CLINICAL STUDY PROTOCOL

Interventional Drug or Biologic

Examining the Effect of Varying Water Content of E-liquids on Sensory Experience

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Synopsis

Primary Objective

The primary objective of this study is to examine whether (a) sensory irritation and (b) appeal are decreased in e-liquids containing flavor aldehydes (i.e. vanilla, cherry, cinnamon) compared to e-liquids not containing flavor aldehydes (i.e. grape) when the water concentration is increased from the baseline water concentration to the following levels; 5% total water volume, 10% total water volume, 20% total water volume.

Secondary Objective (if applicable)

At each water concentration, we will examine if there are differences in perception of sensory irritation and appeal across the distinct aldehyde containing flavors.

Study Duration

2 years to complete study from HIC submission; we anticipate the subject enrollment period will be 8 months (approximately 1 week/participant). Enrollment began in August 2022, so anticipated subject enrollment will end in March 2023.

Study Design

Within subjects' human laboratory experiment

Number of Study Sites

1; Yale School of Medicine. Yale sites include Connecticut Mental Health Center (intake visits) and John B. Pierce Laboratory (lab sessions).

Study Population

Population is regular e-cigarette users who report use of both nicotine and flavors in their e-cigarettes. We are assessing response to alterations of water level in e-cigarettes among regular users as a potential regulatory tool, so current participants must have regular nicotine experience.

Number of Participants

30; pilot study

Primary Outcome Variables

(1) Change in irritation at each water level as measured by the Generalized Labeled Magnitude Scale (gLMS)

(2) Change in appeal at each water level as measured by the Labeled Hedonic Scale (LHS), Drug Effects Questionnaire (DEQ)

Secondary and Exploratory Outcome Variables (if applicable)

(1) At each water level, differences in **irritation (gLMS)** and **appeal (LHS, DEQ)** between flavors (to examine if there are differences in effect of water level on irritation/appeal by aldehyde flavor)

Abbreviations

Abbreviation	Explanation
LHS	**Labeled Hedonic Scale**
gLMS	**Generalized Labeled Magnitude Scale**
TCORS	**Tobacco Centers for Regulatory Science**
e-cig	**E-cigarette**

Glossary of Terms

Glossary	Explanation

APPROVED BY THE YALE UNIVERSITY IRB 11/15/2022

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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 background (2.2) for all relevant information.

2.1.2 Clinical Experience

See background (2.2) for all relevant information.

2.2 Background/prevalence of research topic

Most US tobacco products contain a wide variety of flavorants, with an estimated 15000+ flavors in e-cigarettes (e-cigs) alone.[1] Flavors may enhance the appeal of tobacco products by their positive attributes (e.g. aroma/taste of sweet flavors) and/or by their ability to ameliorate nicotine/tobacco harshness (e.g. cool flavors)[1-4].

While the Family Tobacco Prevention and Tobacco Control Act (FSPTCA) banned the sale of cigarettes with added artificial and natural flavors in 2009, flavors are currently produced and available in electronic cigarette products. Even new FSPTCA regulations (introduced in May 2016) do not include any of the other assortments of flavors or sweeteners in any of the other tobacco products (e.g., e-cigs). One potential reason for the limited action on flavors is the limited scientific evidence on the influence on flavors and sweeteners on nicotine's abuse potential. To support the regulation of tobacco products, the FDA requires rigorous scientific information on which to base their rulings and support these rulings in legal challenges. These regulatory decisions are further complicated by the multitude of flavors available in tobacco products and the constant innovation by the tobacco industry to use flavorant chemicals to enhance and maintain appeal for these products.

As flavors continue to be available in these products and used by e-cigarette users, one aim of researchers is to develop ways to reduce potential toxicity of these products. Flavor chemicals in e-liquids may expose users to respiratory harms.[2,3] While many flavor chemicals used in e-liquids are designated as GRAS (generally recognized as safe), this designation applies to oral ingestion, not inhalation.[4] Levels of flavor chemicals in e-cigarettes vary widely, with flavors accounting for as much as 34% of the e-liquid.[5] Currently, there are no regulations to mitigate risk associated with flavor chemicals in e-cigarettes.

E-cigarettes contain many different ingredients to produce flavors. A common class of e-liquid flavor chemicals are called aldehydes. Aldehyde flavor constituents are used in many popular flavors.[5,6] For example vanillin produces a vanilla flavor and is added to many sweet and dessert e-liquids, benzaldehyde produces the popular fruit cherry flavor and is used in many fruit/sweet e-liquids, and cinnamaldehyde produces a cinnamon flavor, which is added to dessert flavored e-liquids, spice flavored e-liquids, and fruit e-liquids (e.g. cinnamon apple).

