Submitted to IRB: 08/16/21
Approved by IRB: 09/13/21

CENIC II REN - PROJECT 3:

EFFECTS OF VERY LOW NICOTINE CONTENT CIGARETTES AND E-CIGARETTE CHARACTERISTICS ON SMOKING IN ADOLESCENTS

STUDY PROTOCOL

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Abbreviations

- AE: Adverse Event
- BP: Blood pressure
- BPM: Beats per minute
- CENIC: Center for the Evaluation of Nicotine in Cigarettes
- CES: Cigarette Evaluation Scale
- CESD: Center for Epidemiological Studies Depression Scale
- CO: Carbon monoxide
- COPD: Chronic Obstructive Pulmonary Disease
- CPD: Cigarettes per day
- DSMB: Data Safety Monitoring Board
- FDA: Food and Drug Administration
- FTND: Fagerström Test for Nicotine Dependence
- HR: Heart rate
- LMP: Licensed Medical Provider
- MNWS: Minnesota Nicotine Withdrawal Scale
- NDSS: Nicotine Dependence Syndrome Scale
- NIDA: National Institute on Drug Abuse
- NMR: Nicotine metabolite ratio
- NNC: Normal nicotine content
- PATH: Population Assessment of Tobacco and Health
- PI: Principal Investigator
- PPM: Parts per million
- QSU: Questionnaire of Smoking Urges
- RNC: Reduced nicotine content
- SAE: Serious Adverse Event
- SAETRS: Serious Adverse Event Tracking and Reporting System
- TLFB: Timeline Follow Back
- TNE: Total Nicotine Equivalents
- VLNC: Very low nicotine content
- WISDM: Wisconsin Index of Smoking Dependence Motives

Objective and Background

Objective

Project 3 will use laboratory methods with adolescent daily smokers to examine the effects of nicotine content in cigarettes on the reinforcing value and positive subjective effects of cigarette smoking. We will examine how concurrent availability of vaping devices and key vaping characteristics (nicotine concentration and flavors) influence preference to use these products and subjective responses (e.g., liking, satisfaction, sensory effects, outcome expectancies, and perceptions of harm).

Nicotine, tobacco, and adolescents

Cigarette smoking is one of the leading causes of preventable death worldwide (WHO, 2013). Smokers on average lose ten years of life expectancy compared to nonsmokers (Thun et al., 2013). Adolescence is a critical developmental period for tobacco initiation and progression to regular use and dependence, and the vast majority of adult cigarette smokers began smoking in adolescence. Because early age of smoking initiation is associated with greater lifetime exposure to tobacco smoke, those who begin smoking during adolescence are at greater risk for developing tobacco dependence and smoking-related disease and death compared with adult initiators (Jha et al., 2013; Kendler et al., 2013; Marshall et al., 2006). In addition to these risks over the life course, prolonged exposure to nicotine during adolescence is harmful for brain development and can result in prefrontal cortex deficits and changes in behavioral risk factors such as impulsivity that may be permanent (Goriounova et al., 2012; Musso et al., 2007). Comprehensive tobacco control policies that reduce risk of tobacco use in adolescence are critically needed to minimize death, disease, and other harms caused by tobacco use.

Reducing nicotine to reduce the addictiveness of combusted tobacco

The passage of the Family Smoking Prevention and Tobacco Control Act empowers the FDA to assert product standards to reduce harmful constituents in tobacco products, including the authority to reduce (but not eliminate) nicotine (US Congress, 2009). One product standard that has the potential to dramatically reduce smoking-attributable mortality and morbidity is to mandate a reduction in nicotine in all cigarettes to minimally addictive levels (Benowitz & Henningfield, 1994; Henningfield et al., 1998; Tengs et al., 2005; Zeller, Hatsukami et al., 2010). This regulatory strategy has the potential to impact the pattern of cigarette smoking over the lifetime, in part by making cigarettes less reinforcing for young people, decreasing the number of adolescent users who go on to become lifelong smokers (Benowitz & Henningfield, 2013). Like adults, for adolescent smokers, the level of nicotine in very low nicotine content (VLNC) cigarettes may be below the reinforcing threshold of nicotine, thus failing to maintain smoking behavior over the longer term (Sofuoglu & LeSage, 2012; Schassburger et al., under review). While many factors contribute to smoking initiation and persistence, addiction to nicotine is believed to be necessary to sustain tobacco use (US DHHS, 2014).

Effects of nicotine reduction in cigarettes

Nearly all of the studies of reduced nicotine content in cigarettes have been conducted with adults. In several studies, adult cigarette smokers who were switched to using VLNC cigarettes reduced their smoking rate (Donny et al., 2014; 2015). Non-treatment seeking smokers who used VLNC cigarettes in an inpatient unit for 11 days reduced their average cigarettes per day (CPD) by 5 cigarettes (Donny et al., 2007). Among adult smokers who were motivated to quit, switching to VLNC cigarettes for 6 weeks also reduced CPD (Hatsukami et al., 2010). In contrast, among adult smokers not motivated to quit, *gradual* reduction in the nicotine content of cigarettes did not result in CPD reductions (Benowitz et al., 2007; 2015). However, in this and other studies (Donny et al., 2015; Hatsukami et al., 2010), VLNC cigarettes greatly reduced exposure to nicotine, and smokers of VLNC cigarettes also tend to show

