four product-use order sequences in a 1:1:1:1 ratio (n = ~8 subjects from each subpopulation in each sequence: A B D C, B C A D, C D B A, D A C B). Subjects will also be randomized by sex.

5.4 Other Clinical Considerations

5.4.1 Study Restrictions

The following restrictions must be observed during the study:

- No food or beverages are allowed while at the clinical site except as provided by the investigator at the clinical site or approved by the investigator in consultation with the sponsor (Section 5.4.2).
- No food or beverages containing alcohol are to be consumed in the 48 hours before each Check-in or while at the site.
- No other tobacco or nicotine containing products (other than the test products) will be used while at the research site

Subjects will be informed as to the clinic areas where product use will be allowed. Furthermore, subjects will be instructed to only use the supplied product and not to share the supplied product with anyone. Sharing or exchanging of products with another subject is not allowed and may result in dismissal from the study at the discretion of the investigator or sponsor.

5.4.2 Meal Schedule

Standard meals and snacks will be served at appropriate times (not to occur during exhaled breath collections) as determined by the clinic while subjects are at the clinical site. All subjects will receive the same meals/snacks. Time of meals and snacks (start and stop) will be recorded.

Water will be provided as needed during the study, and subjects will be encouraged to maintain their usual hydration habits.

6.0 Study Materials

6.1 Study Products

The test EVPs that will be used in the study are listed in Table 2. The test products consist of unbranded, open-label, CVR2.6.8 e-vapor. Each consists of a standard battery (BVR2.3) and cartridge (CVR2.6.8). Sham products will consist of inactivated BVR2.3 batteries and empty CVR2.6.8 cartridges. During the 12-hour ad libitum use session, subjects will use the "BVR2.3 REV05 w/ topography v2 batteries" [VR0017D0002] with the assigned test EVP cartridge.

6.1.1 Study Product Accountability

All study products will be provided by the sponsor. Site staff will coordinate shipping of all products from the sponsor or designee. The site will document the contents of all shipments received, the total amount of product used during the study, and the total number of unused product remaining at the end of clinical conduct.

Individual product in-clinic dispensing logs will be maintained by the site for each subject. The logs must include the subject number, date and time each product was dispensed and returned.

6.1.2 Study Product Storage

ALCS will be responsible for ensuring that the quality of the test products provided is adequate for the duration of the study. All study products will be stored in a locked, limited-access area in the site. The products should be stored at room temperature (defined as 15°C to 25°C [59°F to 77°F]; brief excursions to 30°C are acceptable). A

sufficient supply for each subject may be transferred and kept in a secure area in the clinic (e.g., locked drawer or cupboard) each day as necessary, with appropriate documentation of transfer noted as described in Section 6.1.1.

6.1.3 Blinding

This section is not applicable since this will be an open-label study.

6.1.4 Study Product Dispensing

Packaging and labeling details will be provided by the sponsor in a standalone document at a later date.

During use, all cartridge weights will be recorded before and after use (within 90 minutes, except for in between exhaled breath samples, where the weight will be recorded before the next sample is collected). The type of cartridge, time dispensed, and returned time will be recorded for each subject. In addition, dispensing during the ad libitum portion of the study will follow instructions listed in section 5.2.15.

6.1.5 Study Product Retention and Return/Destruction

Ten cartridges of each test product will be retained at the clinical site until finalization of the clinical study report. All other unused study product will be returned to the sponsor or destroyed at the direction of the sponsor. All returns or destruction of products will be documented. Upon finalization of the clinical study report, the sponsor will notify the site regarding the return or destruction of the retained products.

Unused product can only be destroyed after being inspected and reconciled by the study monitor or other sponsor designee.

7.0 Data Management

Data management activities will be detailed in the data management plan. Each vendor involved with this study will adhere to Good Documentation Practices and their standard operating procedures covering their respective activities relevant to participation in this study. The investigator will ensure that all data related to the conduct of this study at their site are attributable, legible, contemporaneous, original,

accurate, enduring, and readily accessible. Relevant standard operating procedures will be available for review and auditing purposes as required.