Aldehydes are sensory and respiratory irritants and activate the sensory receptor TRPA1, an ion channel known to contribute to inflammation and cytotoxicity in airway tissue.[7,8] Aldehydes can undergo chemical reactions with the solvents that form the base on e-liquids; propylene glycol (PG) and vegetable glycerin (VG) to form chemical adducts named *flavor*

aldehyde PG/VG acetals (referred to as acetals henceforth). Recent studies have shown that acetals form under typical storage conditions[9], are present in several commercial e-liquids including popular JUUL products,[3,9-11] and can efficiently transfer to e-cigarette aerosols.[9] Acetals have been shown to cause **both** greater sensory irritation and cytotoxicity at the sensory receptor TRPA1 than their parent aldehydes in-vitro, suggesting that this chemical conversion can have negative health implications for users.[3,9] **One way to measure user experience of irritant effects would be to have users' rate associated sensory irritation experienced.**

Preliminary (unpublished) evidence from our chemistry lab at the Yale Tobacco Center of Regulatory Science (TCORS) have indicated that it may be possible to reduce acetal formation simply by adding water to e-liquids. This reduces acetal formation by shifting the chemical equilibrium of the formation reaction. Water is physiologically innocuous to inhale via e-cigarette and adding water to e-liquids may be an elegant way to reduce the sensory irritation and cytotoxicity of e-liquids that contain flavor aldehydes. However, adding water also inherently dilutes the flavor and nicotine concentration of the product, which may reduce the appeal overall. This could be a negative outcome if trying to use flavors as a tool to shift combustible cigarette users to potential reduced harm e-cigarettes. However, if irritation is reduced and appeal is not significantly reduced, a regulation requiring a fixed level of water to e-liquids could be a simple and effective tool to reduce potential toxicity in e-liquids with these flavors. *The goal of the proposed study is therefore to determine if the sensory irritation (a marker of potential toxicity) of commercial e-liquids, can be reduced by increasing their water content without adversely affecting flavor appeal in adult e-cigarette users*.

3 Rationale/Significance

3.1 Problem Statement

Electronic cigarettes (e-cigarettes), once considered an emerging tobacco product, are now the most commonly used tobacco product among youth and growing in popularity among adults.[12,13] Some data suggests that shifting combustible cigarette users to e-cigarette use may have a net positive population benefit.[14] An important facet of e-cigarette appeal is the availability of flavors,[15,16] including non-tobacco flavors like fruit, dessert, candy, and mint.[17] Non-tobacco flavors may be important for encouraging product switching among combustible tobacco users.[18,19] However, flavor chemicals in e-liquids may expose users to respiratory harms.[2,3] While many flavor chemicals used in e-liquids are designated as GRAS (generally recognized as safe), this designation applies to oral ingestion, not inhalation.[4] Levels of flavor chemicals in e-cigarettes vary widely, with flavors accounting for as much as 34% of the e-liquid.[5] Currently, there are no regulations to mitigate risk associated with flavor chemicals in e-cigarettes.

A common class of e-liquid flavor chemicals are aldehydes (e.g. vanillin producing a vanilla flavor; benzaldehyde producing cherry flavor; cinnamaldehyde producing cinnamon flavor).[5,6] Aldehydes are sensory and respiratory irritants and activate the sensory receptor TRPA1, an ion channel known to contribute to inflammation and cytotoxicity in airway tissue.[7,8] Aldehydes can undergo chemical reactions with the e-liquid solvents propylene glycol (PG) and vegetable glycerin (VG) to form chemical adducts named *flavor aldehyde PG/VG acetals* (referred to as acetals henceforth). Recent studies have shown that acetals form under typical storage conditions[9], are present in several commercial e-liquids including popular JUUL products[3,9-11] and can efficiently transfer to e-cigarette aerosols.[9] Acetals have been shown to cause **both** greater sensory irritation and cytotoxicity at the sensory receptor TRPA1 than their parent aldehydes in-vitro, suggesting that this chemical conversion can have negative health implications for users.[3,9]

This study is based on preliminary evidence from our lab that adding water to e-liquids containing flavor aldehydes reduces acetal formation by shifting the chemical equilibrium of the formation reaction. Reducing acetal formation through the addition of physiologically innocuous water may be an elegant way to reduce the sensory irritation and cytotoxicity of e-liquids that contain flavor aldehydes. However, it is possible that adding water might negatively impact the potential value of these flavors for switching from combustible cigarettes by diluting flavorant and nicotine concentrations", potentially resulting in lower delivery per puff. *The goal of the proposed study is therefore to determine if the sensory irritation (a marker of potential toxicity) of commercial e-liquids, can be reduced by increasing their water content without adversely affecting flavor appeal in adult e-cigarette users*.

3.2 Purpose of Study/Potential Impact

The goal of the proposed study is to determine if the sensory irritation (a marker of potential toxicity) of commercial e-liquids containing aldehydes compared to a control non-aldehyde

containing e-liquid, can be reduced by increasing their water content without adversely affecting flavor appeal in adult e-cigarette users. Given that adding water to e-liquid is an innocuous manipulation, if irritation is reduced and effect on appeal is limited, this could be a tool for regulation to reduce harm associated with e-cigarettes.

