# UNIVERSITY OF VERMONT TOBACCO CENTER OF REGULATORY SCIENCE

## Study 2

## Low Nicotine Content Cigarettes in Vulnerable Populations: Pregnant Women

## (Extended Exposure)

## Statistical Analysis Plan

**Version: 1**

**Written to coincide with the Study 2 portion of the protocol dated 5/3/2022**

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## Approved by:

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## 1. Abbreviations and Definitions

VLNC: Very low nicotine content

CO: Carbon monoxide

BPM: Beats per minute

MINI: Mini International Neuropsychiatric Interview

IVR: Interactive Voice Response

CPT: Cigarette Purchase Task

## 2. Introduction

This document will serve as the Statistical Analysis Plan for the University of Vermont Tobacco Center of Regulatory Science study of extended exposure to cigarettes with very low nicotine content conducted with pregnant women. It describes the planned statistical analysis for evaluating the chronic effects of very low nicotine content (VLNC; 0.4 mg/g tobacco averaged across menthol and non-menthol products) research cigarettes (Spectrum cigarettes manufactured by 22nd Century Group, Clarence, NY). Assignment to a menthol or non-menthol product will be based on a participant's reported usual brand. Details for the proposed analysis of the primary, secondary, and exploratory endpoints are provided.

## 3. Trial Objectives

The primary objective of this study is to evaluate the effects of extended exposure to VLNC cigarettes in socioeconomically disadvantaged pregnant women. Parallel studies have been conducted with socioeconomically disadvantaged women of reproductive age, opioid-maintained smokers, and smokers with affective disorders. The study will use a two-condition, parallel-groups design. After a baseline period in which daily smoking rate and other baseline characteristics are assessed, participants will be randomly assigned to one of two cigarette conditions for the 12-week experimental period, namely VLNC cigarettes (0.4 mg/g tobacco) or each participant's reported usual brand (UB).

## 4. Trial Design

This is a randomized, multi-center, parallel-groups design. Sample size is based on 90 participants completing the entire study protocol. The VLNC cigarettes to be used throughout this trial are as follows:

Table 1. Description of cigarettes used in this trial.

| Condition | TPMF Code | Type* | Specifications Nicotine Yield | Specifications Tar Yield | Specification Range for Nicotine Yield | Specifications Nicotine Content |
|---|---|---|---|---|---|---|
| 2 | NRC102 | VLN | 0.03 ± 0.01 | 9 ± 1.5 | 0.02 - 0.04 | 0.37 ± 0.01 |
| 2 | NRC103 | VLN-Men | 0.03 ± 0.01 | 9 ± 1.5 | 0.02 - 0.04 | 0.39 ± 0.00 |
| 3 | N/A | Usual Brand | N/A | N/A | N/A | N/A |
| 3 | N/A | Usual Brand-Men | N/A | N/A | N/A | N/A |

| *Legend: | |
|---|---|
| VLN | Very Low Nicotine |
| VLN-Men | Very Low Nicotine-Menthol |

Usual brand cigarettes will serve as the comparator of interest, unless otherwise specified.

### 4.1. Condition Assignment/Randomization

The study consists of two baseline assessments, separated by one to three weeks, 12 weeks of extended exposure to research or usual brand cigarettes, with assessments completed weekly, an abstinence assessment the day following the Week 12 visit and a final assessment 30 days after completing the study.

Participants will be randomly assigned to one of the two conditions, with randomization stratified by site and menthol preference. The lead statistician will create a randomization schedule for each site, amounting to at least 150% of expected enrollment at that site. The excess randomization codes will be used in the event that a site will have to enroll extra participants due to unexpectedly slow enrollment at another site.

The assumptions needed to generate the randomization schedule for this study are summarized in Table 2. The final randomization sequences were generated January 18, 2022. There are two major types of assumptions: 1) the proportion of participants recruited from each site, and 2) the proportion of recruited participants at each site who smoke menthol/non-menthol cigarettes. The recruitment goal for this study is 111 people. A sample size of 90 completers is proposed to test the primary aim in Study 2. Anticipating 15% attrition, and five pilot participants (3 at UVM, 2 at the University of Kentucky [UK]), 111 participants will be enrolled across both sites (74 at UVM, 37 at UK).