7.1 Data Collection

An electronic case report form (eCRF) will be used in this study for all subjects who sign an ICF. Study site staff will use their own source data collection forms (except where noted as to be provided) or methods to capture required study information. Pertinent source data will be transcribed to eCRFs by study staff and the CRFs will be signed by the site investigator. The eCRF entry guidelines will be provided.

7.2 Data Validation

The data will undergo validation as described in the edit-check plan (or data validation plan) and data management plan. The study site will conduct a 100% quality control check of the source data. Furthermore, a quality control check of the source data against the eCRF will be followed as described in the data management plan. After or during entry into the database, edit checks will be performed as a validation method to check for errors and discrepancies such as missing data, data inconsistencies, and inappropriate data ranges. Data queries will be sent to the applicable site for resolution. Once all queries are resolved and applicable updates are made, edit checks will be rerun until all data are deemed clean.

7.3 Data Coding

The most current version of the Medical Dictionary for Regulatory Activities at the time of the first screening for the study will be used to code AEs and concomitant medications, and this version will be used for the entire study. Coding will be completed by a qualified member of the Cato Research.

7.4 Database Lock

Once all queries have been resolved, the clinical database has been deemed to be complete and clean, and appropriate prelock review of tables, figures, and listings has been done, the data manager will initiate the database lock approval and signature process. The sponsor will be required to provide database-lock approval.

Any changes to the data after database lock will be documented and approved by the sponsor before the database is updated.

7.5 Data Transfer

Upon completion of the study, the data will be transferred to the sponsor in SAS format with supporting SAS documentation, including the SAS programming codes according to the specifications of the sponsor. Subject names, 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.

8.0 Adverse Event Guidelines

8.1 Adverse Events

The following is the definition of an **AE**:

> Any unfavorable and unintended sign (including an abnormal laboratory finding[a]), symptom, or disease[b] temporally associated with the use of a study product, **whether or not** related to the study product.[12,13]
>
> [a] For this study, a laboratory AE 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.

All AEs occurring during this clinical trial after the subject has signed the ICF must be recorded in the CRF, including the date and time of onset and outcome of each event. Any condition existing prior to the use of a study product will be documented as a concomitant disease or condition and should be reviewed to determine whether or not the subject meets the inclusion and exclusion criteria.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE.

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**[13] will be used:

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

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

Severe: The AE is incapacitating and requires medical intervention. 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.

Each AE will also be assessed by the investigator for **relationship to study product (causality)** using the following grades of certainty[14,15] (the strength of a causal association may be revised as more information becomes available):

Not related: Clearly and definitely due to extraneous cause (eg, disease, environment).

Unlikely:
a. Does not follow a probable temporal (ie, time) sequence from use of study product.
b. Does not follow a known pattern of response to the study product.
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 product is re-administered.

Possible:
a. Follows a reasonable temporal (ie, time) sequence from use of study product.
b. Follows a known pattern of response to the study product.
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 (ie, time) sequence from use of study product.

b. Follows a known pattern of response to the study product.

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) (ie, disappears or decreases when the study product is stopped or reduced).

e. Rechallenge information is **not** required to fulfill this definition.

Definitely: a. Follows a reasonable temporal (ie, time) sequence from use of study product.

b. Follows a known pattern of response to the study product.

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) (ie, disappears or decreases when the study product is stopped or reduced).

e. Recurs with re-exposure to study product (rechallenge). Note: Re-exposure of the subject is NOT required, but the "certainly/definitely related" category may only be used when recurrence is observed.

8.2 Serious Adverse Events

The following is the definition for an **SAE.**

An SAE is any adverse study experience 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. [13]

[a] "Life-threatening" means that the subject was at immediate risk of death at the time of the SAE; it does not refer to an 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.

