CLINICAL STUDY PROTOCOL

Interventional Drug or Biologic

Sex Differences in E-Cigarette Flavor Sensory Perception As It Relates to Appeal and Reinforcing Efficacy Among Adult Smokers

Protocol Number

2000032981

Protocol Version

9-24-2024

Version # 8

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Study Phase (1, 2, 3, or 4-postmarketing): N/A

Confidentiality Statement:

REVISION HISTORY:

Revision #	Version Date

Synopsis

Primary Objective The primary objective is to examine the influence of sex on sensory effects, appeal, and reinforcing value of nicotine containing e-cigs in popular flavor components; sweet and cooling.
Secondary Objective (if applicable) The secondary objective is to examine the influence of sex on craving reduction of nicotine containing e-cigs in popular flavor components; sweet and cooling. 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. 2 years to complete entire study, approx. 2 weeks/participant
Study Design Within subject's human laboratory examination
Number of Study Sites 1; Yale School of Medicine
Study Population Adult combustible tobacco users (at least 1 cigarette/day for at least 25 of the past 30 days), with recent e-cig experience, 50% female
Number of Participants 69 anticipated, 60 completers
Primary Outcome Variables Appeal by Sex: Labeled Hedonic Scale (LHS) Ratings of Liking Taste Sensory Effects by Sex: Generalized Labeled Magnitude Scale (gLMS) Ratings of Sweetness, Coolness, Irritation/Harshness, Bitterness Reinforcing Efficacy by Sex: Multiple Choice Procedure (MCP); E-cigarette Purchase Task (eCPT)

Secondary and Exploratory Outcome Variables (if applicable)

Craving Reduction by Sex

Puffing Behavior in Ad-lib Session

E-cigarette Evaluation Questionnaire (exploratory)

Effect of estrogen/progesterone levels

Abbreviations

Abbreviation	Explanation
LHS	**Labeled Hedonic Scale**
gLMS	**Generalized labeled magnitude scale**
MCP	**Multiple Choice Procedure**
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 popular e-cig flavor components (i.e. those that produce cooling and sweetness) on sensory perception, appeal, and reinforcing efficacy of e-cigs by sex.

Sex and Gender: In the current proposal, we plan to focus on differences by sex, rather than gender. Sex, refers to the biological distinctions between males and females, while gender refers to socially constructed roles and behaviors, which occur in historical and cultural contexts and are subject to change by societal expectations and time.[1] Sex and gender overlap and are both relevant when characterizing tobacco product use and behavior.[2] However, largely the tobacco literature does not distinguished sex and gender. In the current proposal, we plan to conduct research examining differences by sex as a biological variable. Although not directly assessing gender in the proposed research strategy, the career development plan includes training opportunities (e.g., coursework, seminars) aimed at examining the relationship of sex and gender and how they relate to tobacco product use, with the goal of using this information to design future projects.

Sex Differences in Response to Tobacco Products: There is a well-established body of research demonstrating evidence of sex differences in response to tobacco products; namely that female tobacco users are less sensitive to changes in nicotine dose than males and more sensitive to changes in non-nicotine sensory cues of the smoking experience.[3-6] In cigarette cessation trials using nicotine replacement therapy, there is a pattern of poorer cessation outcomes among females.[7-12] In examinations in which nicotine content was altered, both in nasal spray or cigarettes, females demonstrated less nicotine discrimination compared to males.[13-15] In a study in the general population of smokers, reduction in cigarette nicotine content did not affect the subjective experience of smoking for females but did for males.[13] Experimental studies suggest females may have greater attention to sensory cues of smoking compared to males.[16-20] Importantly, research has shown females reduced smoking behavior more so than males as a function of blockade of olfactory smoking stimuli (i.e. nose plugs), which mute olfaction and blunt taste cues. This finding was not observed when visual smoking stimuli was blocked, indicating the specific relevance of olfactory cues.[21] For females, this heightened response to sensory stimuli related to olfaction and taste is supported by broader evidence from the chemosensory literature that indicate that females tend to have superior identification of and sensitivity to odors and tastes than males,[22-24] although evidence suggests this sensitivity may decrease somewhat following menopause.[22,25] Taken together, this literature provides support for the hypothesis that chemosensory cues are especially relevant to females in comparison to males in the assessment of nicotine/tobacco products.