3.2.1 Potential Risks

Participation in this study is thought to involve a minor increase over minimal risk. Potential risks and protections against risks are detailed below.

(1) E-cigarette Exposure: All participants will be required to have current regular experience with nicotine containing flavored e-cigarettes. Given this, the likelihood of adverse experience with study e-cigarettes/e-liquids is low. Additionally, we will only expose participants to two puffs per flavor x water level manipulation for a total of 32 puffs (16 puffs/lab session) across the study. 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. Additionally, we will only allow participants with experience with flavored e-liquids. We will confirm at intake they do not have known allergies to either of these products or e-liquid flavors.

(2) E-Liquid Water Manipulation: Some of the e-liquids participants will use will have water added to liquid at levels, so total water level of the e-liquid is 5% total volume, 10% of total volume, 20% total volume. Unmanipulated e-liquids contain some level of water. There is no evidence that the addition of water will produce any additional harm to e-cigarette users or that e-cigarette device will heat water to a level that produces harm.[20] Evidence suggests that addition of water may reduce irritation associated with e-cigarette use.

(3) Nicotine Exposure: E-liquids will contain moderate levels of nicotine salts (36mg/ml). Common side effects of nicotine include 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.

(4) Flavor Exposure: We will expose participants to four different e-liquids flavors; cinnamon, cherry, vanilla, and grape e-liquid. All e-liquids are commercially available currently, except for the cinnamon e-liquid, which is currently only available for research use. This cinnamon e-liquid was developed to mirror commercially available cinnamon products. Cinnamon flavoring is known to have health impacts at levels much higher than participants will be exposed to in this study.[21] 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 four flavors used. In the consent form, we will not state the flavors being used, but will note that one flavor is not currently commercially available.

(5) Urine/Saliva Collection: Urine/saliva collection at intake and during the study for females and should add no risks other than those normally associated with these procedures.

(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.

Protections Against Risk: We will reduce risks by:

- Requiring participants to be regular nicotine & flavor e-cigarette users.
- Administering briefest possible exposure needed for our outcomes to each study product (2 e-cigarette puffs/product)
- Using well-defined inclusion/exclusion criteria to rule out participants with pre-existing medical conditions
- Providing participants with written information on reducing e-cigarette use at the end of study participation.
- Using study staff who have expertise conducting tobacco research and working with adults and who are sensitive to the issues that may arise in working with e-cigarette 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.

- Use the Yale TCORS Independent Data Safety Monitoring Board including experts in the field of tobacco use behaviors and challenge studies (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 statistical expert (Dr. Hanga Galfalvy, Assistant Professor of Neurobiology, Columbia University) .

3.2.2 Potential Benefits

The proposed project will provide the FDA with important data regarding how adding an innocuous constituent, water, to e-cigarettes that contain flavors chemicals comprised of aldehydes, could have the ability to reduce user' irritation. E-liquids with flavor chemicals containing aldehydes are popular and common but may have potential associated toxicity that produces user experienced irritation. Preliminary data from our chemistry lab suggests adding water may be one way to reduce potential toxicity. Regulating the level of water in e-liquids is a relatively simple regulation for the FDA to implement. However, it is possible that adding water might negatively impact the potential value of these flavors for switching from combustible cigarettes by diluting flavorants and nicotine concentrations, potentially resulting in lower delivery (and thus appeal) per puff. Study outcomes could inform potential future legislation aiming at addressing certain health concerns of vaping products, such as a mandate for a minimum amount of water present in e-liquids.

An additional benefit in this study is to participants who are current THC/CBD vapers. They may be at additional risk for EVALI given their THC/CBD vaping, but are not currently being monitored. During the study, they will be monitored for EVALI related symptoms.

4 Study Objectives

4.1 Hypothesis

We hypothesized that as total water volume is increased in nicotine e-liquids containing flavor aldehydes, sensory irritation experienced by the e-cigarette user will decrease. We do not expect to see this decrease in the control e-liquid (grape e-liquid), which does not contain flavor aldehydes.

4.2 Primary Objective

The primary objective of this study is to examine whether (a) sensory irritation and (b) appeal are decreased in e-liquids containing flavor aldehydes (i.e. vanilla, cherry, cinnamon) compared to e-liquids not containing flavor aldehydes (i.e. grape) when the water concentration is increased from the baseline water concentration to the following levels; 5% total water volume, 10% total water volume, 20% total water volume.

4.3 Secondary Objectives (if applicable)

At each water concentration, we will examine if there are differences in perception of sensory irritation and appeal across the distinct aldehyde containing flavors.

