CLINICAL STUDY PROTOCOL

Interventional Drug or Biologic

Examining Appeal and Addiction Potential of Novel E-cigarette Constituents among Adults

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Synopsis

Primary Objective The primary objective of this study is to examine the appeal and addiction potential of e-cigs containing WS-3, WS-23 (synthetic coolants), with and without menthol, and nicotine. A pilot study will determine appropriate concentrations of WS-3, WS-23, and menthol.
Secondary Objective The secondary objective is to examine the interactive effects of different concentrations of WS-3, WS-23, and menthol on appeal and addiction potential. Exploratory effects aim to examine the influence of predictors including sex, combustible tobacco use, and nicotine dependence. Examine the feasibility and accuracy of a wearable smartband to detect e-cigarette puffing behavior. While in the lab for the e-cigarette use sessions, we will have participants wear a small smartband on their wrist, on the same hand they use to vape the e-cigarette. They will put on the smartband prior to starting the vaping session. The silicone smartband is small and lightweight, like a smartwatch or fitbit. The smartband collects movement data from geospatial sensors as the participant moves their arm and hand. The sensor data will be used to measure the movement during the directed e-cigarette puffing during the lab session. There are no changes to the lab procedure or puffing instructions.
Study Duration Approx. 3 years to complete entire study, approx. 2 weeks/participant for the pilot study, 4 weeks/participant for the main study
Study Design Within subject's human laboratory examination
Number of Study Sites 1; Yale School of Medicine
Study Population Adult e-cigarette with recent e-cig experience (past-month use), 50% with current cigarette use (equal number using menthol and non-menthol cigarettes).
Number of Participants 30 participants for the pilot study, 66 participants for the main study. Up to 33 exclusive e-cigarette users enrolled in the main study will be recruited for oral microbiome and inflammatory marker assessments.
Primary Outcome Variables Liking/Wanting: Drug Effects Questionnaire Labeled Hedonic Scale (LHS): Flavor/Sensory Perception

Generalized Labeled Magnitude Scale (gLMS): Ratings of Flavor Intensity, Sweetness, Coolness, Irritation/Harshness, Bitterness
Secondary and Exploratory Outcome Variables (if applicable) E-Cigarette Craving: The Yale Craving Scale Salivary Nicotine/Cotinine Levels Oral microbiome, inflammatory markers, and self-reported oral health status in exclusive e-cigarette users, compared by past-month e-cigarette flavor use.

Abbreviations

Abbreviation	Explanation
LHS	**Labeled Hedonic Scale**
gLMS	**Generalized labeled magnitude scale**
e-cig	**Electronic cigarette**
TCORS	**Tobacco Center for Regulatory Science**

Glossary of Terms

Glossary	Explanation

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1 Introduction

1.1 Introductory Statement

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to ICH GCP guidelines, and according to CFR 21 Part 312, other applicable government regulations and Institutional research policies and procedures.

2 Background

2.1.1 Preclinical Experience

See 2.2 for relevant preclinical information.

2.1.2 Clinical Experience

See 2.2 for relevant clinical information.

2.2 Background/prevalence of research topic

The overall goal of this proposal is to investigate the impact of cooling components of WS-3, WS-23, and menthol on the appeal and addiction potential of nicotine-containing e-liquids among adults.

Menthol and Novel Cooling Constituents: There is a long history of including cooling flavorants like menthol in tobacco products.[1] Menthol is characterized as a flavor constituent with a minty taste and aroma that produces cooling and analgesic sensory effects in the mouth and throat, and is one of the most common additives in nicotine and tobacco products.[2] For example, most cigarettes, even those characterized as non-menthol, contain some amount of menthol.[3] Menthol tobacco product advertising has targeted youth, young adults, and minority communities, and menthol's sensory effects have been found to facilitate cigarette smoking initiation and nicotine intake.[4-6] Further, use of menthol cigarettes is associated with greater dependence and lower rates of quitting smoking.[7,8] Individuals from minority communities smoke more menthol cigarettes and also have a greater risk for morbidity and mortality from smoking-related illness.[9,10] In April 2022, the FDA announced a proposed rule to prohibit menthol as a characterizing flavor in combustible tobacco products. While this proposed rule does not extend to non-combustible tobacco products, it suggests that the FDA could utilize flavor restrictions to reduce the appeal of e-cigarettes to naïve users. In fact, they did ban flavors in cartridge-based e-cigarette devices based on their appeal to high school youth in February 2020.

Menthol is commonly added to e-cigarettes, both in products advertised as menthol-flavored and those that not advertised as menthol-flavored.[11,12] Menthol has concentration-dependent taste and olfactory effects. Menthol can also be harsh at high concentrations and therefore depending on the level of menthol added to e-cigarettes, menthol can reduce or enhance the sensory experience of irritation and harshness.[13] In e-cigarettes, even very low concentrations of menthol have been shown to enhance sensory appeal,[14] while also increasing the sensation of cooling in the mouth and throat.[15] Laboratory examinations with youth and adults have shown that menthol can reduce the aversive sensory effects of nicotine.[14,16,17] Importantly, nicotine and menthol in e-cigarettes can interact, such that high (but not low) concentrations of menthol may enhance e-cigarette liking/wanting for higher and harsher (but not lower) nicotine concentrations.[14] In sum, menthol in e-cigarettes may enhance e-cigarette appeal via its cooling effects and via reducing harshness/irritation from nicotine and tobacco and altering reinforcement from nicotine.

In line with findings from human laboratory studies, survey evidence from youth and young adult e-cigarette users observed that e-cigarettes that contain cooling components are popular

and that cooling may play a role in e-cigarette use behavior.[18,19] Among youth, reporting use of cooling e-cigarettes was associated with more frequent e-cigarette use and greater likelihood of use of nicotine containing e-cigarettes. Recently, e-cigarettes that are labeled as ice-flavored, which typically indicates a combination of fruity/sweet and cooling flavors, have become very popular and among young adults were associated with more frequent e-cigarette use.[19] Our TCORS Laboratory core analyzed a large number of e-cigarettes labeled as containing "ice" flavors and observed these e-liquids contained synthetic coolants called Wilkinson-Sword (WS)-3 or WS-23.[20] These synthetic coolants, which are odorless and tasteless, were found in 13 of 14 Puffbar e-liquid flavors (i.e., a popular disposable e-cigarette brand) and 24 of 25 other e-liquids marketed as cooling. Many of the tested e-liquids contained WS-3 and/or WS-23 in combination with menthol, while others contained only WS-3 and/or WS-23. In addition to our findings, other researchers have also observed the presence of WS-3 and WS-23 in Puffbar and JUUL products.[21] The included concentrations of WS-3 and WS-23 varied widely and had no discernable correlation with nicotine or menthol concentrations. Interestingly, our group found the menthol levels detected in these e-liquids were much lower (0.03-2.34%)[22,23] than those detected in earlier examinations of commercial products that contained menthol (3.5%).[13] This evidence suggests that as devices and nicotine formulations have evolved, manufacturers may also be changing the levels of menthol in e-liquids and adding other compounds like WS-3 or W-23 to produce cooling, perhaps with the intent of avoiding FDA regulations focused on menthol, or to address changes in sensory perceptions related to new nicotine formulations.

WS (or Cyclohexanecarboxamide,N-Ethyl-5-Methyl-2-(1-Methylethyl)) compounds are non-volatile, odorless and tasteless menthol derivatives which were initially synthesized to produce cooling in shaving products without a burning sensation in the eyes.[24,25] These compounds are commonly used as cooling additives with WS-3 being the most popular.[23] Importantly, WS-3 evokes a sensory experience of cooling by activating the cold/menthol receptor TRPM8 in the oral cavity and upper airways, with greater efficacy than that of menthol.[25-28] Notably, most of the evidence on synthetic coolants like WS-3 has examined their use in oral or skin applications. There are no investigations in humans isolating the effects of how synthetic coolants may influence sensory experience following inhalation via e-cigarettes, or what their effects are when they are combined with different concentrations of menthol, or with nicotine.

Importantly, if these coolants are able to replicate the cooling and irritation-reduction experience of menthol without a characterizing flavor (i.e., odor) they could potentially be used as substitutes for menthol (in the event of a menthol or characterizing flavor ban) or used in addition to menthol to enhance menthol's cooling and analgesic effects. Therefore, it is critical to understand user experiences to these synthetic coolants. We therefore propose to conduct the first study of the influence of multiple commercially available concentrations of the synthetic coolant WS-3 on the appeal and addictive potential of e-liquids when used with and without commercial levels of menthol, among adults who use e-cigarettes alone or with combustible tobacco products.

3 Rationale/Significance

3.1 Problem Statement

There is little data on how the synthetic coolants WS-3 and WS-23 affect appeal and sensory perceptions of e-cigarettes and how its effects compare to menthol.

3.2 Purpose of Study/Potential Impact

The overall goal of this proposal is to investigate the impact of synthetic cooling components from comparable concentrations of WS-3, WS-23, and menthol, on the appeal and addiction potential of nicotine-containing e-liquids among adults. This proposal will provide valuable evidence to support the FDA's nicotine and flavor regulatory frameworks and generate additional scientific data to inform future research and regulations concerning these synthetic constituents. We propose to conduct two experiments. Firstly, a pilot sensory experiment will examine cooling from comparable concentrations of WS-3, WS-23, and menthol to determine appropriate concentrations to use in the main study. Secondly, the main study will characterize the appeal and addiction potential of nicotine-containing e-liquids that contain WS-3, WS-23, and menthol. This proposal will be the first to examine the influence of multiple commercially available concentrations of the synthetic coolants WS-3 and WS-23 on the appeal and addictive potential of e-liquids when used with and without commercial levels of menthol, among adults who use e-cigarettes alone or with combustible tobacco products.