reduced exposure to NNK, a tobacco-specific lung carcinogen (Donny et al., 2015; Hatsukami et al., 2010). Smokers who were switched immediately to VLNC also report reduced dependence (Donny et al., 2015; Hatsukami et al., 2010; 2013), which was not observed when nicotine content was gradually reduced (Benowitz et al., 2007; 2015). Across studies, concerns about the potential for VLNC to result in harmful compensatory smoking were not supported. While these findings support the idea that nicotine reduction may reduce cigarette smoking in adults, studies have been complicated by non-compliance, with biomarker data suggesting participants do not fully comply with instructions to refrain from usual brand product use. Noncompliant use of usual brand cigarettes, even at low levels, may obscure the full benefit of VLNC cigarettes on reductions in CPD, toxicant exposure, and eventual cessation (Dermody et al., 2014), and may minimize the aversive effects of abstinence from nicotine. Pervasive non-compliance with VLNC cigarettes (significantly associated with younger age in CENIC Project 1; Nardone et al., 2015) suggests that, if alternative combusted nicotine products were not easily accessible, smokers may turn to alternative non-combusted nicotine products.

Adolescents and VLNC cigarettes

No comparable studies involving adolescents have been published, so it is not known whether benefits and risks of VLNC cigarettes would be similar or different in young smokers. However, using data from the first phase of CENIC (Donny et al., 2015), we compared the effects of reduced nicotine content in cigarettes on CPD over 6 weeks among 18-24 year old participants vs. all other participants (25+ years); whereas older participants showed clear nicotine dose effects on CPD (with lower nicotine dose associated with fewer CPD), effects of nicotine dose in younger participants (who were lighter smokers on average) were less dose-dependent, suggesting that, in adolescents, reducing the nicotine content of cigarettes alone may not be sufficient to decrease smoking rates.

In adolescent smokers, the only published data are on acute effects of VLNC, and those are limited. In a laboratory study comparing the effects of VLNC vs. NNC (Kassel, Evatt et al., 2007), adolescents randomized to smoke a single NNC or VLNC cigarette rated the cigarettes equal in "harshness" and "strength" but found the VLNC less "pleasant" than the NNC cigarette. Adolescents in both conditions reported reductions in negative affect from pre to post smoking, but this reduction was greater in the NNC condition than in the VLNC condition; a secondary analysis showed some compensatory smoking (i.e., greater number of puffs per cigarette) in the VLNC condition (Kassel, Greenstein et al, 2007). Cigarette effects were moderated by baseline nicotine dependence severity such that those with high dependence in the NNC condition reported the largest pre- to post-smoking decrease in negative affect, compared with all other conditions. Kassel and colleagues highlighted the value of laboratory studies for studying causal mechanisms involved in adolescent smoking; this and other laboratory studies (Bidwell et al., 2012; Colby et al., 2010) have provided strong support for negative reinforcement (smoking for relief of negative affect, craving, and other effects of withdrawal) as an important factor maintaining smoking among adolescents. Although VLNC cigarettes appear to acutely provide some withdrawal relief in adolescent smokers, they may be less effective than NNC cigarettes, suggesting that a reduced-nicotine standard for cigarettes may lead to adolescents' seeking alternative nicotine products. Furthermore, tendency to use alternative nicotine products may be moderated by level of dependence or heaviness of smoking.

Reducing the nicotine content of combusted products: the role of e-cigarettes

VLNC studies to date have not manipulated other tobacco/nicotine product use or availability, so we do not know the effects of nicotine reduction on smoking in the context of other products. But if the appeal of and reinforcement from reduced nicotine content combusted products is low, smokers will likely seek alternative sources of nicotine that may partially substitute for cigarettes. In addition to delivering

nicotine effectively, e-cigarettes (e-cigs) provide other sensorimotor stimuli similar to smoking, which can maintain use behavior even when nicotine is absent (Caggiula et al., 2001; Rose, 2006). These sensorimotor stimuli may make e-cigs a particularly good substitute for combusted cigarettes. If reducing cigarette nicotine content reduces the relative reinforcing effects of cigarettes relative to e-cigs, smokers may partially or completely switch from smoking to using e-cigs. Our aim is to understand how specific e-cig characteristics influence their reinforcement value relative to that of NNC and VLNC cigarettes in adolescents. We chose to focus on e-cigs based on data from the National Youth Tobacco Survey (Arrazola et al., 2015) showing that e-cigs are the most highly prevalent tobacco product used by adolescents in the U.S., outpacing traditional cigarettes. Having tripled in prevalence from 2013 to 2014, 13.4% of high school students reported past 30-day e-cig use, while use of cigarettes in the same period had declined to 9.2%. Concurrent use of $\geq$ 2 tobacco products is also common in youth (51.6% of all tobacco users), and cigarettes and e-cigs are the most common combination. Given the changing landscape in tobacco product use in youth, studies involving adolescent smokers must consider tobacco products in the context of e-cig availability.