4.4 Exploratory Objectives (if applicable)

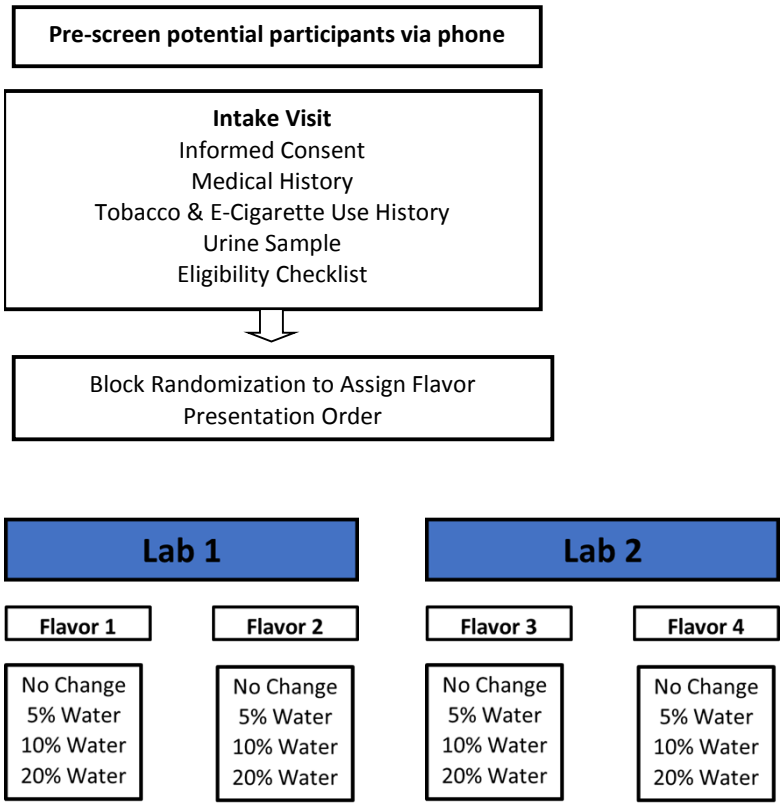
N/A

5 Study Design

5.1 General Design Description

The study is a within subjects' laboratory study, in which participants will complete two laboratory sessions under double blind conditions. Across the study, participants will be exposed to four different e-liquid flavors at a single fixed nicotine concentration (36mg/ml nicotine salt formulation). Three of the e-liquids flavors will be aldehyde containing e-liquids and one flavor will be a non-aldehyde containing flavor and serve as a control. Each flavor will be presented with the follow manipulations to its water concentration; (1) flavor as commercially produced, (2) water added so 5% total water volume achieved, (3) water added so 10% total water volume achieved, (4) water added so 20% total water volume achieved. In each lab session, under double blind conditions, participants will sample two of the four flavors at each of the 4 water volumes (i.e. no change to water volume, 5% water volume, 10% water volume, 20% water volume). Participants will be randomized to flavor order and water concentration for each flavor will be presented from hypothesized least irritating to hypothesized most irritating, as is recommended with chemosensory studies.

Figure 1. Diagram of Study Procedures



5.1.1 Study Date Range and Duration

The expected duration of the total study from recruitment 2 years to complete study from HIC submission; we anticipate the subject enrollment period will be 8 months (approximately 1 week/participant). Enrollment began in August 2022, so anticipated subject enrollment will end in March 2023. The expected duration/participant is approximately 1-2 weeks to complete intake appointment(s), lab 1 and lab 2.

5.1.2 Number of Study Sites

The study will take place at Yale University School of Medicine. Study sites will include the Connecticut Mental Health Center (Rm S201) for intake appointments and Pierce Laboratories (290 Congress Avenue, New Haven, CT), which are equipped with ventilated research chambers utilized for smoking and vaping research studies.

5.2 Outcome Variables

The outcome variables used in this study are **irritation**, as measured by the generalized Labeled Magnitude Scale (gLMS)[22,23] and **appeal**, as measured by the Revised Drug Effects Questionnaire[24] (measuring liking & stimulation), the Labeled Hedonic Scale (LHS) to measure overall liking, the gLMS to measure sweetness, and the Multiple Choice Questionnaire (MCQ; e-cig value).[25]

5.2.1 Primary Outcome Variables

(3) Change in irritation at each water level

(4) Change in appeal at each water level

5.2.2 Secondary Outcome Variables (if applicable)

(2) At each water level, differences in **irritation** and **appeal** between flavors (to examine if there are differences in effect of water level on irritation/appeal by aldehyde flavor)

5.2.3 Exploratory Outcome Variables (if applicable)

N/A

5.3 Study Population

Participant will be healthy adult (21 or older) regular (at least 4x/week) e-cigarette users who are users of nicotine containing e-cigarettes (urine cotinine >200 ng/ml).

5.3.1 Number of Participants

The current study is a pilot study and we aim to enroll 30 subjects. Based on previous pilot experimental work with e-cigarettes in our group, 30 has been sufficient for by our biostatistical staff for determining large effects by water level.