3.2.1 Potential Risks

Participation in this study is thought to involve a greater than minimal risk. Potential risks and protections against risks are detailed below:

(1) E-cigarette Exposure: All participants will be asked to use e-cigarettes in human laboratory sessions and will use study e-cigarettes only in the laboratory (i.e., will not take them outside of the laboratory). All participants will be required to have experience with e-cigarettes. Given this, the likelihood of adverse experience with study e-cigarettes/e-liquids is low. Participation is voluntary and participants can stop anytime they want and/or if they experience adverse events from nicotine or flavor exposure. There have been reported cases of severe pulmonary illness linked to vaping or e-cigarette use (i.e., E-Cigarette or Vaping Associated Lung Injury; EVALI). These cases were first reported in August 2019. These cases included symptoms such as cough, shortness of breath, chest pain, nausea, vomiting, stomach pain, diarrhea, fever, chills, or weight loss. Some patients reported symptoms to have occurred over a few days and some reported to have occurred over a few weeks. Vaping-related disorders have ranged from mild to severe with hospitalization, intensive care with breathing machines and in some cases death. In most cases, but not all, people experiencing these symptoms were using cannabidiol (CBD) and marijuana (THC) e-liquids, and/or using e-cigarette devices and e-liquids that were mixed at home or purchased off market (such as purchasing an e-liquid or device on the street, not from a licensed retailer).

Laboratory data shows that Vitamin E Acetate, an additive in some THC-containing e-cigarette or vaping products, is strongly linked to EVALI.

Products used in our study are purchased only from a licensed retailer and do not contain CBD, THC, or Vitamin E Acetate, so the risks of developing EVALI as a result of study participation are highly unlikely. However, we will monitor for EVALI symptoms at intake and at each lab session using an assessment checklist developed in response to EVALI by our lab, the health assessment checklist (included in study measures). Any study participant reporting current THC/CBD vaping and report of mild or greater EVALI-related symptoms (i.e., cough, shortness of breath, chest pain, nausea, vomiting, stomach pain, diarrhea, fever, chills, or weight loss) without non-EVALI reasonable and proximal cause will be ineligible or withdrawn and recommended.

Additionally, e-cigarette liquids contain propylene glycol and vegetable glycerin, which some people may be allergic to. As participants are current e-cigarette users', so all participants will have prior exposure to these constituents. We will confirm at intake they do not have known allergies to either of these products or e-liquid flavors.

(2) Flavor Exposure: We will expose participants to three different e-liquids flavorants; synthetic coolants WS-3, WS-23 and menthol. All products are widely available commercially and commonly added to popular e-liquids. Menthol, WS-3 and WS-23 administration is reported to produce cooling sensory effects in the mouth and throat.[25-28] In the current study, we will be examining doses of menthol, WS-3 and WS-23 in e-cigarettes that are in line with commercially available doses.[23] In spite of ubiquitous use of menthol in a wide range of products, only few cases of menthol poisoning have been described in the literature in case studies, none related to tobacco or e-cigarette use and an extremely high level of menthol would need to be ingested in a short amount of time to risk toxic effects.[29] Menthol poisoning reported to cause ataxia, confusion, coma, nausea, and vomiting. We are not aware of any cases of poisoning reported for WS-3 and WS-23. Participants will be told that if they feel any adverse effects during the laboratory sessions or want to stop for any reason, they are free to do so. Additionally, we will exclude anyone that reports allergies to any of the flavors used (menthol, WS-3, WS-23, other synthetic coolants). We will also monitor for adverse events.

(3) Nicotine Exposure (applicable for the main study only): E-liquids will contain nicotine in nicotine salt formulation (59mg/ml) that is commonly available in popular commercial e-cigarettes. Common side effects of nicotine include irritation/harshness experienced in the throat, nausea, vomiting, heartburn, and elevated heart rate and blood pressure. Toxic doses of nicotine may cause abdominal pain, hypersalivation, diarrhea, dizziness, confusion, hearing and vision problems, syncope, seizures, hypotension, irregular pulse, and death. However, these toxic effects occur much higher than those that will be used in our studies. Moreover, by recruiting adults who already have experience with e-cigarettes and nicotine, we will further mitigate the risk of these side effects.

(4) Urine & Saliva Collection: Urine and saliva collection will occur at intake and during laboratory sessions and should add no risks other than the time taken to complete them.

(5) Rating Scales and Assessments: These are all noninvasive and should add no risk. The major disadvantages are the time taken to complete them, and possible breach of confidentiality. Our past experience with these measures indicates that they are acceptable to subjects.

(6) Limits to confidentiality: All participants will be specifically told that we will not reveal any personal information collected as part of the research procedures, including their reported use of e-cigarettes and other substance use history. However, there is always the possibility that participation in this study may make others, such as friends and family members, aware of their tobacco use status. They will be told that if they do not feel comfortable with this, then they should not participate in the project. They will also be told that if they report any information to us about abuse or homicidal/suicidal behavior, we will report this information to the appropriate authorities.

(7) COVID & Tobacco Product Use: The following information will be listed in the consent. Although the science on the relationship between getting COVID and using tobacco products like cigarettes and e-cigarettes is still not clear, tobacco products can lead to respiratory conditions like asthma. Therefore, if participants use tobacco products and become infected with COVID-19, there is a potential that participants could have worse health outcomes. Although scientific evidence is incomplete, some studies have suggested that use of e-cigarettes may add to the risk of getting COVID -19 and may contribute to the severity of illness if infected by the virus. We recommend that participants follow the CDC guidelines for the most up to date information about ways to reduce their risk for exposure to COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/index.html. If participants develop symptoms for COVID-19 (fever, cough, shortness of breath, difficulty breathing, muscle aches, sore throat, congestion or runny nose, nausea or vomiting, diarrhea, new loss of taste or smell) or have known or suspected exposure, participants will be instructed to self-isolate and contact their healthcare provider to discuss obtaining a test for COVID-19. Participants will be instructed to inform research staff if they develop any of these symptoms or have known/suspected exposure to COVID-19. If participants become infected with COVID-19, we recommend they stop using all tobacco products.

Protections Against Risk: We will reduce risks by:

(1) Risk of E-cigarette, Nicotine, and Flavor Administration: All participants will self-report on their physical and mental health and we will continue to monitor their health closely during the study. We will monitor heart rate, blood pressure, lung spirometry (measures pulmonary function) and pulse oximetry (measurement of the oxygen in the blood). Participants will also fill out a health assessment checklist to monitor health symptoms (e.g., headaches, dizziness, fainting, nausea, diarrhea) and severity of these symptoms that could be related to nicotine exposure/vaping illness. The e-cigarette lab sessions will be conducted by trained research staff who are sensitive to the smoking population and trained to monitor any potential adverse effects. If they experience any side effects or concerns during the study, we ask them to let us know. Participants will be told that they are able to stop the study at any point. If they feel any discomfort or need to stop for any reason, they can let the research team know. We will also provide

participants with referral sources to quit vaping or smoking (if applicable) if they are interested. We have used similar procedures in prior vaping studies. Subjects will be told that participation is voluntary, and they can stop anytime if they want to and/or if they experience adverse events from nicotine or flavor exposure. The participants are current e-cigarette users, and are, therefore, already self-administering nicotine at doses similar to what is administered in the current studies. The e-cigarettes and e-liquid pods that we will use in the current study are commercially available and the e-liquids were mixed at Yale Pharmacy and do not contain CBD or THC. At this time, we don't know what the risks associated with the use of the e-cigarettes and e-liquids, flavors, etc. that we use in this study are, and who might develop symptoms. The e-liquids we are giving participants contain nicotine, solvents, and flavorings.

(2) Risks associated with rating scales and questionnaires: Careful efforts aimed at maintaining confidentiality will be made and subjects will be reminded that they can choose not to answer any questions they do not feel comfortable answering.

(3) Limits to Confidentiality: Research data will be collected using in-person interviews, assessments, objective measures of smoking behavior, and self-reports. All identifiable information (names and demographic information) collected on paper will be stored in a locked file cabinet and all identifiable information housed online will be stored on secured password protected servers (e.g., Redcap, Yale Shared Drive) only accessible to research staff. All participants will be assigned a study participant ID made up of numbers and letters. A list of IDs and the corresponding names will be maintained by the Principal Investigator and stored in a locked research cabinet. All other research data (interviews, assessments, objective measures of smoking behavior, and self-reports) will not contain identifiable information and will be labeled only with the subjects' unique numerical indicator. Several steps will be taken to safeguard the confidentiality of subjects and their data. Right to privacy for participation in this research will be protected through coding of data and proper storage of research records. Any information published because of the study will be in aggregate and such that it will not permit identification of any participant. We are not directly assessing incidents of child abuse or elderly abuse. However, if this information is disclosed by a participant or volunteer in the context of this research, a report will be made to the Department of Child and Families Services or other agency as required by law. Subjects will be informed of this limit to confidentiality as it is stated in the informed consent document. Individually identifiable health information will be protected in accordance with the Health Insurance Portability and Accountability Act of 1996 and by additional protections of substance abuse treatment records afforded under Code of Federal Regulations (CFR) Part 2, Subpart E. All research personnel will be trained on human subjects' protection and HIPAA procedures.

(4) In general, to minimize risk in this study we will:

- Obtain consent
- Recruit adult e-cigarette users
- Use well-defined inclusion/exclusion criteria

- Use study staff who have extensive training conducting tobacco and substance use research and working with adults and who are sensitive to the issues that may arise in working with tobacco users.
- Protect right to privacy through coding of data and proper storage of research records.
- Obtain a certificate of confidentiality from NIH to further protect the research records of these participants.

3.2.2 Potential Benefit

We expect that the results of the study will benefit science and others through increasing our knowledge of how flavor constituents alter the appeal and addiction potential.

4 Study Objectives

4.1 Hypothesis

We aim to examine the impact of cooling components of WS-3, WS-23, and menthol on the appeal and addiction potential of nicotine-containing e-liquids among adults. We hypothesize that any addition (low, high) of WS-3, WS-23, or menthol will result in similar increases in addiction potential, and that when WS-3, WS-23, and menthol are combined they will produce additive effects relative to menthol alone. We also hypothesize similar changes in appeal outcomes.