Flavors & Tobacco Products: Flavor is a salient part of the sensory experience of nicotine/tobacco products. Flavor is not experienced only as taste, but also as the chemesthesis (i.e. chemical sensations in the mouth), orthonasal (perceived via nostrils) and retronasal (perceived via oral cavity) olfaction. Flavors have historically been added to tobacco products to increase appeal, enhance ease of product use, and to mask the harshness of nicotine.[26] E-cig flavor selection is incredibly diverse with recent reports identifying over 15,000 individual e-cig flavors.[27] In e-cigs, flavor components have unique contributions; sweet tasting e-liquids may contribute to product appeal, increase wanting and liking when paired with nicotine, and potentiate the reinforcing effects of e-cigs.[28-33] Cooling flavors contribute to appeal,[34-36] yet also mask nicotine-related irritation and harshness.[34,35] Both e-cig flavor components are popular among adult combustible and former combustible users, as well as youth.[37-40] Among former combustible users using e-cigs, most report using non-tobacco flavors.[41] Understanding use of e-cig flavors is necessary as flavors are an important target for tobacco regulatory policy. Given their popularity with youth, flavor restrictions are a potential measure aimed at reducing youth e-cig use, however this may have unintended consequences if specific e-cig flavor components, such as components producing sweet or cooling encourage switching from combustible product use.

Sex Differences in Flavored Tobacco Product Use: Sex differences appear in flavor use of tobacco products. In combustible products, flavors have been used by tobacco companies to specifically target females[42] and flavored tobacco use is more prevalent among females.[43-45] Although e-cig use is more prevalent in males, there is data demonstrating that in e-cigs both (a) non-tobacco flavors are used at a higher rate by females and (b) that females value availability of flavor.[3,37,46-50] There is limited direct experimental evidence evaluating sex differences in response to e-cig flavors. In a study evaluating e-cig uptake among cigarette smokers, females had higher ratings of liking and more nicotine intake when given an e-cig flavor that matched their usual brand cigarette.[51] Males did not show this pattern. Other experimental work has shown that females have higher ratings of liking of menthol e-cig flavoring than males[52,53] and higher ratings of overall sensation intensity at low menthol levels.[54] These data suggest that for e-cigs, flavor may be more relevant for females, but this topic is underexplored. Among combustible users, there is a need to examine how popular e-cig flavor components subject to regulations differ by sex in sensory perception, appeal, and reinforcing efficacy. Many studies of sex in the e-cig literature treat sex as a secondary outcome and do not distinguish sex and gender.[51] This proposal therefore addresses a critical gap in evaluating sex differences of flavor's impact e-cig appeal, use, and reinforcement.

E-cigs are not an FDA approved cessation aid, but there is evidence among cigarette smokers that these products could be used as tools to transition away from combustible cigarette smoking.[55,56] Given cigarette smoking is a leading cause of preventable death among U.S. adults and contributes to half a million deaths annually,[57] e-cigs could serve as a harm reduction tool for smokers. For combustible users, flavors have been shown to be an appealing component of e-cigs. However, e-cigs are not harmless and flavors are also established sources of appeal among youth and non-users.[58-61] The FDA has the authority to

regulate the flavors of these products and could limit flavor availability if it was in the interest public health. Unflavored or e-cigs with limited flavor availability may still serve as a potential harm reduction tool, but given the relevance of sensory cues to females, restricting flavors may uniquely disadvantage female cigarette smokers, a population already established to have lowered cessation.[8] Importantly, this may be especially relevant for females of reproductive age who because of their potential to become pregnant and use combustible products during pregnancy, which are associated with poor fetal outcomes,[62-64] are a population whom regulatory efforts must consider. This proposal will provide critical evidence to the FDA by examining influence of e-cig flavor components among male and female smokers.

3 Rationale/Significance

3.1 Problem Statement

Although there have been many investigations of e-cig flavor use, very few explicitly focus on evaluating the sex differences in flavor use.

3.2 Purpose of Study/Potential Impact

The overall goal of this proposal is to investigate the impact of popular e-cig flavor components (i.e. those that produce cooling and sweetness) on sensory perception, appeal, and reinforcing efficacy of e-cigs by sex. This proposal will provide additional scientific data to inform regulation of tobacco products by addressing the FDA's Behavior and Addiction scientific interest areas and will provide information of a priority population; women of reproductive age. This proposal seeks to understand sex differences in the perception, appeal, and reinforcing efficacy of popular flavor constituents in e-cigs in adult cigarette smokers. This proposal will investigate differences in how flavors alter reinforcement from e-cigs among male and female smokers. Additionally, as a secondary outcome, we will examine the additive role e-cig flavors play in craving reduction. To isolate the additive effect of flavor, we will examine flavors in e-cigs with a fixed, commonly used nicotine concentration.

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 e-cig exposure at least 12 times ever OR any use within the past 3 months). 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) **Nicotine Exposure:** E-liquids will contain moderate levels of nicotine salts (36mg/ml). 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 at doses 40 to 50 times 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.