5.3.2 Eligibility Criteria/Vulnerable Populations

In order to eligible to participate in the study, individuals must meet all of the follow criteria:

- At least 21 years old (legal e-cigarette use age)
- Able to read and write
- Report at least 6 months of e-cigarette use
- Report e-cigarette use at least on average 4 days/week in the past month
- Report use of flavored e-cigarettes (i.e. cannot be using unflavored e-cigarettes)
- Report use of nicotine containing e-cigarettes
- Urine cotinine levels of at least 200ng/mL
- Do not plan to discontinue e-cigarette use or other current tobacco product use (e.g. cigarettes) during study duration
- Spirometer reading with an FEV1 of ≧70%.

An individual who meets any of the following criteria will be excluded from study participation:

- Currently pregnant, breastfeeding, or trying to become pregnant
- Medical conditions including chronic and untreated acute pulmonary conditions, that in the investigators view will increase risk of respiratory problems among participants
- Known hypersensitivity to propylene glycol, vegetable glycerin, and/or e-liquid flavorants
- Current use of illegal substances, not including cannabis, as determined by both self-report and a urine drug screen at intake.
- *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
- Report of greater than “without any difficulty” on any item of the PROMIS Physical Function Short Form
- Any significant current medical condition such as neurological, cardiovascular, endocrine, renal, or hepatic pathology that would increase risk or would interfere with/mimic tobacco abstinence.
    - Medical conditions increasing risk of respiratory problems in nonpatient populations: Chronic Pulmonary Conditions;
    - Untreated, unresolved, acute pulmonary conditions (recurring bronchitis and Reactive airway disorder, as examples).
- Current diagnosis of an untreated psychiatric condition
- Not fully vaccinated for COVID-19

6 Methods

6.1 Treatment

6.1.1 Identity of Investigational Product

The investigational tobacco products being used in the study are the following e-liquids in 36mg nicotine salt concentration: (1) DuraSmoke Red Label (50/50 propylene glycol/vegetable glycerin base) Cherry flavor liquid, (2) DuraSmoke Red Label ((50/50 propylene glycol/vegetable glycerin base) Vanilla flavor liquid, (3) DuraSmoke Red Label ((50/50 propylene glycol/vegetable glycerin base) Grape flavor liquid, and (4) American E-liquids Store (50/50 propylene glycol/vegetable glycerin base) Cinnamon Flavor Liquid. All of these products will be administered in a Suorin iShare Pod System E-cigarette. All of these products are currently commercially available, except American E-liquids Store (50/50 base) Cinnamon Flavor Liquid, which is currently only available as a research product. However, this flavor was a previously commercially available e-liquid and was developed the formulation has not changed. It is not currently sold by American E-Liquids due to lack of popularity. In the current study, these e-liquids will be manipulated by adding water to each product, so that water concentration in each of the four flavors equals the following amounts; (1) 5% of the total e-liquid concentration, (2) 10% of the total e-liquid concentration, (3) 20% of the total e-liquid concentration. Additionally, e-liquids will be presented without added water. Participants will sample (i.e. take 2 directed puffs of each) the four flavors unaltered and in each of the listed water dilutions in randomized order. FDA has reviewed the proposed use of the products and does not have concerns with the use of the investigational tobacco products as described in the protocol. FDA issued a No Concerns letter to Investigators on 2/3/22.

6.1.2 Dosage, Administration, Schedule

6.1.3 Water Concentration Manipulation:

E-liquid water content of each e-liquid container will be quantified by Karl Fischer (KF)-titration or by gas chromatography/thermal conductivity detection (GC-TCD). Once the water content of the unaltered product has been determined, the difference to reach 5%, 10%, or 20% water content will be added to the e-liquid in question. For example, if the water content was measured at 1%, 4% will be added (all percent values are weight-based). To do so, weighed amounts of e-liquids will be added to sterile, amber glass bottles (30mL), and the appropriate amount of sterile, deionized water will be added to reach the desired water content level (5%, 10%, 20%). This preparation will take place at the Center for Green Chemistry and Green Engineering (Co-PI Hanno Erythropel's lab location). The resulting 4 bottles per flavor (native water content, 5%, 10%, 20%) will then be transferred to the testing location and stored in a cool and dry location until use. For subject testing, the needed quantity of approx. 0.5mL of e-liquid will be pipetted from the storage container to the e-cigarette device for testing. 30mL of each e-liquid manipulation is sufficient to conduct procedures with all 30 anticipated pilot participants.

E-liquid Administration/Participant Instructions/Pausing due to AEs: Participants will be administered e-liquid in an observed laboratory setting in which they are instructed to take two directed puffs of each e-liquid at each water concentration. Between 2-puff puff bouts, participants will be required to wait 10-minutes before engaging in next puff bout. Participants are not allowed to leave the site with product. There will no changes in nicotine concentration (only flavor administered) and all participants will sample all flavor/water concentration manipulations. The lab sessions will be conducted by trained research staff who are sensitive to the adolescent and smoking 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. 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; the funder of

this pilot) 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.