4.2 Primary Objective

Examine addiction potential (liking/wanting) and appeal (overall flavor appeal, intensity, sweetness, bitterness, irritation, cooling), following a single exposure (5 puffs) to nicotine-containing e-cigs (~59 mg/ml nicotine salt, commonly used in commercial e-cigs), with one of three WS-3 concentrations (none, low, high) and one of the three WS-23 concentrations (low, high, 4%) in combination with one of three menthol concentrations (none, low, high).

4.3 Secondary Objectives (if applicable)

Examine the interactive effects of different concentrations of WS-3, WS-23, and menthol on appeal and addiction potential.

4.4 Exploratory Objectives (if applicable)

An exploratory objective of this study is to examine the influence of predictors such as sex, combustible tobacco use, and nicotine dependence on study outcomes.

Another exploratory objective is to examine oral microbiome, salivary inflammatory markers and self-reported oral health status in exclusive e-cigarette users and compare the differences among users of different e-cigarette flavor groups (1. Mint/menthol, 2. fruit/sweet, 3. tobacco). We hypothesize that users of mint/menthol or fruit/sweet flavors will have higher levels of anaerobic bacteria in their oral microbiome, greater inflammatory markers in saliva, and poorer oral health compared to tobacco flavor users.

5 Study Design

5.1 General Design Description

Pilot study: This pilot study will establish low and high concentrations of WS-3, WS-23, and menthol concentrations to test in the main study. 30 adult e-cigarette users will participate in one laboratory session during which they will be exposed to e-liquids containing all the conditions in Table 1 without nicotine. Participants will receive a two-puff exposure to each condition; each exposure will be separated by 10 mins to allow the flavor to dissipate, similar to our prior work on menthol.[13,14] Each session will begin with familiarization with the sensory rating scales used in the current study[30,31] and training in how to puff the lab e-cig in a directed and standardized way. WS-3, WS-23 and menthol will be presented in blocks (block order will be counterbalanced) with each block presenting increasing menthol/WS-3/WS-23 concentrations as in our earlier work.[13] Participants will rate *"coolness"* using the generalized Labeled Magnitude Scale (gLMS), an established category ratio scale with 7 semantic labels: "no sensation", "barely detectable", "weak", "moderate", "strong", "very strong", and "strongest imaginable sensation of any kind", positioned quasi-logarithmically based on their semantic magnitude.[32,33] Using averages of participant ratings, we will select a low concentration of each constituent that is rated as more than "barely detectable" but less than "moderate" to ensure that cooling is weak but clearly perceptible. For the high concentration of each constituent, we will select a concentration that on average falls between "strong" and "very strong" to identify a strong cooling sensation while avoiding sensations that would also be uncomfortable. We will also determine *overall flavor intensity, sweetness, and harshness/irritation* using the gLMS. During the lab sessions, we may have participants wear a small smartband on their wrist, on the same hand they use to vape the e-cigarette. The smartband collects movement data from geospatial sensors as the participant moves their arm and hand. The sensor data will be used to measure the movement during the directed e-cigarette puffing during the lab session. The smartband is only being used for data collection and is not being evaluated/used as a medical device. There are no changes to the lab procedure or puffing instructions.

Table 1. Pilot Study Design

	Concentration 1	Concentration 2	Concentration 3	Concentration 4	Concentration 5	Concentration 6
WS-3 Block	0.05%	0.1%	0.5%	1.0 %	2.0 %	-
WS-23 Block	0.05%	0.1%	0.5%	1.0 %	2.0 %	4.0%
Menthol Block	0.05%	0.1%	0.5%	1.0 %	2.0 %	-

Table 2. Main Study Experimental Design

	No WS-3	Low (0.1%) WS-3 Concentration	High (2.0%) WS-3 Concentration	Low (0.1%) WS-23 Concentration	High (2.0%) WS-23 Concentration	4% WS-23 Concentration
Low (0.1%) Menthol	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}
High (2.0%) Menthol	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}
No Menthol	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}	S_1-S_{66}

S=Subject. All subjects (1-66) will be exposed to all conditions in the adjacent table.

Main Study: The purpose of the main study will be to determine sensory responses following exposure to e-liquids containing nicotine and WS-3, WS-23, and menthol. This study will recruit 66 adult e-cigarette users (50% with current cigarette use, of which 50% will use menthol cigarettes), to participate in 3 laboratory sessions, separated by at least 24 hrs. At the intake visit, participation in the oral microbiome and inflammatory marker testing will be optional for exclusive e-cigarette users (up to 33). Specifically, they will be asked to provide saliva and urine samples for oral microbiome and inflammatory marker testing and complete a brief survey about their oral health status. Additionally, participants will be asked to submit their most used e-cigarette device/pods from the past-month for analysis. These will be used as exploratory assessments. During the lab visits, participants will receive all eighteen conditions shown in Table 2 below and will be asked to abstain from tobacco use for two hours prior to session start (to control for time since last tobacco exposure). Participants will be randomized to receive one of the 3 menthol conditions (no-menthol, low menthol, high menthol, levels determined by the pilot study above) in Lab 1 and will receive the alternative conditions in randomized order in Lab 2 and Lab 3. During each lab session the menthol condition will be paired with three WS-3 concentrations: no WS-3, low (0.1%) WS-3, or high (2.0%) WS-3 and three WS-23 concentrations: low (0.1%) WS-23, high (2.0%) WS-23, or 4% WS-23 (concentration determined by pilot study) in randomized order. All e-cigarettes will contain nicotine salt at a nicotine concentration of ~59mg/ml, commensurate with levels in popular commercially available nicotine salt e-cigarettes.[34-36] Each exposure will consist of a 5-puff bout (each puff 3-sec long and separated by 30 sec.) collected via a puff topography device (an apparatus used to measure puffing behavior) and will be separated by 30 mins to allow any sensory effects to dissipate between exposures. To precisely measure puffing behavior, we will utilize a validated electronic cigarette topography device (eTop). This device connects to a computer with software designed to measure puffing behavior. An e-cigarette is inserted into a silicone mouthpiece and the mouthpiece is connected to tubing which feeds into the device. As a participant inhales this device measure puffing behavior including number of puffs, puff volume, puff duration, puff flow rate (a calculation of volume/time). These indices help us understand vaping behavior and make it possible to compare e-cigarette exposure across participants. Topography mouthpieces are sanitized in between subjects and inhaling e-cigarettes via topography devices present no increased risk to participants. At the end of each of the three lab sessions, participants will choose between the 6 e-liquids they sampled in the lab using a free-choice paradigm (~30 mins).

Each session will begin with administration of baseline questionnaires assessing withdrawal and craving, collection of samples to assess nicotine levels, familiarization with the sensory and hedonic rating scales[30,31] and training in how to puff the lab e-cig. Training will be conducted using e-cigs that contain the base liquid of 50% propylene glycol (PG)/50% vegetable glycerin (VG) with no nicotine. Similar to our earlier trials[14,16], after each exposure, participants will rate addiction potential (*liking/wanting*: Drug Effects Questionnaire (DEQ)[14]; and appeal (*overall flavor appeal:* Labeled Hedonic Scale (LHS)[31]; *flavor intensity*: overall flavor intensity, irritation, coolness, sweetness, bitterness: generalized Labeled Magnitude Scale (gLMS[30,32]). Salivary nicotine samples will be collected pre-sessions and after each flavor exposure to assess potential impact of nicotine levels on responses, with participants

rinsing their mouths before providing each sample. A brief description of the e-cigarette device and all ingredients that will be used for the pilot and main studies is included in Table 3.

Table 3. Description of all investigational tobacco products.

Ingredients or products	Manufacturer name	Concentrations (mg/ml or %)
E-cigarette device	Suorin iShare™	-
PG	JT Baker: Propylene Glycol, U.S.P. - F.C.C.	50%
GL	Fisher Scientific: Glycerol, USP/FCC	50%
Nicotine Salt	Nicotine River: PurNic Smooth Nicotine Salt 100mg/ml in 50PG/50GL	0 or 59mg/ml
Menthol	Sigma-Aldrich: L-Menthol	0.05%, 0.1%, 0.5%, 1.0%, and 2.0%
WS-3	Sigma-Aldrich: WS-3	0.05%, 0.1%, 0.5%, 1.0%, and 2.0%
WS-23	Vigon: 503872	0.05%, 0.1%, 0.5%, 1.0%, 2.0%, and 4.0%

5.1.1 Study Date Range and Duration

The expected duration of this study is 09/2023 – 09/2026 (3 years). The expected duration/participant is 3 weeks to complete the intake and single laboratory session for the pilot study and 5 weeks to complete the intake + 3 laboratory sessions for the main study. Recruitment and enrollment for the main study will start after the completion of the pilot study (tentatively by the end of year 2024), meaning after all 30 pilot subjects have completed their lab sessions and the arm is closed.

5.1.2 Number of Study Sites

The study will take place at the Yale University School of Medicine. Study sites will include the Connecticut Mental Health Center (CMHC; 34 Park Street, New Haven, CT) or the Substance Abuse Treatment Unit (SATU;1 Long Wharf Dr., New Haven) for intake appointments and Pierce Laboratories (290 Congress Avenue, New Haven, CT) or West Campus Research Unit (WCRU; 500 West Campus Dr., West Haven, CT), which are equipped with ventilated research chambers utilized for smoking and vaping research studies.

5.2 Outcome Variables

The outcome variables in this study are appeal, sensory effects, and reinforcing value (detailed below).

5.2.1 Primary Outcome Variables

Liking/Wanting: Drug Effects Questionnaire

Labeled Hedonic Scale (LHS): Flavor/Sensory Perception

Generalized Labeled Magnitude Scale (gLMS): Ratings of Flavor Intensity, Sweetness, Coolness, Irritation/Harshness, Bitterness

5.2.2 Secondary Outcome Variables (if applicable)

E-Cigarette Craving: The Yale Craving Scale

Salivary Nicotine/Cotinine Levels

5.2.3 Exploratory Outcome Variables (if applicable)

E-cig Appeal: E-cigarette Evaluation Questionnaire (exploratory)

Examine impact of sex, combustible tobacco use and nicotine dependence on experiences from WS-3, WS-23, menthol and nicotine.