(3) Nicotine Withdrawal: Participants in both studies will be asked to engage in overnight abstinence (6-8 hours of abstinence) from nicotine and tobacco products. Symptoms include nicotine craving, mild anxiety, restlessness, irritability, difficulty concentrating, loss of energy and excessive hunger. Although uncomfortable, these are normal symptoms people experience when abstaining from tobacco products and are not regarded as harmful.

(4) Flavor Exposure: We will expose participants to three different e-liquids flavors; unflavored e-liquid, strawberry e-liquid, and menthol e-liquid. Menthol administration produces typical sensory effects in the mouth and throat. The doses of menthol that are found in cough drops, ranging 1 to 10 mg are regarded to be safe. 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 following very high doses of menthol ingestion, 200 mg or more. Menthol poisoning reported to cause ataxia, confusion, coma, nausea, and vomiting. However, these toxic effects occur at doses 20 to 30 times that which will be used in our studies. We are not aware of any effects from the other flavors being used in this study but participants will be told that if they feel any adverse events or want to stop they are free to do so. Additionally, we will exclude anyone that reports allergies to any of the flavors used (strawberry, menthol). We are not aware of any toxic effects from the sweet flavor (strawberry) being used in this study but participants will be told that if they feel any adverse events or want to stop they are free to do so. We will also monitor for adverse events.

(5) Breath, Urine Collection: Urine collection will occur at intake and during laboratory sessions and should add no risks other than the time taken to complete them.

(6) 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.

(7) 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 be required to report this information to the appropriate authorities.

(8) 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 ecigarettes 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. Participants will be informed that if they experience any symptoms (such as abdominal pain, nausea, vomiting, diarrhea, cough, shortness of breath, chest pain) or have other concerns, that they should let us know and let their doctor know right away. They will be sent to the emergency room right away if their symptoms increase rapidly. The e-cigarette lab sessions and appointments 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 smoking if they are interested. We have used similar procedures in earlier work and have not observed any incidences of adverse events. 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 cigarette smokers, and are, therefore, already self-administering higher doses of nicotine. The e-cigarettes and e-liquid pods that we will use in the current study are purchased only from a licensed retailer 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) will be stored in a locked file cabinet. 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 cigarette smokers with some e-cigarette experience
- Use well-defined inclusion/exclusion criteria
- Use study staff who have extensive training conducting tobacco research and working with adults and who are sensitive to the issues that may arise in working with combustible 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 address critical gaps in e-cigarette research by evaluating the impact of flavors on the sensory perception, appeal, and reinforcing efficacy and differences observed by sex. It will elucidate the relationship between use of e-cigarette by sex and flavor.

4 Study Objectives

4.1 Hypothesis

We aim to examine sensory perception, appeal, and reinforcing value of (a) cooling and (b) sweet flavor in nicotine containing e-cigs by sex. **We hypothesize that cooling and sweet flavor components will have the greater ratings of sensory perception/appeal/reinforcing efficacy than the unflavored e-liquid and these effects will be greater in females**

4.2 Primary Objective

The primary objective of this study is to determine if sex (female, male) influences the appeal, sensory effects, and reinforcing value of cooling and sweet flavors in nicotine containing e-cigs.

4.3 Secondary Objectives (if applicable)

The secondary objectives of this study include examining if craving reduction is influenced by sex in sweet and cooling flavor e-liquids. Another secondary objective is to determine if ad-libitum (ad-lib) puffing behavior of sweet and cooling e-cigs differs by sex.

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.

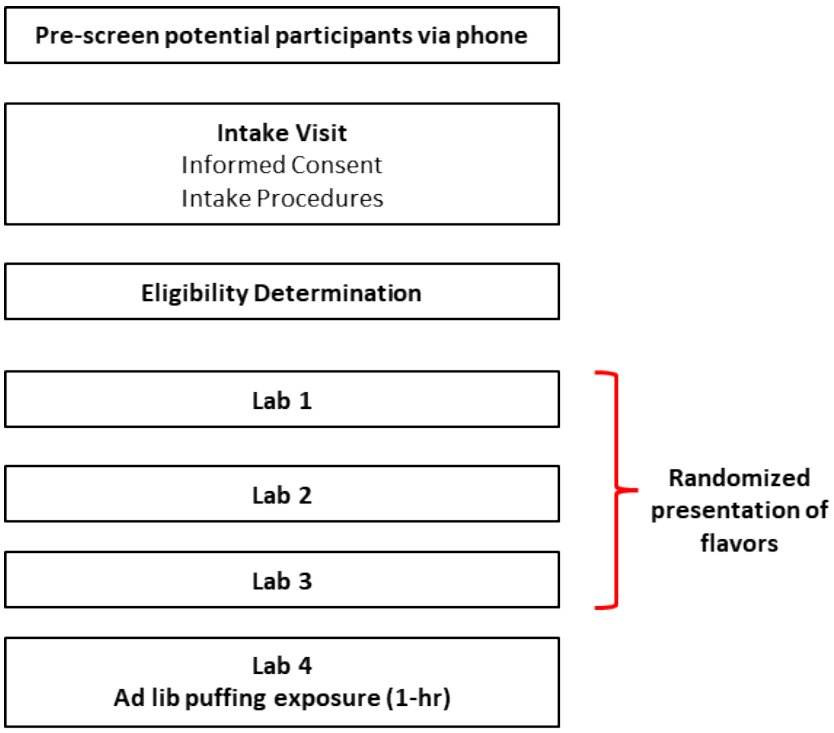
4.4 Exploratory Objectives (if applicable)