6.1.4 Method of Assignment/Randomization

All participants will receive every flavor/water concentration (4 e-liquids flavors at 4 water concentrations = 16 total manipulations). Participants and research assistants will be blinded to flavors administered. We will use block randomization to determine order flavors are presented in. Water concentration will be presented from hypothesized least irritating to most irritating (20% water, 10% water, 5% water, no alteration).

6.1.5 Blinding and Procedures for Unblinding

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. Pods will be numbered from 1-4 for each participant corresponding to the participant. Water concentration (which is not blinded for researchers) will be labeled A, B, C, D, with A representing "No water concentration change", B representing "5% water volume", C representing "10% water volume", and D representing "20% water volume". Participants will be blinded to water concentration. In the event of a serious adverse event, the PI, will consult with Yale Tobacco Center for Regulatory Science (TCORS; the funder of this pilot) 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.

6.1.6 Packaging/Labelling

We obtain all of our e-liquids from American e-Liquid Store, which manufactures all its products in Wauwatosa, WI and reports being the first e-liquid manufacturer in the US to obtain the International Organization for Standardization (ISO) 9001:2008 and Current Good Manufacturing practices (cGMP) certification. The e-liquid mixtures (propylene glycol [PG], vegetable glycerin [VG] and either cinnamon, grape, vanilla, or cherry flavorings 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.

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.

6.1.7 Storage Conditions

In order to ensure that we have adequate amount of e-liquid, of the same constituency, we will purchase large quantities of these solutions prior to starting these experiments which will be stored at and dispensed at John Pierce Labs. Pods will be filled with e-liquid mixtures at John Pierce Labs.

After the e-liquids are received, they will be delivered and stored in a controlled and dark laboratory environment housed in the analytical core of the Yale TCORS (PI: Zimmerman), which is a secured building. The liquids will be kept in a locked storage container in a drawer within the lab room before manipulation. Dr. Erythropel, chemist on the analytical core of the TCORS, and co-PI of this pilot (on a non-human project arm not detailed in the current protocol), will perform water manipulations (see above 6.1.2) to e-liquid containers. Once complete, e-liquids will be immediately transported to Pierce Labs, which is a secure building, where they will be delivered and stored in appropriate light/temperature conditions.

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 and they will then go to Pierce labs and open the envelope and fill the sixteen pods with the appropriate e-liquids. First, they will label each pod with the study ID number and 1A, 1B, 1C, 1D, 2A, 2B, 2C, 2D if Lab 1 or if 3A, 3B, 3C, 3D, 4A, 4B, 4C, 4D if Lab 2. 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.

6.1.8 Concomitant therapy

N/A

6.1.9 Restrictions

There are no restrictions.

6.2 Assessments

6.2.1 Efficacy

To evaluate the efficacy of manipulating water content on irritation reduction and its potential impact on appeal, we will use the following assessments:

1. **General Labeled Magnitude Scale (gLMS):** gLMS ratings will be obtain following each 2-puff puff bout. Participants will rate overall intensity, sweetness, coolness, irritation 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 magnitudes[22]. 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 irritation will be used to assessed change in irritation (primary outcome), and intensity, sweetness, and coolness will be used to assess change in overall appeal (primary outcome).**
2. **Labeled Hedonic Scale (LHS)[23]** : Liking will be assessed after following each 2-puff puff bout using the LHS scale. 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).**
3. **E-cig Effects (Adapted from Drug Effects Questionnaire (DEQ[26]):** A modified version of the Drug Effects Questionnaire [24,27] will be used in which participants will rate

acute responses to the e-cig on a 0 to 100 mm scale, from “not at all” to “extremely.” Following each 10-puff bout, we will assess E-cig Liking/Wanting (average of “I feel good e-cig effects”, “I want more of that e-cig I received”, “I feel the e-cig strength” and “I like the e-cig effect”). At baseline and following each fixed-dose bout, we will also assess 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”). **E-cig Effects will be used to assess change in overall appeal (primary outcome).**

4. **Multiple Choice Questionnaire to assess e-cig value (Adapted from Multiple Choice Procedure (MCP)[25,26]**: At the end of each 2-puff puff bout, 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. At the end of each lab session, participants will be given a choice between another 10 e-cig puffs or one of the monetary amounts they picked in the MCPs completed earlier. **MCP will be used to assess change in overall appeal (primary outcome).**

6.2.2 Safety and Pregnancy-related policy

Women who report pregnancy, trying to become or breastfeeding will be excluded from the study. All participants will be informed if pregnancy status changes to inform staff. Participants will be given a urine pregnancy test at every visit.

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 minor increase over 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 collection will be done at study intake to assess cotinine levels, illicit drug use, and pregnancy status. Urine collection will be done at Lab 1 and Lab 2 for females to determine pregnancy status. Saliva collection will be done at intake to assess nicotine/cotinine levels.