Comparative difference among exclusive e-cigarette users of different flavor groups (menthol or mint, fruit /sweet, tobacco), between oral microbiome constitution and inflammatory markers and self-reported oral health.

5.3 Study Population

Participants will be females and male adult e-cigarette users aged 18 years or older.

5.3.1 Number of Participants

We aim to recruit 30 adults for the pilot study and 66 adults for the main study (33 with current cigarette use, 16 reporting menthol cigarette use). In the main study, we will recruit a subset of eligible exclusive e-cigarette users (up to 33) for oral microbiome and inflammation assessment.

5.3.2 Eligibility Criteria/Vulnerable Populations

For the pilot study:

Inclusion Criteria:

- 18 years or older
- Able to read and write
- Use e-cigarettes containing nicotine
- Have used e-cigs at least 30% of days/past month (at least 8 days on 28-day timeline followback)
- No strong dislike of the menthol flavor
- At least 50% have used only e-cigs with no combustible tobacco products (e.g., cigarettes, cigars, cigarillos) in the past month
- Urine cotinine ≥200ng/ml
- Willing to abstain from tobacco/nicotine use 2 hrs prior to sessions
- Not currently quitting/trying to quit nicotine/tobacco use

Exclusion Criteria:

- Current criteria for moderate or severe cannabis and alcohol use disorder as per DSM-V criteria
- Current criteria for mild, moderate or severe substance use disorder on any other psychoactive substances as per DSM-V criteria except for tobacco use disorder
- Use of psychoactive drugs including anxiolytics, antidepressants, and other psychostimulants unless prescribed and stable for two months

- Current diagnosis of any severe and untreated psychiatric disorder
- Any significant current medical condition such as neurological, cardiovascular, endocrine, renal, or hepatic pathology that would increase study risk
- Any blood pressure values with systolic <100 or >160 mmHg or diastolic <65 or >100 mmHg
- Allergy to menthol, PG/VG, WS-3, or WS-23
- If of childbearing potential: pregnant, trying to become pregnant or breastfeeding
- Uncontrolled asthma (defined as <20 on Asthma Control Test AND/OR endorsement of “yes” to environmentally induced bronchospasm that requires prescription Epipen Medical conditions, including chronic and untreated acute pulmonary conditions, that in the investigators view will increase risk of respiratory problems among participants
- Report of greater than “without any difficulty” on any item of the PROMIS Physical Function Short Form
- For current (past-month use) cannabis vapers: Any report of mild or great EVALI-related symptoms (i.e., cough, shortness of breath, chest pain, nausea, vomiting, stomach pain, diarrhea, fever, chills, or weight loss) without non-EVALI reasonable and proximal cause
- Dislike of menthol flavor

For the main study:

Inclusion Criteria:

- 18 years or older
- Able to read and write
- Use e-cigarettes containing nicotine
- Have used e-cigs at least 30% of days/past month (at least 8 days on 28-day timeline followback)
- Urine cotinine ≥200ng/ml
- Willing to abstain from tobacco/nicotine use 2 hrs prior to sessions
- Not currently quitting/trying to quit nicotine/tobacco use
- *For cigarette using participants only*: use of cigarettes at least 30% of days/past month (at least 8 days on a 28-day timeline followback)
- A subset of eligible participants (exclusive e-cigarette users only) for oral microbiome assessment must be willing to refrain from eating, drinking (except water) or brushing their teeth for 2 hour before saliva collection.

Exclusion Criteria:

- Current criteria for moderate or severe cannabis and alcohol use disorder as per DSM-V criteria
- Current criteria for mild, moderate or severe substance use disorder on any other psychoactive substances as per DSM-V criteria except for tobacco use disorder
- Use of psychoactive drugs including anxiolytics, antidepressants, and other psychostimulants unless prescribed and stable for two months

- Current diagnosis of any severe and untreated psychiatric disorder
- Any significant current medical condition such as neurological, cardiovascular, endocrine, renal, or hepatic pathology that would increase study risk
- Any blood pressure values with systolic <100 or >160 mmHg or diastolic <65 or >100 mmHg
- Allergy to menthol, PG/VG, WS-3, or WS-23
- If of childbearing potential: pregnant, trying to become pregnant or breastfeeding
- Uncontrolled asthma (defined as <20 on Asthma Control Test AND/OR endorsement of “yes” to environmentally induced bronchospasm that requires prescription Epipen Medical conditions, including chronic and untreated acute pulmonary conditions, that in the investigators view will increase risk of respiratory problems among participants
- Report of greater than “without any difficulty” on any item of the PROMIS Physical Function Short Form
- For current (past-month use) cannabis vapers: Any report of mild or great EVALI-related symptoms (i.e., cough, shortness of breath, chest pain, nausea, vomiting, stomach pain, diarrhea, fever, chills, or weight loss) without non-EVALI reasonable and proximal cause
- For oral microbiome and inflammation assessment, participants who report a medical condition such as diabetes, uncontrolled hypertension, renal and hepatic disorders, antibiotics use in the past 3 months, oral cancer diagnosis, or who are undergoing active treatment for any medical condition

6 Methods

6.1 Treatment

6.1.1 Identity of Investigational Product

Products used in this study are investigational tobacco products including 16 different e-liquids and 1 e-cigarette device for the pilot study and 18 different e-liquids and 1 e-cigarette device for the main study. The e-cig device to be used will be the Suorin iShare™ pod device. iShare characteristics resemble the popular JUUL device in terms of power output, shape, and size, but with the benefit of refillable cartridges. The Yale TCORS lab core has demonstrated its near 100%-effectiveness in delivering nicotine to users using a custom-made vaping machine. Nicotine concentrations will be 0 mg/ml in the pilot study and will be 59 mg/ml nicotine salt (nicotine benzoate) in the main study, a widely used and well tolerated nicotine salt formulation and concentration, similar in strength to a 5% JUUL product. PG/VG levels for the study e-liquids will be 50:50 PG/VG, a ratio typical of nicotine salt e-liquids and used in previous studies by our group.

E-liquids will be created upon review of the (Investigational Tobacco Product) ITP application by the FDA; Dr. Krishnan-Sarin leads Yale TCORS ITP applications and will oversee this process. All e-liquids will be created via the Yale Investigational Drug Service (IDS) Pharmacy under the direction and supervision of Dr. Patel Prashant (Manager, Investigational Drug Service Pharmacy). E-liquid recipes have been developed via the Yale Laboratory Core and with consultation of Dr. Tony Pace, a chemist with American E-

liquids/Vaping Direct and collaborator in research with our group with years of experience creating e-liquids for research.[27] E-liquid ingredients will be purchased by our group, then shipped to our research team and upon receipt be transported to and stored in the Yale IDS Pharmacy (see 6.1.6) and created in 30mL batches as needed by Yale IDS. A research assistant/associate/fellow will document the receipt from IDS. E-liquid ingredients all meet the requirements of United States Pharmacopeia (USP) or Flavor and Extract Manufacturers Association of the United States (FEMA) “GRAS” (generally recognized as safe) have been reviewed by Yale IDS and the Yale Tobacco Center of Regulatory Science. Additionally, FDA has reviewed the proposed ITP of all e-liquids prior to study start. The e-liquids being created will be produced with three flavors; (1) menthol, (2) WS-3 and 3) WS-23 to create 16 e-liquids for the pilot and 18 e-liquids for the main study. Pilot e-liquids will contain no nicotine and main study e-liquid will contain a fixed amount of nicotine. E-liquid nicotine and flavor profiles will be confirmed and characterized by the Yale TCORS lab core.[38]

6.1.2 Dosage, Administration, Schedule

Regarding the pilot study, participants will receive two puff exposures to each condition; each exposure will be separated by 10 mins to allow the flavor to dissipate. All e-cigarette pods will be filled to 0.5mL of e-liquid.

Regarding the main study, e-cigarette pods will be filled with approximately 0.9mL of e-liquid, which in previous trials has produced at least 30 puffs. Participants will be randomized to receive one of the 3 menthol conditions (no-menthol, low menthol, high menthol, levels determined by the pilot study above) in Lab 1 and will receive the alternative conditions in randomized order in Lab 2 and Lab 3. During each lab session the menthol condition will be paired with three WS-3 concentrations: no WS-3, low (0.1%) WS-3, or high (2.0%) WS-3 or three WS-23 concentrations: low (0.1%) WS-23, high (2.0%) WS-23, or 4% WS-3 (concentration determined by pilot study) in randomized order. All e-cigarettes will contain nicotine salt at a nicotine concentration of ~59mg/ml, commensurate with levels in popular commercially available nicotine salt e-cigarettes.[55-57] Each exposure will consist of a 5-puff bout (each puff 3-sec long and separated by 30 sec) via a puff topography a device (an apparatus used to measure puffing behavior) and will be separated by 30 mins to allow any sensory effects to dissipate between exposures. The e-cig pods containing e-liquids will be weighed before and after the session.

Participants will be told they can discontinue puffing behavior at any time if they experience discomfort. The lab sessions will be conducted by trained research staff who are sensitive to the population and trained to monitor any potential adverse effects. Heart rate, blood pressure, and pulse oximetry will be assessed at baseline and every in-person visit for safety. If they experience any side effects or concerns during the study, we ask them to let us know. Blood pressure will be taken before and after each e-cigarette exposure during laboratory sessions. A blood pressure and heart monitoring form will be used to assess if blood pressure or heart rate is above or below the following threshold (>180, <100 systolic, >110, <60 diastolic) sessions will be paused and potentially discontinued if blood pressure is unable to return to in range values. If blood pressure is above or below these thresholds, blood pressure will be repeated 2 times to confirm value. Additionally, we will assess blood pressure across

laboratory sessions and contact the study physician if necessary if any concerning trends (with or without threshold blood pressure occur). Participants will be told that they are able to stop the study at any point. If they feel any discomfort or need to stop for any reason, they can let the research team know. In the event of a serious adverse event, the PI (Dr. Suchitra Krishnan-Sarin), will consult with Yale Tobacco Center for Regulatory Science (TCORS) co-Is, along with center chemosensory expert Dr. Barry Green to determine if blind needs to be broken. Given all study participants are administered all e-liquids, the study will be paused during this period if this occurs.