Nicotine and adolescent smoking

Compared to adults, adolescents tend to be lighter and more intermittent smokers, have shorter smoking histories, lower levels of exposure to CO and cotinine, and are less dependent on nicotine on average (Colby, 2015; Colby et al., 2000a, b; Lewis-Esquerre, Colby et al., 2005; Mermelstein, Colby et al., 2002). Despite having less experience with smoking, adolescents very early in their smoking careers (having smoked as few as 10 cigarettes lifetime) self-administer physiologically active doses of nicotine, with no differences found between non-daily and daily smokers (Corrigall et al., 2001). Smoking topography appears similar to that of adults in terms of total puff volume, but adolescents tend to take smaller, more frequent puffs compared with adults (Corrigall et al. 2001; Zack et al., 2001). In adolescent daily smokers (Wood et al., 2004), CO boost and nicotine boost from smoking a single cigarette were comparable on average (though more variable) to published data from adult smokers. Like adults, adolescents regulate their nicotine intake while smoking by varying their smoking topography (Kassel et al., 2007; Veilleux, 2011). Smoking abstinence in adolescents (as in adults) increases craving, withdrawal, and negative affect and smoking reinstatement reverses these symptoms to baseline levels (Bidwell et al., 2012; Colby et al., 2010). Zack and colleagues (2001) found that smoking abstinence impaired inhibitory information processing in adolescents, and smoking reinstatement improved it in a manner similar to adult smokers, but daily frequency of smoking was an important moderator. In sum, the role of nicotine in maintaining adolescent cigarette smoking appears similar to that for adults, yet overall exposure to nicotine tends to be lighter and more variable during adolescence. It is not entirely clear, then, whether or why younger participants should respond differently to VLNC cigarettes compared with adults, yet data from the first phase of CENIC (above) suggest they may.

Adolescents and e-cigarettes

The rapid emergence of e-cigs as an alternative tobacco product has been controversial and contentious in the fields of public health and tobacco control. Although e-cigs have lower levels of toxicity compared with combusted cigarettes (Goniewicz et al., 2014; Harrell et al., 2014), nicotine exposure in youth carries risk: e-cigs may inhibit smoking cessation, e-cigs may re-normalize traditional smoking (by reintroducing tobacco product use into smoke-free places), and e-cigs' non-tobacco flavors like candy and fruit may appeal to youth and cause e-cigs to serve as a "gateway" to other tobacco products (Grana et al., 2014; Klein, 2015). There is emerging evidence that e-cig use among never-smoking adolescents prospectively predicts increased susceptibility to cigarette smoking, and greater likelihood of combusted cigarette use (Leventhal et al., 2015; Primack et al., 2015), though whether this is a causal effect or the

result of shared risk factors for using tobacco products generally is not yet known (Barrington-Trimis et al., 2015). Among adolescent current smokers (at any level), e-cig use was associated with greater intentions to quit smoking (Dutra & Glantz, 2014); however, among ever-smokers, e-cig ever use and current use were associated with lower rates of past 30-day smoking abstinence. Data from these studies provide invaluable descriptive information about rates and trends in e-cig and dual use. Our proposed study aims to provide causal information by experimentally manipulating the effects of product characteristics on choices to smoke cigarettes, use e-cigs, or abstain from tobacco products in a laboratory setting.

Product characteristics

Depending on device, e-cigs can deliver as much or more **nicotine** than a traditional cigarette with relatively rapid absorption in adult experienced users (Spindle et al., 2015; Ramoa et al., 2015). Even naïve users (< 5 lifetime episodes of e-cig use, with minimal instructions on using the device) can attain dose-related plasma nicotine levels similar to those delivered via cigarettes, from their first bout of use (Lopez et al., 2015). In both types of users, e-liquid nicotine concentration and user plasma nicotine concentration are directly related. Like cigarettes, e-cigs can suppress cigarette abstinence symptoms (Bullen et al., 2010; Vansickel et al., 2012), suggesting that e-cigs may function as both positive and negative reinforcers. No lab studies with adolescents have been published yet, but survey data show a relationship between preferred nicotine concentration in e-cigs and cigarette smoking status (Camenga et al., 2015). Use of non-nicotine e-cigs was prevalent among nonsmokers (43%), but infrequent among non-daily smokers (10%), and not observed among daily smokers (0%). Conversely, use of e-cigs with higher nicotine concentration (18-24 mg/mL) was prevalent among daily smokers (49%) and infrequent among non-daily smokers (13%) and non-smokers (8.4%). Similarly, Krishnan-Sarin et al. (2015) found that adolescent cigarette smokers were more likely to initiate with nicotine-containing e-cigs while nonsmokers and former smokers were more likely to initiate with nicotine-free e-cigs.