An exploratory objective of this study is to examine how female hormones (progesterone and estrogen) are associated with e-cig outcomes.

5 Study Design

5.1 General Design Description

Participants will complete 4 randomized, double blinded e-cig exposure laboratory sessions under 6-8 hour abstinence, in which they will sample an e-cig with a fixed nicotine concentration (36 mg/ml) of nicotine salt formulation in three flavors: (1) a sweet flavor, (2) a cooling flavor, and a (3) an e-cig with no added flavoring. Participants will abstain from nicotine and tobacco products 6-8 hours prior to each session. At Lab session 1, prior to data collection, participants will engage in a 15-min training session of the psychophysical rating tasks. For Lab sessions 1-3, participants will engage in one directed puffing bout (10 puffs, 3 sec puffs with 30 second interpuff intervals). Following the puffing bout, participants will complete in order measures of sensory perception including overall liking/disliking and the intensities of sensations of sweetness, bitterness, cooling, and irritation/harshness, and indicate whether irritation/harshness is felt in the mouth, throat, or both. Sensory measures will then be followed by measures of general appeal and reinforcing efficacy, and finally a post-exposure measure of craving (collected 15 min after exposure). At each lab session, we will collect baseline assessments including, urine sample (to collect nicotine and in females test for pregnancy and luteinizing hormone [LH] testing), heart rate, blood pressure, pulse oximetry, a health assessment checklist and health changes questionnaire, and for females, date of last period. Each flavor will be tested in separate sessions, under double blinded condition and presented in a randomized order. In Lab 4, participants will complete a session in which they can use any of the e-liquids they had tried in prior sessions during a 60-minute period. The 1-hr puffing session in laboratory 4 will be video recorded. The purpose of this is to assess puffing behavior. Like all electronic data in this study, videos will be stored on a secure server and only accessible to study staff. Once video data is coded by research team, it will be deleted. All flavors will be available and participants will be left alone to freely choose which they will use. Number of puffs and duration will be coded by a researcher and e-cig tanks containing e-liquids will be weighed before and after the session. 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. There are no changes to the lab procedure or puffing instructions.

5.1.1 Study Date Range and Duration

The expected duration of this study is 07/2023 – 07/2025 (2 years). The expected duration/participant is 2 weeks to complete the 4 laboratory sessions.

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 (Rm S201) for intake appointments and Yale New Haven Hospital Affiliated site West Campus Research Center (West Haven, CT) and Pierce Laboratories (290 Congress Avenue, New Haven, CT) for lab sessions, both 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). Primary outcomes will be collected at Labs 1-3 and Lab 4 will include secondary outcomes.

5.2.1 Primary Outcome Variables

Appeal: Liking/disliking of e-cig, measured by the LHS

Ratings of Sensory Perception (sweetness, harshness/irritation, cooling, bitterness): measured via gLMS

Reinforcing Value: Multiple Choice Procedure (MCP); E-cig Purchase Task

5.2.2 Secondary Outcome Variables (if applicable)

Craving Reduction: Questionnaire of Vaping Craving

Puffing Behavior: Number of ad-lib puffs, change in e-cig pod weight

5.2.3 Exploratory Outcome Variables (if applicable)

E-cig Appeal: E-cigarette Evaluation Questionnaire (not yet validated, so exploratory)

Level of Female Hormone influence of appeal: In females, examine impact of progesterone and estrogen and time of menstrual cycle on ratings.

5.3 Study Population

Participants will be females and male adult combustible tobacco product users at least 21 years of age to 44 years of age (to restrict examinations to women of reproductive age and to account for age related olfactory/taste deficits that could confound findings by sex).[65]

5.3.1 Number of Participants

We aim to recruit 69 adults to have 60 completed participants (30 female, 30 male).