6.1.3 Method of Assignment/Randomization

Participants will be randomized to order of menthol conditions and WS-3 or WS-23 conditions.

6.1.4 Blinding and Procedures for Unblinding

E-cig menthol conditions, WS-3, and WS-23 conditions will be double-blinded and letter coded. Letter codes will differ by participant. A researcher not administering lab session procedures will be responsible for filling e-liquids pods. Our biostatistician, Ralitza Gueorguieva, will produce a randomization chart that will be given to the designated researcher and stored in a locked cabinet.

In the event of a serious adverse event, the PI (Dr. Suchitra Krishnan-Sarin), will consult with the project Co-Is and DSMB to determine if blind needs to be broken. Given all study participants are administered all e-liquids, the study will be paused during this period if this occurs.

6.1.5 Packaging/Labelling

All e-liquids will be created by the Yale IDS Pharmacy as described in 6.1.1. All ingredients sourced will be USP or FEMA GRAS, assessed by the Yale TCORS Lab Core and Yale IDS Pharmacy. All e-liquids will contain nicotine, propylene glycol and vegetable glycerin. The e-liquid mixtures (propylene glycol [PG], vegetable glycerin [VG] and, menthol, WS-3, and WS-23 conditions will consist of concentrated flavor liquids added to a base liquid which will consist of a commonly used ration of 50% PG and 50% VG. PG is the original base liquid and is believed to produce a throat sensation (“throat hit”) which mimics the feel of smoking a cigarette. VG is included to enhance the volume of vapor production, giving a greater sensory illusion of smoking. The Yale IDS Pharmacy has consulted with PI on this project.

E-cigarette device to be used is the Suorin iShare auto battery and Suorin iShare replacement pods will be used. This e-cigarette is similar in size and function to other commercially available more popular pod style devices. A new e-cigarette device will be used for each subject, and new pods will be used for each experiment.

E-liquid nicotine and flavor profiles will be confirmed and characterized by the Yale TCORS lab core.[38] These products do not have an expiration date but will be tested at regular intervals to confirm not change in product composition, as is done in HIC 200023077. Once purchased, if unopened, ingredients will be stored for 3 years or until expiration dates (whichever is sooner, per Yale IDS Pharmacy). Raw ingredients will only be opened at the

Yale IDS Pharmacy. Once opened, ingredients will be disposed of after 1 year (per Yale IDS Pharmacy). Once mixed and dispensed, e-liquids will be disposed of after 90 days of use (per Yale IDS Pharmacy).

6.1.6 Storage Conditions

In order to ensure that we have adequate amount of e-liquid, of the same constituency, we will purchase large enough quantities of ingredients to create e-liquids for the entire study prior to starting these experiments which will be stored at and dispensed at Yale IDS Pharmacy. All raw ingredients (nicotine, propylene glycol, vegetable glycerin, flavorants) will be stored unopened in a locked cool and dry place onsite until recruitment begins. Ingredients will be transported to Yale IDS Pharmacy prior to recruitment start. Once recruitment begins and e-liquids are needed, Yale Pharmacy will dispense e-liquid in 30mL batches. Once complete, e-liquids will be immediately transported to Pierce Labs, which is a secure building accessible only by key code, where they will be delivered and stored in appropriate light/temperature conditions. E-liquids will be delivered and stored in a controlled and dark laboratory environment housed in John Pierce Labs and/or West Campus Research Center. Pods will be filled with e-liquid mixtures at John Pierce Labs.

On the day prior to the lab sessions, a research assistant/associate/fellow who is not involved in the actual conduct of the lab sessions will fill the e-cig pods with the appropriate doses. The information on randomizations to menthol, WS-3, and WS-23 condition orders for lab sessions will be generated by study biostatisticians prior to study start. This information is stored on a randomization sheet that has each participant's information. The individual filling the pods will be notified of which subject ID number needs to be filled. Each 30mL bottle of e-juice will be shaken vigorously prior to filling the pods. Next, they will open the top of the pod and using a mechanical pipette will load 0.9ml into the pipette from the e-juice bottle and carefully transfer it into the pod for the main study (0.5ml will be loaded to the pods for the pilot study). Each pod is then stored in a separate airtight bag to avoid cross contamination of flavors. The individual bags are then stored together in a larger bag labeled with the study ID number and stored back in the container until the lab is set to begin. Once the research assistant is ready to begin each lab, they will open the container, take the appropriate pod(s) out and leave the others. At the end of the lab, they will return the pod(s) used that day to their proper bag and again return it to the box.

A research assistant/associate/fellow will maintain complete and accurate records to account for the receipt, use, and disposition of these pods with e-liquids, as well as the e-cigarette devices, to ensure that these investigational tobacco products (ITPs) are not commercialized. All post-study ITPs from the lab will be collected, properly transferred, and disposed of to the head of Plant Operations at Connecticut Mental Health Center (CMHC; 34 Park Street, New Haven, CT) at the end of the study, with the PI signing off to ensure compliance and prevent commercialization. Regarding the remaining e-liquids stored but not used in the Yale IDS Pharmacy, they will be disposed of by the Yale IDS Pharmacy following the policy on the use of controlled substances in research. This commitment underscores our dedication to adhering to FDA guidelines and maintaining the integrity of our research.

6.1.7 Concomitant therapy

N/A

6.1.8 Restrictions

There are no restrictions.

6.2 Assessments

6.2.1 Efficacy

- **Generalized Labeled Magnitude Scale (gLMS)[30,32,33]:** Participants will rate overall intensity, sweetness, bitterness, coolness, irritation, mintiness, and smell using the gLMS, a category ratio scale with 7 semantic labels: “no sensation”, “barely detectable”, “weak”, “moderate”, “strong”, “very strong”, and “strongest imaginable sensation of any kind”. Labels are positioned quasi-logarithmically according to their empirically determined semantic magnitudes. The gLMS will be displayed on a monitor via a custom program. The scale produces ratio-level data equivalent to that produced by the method of magnitude estimation.[30] Intensity ratings are typically normalized via log-transformation prior to parametric statistical analyses.
- **The Yale Craving Scale[39]:** Based on the gLMS and displayed in the same way, this scale will be used to measure e-cigarette craving. Ratings will be evaluated for normality and transformed as needed.
- **Labeled Hedonic Scale (LHS)[31]:** A bipolar scale with “neutral” in the middle and 5 symmetrical semantic labels: “like/dislike slightly”, “like/dislike moderately”, “like/dislike very much”, “like/dislike extremely”, “most liked/disliked imaginable”, positioned on the scale per their semantic magnitude. The LHS, also displayed on a computer monitor, yields ratio-level data on liking/disliking and typically does not require log-transformation.
- **Drug Effects Questionnaire (DEQ):** A modified version of the DEQ[14,37,40] will be used to assess Liking/Wanting (average of “I feel good e-cig/pouch effects”, “I want more of that e-cig/pouch I received”, “I feel the e-cig/pouch strength” and “I like the e-cig/pouch effect”), and Stimulant effects (average of “I feel energized” and “I feel high”), and Nicotine Withdrawal (average of “I feel sleepy”, “I feel angry”, “I feel irritable”, “I am having difficulty concentrating”, “I feel restless” and “I feel hungry”), on a 0-100 mm scale, from “not at all” to “extremely.”
- **Urine Cotinine and Nicotine levels:** Urine cotinine levels are accurate indicators of tobacco use[41,42] and will be obtained at intake to verify eligibility and measure baseline nicotine and cotinine levels. Additionally, urine samples collected at intake will be used for total nicotine equivalents assessment for oral microbiome and inflammation analysis for up to 33 exclusive e-cigarette users enrolled in the main study. Participation in the oral microbiome and inflammation assessments is optional.
- **Nicotine Equivalents:** Salivary samples for nicotine and cotinine levels will be obtained at baseline and following exposures. To avoid buccal nicotine contamination, participants will rinse their mouths prior to providing each sample.

- **Demographic Information (at Intake):** Age, race, socioeconomic status, marital status, educational and occupational levels, and medical history will be assessed with interviews and self-report forms.
- **Tobacco Use History Questionnaire (at Intake):** Self reports questions used in Population Assessment of Tobacco and Health (PATH) Study and other standardized measures.
- **Timeline Follow Back Interview[43,44] (at Intake):** Use of e-cigarettes, cigarettes, and other tobacco products will be examined using a 28-day TLFB at intake, and follow-up appointments to determine eligibility. Test-retest reliability is high in adult tobacco users.[38]
- **Nicotine Dependence (at Intake):** The 8-item PROMIS measure developed for cigarettes[45] and adapted for e-cigs,[46] with good internal consistency & measurement invariance across sex and race will be used**.**
- **Self-reported Oral Health Status (at intake):** Exclusive e-cigarette users (up to 33) will answer questions related to oral health, such as '*In the past-month, did you observe any bleeding after brushing or flossing, or due to other conditions in your mouth*. (Yes/No)', '*In the past-month, did you notice pain in your mouth and how often* (if yes)', '*In the past-month, did you notice sores inside your mouth', 'In the past-month did you notice dark spots on your teeth'.* The responses to self-perceived oral health will be examined to explore differences based on flavor of e-cigarette used in the past. Participation in this assessment is optional for this subset of participants.
- **Oral Microbiome (at intake):** Participation in this assessment is optional. Three saliva samples from exclusive e-cigarette users will be collected at intake and analyzed for oral microbiome (bacteria, viruses, and fungi) and markers of inflammation.
- **Puff Topography (at lab):** Puff topography will be measured using a device that was developed for e-cigarettes[47,48] and adapted for the disposable e-cigarette device to be used in this study during the three lab sessions. This device includes a mouthpiece in which the e-cigarette device is inserted into and the participant uses the mouthpiece to inhale via the e-cigarette. It does not change the puffing or introduce an additional risk. For each vaping session, the puff topography device uses computer software to determine the parameters of each puff. The software converts signals to airflow (ml/sec) and integrates the flow data, producing measures of puff volume, duration, number, and interpuff interval (IPI). Puff topography will be collected during the directed puff procedures to assess consistency in puffing behavior across participants. Topography mouthpieces are sanitized in between subjects and inhaling e-cigarettes via topography devices present no increased risk to participants.