Participants will be recruited from the University of Vermont (UVM) and the University of Kentucky (UK). In the acute exposure portion of this project, 19% of the smokers at UVM used menthol and 81% smoked non-menthol cigarettes. Based on data from previous studies conducted at UK, menthol was assumed to be the preferred flavor for 33% of the UK participants. For ease of calculations, the UVM estimate was rounded to 20% menthol smokers.

The assumption for the extended exposure study is that 67% of the smokers recruited will come from UVM, with the remaining 33% from UK. From this it follows that 74 participants will be recruited at UVM (=0.67*111) and 37 at UK (=0.33*111).

The estimated numbers of menthol and non-menthol smokers to be recruited were calculated as the products of the proportions of menthol and non-menthol smokers and the recruitment numbers. For UVM, this was calculated as 0.20*74 or 15 menthol smokers, and 0.80*74 or 59 non-menthol smokers, rounded to the nearest integers. For UK, this was calculated as 0.33*37 or 12 menthol smokers, and 0.67*37 or 25 non-menthol smokers, rounded to the nearest integers. In order to allow an oversupply of randomization codes, the number of codes generated is to be at least 150% of the recruitment goal. To conform to the number of participants in each randomization block (which is 4) the number of randomization codes used is a multiple of 4. Thus, in order to allow for 15 UVM menthol smokers, 24 randomization codes were generated (15*1.5 = 22.5, next higher multiple of 4 = 24). In order to allow for 59 UVM non-menthol smokers, 92 randomization codes were generated (59*1.5 = 88.5, next higher multiple of 4 = 92). In order to allow for 12 UK menthol smokers, 20 randomization codes were generated (12*1.5=18, next higher multiple of 4 = 20). In order to allow for 25 UK non-menthol smokers, 40 randomization codes were generated (25*1.5 = 37.5, next higher multiple of 4 = 40).Based on the calculations above, a total of 176 randomization codes were initially generated for this study.

Table 2. Assumptions used in developing the randomization schedule.

| | Menthol | Recruitment Percent | Total Recruitment n | Menthol Recruitment n | Menthol Randomization n | Non-Menthol Recruitment n | Non-Menthol Randomization n | Randomization Goal (150%) | Final Randomization n |
|---|---|---|---|---|---|---|---|---|---|
| UVM | 20% | 67% | 74 | 15 | 24 | 59 | 92 | 111 | 116 |
| UK | 33% | 33% | 37 | 12 | 20 | 25 | 40 | 56 | 60 |
| Total | | | 111 | 27 | 44 | 84 | 132 | 167 | 176 |

### 4.2. Sample Size

Sample size was determined using power analysis for hypothesis tests related to the Primary Aim of Study 2, specifically to detect a significant difference between the VLNC condition and the UB condition in the primary endpoint, cigarettes per day (CPD), at the end of the trial. Effect size estimates were based on our previous studies of normal nicotine cigarettes (NNC) and VLNC use in disadvantaged women of childbearing age and estimates of CPD in our completed study on smoking topography in pregnant women and women of childbearing age. In that study, pregnant women smoked an average of 12.1 CPD, while preliminary data from our trial of women of childbearing age suggest a decrease of 5 CPD in women smoking VLNC compared to those smoking NNC. A sample size of 45 completers per condition will provide 80% power to detect statistically significant differences in smoking rate, with significance level alpha = 0.05.

## 5. Study Populations

### Inclusion/Exclusion Criteria

Inclusion Criteria:

1) Pregnant women ages 21-44 years who have < an Associate's degree
2) Have appropriate equipment to complete face-to-face video assessments. For participants who do not have smartphone, research staff will explore potential alternative plans (e.g., provide inexpensive Android phone)
3) Have a positive urine cotinine dipstick
4) Be without current (within the past year) serious mental disorder that would interfere with study results or completion as determined by the licensed medical professional or PI
5) Be sufficiently literate to complete the research-related tasks;
6) Be in good physical health without serious illness or change in health or medication in the past three months as determined by the licensed medical professional at each site;
7) Report no significant use of other tobacco or nicotine products within the past month (more than 9 days in the past 30).
8) Provide verification of gestational age ≤ 25 weeks from OB/GYN at time of enrollment