5.3.2 Eligibility Criteria/Vulnerable Populations

Inclusion Criteria:

- 21-44 years old (to restrict examinations to women of reproductive age and to account for age related olfactory/taste deficits that could confound findings by sex.[65])
- Able to read and write
- Current cigarette smoking (at least 1 cigarette/day for at least 25 of the past 30 days).
- Recent e-cig exposure (12 times in lifetime or use in the past 3 months).
- Urine cotinine ≥200ng/ml
- Willing to abstain from tobacco/nicotine use 6-8 hrs prior to sessions
- Not currently quitting/trying to quit nicotine/tobacco use

Exclusion Criteria:

- Self-reported history of untreated chronic medical conditions/severe psychiatric conditions (defined as ongoing, active symptoms of a medical/psychiatric condition with no current or stable treatment; e.g. report of depression symptoms, but no treatment such as medication or behavioral treatment)
- Use of psychoactive drugs unless prescribed and stable for at least 2 months (<2 months of use)
- Current non-prescription, illicit drug use (besides cannabis; determined by urine drug screen)
- Current criteria moderate/severe alcohol or cannabis use disorder defined by DSM-5 criteria
- Current criteria for mild, moderate, or severe other SUD (besides nicotine) defined by DSM-5 criteria
- Food/chemical allergies relevant to e-cigs and selected flavors including menthol and/or strawberry product or agent
- Dislike of strawberry or menthol flavor
- If female: pregnant, trying to become pregnant, breastfeeding, or postmenopausal
- For current 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
- <20 on the Asthma Control Test (ACT)
- Report of greater than “without any difficulty” on any item of the PROMIS Physical Function Short Form
-

6 Methods

6.1 Treatment

6.1.1 Identity of Investigational Product

Products used in this study are investigational tobacco products including 3 different e-liquids and 1 e-cigarette device. 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 36 mg/ml nicotine salt (nicotine benzoate), a widely used and well tolerated nicotine salt formulation and concentration, similar in strength to a 3% JUUL product. PG/VG levels for the study e-liquids will be 50:50 PG/VG, a ratio typical of nicotine salt e-liquids.

E-liquids will be created upon ITP application approval from FDA; Dr. Krishnan-Sarin leads Yale TCORS ITP applications and will mentor 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-liquids will be created via use of three recipes provided by Dr. Tony Pace, a chemist with American E-liquids/Vapin' Direct and collaborator in research with our group with years of experience creating e-liquids for research.[35,59] E-liquid ingredients will be purchased by our group and stored in the Yale IDS Pharmacy (see 6.1.6) and created in 30mL batches as needed by Yale IDS. E-liquid ingredients (see tables below) 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, all e-liquids will have Investigational Tobacco Product (ITP) Approval prior to study start. The e-liquids being created will be produced in three flavors; (1) menthol, (2) strawberry, and (3) unflavored. E-liquid nicotine and flavor profiles will be confirmed and characterized by the Yale TCORS lab core.[66]

Table 1. Flavorless E-liquid Recipe (per 100mL of e-liquid)

Ingredient	**Quantity Needed (mL)**	**Ingredient Source**	**Ingredient Grade**	**Ingredient Cost**
Nicotine (100mg/mL in 50/50 PG/VG)	36	Nicotine River PurNic Smooth Nicotine Salt 100mg/ml	FEMA/GRAS	$194.99/gallon
PG	32	Fisher Scientific Propylene Glycol, U.S.P. – F.C.C., J.T. Baker	U.S.P	$246.00/500mL
VG	32	Glycerol, Fisher Chemical	U.S.P	$503.50/1L

Table 2. Strawberry E-liquid Recipe (per 100mL of e-liquid)

Ingredient	**Quantity Needed (for 100mL)**	**Ingredient Source**	**Ingredient Grade**	**Ingredient Cost**
Nicotine (100mg/mL in 50/50 PG/VG)	36	Nicotine River PurNic Smooth Nicotine Salt 100mg/ml	FEMA/GRAS	$194.99/gallon

PG	12	Fisher Scientific Propylene Glycol, U.S.P. – F.C.C., J.T. Baker	U.S.P	$246.00/500mL
VG	32	Glycerol, Fisher Chemical	U.S.P	$503.50/1L
Strawberry Flavor	20	LorAnn Oils Strawberry Flavor	FEMA/GRAS	$17.50/4 oz.