Much attention has been focused on the thousands of e-cig **flavors** available, which likely increase the appeal of e-cig use in adolescents. Two studies examined the role of flavors in the appeal of e-cigs adolescent never-smokers. In one, Pepper et al. (2013) found that 18% of those who had never used e-cigs were willing to try them, but willingness to try a “plain” vs. a flavored e-cig did not differ. Shiffman et al. (2015) found that non-cigarette flavors were more appealing to adult *smokers* (some of whom had used e-cigs) than to adolescent non-smokers with no e-cig experience. By contrast, in adolescents who had tried e-cigs, flavored e-cigs appear to be more appealing than cigarette-flavored e-cigs. Camenga et al. (2015) showed that, among lifetime e-cig users, current e-cig users, and dual cigarette/e-cig users alike, fruit is by far the most widely used flavor, candy is next most popular, while cigarette flavors are favored by less than 10% of each group. Krishnan-Sarin et al. (2015) reported that sweet flavors were preferred by 56.8% of 953 lifetime adolescent e-cig users, compared to only 3% who preferred tobacco flavor. Kong et al. (2015) found that “appealing flavors” was among the top reasons (43.8%) to try e-cigs in adolescents and young adults (along with curiosity and peer influence). In unpublished data from an adolescent cohort study at Brown (R01 AA016838, Jackson, PI; Colby Co-I), of 44 adolescents who reported past 30-day e-cig use, 70% endorsed "they come in flavors I like" as a reason. Thus, among adolescents who have tried e-cigs, availability of non-cigarette flavors may be a key factor that affects e-cig use and the relative reinforcing effects of e-cigs.

Overall Study Design

A 2 x 2 x 2 laboratory-based study (*N*=120) will be conducted with adolescent daily smokers, using a **choice procedure** to determine the effects of very low nicotine content on cigarette smoking, and how

the effects of VLNC are influenced by the concurrent availability of e-cigs varying in **nicotine concentration** and **flavors.** These procedures involve a sampling phase, in which the participant samples and rates the two options, followed by choice (preference) phase, consisting of a series of discrete trials in which the participant can choose between the two options (or choose to forgo either option). The option that is chosen more often is considered to have greater relative reinforcing efficacy (Comer et al., 2008). In addition to having strong internal and predictive validity (Carter et al., 2009), choice procedures have ecological validity as they mimic situations in which smokers choose which product to buy or use among those concurrently available in the natural environment. We considered a free-access procedure in which both options are made concurrently available and *ad libitum* consumption is measured, however choice procedures are generally more sensitive than free access procedures to differences among reinforcers (e.g., Griffiths et al., 1986; Shahan et al., 1999). Participants will complete **six sessions**, including the **Screening/Baseline (BL)** session, during which eligibility will be confirmed and background questionnaires will be completed. Participants will then complete five lab sessions, a minimum of 48 hours apart; each session will follow overnight smoking abstinence.

In **Lab Session 1**, only combusted cigarettes (either NNC or VLNC) will be available. This session will always occur first because, conceptually, the no-e-cig session is the baseline condition in this study; scheduling this session first ensures that smoking choices in this condition will not be affected by any potential carryover effects of e-cig exposure on smoking choices. During the sampling phase of the choice procedure ("Preference Task"), participants will sample and then rate the cigarette using the Product Rating forms. In the preference phase, participants will be prompted via computerized instructions to make a series of 10 choices. Each choice will be to either take 2 cigarette puffs or to abstain from puffing; a total of 20 cigarette puffs is available. After completing the choice task, participants will practice using the vaping devices and sample the e-liquid flavors available. They will select which cigarette and non-cigarette flavored e-liquids they want to use during Sessions 2-5.

Lab Sessions 2-5 will each include a preference task in which two products are available. In the sampling phase, participants will sample and then rate the same combusted cigarette type from Lab Session 1 and will also sample and rate an e-cig with the assigned combination of characteristics for that session (i.e., 3 mg/mL nicotine + cigarette flavor; 3 mg/mL nicotine + non-cigarette flavor; 18 mg/mL nicotine + cigarette flavor; or 18 mg/mL nicotine + non-cigarette flavor). E-liquid nicotine concentration will be administered under double-blind conditions; flavors, by necessity, will not be blinded. In the preference phase, participants will be prompted via computerized instructions to make a series of 10 choices. Each choice will be to either take 2 cigarette puffs, 2 e-cig puffs, or to abstain from puffing; again, a total of 20 puffs (from either or both products) is available (tasks are described in greater detail below). Between lab sessions, participants will be instructed to return to their usual tobacco use patterns.

Design Schematic					
NNC cigarettes (*n*=60): 5 sessions			**VLNC cigarettes (*n*=60):** 5 sessions		
	e-liquid flavors			**e-liquid flavors**	
e-liquid nic concentration	cigarette	non-cigarette	**e-liquid nic concentration**	cigarette	non-cigarette
3 mg/ml	x	x	3 mg/ml	x	x
18 mg/ml	x	x	18 mg/ml	x	x
No e-cig	x		No e-cig	x	