Exclusion Criteria:

1) Exclusive use of roll-your-own cigarettes;
2) Planning to quit smoking in the next 30 days;
3) A quit attempt in the past 30 days resulting in greater than 3 days of abstinence;
4) Currently taking anticonvulsant medications including:
    a. Phenytoin [Brand Name: Dilantin]
    b. Carbamazepine [Brand Name: Tegretol, Carbatrol, Equetro, Epitol]
    c. Oxcarbazepine [Brand Name: Trileptal]
    d. Primidone [Brand Name: Mysoline]
    e. Phenobarbital
5) Currently seeking smoking cessation treatment;
6) Have used nicotine replacement, bupropion or other pharmacotherapies as cessation aids in the past month (bupropion will be allowed for treatment of depression);
7) Symptoms of psychosis, dementia or mania;
8) Suicidal ideation in the past month (score > 1 on the Beck Depression Inventory question 9 or endorse question 4 and/or 5 on the Mini International Neuropsychiatric Interview (MINI) suicide subscale);
9) Reporting a plan or attempt to commit suicide, which is assessed on question A3g of the MINI Neuropsychiatric Interview Major Depressive Episode Module. Thoughts of suicide without an intent or plan is not an exclusion criterion;
10) Suicide attempt in past six months (endorse question 6 on the MINI suicide subscale with suicide attempt in the past six months);
11) Participation in another research study in the past 30 days
12) Reporting symptoms of COVID-19
13) Positive toxicology screen for any of the following drugs will be grounds for exclusion: cocaine, opiates, methadone, oxycodone, buprenorphine, benzodiazepines, barbiturates, amphetamines, methamphetamines, MDMA and PCP.
    a. Marijuana will be tested but will not be an exclusionary criterion. Participants will be discouraged from smoking marijuana during the study.
    b. Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines will not necessarily be excluded.
    c. Participants failing the toxicology screen will be allowed to re-screen once, if they are still eligible for the screening at the time of the re-screen. These participants will need to be re-consented before being re-screened to ensure they have received adequate informed consent.
14) Systolic blood pressure < 80 or ≥ 140 mmHg;
    a. Participants failing for blood pressure will be allowed to re-screen once.
15) Diastolic blood pressure < 50 or ≥ 90 mmHg;
    a. Participants failing for blood pressure will be allowed to re-screen once.
16) Breath CO > 50 ppm;
17) Heart rate greater than or equal to 110 bpm or less than 45 bpm;
    a. Participants failing for heart rate will be allowed to re-screen once.

Women under age 21 are excluded because they cannot legally buy cigarettes. Those with unstable medical, psychiatric, or medication conditions (condition and/or medication changes in the past three months) are excluded as these symptoms could affect a participant's ability to complete the study. Examples include but are not limited to the following: angina, stroke, heart attack which occurred since phone screening, blood clots in the arms or legs for which the individual is undergoing active medical treatment, cancer requiring active chemotherapy or radiation therapy, severe shortness of breath caused by conditions such as uncontrolled asthma, COPD, or arrhythmia, active untreated infection such as pneumonia, active untreated endocrine disorder such as hyperthyroidism. Individuals with baseline CO readings greater than 50 ppm, those with blood pressure or heart rate readings that are out of range (acceptable ranges are systolic: 80-139 mmHg; diastolic: 50-89 mmHg; heart rate: 45-109 bpm) and anyone who has attempted suicide in the past 6 months will be excluded from the study for safety concerns. Individuals who smoke 'roll your own' cigarettes exclusively will be excluded from the study because we will be unable to standardize their baseline smoking behavior. Individuals who have recently participated in a research study will be excluded as participation may have changed their smoking behavior, which may preclude a stable smoking baseline. Because participants are required to complete portions of the protocol independently, they will need to be able to independently read and comprehend the study materials.