6.3 Study Procedures

Prescreening

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 be asked to complete intake in two parts; the first remotely (via Zoom) and an in person session to collect biochemical specimens. If participants do not have the necessary technology to complete the first part of the intake remotely, the full intake will be done in person. All intakes will conducted at CMHC (34 Park Street). At intake, participants will be consented via Redcap consent. Informed consent will be obtained while participant is in Zoom or in person. If on Zoom, participant will be instructed to be in a private setting away from others. Research staff will administer consent from a private room. If in person, consent will be obtained in a private room. Participants will have as much time as needed to consider consent.

If participant provides consent, they will provide identifying information (to be kept separately from deidentified study materials in a locked cabinet). At intake participants will complete the following assessments:

- **30-day Timeline Follow Back** assessing e-cigarette use, other tobacco product use, marijuana use, and alcohol use,
- **E-cigarette/Tobacco Use History** assessing e-cigarette use history and tobacco use history, products used, flavors tried
- **Demographic Questionnaire**
- **E-Cigarette Dependence Scale (EDS)**[28]
- **Medical History** assessing psychiatric and medical conditions
- **Asthma Control Test,**[29] participants will be asked to rate their asthma symptoms on a 1-5 scale, from “all of the time” to “none of the time”. This five-question health survey is used to measure asthma control in individuals 12 years and older.
- **FACIT cough questionnaire,** a single item assessing cough severity
- **PROMIS Fatigue,**[30] we will use the 7-item PROMIS measure of fatigue which rates fatigue from 1=” never” to 5=” always”.
- **PROMIS Dyspnea,**[31] We will use the 5-item PROMIS measure of dyspnea which rates shortness of breath from 1-10. Items include: shortness of breath in general, intensity of shortness of breath, frequency of shortness of breath and duration of shortness of breath.
- **EVALI Health Assessment checklist,** a checklist of health symptoms and severity of these symptoms that could be related to nicotine exposure/vaping illness

Additionally, at the in-person portion of the intake, we will collect a urine sample and test cotinine level (a byproduct of nicotine) using a dichotomous dipstick (Countrywide Testing, 1 panel quickscreen cotinine/nicotine test dipcard – (COT200) DCT-114), illicit drug use, and if female, pregnancy. We will do a brief physical and collect blood pressure, spirometry, pulse oximetry, heart rate, and breath carbon monoxide (CO)

Lab Visits

See list of assessments in Table 1. Participants will be asked to abstain from e-cigarette use and other tobacco product use two hours prior to the session, which will be assessed via self-report. At session arrival, participants will complete baseline questionnaires (see Visit Schedule below), heart rate, blood pressure, spirometry, and pulse oximetry. After

completion of baseline assessments, at Lab 1, participants will be familiarized with the sensory and hedonic scales and trained in how to puff the e-cigarette (using non-nicotine and non-flavored e-liquid). Participants will then begin testing procedures for their assigned Flavor 1, which will be administered under double-blinded randomized conditions (see above). For each flavor, participants will take two puffs of the flavor when diluted by four levels of water concentration. Participants will be instructed to take two 3-second puffs with a 30 second inter puff interval. Each two-puff bout will be separated by 10-minutes. These procedures are adapted from our recent work (HIC #2000023077), with the number of puffs is reduced as we are not examining the effects of nicotine and only sensory effects of flavor. Per our current practice we will record timed instructions for each puff bout and participants will be trained to follow these directions. Following each puff exposure participants will complete the General Labeled Magnitude Scale (gLMS) for measures of overall intensity, irritation, coolness and sweetness, the Labeled Hedonic Scale (LHS) for measures of liking, the E-Cig Effects Questionnaire for measures of e-cigarette drug effects, and the Multiple-Choice Questionnaire to assess e-cigarette value. Participants will complete the above procedures for two flavors and four water concentrations for each flavor during each lab session. For each flavor, water concentration order will not be randomized and will be presented from hypothesize least irritating (20% water concentration) to hypothesized least irritation (e-liquid as commercially available with no water dilution) to a) maintain consistency of presentation of water concentration across flavors and b) to avoid potential irritation effects from hypothesized more irritating products to less irritating products. Following at least 24 hours, participants will repeat procedures with the remaining two flavors. Each lab session will last approximately two hours.

Debrief

At the end of Lab 2, participants will be thanked for their participation and ask to evaluate the four flavors they sampled (i.e. guess what flavors they tried, what nicotine concentration they e-liquids were) and will be given information from the CDC on e-cigarette cessation.