Table 3. Menthol E-liquid Recipe (per 100mL of e-liquid)

Ingredient	**Quantity Needed (for 100mL)**	**Ingredient Source**	**Ingredient Grade**	**Ingredient Cost**
Nicotine (100mg/mL in 50/50 PG/VG)	36	Nicotine River PurNic Smooth Nicotine Salt 100mg/ml	FEMA/GRAS	$194.99/gallon
PG	22	Fisher Scientific Propylene Glycol, U.S.P. – F.C.C., J.T. Baker	U.S.P	$246.00/500mL
VG	32	Glycerol, Fisher Chemical	U.S.P	$503.50/1L
Menthol dissolved in PG (100mg/mL)	10	L-Menthol, Sigma Aldrich	U.S.P	$81.90/100gm

6.1.2 Dosage, Administration, Schedule

E-cigarette pods will be filled with approximately 0.9mL of e-liquid, which in previous trials has produced at least 30 puffs. For Labs 1-3, participants will engage in one directed puffing bout in one of the three e-cig flavors presented in randomized order (10 puffs/3 sec puffs/30 second interpuff intervals), as used in HIC 2000023077. In Lab 4, participants will be told they can use any of the three e-liquids tried in Labs 1-3 during a 60-minute period. Participants will be left alone to freely vape. Number of puffs and duration will be coded by a researcher and 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. A blood pressure and heart monitoring form will be used to assess if blood pressure or heart rate is too high to participate in the session. 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. Danielle Davis), will consult with Yale Tobacco Center for Regulatory Science

(TCORS) co-Is, Dr. Stephanie O'Malley and Dr. Suchitra Krishnan-Sarin, 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

Dr. Ralitza Gueorguieva has developed a randomization plan that stratifies by sex and cigarette menthol status. Participants will be randomized to order of flavor.

6.1.4 Blinding and Procedures for Unblinding

E-cig flavors 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. Danielle Davis), will consult with Yale Tobacco Center for Regulatory Science (TCORS) co-Is, Dr. Stephanie O'Malley and Dr. Suchitra Krishnan-Sarin, 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.**

6.1.5 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 (see proportions and sources in recipes in Tables 1-3.) The e-liquid mixtures (propylene glycol [PG], vegetable glycerin [VG] and either strawberry, menthol, or no added flavor will consist of concentrated flavorings 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.[66] 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 expirations (whichever is sooner, per Yale IDS Pharmacy). 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 quantities of ingredients 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 at Yale IDS Pharmacy unopened until recruitment begins. 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 or West Haven Research Center, which is a secure building, where they will be delivered and stored in appropriate light/temperature conditions. E-liquids will delivered and stored in a controlled and dark laboratory environment housed in John Pierce Labs or West Haven 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 flavor order for lab sessions will be generated by study biostatisticians prior to study start. This information is stored for each participant in an individual envelope and a printed study ID on the cover. These envelopes are stored at Pierce lab along with the purchased e-liquids. The individual filling the pods will be notified of which subject ID number needs to be filled. Each 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 .9ml into the pipette from the e-juice bottle and carefully transfer it into the pod. 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 locked container until the lab is set to begin. Once the research assistant is ready to begin each lab, they will open the locked 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 lock box. None of the e-juice or pods will be transported outside of Pierce lab. The research staff will have the group randomization list and will dispense the appropriate e-liquid needed for each subject.

6.1.7 Concomitant therapy

N/A

6.1.8 Restrictions

There are no restrictions.

6.2 Assessments

6.2.1 Efficacy

Assessments Used to Assess Primary, Secondary, & Exploratory Outcomes:

- Labeled Hedonic Scale (LHS): LHS will be assessed following each puffing bout. The LHS is 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 according to their semantic magnitude. The LHS yields ratio-level data on the magnitude of liking/disliking of sensation equivalent to that produced by magnitude estimation (ME). This scale will also be displayed on a computer screen as above and numerically transformed. LHS will be used to assess change in overall appeal (primary outcome). **LHS will assess difference in liking across the 3 flavors and differences in LHS at each flavor by sex.**
- Generalized Labeled Magnitude Scale (gLMS): gLMS will be assessed following each puffing bout. Participants will rate overall intensity, sweetness, coolness, irritation, and bitterness using the gLMS which is a category ratio scale with 7 semantic labels: “no sensation”, “barely detectable”, “weak”, “moderate”, “strong”, “very strong”, and “strongest imaginable”. The labels are positioned quasi-logarithmically according to their empirically determined semantic magnitudes22. The

gLMS will be displayed on a monitor via a custom LabView® program, and participants will make their ratings using a mouse. Ratings are numerically transformed and then used for statistical analyses. **gLMS will be used to assess differences in overall sensory effects (primary outcome).**

- Multiple Choice Procedure (MCP): Following each lab session, participants will be asked to make discrete hypothetical choices between 10-puffs of the e-cig they had just used or a series of 44 monetary values ($0.25-$15.06). The minimum monetary value at which money is chosen over the e-cig puffs is a contingency-based estimate of e-cig value. **MCP will be used to assess difference in overall reinforcing value (primary outcome).**