### 5.1. Full Analysis Population

Participants will be randomized to a study condition after the completion of the first baseline visit in order to allow study staff adequate time to compile the supply of the appropriate cigarettes sufficient to cover 100% of their reported baseline CPD. Thus, randomization begins after the first baseline visit and is completed at the second baseline visit when participants receive research or usual brand cigarettes. For data analyses, the full analysis population will include participants completing the entire randomization process. Participants will be analyzed based upon the dose to which they were assigned, regardless of protocol violations and/or compliance to condition assignment. Our primary results will be based on this intention-to-treat population (i.e. randomized to a condition and received cigarettes).

### 5.2. Per-Protocol Population

A separate per-protocol population will also be used for this study, with the results from these analyses compared to those of the full analysis population in order to examine whether missing data affects the outcomes of interest. Participants who complete the 12-week protocol will represent the per-protocol population.

### 5.3. Safety Population

All participants receiving any study cigarettes will be included in the safety population. Participants who may have attended the screening and first baseline visit, and have been randomized, but did not actually receive any cigarettes will be excluded from the

safety population.

## 6. Trial Endpoints

### 6.1. Primary Endpoints

The Primary Aim of the extended exposure study is to compare the effects of cigarettes varying in nicotine yield on smoking rate. The primary endpoint is average CPD during Week 12. Additional measures addressing this aim are breath CO level, urinary cotinine concentration, nicotine dependence (Fagerström Test for Nicotine Dependence, Wisconsin Index of Smoking Dependence Motives) scores, nicotine withdrawal (Minnesota Nicotine Withdrawal Scale, Questionnaire of Smoking Urges), and the Cigarette Purchase Task (CPT).

- CPD will be collected by daily Interactive Voice Response (IVR) to assess cigarette use in the days since the last interview. This will yield a continuous record of cigarette use throughout the study. A weekly average will be obtained for analysis by averaging the daily CPD reports.
- Breath CO
  - Collected at baseline and weekly for the duration of the study
- Urinary cotinine concentration
  - Collected at the second baseline, Weeks 6 and 12
- Nicotine dependence and withdrawal
  - Collected at screening, the first baseline, every other week (even weeks for nicotine dependence, odd weeks for nicotine withdrawal) until study completion and the abstinence visit
- CPT
  - Collected at the second baseline and Weeks 2, 6, 12 and the abstinence visit for usual brand cigarette
  - Collected at Weeks 2, 6, and 12 and the abstinence visit for study cigarette
  - Breakpoint: the lowest price at which cigarette consumption is 0.
  - Elasticity of demand: the sensitivity of cigarette consumption to price increases.
  - Omax: the maximum daily expenditure for cigarettes.
  - Pmax: the price at which cigarette expenditure is maximized.
  - Intensity: cigarette consumption at the lowest price ($0 cost).

### 6.2. Secondary Endpoints

- Measures of depression and anxiety
  - Collected at the second baseline and Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12

    - Beck Depression Inventory
    - Overall Anxiety Severity and Impairment Scale
- Adherence to assigned tobacco products
    - Collected through the daily IVR system
- Use of multiple nicotine products.
    - Collected through the daily IVR system
- Biomarkers of exposure to tobacco carcinogens
    - Collected at the second baseline and Weeks 6 and 12
- Study retention
- Quit attempts and spontaneous quitting
    - Assessed at weekly visits and at the 30-day post-intervention assessment

### 6.3. Exploratory Endpoint

- None at this point.

### 6.4. Safety Endpoints

- Adverse events (AEs)
- Serious adverse events (SAEs)
- Birth outcomes
    - Live birth
    - Birth weight
    - Mother's gestational age at delivery
    - Type of nursery admission

## 7. Statistical Analysis

### 7.1. General Approach

To examine these Aims, repeated measures analysis of variance (ANOVA) will be used for outcomes that are measured on multiple occasions from baseline to the end of the intervention phase. Each repeated measures ANOVA model consists of five terms: cigarette condition, visit, interaction between cigarette condition and visit, a random effect for study site, a random participant effect (between-subject error), as well as random error (within-subject error). Variance parameters will be estimated using restricted maximum likelihood method with the Satterthwaite approximation. In the event of a statistically significant condition effect or a condition-by-visit interaction, post-hoc tests will be conducted in order to explore the nature of the significant findings.