Table 1. List of Study Assessments

	Pre-screening (Pre-consent)	Intake	Lab 1 Baseline Assessments	Lab 1Assessments Following each E-Liquid Administration	Lab 2 Baseline Assessments	Lab 2 Assessments Following each E-Liquid Administration
Contact Info & Eligibility Review	X					
Informed Consent		X				
Identifying Information		X				
Demographics		X				
30-day Timeline Follow-back		X				
Tobacco & E-Cigarette Use History		X				
Medical History		X				
E-Cigarette Dependence Scale		X				
Cotinine & Urine Drug Screen		X				
Asthma Control Test		X	X		X	
PROMIS Fatigue		X	X		X	
PROMIS Dyspnea		X	X		X	
FACIT Cough Question		X	X		X	

EVALI Health Assessment		X	X		X	
General Labeled Magnitude Scale (gLMS)				X		X
Labeled Hedonic Scale (LHS)				X		X
E-Cigarette Drug Effects				X		X
Multiple Choice Questionnaire				X		X
Heart Rate, Blood Pressure, Pulse Oximetry		X	X		X	
Pregnancy Test		X	X		X	
Debrief						X

6.3.1 Study Schedule

Participants will participate in an intake visit (that will be completed in two parts; remote and in-person as possible). Following intake, they will complete two lab sessions approximately 2 hours each that occur 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

Prescreening

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. Potential participants will be directed to a voluntary link and/or phone number. If interested, participants will be asked if they regularly use nicotine containing e-cigarettes, if those 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.

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 for detailed description of on study (Lab 1 & 2) procedures and assessments administered in Table 1.

6.3.6 End of Study and Follow-up

After study completion, participants will be given written material for cessation. Given low study risk and brief e-cigarette administrations, 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

To examine the primary objective of the study, which is to examine how increasing the concentration of e-liquids with aldehyde flavor chemicals impacts sensory perception of irritation and appeal among current adult e-cigarette users, we will use mixed effects models with water concentration as the within subject factor and each of the measure (e.g. irritation, appeal) as outcomes in separate models. 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. 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.

For the secondary aim, which is to examine differences by flavor for outcomes at each water concentration, we will examine at each water concentration, flavor as the within subject factor and each of the measure (e.g. irritation, appeal) as outcomes in separate models. We will construct confidence intervals for least square means and contrasts between least square means to estimate effect sizes and to inform future studies.

6.4.2 Sample Size Considerations

Given this is a pilot study, formal power analyses were not conducted. However, based on prior pilot data in which brief lab exposure to e-cigarettes were conducted by our group (HIC 2000025687) determined that 30 participants is sufficient to detect large differences of irritation and appeal by water concentration at two sided alpha level of 0.05.

6.5 Planned Analyses

6.5.1 Primary Objective Analysis

To examine the primary objective of the study, which is to examine how increasing the concentration of e-liquids with aldehyde flavor chemicals impacts sensory perception of irritation and appeal among current adult e-cigarette users, we will use mixed effects models with water concentration as the within subject factor and each of the measure (e.g. irritation, gLMS and appeal, LHS) as outcomes in separate models. 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 aim, which is to examine differences by flavor for outcomes at each water concentration, we will examine at each water concentration, flavor as the within subject factor and each of the measure (e.g. irritation, appeal) as outcomes in separate models. We will construct confidence intervals for least square means and contrasts between least square means to estimate effect sizes and to inform future studies.

6.5.3 Exploratory Objectives Analyses (if applicable)

N/A

6.5.4 Safety

See 6.1.2 for treatment of adverse events.

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. All participants will receive the same treatment (within subject design).

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

Given this is a pilot trial, we do not have apriori defined associations between subject characteristics and response to change in water concentration. Additionally, as this is a within subject design subject characteristics that influence e-cigarette perception (i.e. menthol status, heaviness of e-cigarette use, prior flavor experience) are likely not to impact analyses.

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, the water concentration administer, or the nicotine concentration of the product. They will be told that products containing moderate levels of nicotine, flavorings that do or do not contain components considered “flavor aldehydes” and that we will be adding water to some of the formulations they will be trying. 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 ($30 for remote portion, $20 for in person portion). This is commensurate to other e-cigarette studies in our labs. Participants will be compensated $75 for each laboratory session that is approximately 2 hours in length. Additionally, participants are asked to abstain from vaping for 2 hours prior to each lab session, so this compensation is included. Additionally, at in person appointments, participants will be compensated for travel with $25. This is the equivalent of the cost of a rideshare in the greater New Haven area to and from our appointment space. 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, due to increased health monitoring for EVALI symptoms. This is included as an added study benefit. 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, 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 minor increase over minimal risk as participants are all current e-cigarette users using nicotine and flavors. It is unlikely brief exposure to e-cigarettes with or without added water 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 30 participants have completed both 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

This study is funded through the Yale Tobacco Center of Regulatory Science (TCORS) pilot program. All pilots including this one receive FDA/NIH approval before receiving funding. FDA/NIH approval to conduct this pilot was awarded.

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.

APPROVED BY THE YALE UNIVERSITY IRB 11/15/2022

8 Appendices

Appendix #	Title	Section	Topic

9 List of Tables

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1. Hsu G, Sun JY, Zhu SH. Evolution of Electronic Cigarette Brands From 2013-2014 to 2016-2017: Analysis of Brand Websites. *J Med Internet Res.* 2018;20(3):e80.
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