- **Cardiovascular Measures (at intake and lab):** During intake, participants' blood pressure will be recorded using a general home Omron device. To monitor participants' vital signs during directed puffing bouts in the lab session, physiological measures such as heart rate and blood pressure will be recorded at the beginning (6 times) and end of (6 times) of each lab session using the Omron Professional IntelliSense Blood Pressure Monitor, which is clinically validated for professional use and offers enhanced precision. The device will automatically measure triplicate readings (separated by 30-sec breaks) to accurately monitor participants' heart rate and blood pressure.
- **Spirometry Lung Function Measures (at intake and lab):** We will collect participants' spirometry at intake and all lab sessions using the Vitalograph Model 4000 Respiratory Monitor Spirometer (Smoking Motivator Edition). This procedure follows standardized guidelines to ensure accurate and reliable data collection, assessing participants' respiratory function throughout the study.

6.2.2 Safety and Pregnancy-related Policy

For participants of childbearing potential, pregnancy tests will be administered at intake and each experimental session. Persons reporting pregnancy, breastfeeding, or trying to become pregnant will not be enrolled. If a participant becomes pregnant during the study, they will be withdrawn and if desired, provided with local pregnancy resources.

6.2.3 Adverse Events Definition and Reporting

During screening, participants will undergo a review of their medical history, antibiotic use, a brief physical check (e.g., blood pressure, spirometry, heart rate, pulse oximetry, urine drug and pregnancy test) and a list of EVALI-related symptoms check (i.e., cough, shortness of breath, chest pain, nausea, vomiting, stomach pain, diarrhea, fever, chills, or weight loss) to assess health. Participants with chronic and untreated acute pulmonary conditions, current, untreated psychiatric conditions, or any other condition that could interfere with study participation/meets exclusion criteria will not be enrolled. Special attention will be placed on serious mental health issues not being treated and those at risk will be encouraged to seek mental health treatment. Additionally, participants with any report symptoms that resemble EVALI without another reasonable and proximal cause will not be enrolled. Participants who experience these symptoms during the lab sessions will seek consultation with study medical staff and determine whether to complete the lab session. Participants will be terminated from participation if the investigator feels that their health or well-being may be threatened by continuation in the study. All participants will receive educational material of tobacco product use at the end of their study participation. We will measure health status throughout the study by using self-report questionnaires to assess fatigue, dyspnea, asthma and overall health with a checklist of symptoms related to the reported vaping illnesses. These assessments will be administered at every in-person visit. We will also assess heart rate, blood pressure, and pulse oximetry at baseline and every in-person visit.

This protocol presents a greater than minimal risk to the subjects and adverse events are not anticipated. In the unlikely event that such events occur, serious and unanticipated and

related adverse events will be reported in writing within 48 hours to the Yale IRB and NIDA. The initial serious adverse event (SAE) report will be followed by submission of a completed SAE report to both institutions. In the event that a participant either withdraws from the study or the investigator decides to discontinue a participant due to an SAE, the participant will be monitored by the investigator via ongoing status assessment until 1) a resolution is reached i.e., the problem requiring hospitalization has resolved or stabilized with no further changes expected 2) the SAE is determined to be clearly unrelated to the study intervention, or 3) the SAE results in death. Outcome of SAEs will be periodically reported to NIDA. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NIDA.

The principal investigator, Dr. Krishnan-Sarin, will be responsible for evaluating the adverse events and study data at regular intervals and determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol (at Risks to Subjects) or consent form (at Risks and Inconveniences) are required. During the review process, the PI will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. If necessary, PI will consult with study physician. Either the PI or the IRB or the DSMB have the authority to stop or suspend the study or require modifications. The review of all adverse events by the PIs will determine the attribution and grade of severity of the adverse event by using the following scales:

Attribution of Risk Categories:

Definite: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probable: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possible/Potential: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant’s clinical condition, other concomitant events). Although an AE may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Unrelated: The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Grades of Risk:

0: No adverse event or within normal limits

1: Mild adverse event; Events require minimal or no treatment and do not interfere with the participant's daily activities.

2: Moderate adverse event; Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

3: Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect; Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

4: Life-threatening or disabling adverse event

5: Fatal adverse event

Expectedness:

The Principal Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

6.2.4 Pharmacokinetics (if applicable)

N/A

6.2.5 Biomarkers (if applicable)

Urine Cotinine/Nicotine/Other Drug Use/Pregnancy: Urine samples to test nicotine/cotinine will be collected at intake. Urine samples will be tested for pregnancy at each lab session and at intake. A urine drug screen will be conducted at intake. If any positive drug test results (aside from marijuana) are obtained, participants will have their intake appointment rescheduled to conduct another follow-up drug test prior to the lab session. Urine samples from exclusive e-cigarette users enrolled in the main study will be

tested for total nicotine equivalents and used for oral microbiome and inflammation assessments.

Saliva Samples: Salivary samples for nicotine and cotinine levels will be obtained at baseline and following each exposure. Salivary samples for the oral microbiome will only be collected at intake. To avoid buccal nicotine contamination, participants will rinse their mouths prior to providing each sample.

6.3 Study Procedures

Prescreening: We aim to recruit 66 adult participants (33 female, 33 male) using online advertisements, community flyering, and referrals. We will use the centralized recruitment services provided by the Yale TCORS Administrative Core which involves the use of various recruitment techniques including advertising on social media websites (e.g., Facebook, Instagram, Snapchat, Reddit), at local colleges and jobsites and through Craigslist and flyers in the community, and a clinical trials recruitment company, BuildClinical. BuildClinical is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. They have worked with IRBs in the US to ensure we adhere to all the appropriate guidelines and procedures. They utilize study-specific advertisements to engage participants on digital platforms such as Facebook, Google, WebMD, etc. and redirect them to a study-specific landing page should they click it. On the landing page, the person can complete an online pre-screen questionnaire that gets routed into BuildClinical's platform. BuildClinical's Secure Socket Layer (SSL) software encrypts all inputted information and keeps your information private and HIPAA compliant. Their backend servers are stored in the US at some of the most secure data centers in the world. The PI and/or study research staff will be responsible for contacting participants. Potential participants will be directed to a voluntary link containing brief screening questions including information for research team to contact and/or phone number for participants to contact research staff directly. If interested, participants will be asked if they regularly use nicotine containing e-cigarettes, and if they are at least 18. If interested, potential participants will be invited to take part in an intake visit to determine eligibility. Intake visits will be completed in person, as a urine sample is needed. The Yale TCORS centralized repository protocol (HIC# 2000037658) may refer potential participants for other future study recruitment.

Intake Visit: Participants will complete an initial intake appointment to sign informed consent and complete assessments to determine eligibility. Demographic information (age, biological sex, gender identity, race/ethnicity, marital status, socioeconomic status), self-reported medical and substance use history, urine drug, cotinine and pregnancy tests, heart rate, blood pressure, spirometry, and pulse oximetry will be collected. The Structured Clinical Interview for DSM-V (SCID) will be used to assess SUDs, as used in similar trials led by Dr. Krishnan-Sarin (HIC # 200023077). Tobacco use history assessments will assess lifetime/current tobacco product and flavor use, use frequency, nicotine dependence (Fagerstrom Test for Nicotine Dependence[70]), e-cig dependence (E-cig Dependence Scale[71]), perceptions of tobacco product use and risk, measures of usual brand cigarette/e-cig appeal and reinforcing efficacy, and cessation attempt history.

Saliva samples will be collected from exclusive e-cigarette users only (up to 33) at intake from the main study for testing oral microbiome and inflammatory markers. Additionally, participants will be asked to bring their most frequently used e-liquid/device in the past-month to the study visit, for collection and testing by the study team. The e-liquids will be

tested in our lab for flavor and nicotine strength. Participants will receive an additional $10 as compensation for turning in their device/pods which will NOT be returned to the participants. Urine samples will be collected for testing total nicotine equivalents. The saliva and urine samples collected at intake visit will only be used for oral microbiome and inflammation assessment if the participant is deemed eligible for the main study after intake. If not eligible, the samples will be discarded.

Lab Visits: See list of assessments in Table 4. We will collect urine pregnancy test, heart rate, blood pressure, pulse oximetry, spirometry, a health assessment checklist and health changes questionnaire, a craving measure.

The pilot study will establish low and high concentrations of WS-3, WS-23, and menthol concentrations to test in the main study. Participants will attend one lab session during which they will be exposed to e-liquids containing 16 conditions of menthol, WS-3, and WS-23 without nicotine. Participants will receive two puff exposures to each condition; each exposure will be separated by 10 mins to allow the flavor to dissipate. Each session will begin with familiarization with the sensory rating scales and training in how to puff the lab e-cig. WS-3, WS-23, and menthol will be presented in blocks (block order will be counterbalanced) with each block presenting increasing menthol/WS-3/WS-23 concentrations. E-cig pods containing e-liquids will be weighed before and after the session. The TCORS Laboratory Core will prepare the e-liquids and analyze the amount transferred into the vape aerosol using the “vaping/lung machine” developed by the core.