Summary of Measures

MEASURES	SCREEN/ BL	LAB SESSIONS				
		1	2	3	4	5
QUESTIONNAIRES AND MONITORING						
Demographics Questionnaire	X					
Medical History Questionnaire & Follow-Up Questionnaire	X					
MINI Suicide Subscale	X					
Tobacco Use History Questionnaire	X					
Vaping History Questionnaire	X					
Timeline Follow Back (TLFB): Baseline and Lab Session Versions	X	X	X	X	X	X
Contemplation Ladder	X					
Drug Use History Questionnaire	X					
Depressive Symptoms (CES-D)	X					
Fagerstrom Test for Nicotine Dependence (FTND)	X					
Cigarette Dependence Questionnaire	X					
Environmental Tobacco Smoke (ETS) Questionnaire	X					
Smoking/Vaping Identity	X					
Smoking Expectancies Questionnaire	X					
Respiratory Health (ATSQ)	X					
Predicted Behavior Interview						X
Adverse Events		X	X	X	X	X
End of Study Questionnaire						X
LABORATORY PREFERENCE TASK						
Questionnaire on Smoking Urges (QSU)- Brief	X	X	X	X	X	X
Minnesota Nicotine Withdrawal Scale (MNWS)	X	X	X	X	X	X
Positive and Negative Affect Scale (PANAS)	X	X	X	X	X	X
Cigarette Evaluation Scale (CES)	X	X	X	X	X	X
Vaping Expectancies Questionnaire (VEQ)	X					
Vaping Device Utility Questionnaire	X					
Perceived Health Risk Scale (PHRS)	X	X	X	X	X	X
Cigarette Purchase Task (CPT)	X	X	X	X	X	X
# Choices for Cigarette Puffs		X	X	X	X	X
# Choices for E-Cigarette Puffs		--	X	X	X	X
# Choices to Abstain		X	X	X	X	X
CO Boost (Smoke Exposure during Preference Task)		X	X	X	X	X
BIOMARKERS						
Cotinine for NicAlert (or comparable), if applicable (Urine)	X					
Total Nicotine Equivalents (Urine)	X					
Nicotine Metabolite Ratio (Saliva)	X					
Alveolar Carbon Monoxide (Breath CO)	X	X	X	X	X	X
PHYSIOLOGICAL						
Blood Pressure	X	X	X	X	X	X
Heart Rate	X	X	X	X	X	X
Pregnancy (Urine)	X	X	X	X	X	X

Screening/Baseline Visit

Recruitment

Participants will be recruited from Brown University (Co-PIs: Colby & Tidey). Participants will be recruited through flyers, local and school newspapers, bus, social media, online via our recruitment website, Brown and sites.brown websites, Today@Brown, and Craigslist advertisements targeting smokers within the eligibility age range. We will use the digital marketing agency BUMP to help increase

recruitment. Recruitment programs include integrated online advertising campaigns and landing pages designed to maximize engagement with qualifying candidates. Ads will briefly describe the study purpose, that it not intended to be a treatment for smoking and individuals will receive compensation for their time and participation. We will also promote the studies at public, private and charter schools in Rhode Island and Massachusetts via flyers and in-person information sessions. To reach adolescents out of high school, we will promote the study at GED classes, youth job training programs, and other community-based settings and programs using flyers and information sessions.

A Qualtrics contact form can be used for potential participants to provide their name, phone number, and the best time to be contacted via telephone, in response to study ads. The link to the Qualtrics form would be included directly within an electronic ad and/or on the study websites. Adolescents who are interested in participating will complete a telephone screening interview. At in-person recruitment events, adolescents may choose to provide contact information to research staff to be contacted by phone; adolescents responding to ads will call the research office to complete the brief, confidential telephone interview. The screener will assess: tobacco product use history; past 30 day use of tobacco products, alcohol and illicit drugs (not marijuana); pregnancy/breastfeeding; and intention to quit smoking. Research assistants will record the potential participant's responses in REDCap, a web-based data collection platform hosted by the University of Minnesota's Clinical and Translational Science Institute (CTSI). The phone screening data will be used to establish preliminary eligibility. It will not be used for research purposes. Identifying information will be stored separately in a password-protected Access Database on the Center for Alcohol and Addiction Studies (CAAS) server.

Adolescents who meet initial eligibility criteria, and who remain interested after hearing a more detailed study description, will provide contact information for their parent/guardian (minors only) so that parental informed consent can be obtained; 18-20 year olds can provide their own consent. **Minors will not be scheduled without research staff first talking directly to a parent/guardian.** Research staff will call the parent or guardian, describe the study, and answer any questions. Consent forms will be mailed to the parent (with a copy to keep). Adolescents will then be scheduled for the initial in-person session and transportation arranged if necessary. The adolescent (if a minor) will bring the signed consent form to the in-person screening session. Participants will complete informed assent (minors)/consent at the in-person screening session. A researcher will review the assent/consent form in detail, review all study procedures, and answer questions. Participants will receive a copy of their consent form.

Potential participants ages 18-20 will be instructed to bring a valid, state issued photo ID to the screening visit. Acceptable forms of identification include a Driver's License, State Photo ID Card, State Voter ID Card, Passport, or Military ID. If the potential participant does not have a valid, state issued photo ID, the interviewer can provide him/her with information on obtaining one. In addition to this option, we would accept a birth certificate plus a second ID document that has a photo (e.g., student ID). Participants ages 15-17 will have parental confirmation of age. Participants will be given the option of receiving study information or visit reminders by phone, email, and/or text message.

A participant must complete his/her in-person screening session within 30 days of completing the Telephone Recruitment Questionnaire. If the participant is not able to attend the in-person screening visit in that timeframe, he/she will need to complete the telephone recruitment questionnaire again but will maintain the same REDCap ID number in the participant screening database.