An important medical event that may or may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on 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 SAEs, **whether or not** considered study related, must be reported by telephone and by fax to the sponsor and Medical Monitor within 24 hours of the site's learning of the SAE or, at the latest, on the following workday. The contact information is as follows.

Sponsor Medical Contact:

Jianmin Liu, M.D.
Altria Client Services LLC
Phone: 804-335-2441; Cellular: 804-852-4156
Fax: 804-335-2090

Medical Monitor:

Jack Snyder, M.D., Ph.D., RAC, DABT, PMP, CPI
9605 Medical Center Drive, Suite 350
Rockville, MD 20850
Phone: 301-919-0224
Fax: 919-361-2290

The initial SAE report must be as complete as possible, including an assessment of the causal relationship between the event and the study product. Information not available at the time of the initial report (e.g., end date, laboratory values) must be documented on a follow-up SAE report. The SAE report forms and specific procedures for reporting SAEs will be provided separately.

In compliance with Good Clinical Practice (GCP) reporting guidelines, **the investigator must also inform the IRB and the site monitor of an SAE, whether or not** it is considered study related.

8.3 Adverse Event and Serious Adverse Event Follow-up

Each AE, including clinically significant laboratory abnormalities, whether serious or non-serious, will be followed until resolved, determined that follow-up is no longer required at the discretion of the investigator, or lost to follow-up, regardless of whether or not the subject is still participating in the study. Final outcome may be classified as resolved, improved, unchanged, worse, fatal, or unknown (lost to follow-up). When appropriate, medical tests and examinations will be performed to document the outcome of the AE.

8.4 Pregnancy

A positive pregnancy test before randomization will be documented as a screen failure. A positive pregnancy test after randomization will be documented by the investigator on the pregnancy form (provided separately) and will be documented as a protocol deviation in the clinical conduct study report to the IRB. The investigator or designee will discontinue the pregnant subject from the study and will advise her to seek prenatal care and counseling from her primary care provider. If the pregnant subject is a tobacco user, the investigator will refer her to the Quit Assist™ website, which contains citations to a number of third-party information sources, including websites, telephone resources, and other organizations with additional information. Advice given will be documented in the subject's source document.

Pregnancy itself is not an SAE.

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 standard operating procedures and with the subject's consent. This request and the subject's response will be documented in the subject's source document.

9.0 Statistical Methods

9.1 Sample Size Estimation

This study is being conducted to characterize exhaled breath of subjects using the four test products. Due to the limited available data on exhaled breath during e-vapor product use, a sample size of 32 subjects is believed to be appropriate for descriptive purposes.

9.2 Statistical Analysis

Details of the statistical analysis will be provided in the statistical analysis plan (SAP).

SAS, Version 9.3 or higher, will be used for all data presentation and summarization including statistical analyses and tables, figures, and listings.

9.3 Analysis Populations

The Exhaled Breath Population will include all subjects who used any study product and have an exhaled breath measurement.

The Safety Population will include all subjects who used any study product.

If it is determined that a subject was pregnant during the study, all the pregnant subject's safety data will be reported.

9.4 Data Summarization

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). Descriptive statistics (number of observations, mean, median, SD, and range) will be used for continuous data variables and frequency counts (number of observations and percentage) for categorical data variables as described in the SAP.

9.5 Statistical Analysis

9.5.1 Subject Demographics and Baseline Characteristics

Descriptive statistics will be calculated for continuous variables (age, weight, height, conventional cigarette consumption, e-vapor consumption, and BMI) and frequency counts will be tabulated for categorical demographics variables (sex, ethnicity, and race, etc.).

9.5.2 Exhaled Breath Analytes, Puff Topography and Cartridge Weights

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 provided for each analyte level (defined as the difference between the product sample value and the sham sample value), average puff duration, average flow rate, average puff volume, average number of puffs per cartridge, and for cartridge weight change by test product and use session (exhaled breath or ad libitum use). Separate linear mixed model for analysis of variance will be used to estimate effects of test product on the (1) each analyte level in the exhaled breath samples, (2) average puff duration, (3) average puff flow rate, (4) average puff

volume, (5) average puff number per cartridge, and (6) cartridge weight changes. The models will include sequence, study product, and period as fixed effects and subject nested within-sequence as a random effect. A frequency table will be used for the response to the Use the Product Again Questionnaire. Additional details of the statistical analysis will be provided in the statistical analysis plan.