- **E-cig Purchase Task (eCPT):** Following each lab session, participants will complete the eCPT. The eCPT examines abuse liability via purchasing behavior at a series of escalating prices. A version modified from existing literature94 and currently used in a Yale TCORS pilot will be used. **eCPT will be used to examine differences in reinforcing value across flavors/sex.**

- **Modified E-cig Evaluation Questionnaire:** We will also collect the modified E-Cigarette Evaluation Questionnaire following each lab session, a scale designed for cigarettes and adapted for e-cigs. This is an 11-item measure assessing e-cig experience on 5 subscales:[67,68] Vaping Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensations, Craving Reduction, and Aversion. This measure has not been validated in e-cigs, so it will be an exploratory outcome.

- **Questionnaire of Vaping Craving:** For e-cig craving pre and post e-cig administration, we will use the brief Questionnaire of Vaping Craving (QVC), a 10-item measure with three subscales; desire, intent, and anticipation of positive outcome.[69]

- **Ad-lib Puffing Behavior (Lab 4):** Number of puffs of each e-liquid and change in pod weight will be taken at Lab session 4 to examine relative reinforcing efficacy of products.

- **Urinary Nicotine/Cotinine (Labs 1-3): Urine samples will be collected pre and post puffing bouts to assess cotinine and nicotine levels.**

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 and a brief physical (blood pressure, spirometry, heart rate, pulse oximetry, urine drug and pregnancy test) 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. 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 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. Davis, 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 and before and after each lab session. Urine samples will be tested for pregnancy at each lab session and at intake. A urine drug screen will be collected at intake.

6.3 Study Procedures

Prescreening: We aim to recruit 69 adults to have 60 completed participants (30 female, 30 male) using online advertisements, community flyering, and referrals. Potential participants will be recruited via flyers, online recruitment (e.g. Yale Daily News, YCCI website, Craigslist), and/or materials with study information and phone number. 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, if those e-cigarettes are flavored (including flavors like tobacco), and if they are over 21. 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.

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, pulse oximetry, and carbon monoxide (CO) levels will be collected. The Structured Clinical Interview for DSM-V (SCID) will be used to assess SUDs, as used in ongoing 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. For females, we will collect reproductive history (including use of hormonal medication), and date of last menstruation.

Lab Visits: See list of assessments in Table 1. Participants will be asked to abstain from nicotine/tobacco product use overnight as determined by a 50% Carbon Monoxide (CO) reduction or a CO level ≤9ppm, both indicative of overnight abstinence. Additionally, we will collect urine pregnancy test, heart rate, blood pressure, pulse oximetry, spirometry, a health assessment checklist and health changes questionnaire, a craving measure, and for females, date of last menstruation..

At Lab session 1, participants will engage in a 15-min training session of the psychophysical tasks they will use to report sensory experience. For Labs 1-3, participants will engage in one directed puffing bout (10 puffs/3 sec puffs/30 second interpuff intervals), a procedure adapted from Dr. Krishnan-Sarin's studies. 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). Flavors will be tested in separate sessions under double blinded conditions in randomized order. In Lab 4, participants will be told they can use any of the three e-liquids

tried in Labs 1-3 during a 60-minute period. Participants will be left alone to freely vape. Number of puffs and duration will be coded by a researcher and 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. 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. There are no changes to the lab procedure or puffing instructions.

Debrief: Following study completion, participants will be thanked for their participation and ask to evaluate the three flavors they sampled in a set of qualitative questions (i.e. guess what flavors they tried, what nicotine concentration they e-liquids were) and will be given information from the CDC on cigarette smoking and e-cigarette cessation.

Table 1. List of Study Assessments

	Pre-screening	Intake	Lab 1	Lab 2	Lab 3	Lab 4
Contact Info & Eligibility Review	X					
Informed Consent		X				
Identifying Information		X				
Demographic Information		X				
Medical History/Reproductive History		X				
Timeline Follow Back		X				
Urine Drug Screen		X				
Urine Nicotine/Cotinine Sample		X	X	X	X	X
Urine Pregnancy Screen		X	X	X	X	X
Blood Pressure/Heart Rate/Pulse Oximetry		X	X	X	X	X
Spirometry		X	X	X	X	X
Carbon Monoxide		X	X	X	X	X
Tobacco Use History/Dependence Measures		X				

SCID IV: Substance Section		X				
Asthma Control Test		X				
PROMIS Dyspnea		X	X	X	X	
FACIT Cough Item		X	X	X	X	
PROMIS Fatigue		X	X	X	X	
Menstrual Phase Identification Questionnaire		X	X	X	X	X
EVALI Health Assessment		X	X	X	X	
gLMS Questionnaire			X	X	X	
LHS Questionnaire			X	X	X	
MCP Questionnaire			X	X	X	
eCPT Questionnaire			X	X	X	
Modified E-cigarette Evaluation Questionnaire			X	X	X	
Questionnaire of Vaping Craving			X	X	X	X
Number of Ad-Lib Puffs						X

6.3.1 Study Schedule

Participants will complete an intake visit (approx. 2 hrs.). Following intake and eligibility determination, participants will complete 4 lab 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 paper and consent will be administered by trained research staff.