In the main study session, participants will engage in a 15-min training session of the psychophysical tasks they will use to report sensory experience at Lab 1. For Labs 1-3, participants will engage in six directed puffing bouts (5 puffs/3 sec puffs/30 second interpuff intervals) will be separated by 30 mins to allow any sensory effects to dissipate between exposures, a procedure adapted from Dr. Krishnan-Sarin’s previous studies. Puffing will occur using a puff topography device (i.e., an established device in which the e-cigarette is attached to a mouthpiece) to measure the parameters of each puff taken (puff duration, volume, etc.). During the 15-minute training session at Lab 1, participants will familiarize themselves with vaping using a puff topography device. Following bout, participants will complete in the following order measures of overall liking/disliking, intensities of sweetness, bitterness, cooling, and irritation/harshness, and where irritation/harshness is felt (i.e., mouth, throat, both). Sensory measures will be followed by collection of general appeal and reinforcing efficacy measures, and a post-exposure measure of craving (collected 15-min after exposure). Cardiovascular measures, including blood pressure and heart rate, will be recorded both before and after each bout session. Participants will be randomized to receive one of the 3 menthol conditions (no-menthol, low menthol, high menthol, levels determined by the pilot study above) in Lab 1 and will receive the alternative conditions in randomized order in Lab 2 and Lab 3. During each lab session the menthol condition will be paired with three WS-3 concentrations: no WS-3, low WS-3, or high WS-3 and three WS-23 concentrations: low WS-23, high WS-23, or 4% WS-23 (concentration determined by pilot study) in randomized order. E-cig pods containing e-liquids will be weighed before and after the session. Lab sessions will occur no more frequently than every 24-hrs. At the end of

each of the three lab sessions, participants will choose between the 6 e-liquids they sampled in the lab using a free-choice paradigm.

Debrief: Following study completion, participants will be thanked for their participation and will be given information from the CDC on cigarette smoking and e-cigarette cessation.

Table 4. List of Study Assessments

	Pre-screening	Intake (pilot)	Lab 1 (Pilot)	Intake (main)	Lab 1 (main)	Lab 2 (main)	Lab 3 (main)
Contact Info & Eligibility Review	X						
Informed Consent		X		X			
Identifying Information		X		X			
Demographic Information		X		X			
Medical History		X		X			
Timeline Follow Back		X		X			
Urine Drug Screen		X		X			
Urine Nicotine/Cotinine Sample		X		X			
Saliva Sample				X	X	X	X
Urine Pregnancy Screen		X	X	X	X	X	X
Blood Pressure/Heart Rate/Pulse Oximetry		X	X	X	X	X	X
Spirometry		X	X	X	X	X	X
Puff Topography					X	X	X
Tobacco Use History/Dependence Measures		X		X			
SCID IV: Substance Section		X		X			
Asthma Control Test		X	X	X	X	X	X
PROMIS Dyspnea		X	X	X	X	X	X
FACIT Cough Item		X	X	X	X	X	X
PROMIS Fatigue		X	X	X	X	X	X

EVALI Health Assessment		X	X	X	X	X	X
gLMS Questionnaire			X		X	X	X
LHS Questionnaire					X	X	X
Drug Effects Questionnaire					X	X	X
Questionnaire of Vaping Craving					X	X	X
Oral health questionnaire				X			
Urine for nicotine equivalents testing for oral microbiome and inflammatory marker assessment (from up to 33 exclusive e-cigarette users only)				X			
Saliva for oral microbiome and inflammatory marker assessment (from up to 33 exclusive e-cigarette users only)				X			

6.3.1 Study Schedule

For each study, participants will complete an intake visit (approx. 2 hrs). Following intake and eligibility determination, participants will complete the pilot study with a single lab session and the main study with 3 labs sessions, at least 24 hours apart.

6.3.2 Informed Consent

At intake, participants will be read informed consent and any questions will be discussed with researcher. Participants will sign informed consent (via Redcap) and consent will be administered by trained research staff.

6.3.3 Screening

For the pilot study, we aim to recruit 30 adult participants and for the main study, we aim to recruit 66 adult participants (33 female, 33 male) using online advertisements, community flyering, and referrals. Potential participants will be recruited via the centralized recruitment services provided by the Yale TCORS Administrative Core which involves the use of various recruitment techniques including advertising on social media websites (e.g., Facebook, Instagram, Snapchat, Reddit), at local colleges and jobsites and through Craigslist and flyers in the community, and a clinical trials recruitment company, BuildClinical. PI and/or study research staff will be responsible for contacting participants. Potential participants will be directed to a voluntary link containing brief screening questions including information for research team to contact and/or phone number for participants to contact research staff

directly. If interested, participants will be asked if they regularly use nicotine containing e-cigarettes, and if they are at least 18. If interested, potential participants will be invited to take part in an intake visit to determine eligibility. Intake visits will be completed in person and should take approximately 2 hours.

Participants who fail to be eligible for current medical reasons (e.g., just started a new medication, have an upcoming procedure, or have an ailment that has resolved) or have another temporary ineligibility may be rescreened at a later date. PI will assess in concert with study nurse to determine if they are ok to rescreen. Participants will not be told at any intake why they are not eligible to protect inclusion/exclusion criteria and avoid any deceptive tactics for enrollment.

6.3.4 Enrollment

If eligibility criteria are met, participant will be enrolled. PI will deem subjects eligible following review and will assign enrolled subject ID.

6.3.5 On Study Visits

See 6.3 “Study Procedures” above and Table 1.

6.3.6 End of Study and Follow-up

After completion, participants will be provided with written materials on e-cigarette cessation. Brief follow-up phone calls will take place at 1 month and 6 months after study completion to assess their current tobacco use behavior.

6.3.7 Removal of subjects

Subjects may withdraw at time voluntarily for any reason. Once participants have signed consent, they may withdraw at any time by communicating to any member of the research team. Any data that has already been collected can still be used, as necessary to ensure the integrity of the study and/or study oversight. Additionally, the research team may decide to withdraw participants if necessary, including if participants do not follow directions of the study team, the study team decides the study is not in a participant’s best interest, or if a participant becomes pregnant, intends to become pregnant, or begins nursing a child.

No anticipated adverse events are expected that would require participant withdrawal. Adverse events are not anticipated. In the event that a participant either withdraws from the study or the investigator decides to discontinue a participant due to an adverse event, the participant will be monitored by the investigator via ongoing status assessment until 1) a resolution is reached i.e., the problem requiring hospitalization has resolved or stabilized with no further changes expected 2) the SAE is determined to be clearly unrelated to the study intervention, or 3) the SAE results in death. Outcome of SAEs will be periodically reported to NIDA. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NIDA. Withdrawal will be documented in a study participant tracker.

6.4 Statistical Method

6.4.1 Statistical Design

Preliminary analyses will provide an overview of the data (e.g., randomization success, outcome distribution, missingness patterns). Outcome analyses will be intent-to-treat and use mixed-effects models which allow for different numbers of observations/subject, use all available data, and can handle missing-at-random data. If model assumptions will appear to be violated, we will transform the data or fit more flexible generalized linear or nonparametric mixed models. For the pilot study, separate mixed-effects models will examine the influence of different concentrations of menthol, WS-23 and WS-3 on coolness and overall intensity and will be used to select the concentrations of menthol and WS-3 that product the weak but detectable coolness and the maximum coolness. For the main study, separate mixed-effects model will include menthol (0, Concentration 1, Concentration 2), WS-3 (0, Concentration 1, Concentration 2), WS-23 (0, Concentration 1, Concentration 2, Concentration 3) and time within lab as factors, all interactions among those factors and adjust for stratification variables. A combination of random effects and structured variance-covariance matrices will be used to account for within-subject correlations. The best-fitting structure will be selected based on Akaike information criterion (AIC). For the main study, higher liking/wanting for WS-3 (at Concentration 1 or 2) or WS-23 (at Concentration 1 or 2 or 3) combined with menthol (at Concentration 1 or 2), compared to menthol alone, will be supportive of the primary hypothesis. Similar models will examine the other outcomes and interactions of different concentration combinations of WS-3, WS-23 and menthol with nicotine. Exploratory analyses will also consider the impact of sex, combustible tobacco use and nicotine dependence on experiences from WS-3, menthol and nicotine.

6.4.2 Sample Size Considerations

For our pilot study, we will recruit 30 subjects. While we did not conduct a power calculation for this pilot study, we conducted a similar pilot study with 10 participants in our earlier work with multiple menthol concentrations, and do not anticipate any problems with identifying differences in coolness between the WS-3 and menthol concentrations. For the main study, we will recruit 66 subjects. With an anticipated dropout of 10%, 60 subjects are expected to have complete data. Because we have 4 primary hypotheses (each WS-3 concentration X each menthol concentration > menthol concentration alone), we used a Bonferroni-corrected alpha=0.1. With N=60 completers and a SD of paired differences of 25 as observed in our prior data, we have 80% power to detect a difference of 11.4 (moderate effect size d'=.45) in liking/wanting (0-100 scale) between each WS-3 concentration X each menthol concentration and menthol alone using a paired t-test at alpha=0.01.

6.5 Planned Analyses

6.5.1 Primary, Secondary, and Exploratory Objective Analysis

For the pilot study, separate mixed-effects models will examine the influence of 5 different concentrations of menthol and WS-3 on coolness and overall intensity and will be used to select the concentrations of menthol and WS-3 that product the weak but detectable coolness and the maximum coolness. For the main study, separate mixed-effects model will include menthol (0, Concentration 1, Concentration 2), WS-3 (0, Concentration 1, Concentration 2), WS-23 (0, Concentration 1, Concentration 2, Concentration 3), and time within lab as factors, all interactions among those factors and adjust for stratification

variables. A combination of random effects and structured variance-covariance matrices will be used to account for within-subject correlations. The best-fitting structure will be selected based on Akaike information criterion (AIC). For the main study, higher liking/wanting for WS-3 (at Concentrations 1 or 2) or WS-23 (at Concentrations 1 or 2 or 3) combined with menthol (at Concentration 1 or 2), compared to menthol alone, will be supportive of the primary hypothesis. Similar models will examine the other outcomes and interactions of different concentration combinations of WS-3/WS-23 and menthol with nicotine. Exploratory analyses will also consider the impact of sex, combustible tobacco use and nicotine dependence on experiences from WS-3/WS-23, menthol and nicotine.