### 7.2. Describing the Study Population(s)

### 7.2.1. Baseline Characteristics:

Baseline characteristics will be summarized by condition to identify any group imbalances post-randomization. This will include demographic characteristics (age, race/ethnicity), smoking characteristics (CPD, menthol status), and nicotine dependence, as well as the randomization scheme noted above. Continuous variables will be summarized by mean and standard deviation and compared by one-way analysis of variance, with variable transformations conducted as necessary to improve the normality of the distributions. Categorical variables will be summarized by frequencies and percentages and compared using the Chi-squared test.

### 7.2.2. Intention-to-Treat Population:

The intention-to-treat population, defined above in section 5.1 (Full Analysis Population), will serve as the population for all primary analyses.

All participants must have successfully completed the baseline assessment, been randomized, and received at least their first supply of cigarettes. This means that the intention-to-treat population must have, at a minimum, completed the second baseline session.

### 7.2.3. Safety Population

The population for all safety analyses includes all participants who received any study cigarettes, excluding participants who dropped out prior to randomization. Note that participants are randomized at the end of the first baseline visit, but the VLNC condition does not receive research cigarettes until the second baseline visit, so this population effectively includes all participants attending at least the second baseline session.

## 7.3. Primary Endpoint Analysis

### 7.3.1. Primary Analysis

The primary endpoint is CPD during Week 12, compared to the CPD at baseline. While the primary outcome is the mean total CPD, consisting of both study and non-study cigarettes, the analyses will be repeated examining only the CPD contributed by study cigarettes. CPD will be computed as the mean CPD calculated on a weekly basis. Data analysis will use repeated measures analysis of variance, with two conditions and two time points (baseline and Week 12). In addition, the analysis model will include a random effect for site, a random

participant effect and random error (within-subject error). Variance parameters will be estimated using restricted maximum likelihood method with the Satterthwaite approximation.

Because CPD will be measured weekly, additional analyses will be performed including data collected at all time points. The analysis will be conducted in a manner similar to that described above, with the exception that there will be 13 time points: baseline and Weeks 1 through 12. For these analyses, the variance-covariance structure will be specified as first-order autoregressive.

Additional outcomes include the CPT, breath CO level, urinary cotinine concentration, and nicotine dependence and withdrawal scores. These will be examined in a manner similar to that described above for CPD. Natural log transformations will be performed as needed so that ANOVA model assumptions of normality and equal variances hold. Geometric means in original units will be calculated as well. Note that not all outcomes are assessed at weekly in-person visits. The time points at which each outcome is assessed is described in Section 6.

Finally, several assessments will be made at baseline as well as at the abstinence visit, which occurs the day following the Week 12 assessment. At this visit, measures of craving and withdrawal will be completed. The analysis will be similar to that described above for the CPD comparing baseline CPD to that reported at the end of the trial. The analysis will deviate from that described only in the timing of the tasks, with the time points consisting of the baseline and the abstinence visits, rather than the baseline and Week 12 visits reported above.

### 7.4. Secondary Endpoint Analysis

Repeated measures analysis of variance will be used for the analysis of most secondary endpoints. Analyses will conform to the methods outlined above for primary endpoints. These outcomes include use of other nicotine products and biomarkers of exposure to tobacco carcinogens. Use of multiple nicotine products will be collected through the IVR system, as the response to questions asking whether the participant used smokeless tobacco, e-cigarettes or nicotine replacement products. Because responses are recorded as Yes/No, the responses will be aggregated on a weekly basis and are expressed as the number of days that other nicotine products are used.

Biomarkers will be analyzed on natural log scales so that ANOVA model

assumptions of normality and equal variances hold. Geometric means in original units will be calculated as well. Interaction terms between BMI and cigarette condition will be added to these models to explore possible moderating effects of obesity on the relationship between RNC cigarettes and biomarkers.