6.3.3 Screening

We aim to recruit 69 adults to have 60 completed participants (30 female, 30 male) using online advertisements, community flyering, and referrals. Potential participants will be recruited via flyers, online recruitment (e.g. Yale Daily News, YCCI website, Craigslist), and/or materials with study information and phone number. 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, if those e-cigarettes are flavored (including flavors like tobacco), and if they are over 21. 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, have an ailment that has resolved) or have another ineligibility that may be temporary may rescreen 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.

6.3.4 Enrollment

If eligibility criteria is met, participant will be enrolled. PI (Danielle Davis) 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 given written materials on cessation. No follow up sessions will occur.

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

For primary outcomes, mixed models will include sex (female, male; between subjects factor), flavor (sweet, cooling, unflavored; within-subjects factor), and interactions. Models will also adjust for participant menthol cigarette status (menthol cigarette user; non-menthol cigarette user; between subject factor) and abstinence compliance status (between subjects factor).

6.4.2 Sample Size Considerations

Analyses are powered for to detect large effect sizes for differences by sex. For this study, we have more than 80% power to detect large differences between males and females (Cohen's d=0.8) at two-sided alpha level of 0.05 with 60 individuals total (30 males and 30 females). We anticipate 15% attrition based on current investigations in our group with cigarette smokers in e-cigarette lab exposure paradigms, so we will enroll 69 participants.

6.5 Planned Analyses

6.5.1 Primary Objective Analysis

For primary outcomes, mixed models will include sex (female, male; between subjects factor), flavor (sweet, cooling, unflavored; within-subjects factor), and interactions for each primary outcome measure. Models will also adjust for participant menthol cigarette status (menthol cigarette user; non-menthol cigarette user; between subject factor) and abstinence compliance status (between subjects factor). A significant main effect of condition will be considered supportive of our hypothesis. We will construct confidence intervals for least square means and contrasts between least square means to estimate effect sizes and to inform future studies.

6.5.2 Secondary Objectives Analyses

For the secondary objectives, craving reduction (change from baseline to after flavor administration) and difference in puff taking in Lab 4, we will also use mixed models will include sex (female, male; between subjects factor), flavor (sweet, cooling, unflavored; within-subjects factor), and interactions.

6.5.3 Exploratory Objectives Analyses (if applicable)

For hormonal evaluations, we will assess primary outcome measures (appeal, sensory effects) by hormone level as a continuous variable.

For modified E-cig Evaluation Questionnaire, we use mixed models will include sex (female, male; between subjects factor), flavor (sweet, cooling, unflavored; within-subjects factor), and interactions.

6.5.4 Safety

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

6.5.5 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.

6.5.6 Interim Analysis (if applicable)

N/A

6.5.7 Health economic evaluation

N/A

6.5.8 Other

N/A

6.5.9 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.

6.5.10 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 flavors administered or the nicotine concentration of the product. They will be told that products containing moderate 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.

Participants will be compensated $50 for completing both parts of intake. This is commensurate to other e-cigarette studies in our labs. Participants will be compensated $75 for Labs 1-3 (approximately 1 hour in length). Participants will be compensated $100 at Lab 4 as it is slightly longer. All completers will receive a $50 end of study bonus. Additionally, participants are asked to abstain from tobacco use overnight, so this compensation reflect that burden. If participant is unable to provide their own travel, rideshare travel (i.e. Uber) can be provided up to 25 miles away (i.e. an estimated $30/trip). Given the short duration of the study and participant preference in previous vaping studies, all compensation will be provided in cash.

The only potential unknown condition identified by the study team that could be discovered as a result of study procedures would be EVALI-related symptoms, due to increased health monitoring.. . During the study, they will be monitored for EVALI related symptoms. Any endorsement of EVALI symptoms that does not have a clear related and proximal cause will result in withdrawal and participants will be referred to a primary care provider.

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 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 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 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. Davis 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. 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 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.

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 consider 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 60 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 be have any role in study design, conduct, analysis, or publication.

7.17 Funding Source

The study is funded through the Center for Tobacco Products as part of a K01.

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

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