Oral microbiome and inflammatory marker assessment will serve as another exploratory objective in this study, Microbial testing, including DNA extraction, library preparation and sequencing methods, will be performed at the CosmosID lab. The analysis will evaluate alpha and beta diversity across different flavor-use groups, with the significance of group-wise clustering determined using a permutational multivariate analysis of variance (MANOVA). Statistical methods such as ANOVA, t-tests and chi-square will assess between-group differences, while controlling for nicotine concentration based on total nicotine equivalents measured in urine samples from participants.

6.5.2 Safety

See 6.1.2 for treatment of adverse events. Additionally, a health assessment will be collected at every visit.

6.5.3 Analysis of Subject Characteristics

Participant demographics, tobacco product history, e-cigarette history, and e-cigarette dependence level will be collected at intake and used to characterize population.

6.5.4 Interim Analysis (if applicable)

N/A

6.5.5 Health economic evaluation

N/A

6.5.6 Other

N/A

6.5.7 Subsets and Covariates

As noted, we will stratify by menthol cigarette status in randomization and include as a covariate in analyses as that is a known confound to ratings of cooling e-cigs. If necessary, we will also adjust for participant abstinence compliance.

6.5.8 Handling of Missing Data

Outcome analyses will be intent-to-treat and using mixed-effects models. These models allow for different numbers of observations per subject, use all available data, and can handle missing-at-random data. If model assumptions will appear to be violated, we will transform the data or fit more flexible generalized linear or nonparametric mixed models.

7 Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

The study will be conducted in accordance with the conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

Participants will not be told of the menthol or WS-3 or WS-23 conditions administered or the nicotine concentration of the product. They will be told that products containing levels of nicotine and flavorings similar to commercial flavors of products they are using. We will inform participants of this in the consent and indicate that we do not plan to tell them as we do not want prior perceptions of nicotine content or flavors to influence their experience with the product. Participants will be asked not to sign consent if they are uncomfortable with this.

For the pilot, participants will be compensated up to $150: $50 for the intake. For the lab, participants will be compensated $90 in addition to a $10 travel payment if they provided their own travel.

For the main study, participants will be compensated up to $510: $50 for the intake, $115 for Lab 1, $115 for Lab 2, and $115 for Lab 3 in addition to $10 travel payments to the lab sessions when they use their own transportation. Participants can also earn a $35 completion bonus for completing all parts of the study. The above compensation will be provided by cash. Those eligible for the oral microbiome and inflammation assessment who submit their e-cigarette device/pods will receive an additional $10 (cash payment).

For the 1-month and 6-month follow-up phone calls (for the main study), participants will be paid $20 in the form of an Amazon gift card or cash.

No information from this study will be added to participant medical records.

7.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required.

The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year.

A study closure report will be submitted to the IRB after all research activities have been completed.

Other study events (e.g., data breaches, protocol deviations) will be submitted per Yale’s IRB's policies.

7.3 Subject Confidentiality

Subject confidentiality is held in strict trust by the research team. Subject medical record review will be limited to the just the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to collect data for this project. No direct subject identifiers will be entered into study tracker.

Each subject will be assigned a unique study number that is not linked to their identifying information. A master list linking the unique study number to the human subject will be maintained in a locked drawer in PI's office and on an encrypted document on a secure server. Only study staff will have access to this document.

7.4 Deviations/Unanticipated Problems

If the study team becomes aware of an anticipated problem (e.g., data breach, protocol deviation), the event will be reported to the IRB by PI within 24 hours of being aware of event if necessary. Protocol deviations will be documented in Study Deviation Tracker.

7.5 Data Collection

Data will be collected via surveys and questionnaires listed in the Visit Schedule table above. No licensure is required for any administration and all questionnaires will be administered by research assistants who have been trained in administration of sensory tasks. All data will be collected either via paper forms or via Redcap (CRF 11 compliant) with the exception of the gLMS and LHS, which will be collected on custom software for quantification of subjective responses. The data will be downloaded on a monthly basis from Redcap and stored on a secure server. Any paper data will be stored in a locked file cabinet. Data will be stored this way for 7 years after the final data is collected. After this point, the Principal Investigator will oversee the process in which data is destroyed or de-identified.

7.6 Data Quality Assurance

All data forms will be identified with the study ID of the participant and include date/time and study visit. The codes that link the name of the participant and the study ID will be kept confidential by the Principal Investigator in a secured cabinet. Online data will be downloaded on a monthly basis from Redcap and stored on a secure server. Error checking and data validation will occur weekly, and any problems will be queried and resolved immediately. Dr. Krishnan-Sarin will receive monthly data quality reports to check for completeness and accuracy of key demographic and prognostic variables, as well as rates of recruitment and retention.

Multiple measures are in place to ensure the validity and integrity of the data. First, all research staff receive Human Subjects Protection and Good Clinical Practice training. Second, formal training in clinical assessments and procedures will be conducted to ensure quality of the data and help implement and improve recruitment strategies so that enrollment goals are achieved. Third, weekly research staff meetings take place, as a forum for in-service training as well as to discuss questions regarding issues that arise in the research protocols. Lastly, adherence to assessment administration will be monitored and if research drift is observed, the research assistants will be re-trained.

7.7 Study Records

Study records are considered protocol, consents, biosamples, and survey/questionnaires collected as part of the study procedures, as well as investigational tobacco products (ITPs), including records showing the receipt, shipment, or other disposition of the e-liquids, pods, and e-cigarette devices. No medical records are collected as part of this study.

7.8 Access to Source Documents

Source documents include paper and Redcap data collected as a part of the study. Online data will be downloaded on a monthly basis from Redcap and stored on a secure server. Paper data will be stored in a locked cabinet.

7.9 Data or Specimen Storage/Security

The majority of data for this study will be collected, recorded and stored using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies. It includes features for HIPAA compliance including real-time data entry validation (e.g., for data types and range checks), a full audit trail, user-based privileges, de-identified data export mechanism to statistical packages (SPSS, SAS, Stata, and R), and integration with the institutional Active Directory. Access to study data in REDCap will be restricted to the members of the study team with authentication through University NetID credentials.

The REDCap@Yale database and web server are housed on secure platforms that are backed up daily. REDCap@Yale meets the security standards for use with high-risk data as set forth by the Yale Information Security Office.

Identifying information (consent, contact information) will be collected on paper documents and stored in a locked cabinet and/or uploaded to secure server and encrypted. Saliva for inflammatory marker testing and urine samples will be stored in tubes in -80 degree C freezer designated for study samples. Samples for oral microbiome analysis will be stored at room temperature for 30 days. If required to store for longer duration, the samples will be moved to freezer at -80 degree C. All specimens will be labelled only using study ID and no identifying information. Only PI or trained research staff will have access to the specimens.

All participants will be assigned a Study ID following consent and all study documents will use that ID.

7.10 Retention of Records

The data will downloaded on a monthly basis from Redcap and stored on a secure server. Any paper data will be stored in a locked file cabinet. Data will be stored this way for 7 years after the final data is collected. After this point, the Principal Investigator will oversee the process in which data is destroyed or de-identified.

7.11 Study Monitoring

The principal investigator and study team are responsible for monitoring the data, assuring protocol compliance and conducting the safety reviews. Study monitoring will occur weekly. The study team, in consultation with the Yale TCORS Independent Data Safety Monitoring Board (DSMB), will evaluate adverse events and determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol or consent form are needed. Reviews will be held every 6 months.

7.12 Data Safety Monitoring Plan

The study is considering a greater than minimal risk as participants are all current tobacco users using nicotine and flavors. It is unlikely brief exposure to e-cigarettes would present additional risk to this population.
The study team, in consultation with the Yale TCORS Independent Data Safety Monitoring Board (DSMB), will evaluate adverse events and determine whether the adverse event affects Reviews will be held every six months. Following each DSMB meeting written minutes will be prepared and distributed summarizing any recommendations. These written reports will insure timely communication with the PI with preparation of any protocol amendments necessary. After each DSMB meeting, this written report will describe all recommendations including additional safety steps.

a. DMSB Members and affiliation

The Yale TCORS Independent Data Safety Monitoring Board includes experts in the field of tobacco use behaviors and challenge studies (Chair: Dr. Tony George, FRCPC, Professor and Co-Director, Division of Brain and Therapeutics, Dept. of Psychiatry, U of Toronto; Dr. Thomas Brandon, Professor and Chair, Department of Health Outcomes & Behavior, H. Lee Moffitt Cancer Center & Research Institute) and a statistician (Dr. Hanga Galfalvy, Assistant Professor of Neurobiology, Columbia University).

b. Frequency of meetings

Meeting will be held twice a year or more often if requested by the Board.

c. Conflict of interest

The members of the DSMB and all study Investigators have completed Conflict of Interest forms created by Yale's IRB in accordance with NIH guidelines.

d. Protection of confidentiality

Members of the DSMB agree to maintain the confidentiality of all information discussed in the meetings and reports of the DSMB.

e. Monitoring activities (initial and ongoing study review)

The DSMB will review the study information and the plans for review prior to the initiation of the studies. They will provide an ongoing review every six months thereafter based on the attached DSMP report.

f. **Communication plan to IRB, NIDA, and FDA (if applicable)**

The summary of the DSMB meeting will be submitted to the IRB and to NIDA following each meeting.

7.13 Study Modification

All study modifications will be submitted to the HIC and will not be implemented before receiving approval. Old versions of the protocol will be saved for reference.

7.14 Study Discontinuation

There are no anticipated circumstances that would warrant termination. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

7.15 Study Completion

Study completion will be when 66 participants have completed all lab sessions of the study. Once study enrollment is closed, the IRB and FDA will be notified.

7.16 Conflict of Interest Policy

No persons with a conflict of interest will have any role in study design, conduct, analysis, or publication.

7.17 Funding Source

The study is funded through the Center for Tobacco Products and the National Institute on Drug Abuse as part of a Grant #U54DA036151.

7.18 Publication Plan

The principal investigator holds the primary responsibility for publishing study results. The PI will seek to publish the results expeditiously after data analysis.

8 Appendices

Appendix #	Title	Section	Topic

9 List of Tables