Differences between groups in self-reported quit and cigarette reduction rates at the 30 day post-intervention follow-up period will be evaluated using Chi-squared tests, as will be the case for other discrete outcomes such as drop-out rates. Secondary analyses using logistic regression will be completed to identify factors associated with cessation and retention. Candidate factors, in addition to condition, include subjective responses to the products, willingness to pay for the product (CPT), and withdrawal. SAS (SAS Institute Inc., USA) will be used for all analyses.

### 7.5. Exploratory Endpoint Analysis

None planned at this time.

### 7.6. Missing Data

We will test the pattern of missing values using Little's MCAR test, and, assuming that the data are missing at random, we will employ statistical procedures that are based on maximum likelihood estimates, which will allow the inclusion of all subjects without imputation of missing values. The maximum likelihood approach estimates the parameter values that would maximize the probability of observing the data collected. In the event of missing variables, the likelihood for a given individual is the probability of observing the non-missing variables. Thus, the maximum likelihood approach allows the use of data from participants for the time period for which data is available, but not for time periods for which the data is missing. This procedure uses information from earlier time periods to estimate the effects of later time periods, while also accounting for the uncertainty of the projection in the computation of standard errors and test statistics. While maximum likelihood estimation is considered superior to imputation methods for the treatment of missing data in clinical trials, additional analyses will be conducted using several imputation methods to test the sensitivity of these results. Imputations methods will include, but not be limited to, last observation carried forward and multiple imputation with the Markov Chain Monte Carlo (MCMC) method carried out in PROC MI in SAS.

In the case of non-random missing data, we will conduct sensitivity analyses using

multiple imputation with the Markov Chain Monte Carlo (MCMC) method carried out in PROC MI in SAS. If missing data is associated with experimental condition, we will conduct multiple imputation for each experimental condition. At least 10 imputed data sets will be generated, with the experimental-condition effect being assessed in each imputed data set. A final single assessment of experimental condition differences will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS. The results of these analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

### 7.7. Interim Analyses

No interim analyses are planned for this study.

## 8. Safety

Adverse events and birth outcomes will be the only safety data that will be reported.

### 8.1. Adverse Events

Safety data will include the number of adverse events, classified as to category. Categories of events are based on the Medical Dictionary for Regulatory Activities (MedDRA). In addition to the type of event, adverse events will be categorized by severity, whether the adverse event is related to the study products/procedures, and whether the adverse event is expected.

Summary frequencies will be provided based on the overall number of events as well as separately by dose condition. In addition to the number of events of a particular type recorded, the percent of adverse events will be computed with the denominator being the number of participants in the safety population. Thus, the description of events will be reported as events/person. Categorization of the severity of events, relationship to study product/procedures, and expected nature of the event will be also reported as the frequency and percent of participants.

Safety analyses will be reported only to the DSMB and the appropriate Institutional Review Boards, with the exception that any serious adverse event will also be reported to the study sponsor.

Birth outcomes will be reported descriptively.

### 8.2. Clinical Laboratory Evaluations

Laboratory measures will not be considered in the safety evaluations for this study.

### 8.3. Other Safety Measures

Not applicable.

## 9. Pharmacokinetics

Not applicable.

## 10. Other Analyses

Not applicable.

## 11. Reporting Conventions

P-values greater than or equal to 0.001 will be reported to 3 decimals places; those less than 0.001 will be reported as <0.001. The mean, standard deviation, and other statistics will be reported to one decimal place greater than the original value. Quantiles such as median, minimum and maximum will use the same number of decimal places as the original data. Estimated parameters not on the same scale as the raw observations, such as regression coefficients, will be reported to three significant digits.

## 12. Technical Details

This SAP was developed based on the protocol entitled Low Nicotine Content Cigarettes in Vulnerable Populations: Pregnant Women. The version used is number 12, dated May 3, 2022.

For the most part, statistical analyses will be performed using SAS, version 9.4. Data management and cleaning will be performed using tools available in REDCap as well as SAS, version 9.4.

There currently are multiple statisticians working on this study, allowing exchange of ideas as part of their regular workflow. This allows both formal and informal checks of the analyses and code. In addition, the biostatistical staff meet regularly for this study, allowing thorough discussion of all proposed analyses.

## 13. Summary of Changes to the Protocol

No changes to the analyses outlined in the protocol are proposed in this document.