9.6 Safety Analysis

Descriptive statistics will be calculated for the safety parameters. No formal statistical tests are planned for safety data.

Continuous variables will be summarized using number of observations, number of missing, mean, SD, median, minimum, and maximum. Frequency counts will be reported for all categorical data.

Frequencies of subjects with AEs will be summarized by group and overall.

Baseline demographics and tobacco product-use history will be summarized overall.

AE data will be coded (to the lowest-level term) by using the Medical Dictionary for Regulatory Activities. AEs will be listed in by-subject data listings, including verbatim term, preferred term, test product, severity, and relationship to test product. Frequency counts of AEs will be provided by body system, preferred term, and test product. Frequency counts of AEs will also be summarized by severity and relationship to test product.

Observed values and changes from baseline for vital signs and other safety parameters as appropriate will be listed and summarized by test product for each time point using appropriate descriptive statistics.

10.0 Quality Assurance

10.1 Compliance with the Protocol and Protocol Revisions

By signing this protocol, the investigator agrees to conduct the study as described in the protocol. All revisions to the protocol must be discussed with and prepared by the sponsor or designee. The investigator should not implement any change to the protocol

without prior review and documented approval from the IRB of an amendment, except where necessary to eliminate an immediate hazard to study subjects.

If a deviation or change to the protocol is implemented to eliminate an immediate hazard before obtaining IRB approval, the deviation or change will be submitted as soon as possible to the IRB for review and approval as is consistent with IRB procedures, the sponsor, and regulatory authorities (if required). Documentation of approval, signed by the chairperson or designee of the IRB, must be sent to the sponsor.

If an amendment substantially alters the study design or increases the potential risk to the subject, the following will be done:

- The ICF must be revised and submitted to the IRB for review and approval.
- The revised ICF must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment.
- The new ICF must be used to obtain consent from new subjects before enrollment.

10.2 Study Monitoring

Study monitoring details will be provided in a clinical monitoring plan. The responsible study monitor or designee will contact and visit the investigator as necessary. The study monitor will be allowed, upon request, to inspect and verify various records of the study (e.g., source document, ICFs, CRFs) in a manner consistent with GCP and all other applicable state and federal law.

It will be the study monitor's or designee's responsibility to inspect the CRFs to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered. The study monitor will verify that each subject has consented in writing. Where the terms of the informed consent, GCP, 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 or her designee) agrees to cooperate with the study monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

In addition, the sponsor's internal auditors (or designee) and government inspectors may evaluate the study and must be allowed access to CRFs, source documents, and other study files.

The investigator must notify the sponsor promptly of any inspections of the study or activities related to the study scheduled by regulatory authorities, allow the sponsor to be present, and promptly forward copies of inspection reports to the sponsor.

11.0 Administrative Considerations

11.1 Confidentiality

By signing this protocol, the investigator affirms that all information provided to the investigator by the sponsor will be maintained in confidence and such information will be divulged to the IRB only under appropriate understanding of confidentiality with the IRB. Data generated by the study will be considered confidential by the investigator.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with applicable regulatory requirements. By signing this protocol, the investigator agrees that the sponsor, sponsor representatives, IRB, or regulatory agency representatives may consult and/or copy study documents in order to verify CRF data. By signing the consent form, the subject agrees to this process. If study documents are copied during this process of verification, the subject will be identified by a unique code and the full name and other personal identifiers will be masked.[16] At completion of the study (i.e., at issuance of final study report), the final database will be transferred to the sponsor. Subject names, date of birth (except year), and other personal identifiers will be redacted from this data transfer file; any such information removed will be documented at the time of transfer.