Electronic Informed Consent

During the COVID-19 pandemic, modifications will be made to the consent process for participants and parents, if applicable. We will conduct the informed consent/assent process remotely and consent/assent will be provided via an online form in REDCap, hosted by the University of Minnesota. The participant informed consent or assent process will be conducted while the participant is in the laboratory room alone. The research assistant will be interacting with the participant via Zoom from the adjacent control room or an alternate laboratory room. Participants will be provided a copy of the consent/assent form for their review during the consent/assent process. All interview procedures will be conducted during in-person lab procedures in this fashion to comply with social distancing measures (i.e., participant and RA in separate rooms and interacting via Zoom).

In the case of minors who are interested in participation, research staff will call the minor's parent/guardian either by telephone or Zoom. The study information will be provided and any questions that the parent/guardian has will be answered by research staff. If the parent/guardian is willing to consent to their child's participation, a link to an online consent form in REDCap will be sent via email. Research staff will confirm the parent has received the link and complete a consent process with the parent. A copy of the consent/assent form can be saved or printed for their records. If the parent/guardian is unable to complete the consent process at the time, staff may offer to email a copy of the consent form to the parent/guardian and the consent process will be scheduled at a later time. Minor participants will only be scheduled for an in-person screening appointment following consent of their parent/guardian.

Informed Consent Process

Before beginning the informed consent and assent process, participants ages 18-20 will need to produce valid, state issued photo identification. The interviewer will confirm the age and identity of the participant. In order to ensure adequate informed consent or assent, participants will be asked to read the first several lines aloud to determine literacy. The interviewer will then read the consent/assent form aloud to the participant. If the interviewer determines that the participant is not literate, he/she will be dismissed from the study but will receive $35 for attending the screening visit.

In accordance with the National Advisory Council on Drug Abuse (NACDA) guidelines for substance abuse research involving adolescents, a PowerPoint presentation highlighting the major topics in the consent/assent forms (e.g., study procedures, confidentiality, potential risks and benefits) will be reviewed with the participant. At the end of the PowerPoint presentation, the participant will be instructed to read several open-ended questions aloud and discuss the answers with the researcher. Only after the participant and the researcher are fully satisfied that the participant understands the purpose of the study, the confidentiality of the data, the procedures, the risks/benefits and his/her rights as a research participant will the consent or assent form be signed and the participant undergo screening procedures. The participant will be provided with a signed copy of the consent or assent document to take home with her/him.

Screening Procedures

The following physiological measures will be collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) Expired breath carbon monoxide (CO) levels will be assessed using a Smokerlyzer Micro+ CO meter (Bedfont Instruments), a reliable and valid measure of recent smoking. A minimum of two breath samples will be completed for each CO reading and the average of the two will be used as the final CO. However, if the average of the two readings differs by more than 2 ppm, a third sample will be needed. The final CO will then be the average of all three breath samples.

a. NicAlert Strips will be used to assess urinary cotinine levels if a participant's carbon monoxide reading is less than or equal to 5 ppm. NicAlert readings >= 3 will meet study inclusion criterion. A positive result on a comparable cotinine test strip, NicConfirm or an IDTC Rapid Nicotine Test Card, may be used as an alternative. The specific test to be used will be determined based on availability.

2) Pregnancy Tests (HCG detection) will be performed for female participants.
3) Blood pressure and heart rate will be measured using a CritiCare or Omron monitor to help the licensed medical professional determine final participant eligibility.

The following screening questionnaires will be participant-administered via paper and then will be entered into the study databases by the interviewer at the end of the visit:

1) Identifying Information Form will include the participant's Subject Identifier, name, address (including the county of residence), email address, phone number, age, and date of birth.
    a. This form will be entered into the 'Identifying Information Access Database'.
        i. Identifying information will be kept in a locked file cabinet (source document) and in a password protected Access Database (electronic version) separate from all other study data.

The following screening assessments will be administered as an interview and entered into REDCap by the interviewer:

1) Brief Medical History Questionnaire to query current diagnoses, symptoms and past health problems.
    a. Sections of the questionnaire will be entered into REDCap.
    b. The medications section will be transferred to the 'Concomitant Medications' form in REDCap.
2) Medical History Follow-Up Questionnaire, if applicable, to further query current diagnoses, symptoms and past health problems.
    a. This questionnaire is entered as a pdf and sent to Licensed Medical Provider (LMP). It will be a source document only and will not be used as data.
3) The Mini International Neuropsychiatric Interview (MINI) suicide subscale (Sheehan et al., 1997) to evaluate suicide risk.
4) Tobacco Use History and Exposure Questionnaire, which measures variables such as smoking amount, cigarette brand, age of initiation of smoking, number of quit attempts, duration of quit attempts and duration of smoking.
5) Vaping History Questionnaire will assess lifetime and past 30-day use of vaping devices as well as information about the devices and e-liquids used.
6) Drug Use Questionnaire, which measures illicit drug use during the past 30 days.
7) Timeline Follow Back (TLFB) Questionnaire**,** which will assess past 14-day tobacco and nicotine product use as well as alcohol and marijuana use. If a participant's reported cigarette or vaping use on the TLFB conflict with their reporting on the Tobacco Use History and Exposure Questionnaire, the RA will point out the discrepancy to the participant and ask whether they would like to modify their responses on the Tobacco Use History and Exposure Questionnaire or clarify why they are discrepant (e.g., participants had an upper respiratory infection in the past week that resulted in atypical smoking behavior).

The following screening assessments will be administered via Qualtrics or REDCap, web-based data collection platforms hosted by the University of Minnesota's CTSI:

1) Demographic History Questionnaire, which will assess age, gender, ethnicity, race, education, income, marital status, living situation, and employment history.
2) Center for Epidemiological Studies-Depression Scale (CES-D; Radloff, 1977), which measures symptoms of depression
3) Contemplation Ladder (Abrams, et al., 2003) to assess intention to quit smoking.

In the event that the Qualtrics/REDCap websites are not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics/REDCap when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to REDCap.

Eligibility Determination

Research staff will determine initial eligibility after reviewing all eligibility criteria except for the medical/psychiatric history. Researchers will pay ineligible participants $35 for their time spent completing the screening assessments. The LMP will not review the medical history forms of participants who are not eligible for other, non-medical reasons.

If the participant is deemed eligible, then she/he will provide saliva and urine samples, complete the Baseline assessments, and be scheduled for Lab Session 1. They will receive $70 for completing both the Screening and Baseline procedures. Participants will be instructed to use tobacco products as they normally would between the Screening/Baseline visit and the night before Lab Session 1; participants should not use any tobacco products on the day of Lab Session 1.

Final eligibility of the participant will be determined by a licensed medical professional (LMP) after reviewing the Brief Medical History Questionnaire, Medical History Follow-Up Questionnaire and the MINI suicide subscale. If the participant's score on the CESD indicates elevated symptoms of depression, then the CESD will be submitted to the LMP for review as well. The LMP will sign off prior to Lab Session 1 that the participant is medically stable to use the study product(s). If the LMP determines a participant is not medically stable enough to continue in the study, then the researcher will contact the participant prior to Lab Session 1 to cancel the lab visit.

At the end of the Screening/Baseline Visit, the researcher will complete the End of Visit Evaluation Form, which will be entered into REDCap. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, and to note any concerns about the validity of the participant's self-report of tobacco use.

Suicidality/Mental Health Monitoring

Participants who indicate a suicide attempt in the past 2 years on the MINI Suicide Subscale will not be eligible to participate in the study. The participant will be paid $35 and provided with local mental health resources. The LMP/on-site clinician will be available by phone for consultation within 24-hours. If the participant has attempted suicide between 2-10 years ago, then the LMP will have to approve eligibility.

The following suicidality/mental health monitoring protocol is being used by CAAS researcher Dr. Rachel Cassidy who is also conducting an adolescent smoking study:

> If the participant has stated that they are currently feeling suicidal or have thoughts of hurting themselves, the RA should call the study LMP/clinician (Dr. Patricia Cioe, or another CAAS clinician if Dr.Cioe is not available). Specifically, this safety plan will be triggered by a positive response to any item on the MINI Suicide Subscale.

Dr. Cioe will ask the following questions and document the participant's responses. Assess the situation in a calm, matter-of-fact tone. Gather and document as much information as possible. Dr. Cioe will ask the following questions:

- *You mentioned that you have been thinking about death and/or committing suicide. Could you tell me a little more about these thoughts?*

- *Have you ever made any suicide attempts before?* Try to get information on how many times, how long ago, what he/she did, and what happened.

- *Do you have a plan now?* Assess if the subject has a plan, a way to carry out the plan, and an intention to carry out the plan. Try to determine relevant details, such as: Does the subject live alone? Will they have the opportunity to carry out the plan?

- *Do you have a therapist, counselor, or doctor that you talk to on a regular basis?*
  IF YES: *What is that person's name?*

  IF NO: *Have you discussed this with a friend or family member?*
  IF YES: *What is his/her name*

- See if the subject will contract to not hurt themselves.

If the participant has indicated a current plan to commit suicide, but the LMP/study clinician has determined that immediate action is not required (no means or intent to carry out the plan) the LMP/study clinician will call them the next day to check on how they are feeling and provide them with the contact number for the Samaritans.

If the LMP/study clinician indicates that immediate action is required, let them know that the LM/clinician has their very best interest in mind and that also this situation requires that we contact the appropriate personnel in order to help protect their well-being. **Call 911** to inform emergency personnel of the situation at the most appropriate time, either while still in the presence of the participant or immediately after you leave them.

- If the participant reports current intent to commit suicide, contact the participants' parent or guardian on file (if a minor) AFTER calling 911.