By signing this protocol, the investigator recognizes that certain personal identifying information of the Investigator (e.g., name, clinic address, curriculum vitae) may be made part of a regulatory submission and may be transmitted to the sponsor for internal study management purposes or as required by individual regulatory agencies. Additionally, the investigator's name, clinic address, and phone number may be included when reporting SAEs to other investigators and stored in managed regulatory-controlled databases.

11.2 Ethical Conduct and Responsibility of the Investigator

The investigator is responsible for ensuring that the clinical study is performed in accordance with the Declaration of Helsinki and in a manner consistent with the International for Council for Harmonization E6, Guideline for Good Clinical Practice, the corresponding section of the United States Code of Federal Regulations (CFR) governing Protection of Human Subjects (Title 21 CFR Part 50), and IRBs (Title 21 CFR Part 56).

The investigator should ensure that all persons assisting with the study are qualified for the duties assigned, adequately informed and trained about the protocol and amendments to the protocol, the study products, 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. Any personnel change in this list during the course of the study will be documented. All study-related training will be documented.

11.3 Institutional Review Board

This protocol will be reviewed by an IRB. The IRB operations will be in compliance with Title 21 CFR Part 56 and GCP. The study will not be initiated without unconditional IRB approval of the protocol, ICFs, subject recruitment materials, any written information provided to the subjects, and other documents requested by the IRB. The IRB approval should be obtained in writing and clearly identify the study, the documents reviewed, and the date of the review. All revisions or amendments to the protocol must also be approved in writing by the IRB.

Any relevant reports and safety information required during the study-approval period will be submitted to the IRB in compliance with relevant requirements.

11.4 Informed Consent

Investigators will ensure that subjects are informed about the purpose, potential risks, and other pertinent issues regarding this clinical trial in accordance with Title 21 CFR Part 50 and GCP. Subjects will be provided a copy of the ICF, and will be allowed sufficient time to review and ask questions about the study. The ICF will

be signed and dated by the subject and by a member of the clinical staff qualified to conduct the informed consent discussion. Each subject will receive a signed and dated copy of the ICF.

If a revision to the ICF is required, the investigator or designee will fully inform subjects of any new information pertinent to the subject's continued participation in the study and subjects will be required to sign the updated form.

11.5 Termination of Study

The sponsor reserves the right to discontinue this study at any time. The investigator, in collaboration with the sponsor, reserves the right to discontinue the study for safety reasons at any time.

11.6 Study Report

A study report written consistent with the International for Council for Harmonisation guidelines will be provided by Cato Research to the sponsor. The report will include a description of the clinical conduct of the study, safety evaluation, analytical methods and results, and the statistical analysis described in the statistical methodology section of the protocol and the SAP.

11.7 Study Record Retention

The investigator is required to maintain copies of all essential documents and all primary data (e.g., CRFs, laboratory records, data sheets, correspondence, photographs, computer records, photocopied government-issued identification to verify subject age) that are a result of the original observations and activities of the study and are necessary for the reconstruction and evaluation of any study report for the maximum period required by applicable regulations and guidelines or for the period specified by the sponsor, whichever is longer. At minimum, study records will be retained for at least 2 years after the last approval/authorization of a marketing application or until at least 5 years have elapsed since the formal discontinuation of clinical development of the study products.

If the investigator wants to relocate the records or is unable to retain them for the specified retention period, the sponsor must be contacted and notified in writing.

If the investigator withdraws from the study (e.g., relocation, retirement), the records will be transferred to a mutually agreed upon designee (e.g., another investigator). The sponsor will be notified in writing of any such transfer.

All study documents will be made available, if required, to relevant health authorities. The investigator must contact the sponsor before destroying any records associated with the study.

12.0 References

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13. US Department of Health and Human Services, Food and Drug Administration. International Conference on Harmonization; Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (E2A). *Federal Register*. 1995;60(40):11284.

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