Inclusion Criteria:

1) Ages 15-20
2) Daily or near daily smokers for 3 months or more
3) Must have used an electronic nicotine device on a minimum of two lifetime occasions.
4) Breath CO levels > 5 ppm (if $\leq$ 5 ppm, then NicAlert Strip = $\geq$ 3) or positive result on a comparable cotinine test (NicConfirm or IDTC Rapid Nicotine)
5) Speak, comprehend, and read English well enough to complete study procedures

Exclusion Criteria:

1) Unwilling to use research cigarettes or vaping device as part of the study
2) Intention to quit smoking in the next 30 days

3) Using tobacco products (other than cigarettes, vaping devices, or roll-your-own tobacco) ≥ 15 days in the past 30 days.
4) Any medical or psychiatric condition in which participation is likely to post a significant threat to health or for which the condition could interfere with the ability of the participant to fully participants (as determined by the LMP)
5) Self-reported illicit use of any drug expect marijuana ≥ 10 days in the past 30 days
6) Binge drinking alcohol (≥10 days in the past 30 days, ≥4/5 drinks in a 2-hour period (female/male))
7) Pregnant or breastfeeding
8) CO reading > 80 ppm
9) Systolic BP outside the range of 90-159, diastolic BP outside the range of 50-99, or heart rate outside the range of 45-104. Participants failing for any of these criteria will be allowed to re-screen once per criterion.
10) Indicating Yes on Questions 4-6 on the MINI or with a suicide attempt in the past 2 years (if within the past 2-10 years, LMP approval required).
11) Having participated in another research study during the past year in which they were switched to research cigarettes for longer than one week.
12) Self-reported allergies to propylene glycol, vegetable glycerin, nuts, or chemicals used to flavor food products.
13) Adverse reactions when previously using vaping devices.

Those with unstable medical or psychiatric conditions are excluded as these symptoms could affect a participant's ability to complete the study. Examples include but are not limited to the following: angina, stroke, heart attack, blood clots in the arms or legs for which the individual is undergoing active medical treatment, cancer requiring active chemotherapy or radiation therapy, severe shortness of breath caused by conditions such as uncontrolled asthma, COPD, or arrhythmia, active untreated infection such as pneumonia, active untreated endocrine disorder such as hyperthyroidism. The LMP can also exclude participants for conditions in which participation is likely to pose a significant threat to health or for which the condition could interfere with the ability of the participant to fully participate. We will exclude those indicating immediate readiness to stop smoking or plans to quit in the next 30 days, as participation in this study may not lead to reductions in smoking. We will exclude pregnant and breastfeeding women due to the potential harmful effects of tobacco use on developing fetuses and infants. Anyone with current or recent alcohol or drug abuse problems as these factors could independently affect smoking behavior during the study. Individuals with baseline CO readings greater than 80 ppm, those with heart rate or blood pressure readings that are out of range, those with allergies to propylene glycol/vegetable glycerin/nuts/chemical food flavorants and anyone who has attempted suicide in the past two years will be excluded from the study for safety concerns. If an individual has recently participated in a smoking research study that changed his/her smoking behavior this person would be excluded because he/she would not have a stable smoking baseline. Because participants are required to complete portions of the protocol independently in the lab, they will need to be able to independently read and comprehend the study materials. Daily vaping device users are not excluded and individuals must have tried vaping devices on two or more occasions in their lifetime in order to minimize concerns about introducing a novel nicotine product to those who have not previously used it.

Potential risks of participation

1) Survey Questionnaires: The interview will include questions about medical history, drug and alcohol use, and questionnaires about mood. Answering these personal questions could make the participant

feel uncomfortable. However, the questions we ask are commonly used in research and clinical practice and the participant will not be required to answer any question he/she is not comfortable answering. Answers to these questions will be kept confidential.

2) Breach of Confidentiality: The risk of the interview is loss of privacy if other people find out the results. We will work to ensure that the participant's confidentiality is kept.
3) Obtaining blood pressure: The blood pressure cuff might be a little uncomfortable. In obtaining blood pressure, researchers may find out that the participant has abnormal blood pressure.
4) Smoking Cigarettes: All cigarettes are harmful to a person's health. Smoking can lead to severe or fatal medical problems including heart disease, respiratory (breathing) problems and diseases, cancer, diabetes, and other health risks. The study cigarettes do not provide any less risk than the participant's usual brand cigarette.
5) Vaping device: Vaping devices can expose users to several harmful chemicals, including nicotine. Nicotine, which is also in cigarettes, may lead to some of the same diseases as smoking. Exactly what other chemicals are in vaping devices, and how they might be harmful, is not completely understood. Vaping device aerosol is not harmless "water vapor", although it is generally contains fewer toxic chemicals than cigarettes. The most common side effects related to vaping are changes in taste, mucus in throat/sinus, dry mouth, dry cough, throat irritation, sore throat, mouth ulcers, dizziness, headache, and nausea. On rare occasions, batteries from vaping devices have exploded and injured users. However, this is extremely unlikely to happen in this study.
   The U.S. Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) are investigating recent reports of serious lung disease associated with the use of vaping/e-cigarette devices. Many of the illnesses are related to vaping cannabis oil. FDA has advised people to avoid buying vaping products on the street, to refrain from vaping THC oil, and warned against modifying or adding any substance to products purchased at stores. If you use a vaping device/e-cigarette products, you should watch for symptoms such as cough, shortness of breath, chest pain, nausea, vomiting, diarrhea, abdominal pain, fatigue, fever, and weight loss, and get medical attention right away for any health concerns. You can also call your local poison control center at 1-800-222-1222."
6) Nicotine is an addictive chemical. All products containing nicotine can become addictive and lead to longer-term use in the future. Exposure to smoke and nicotine in this project is carefully supervised and limited, so that it is not substantially more than what the participant is exposed to on a daily basis. Only those teenagers who are daily cigarettes smokers will be allowed to participate. Symptoms of too much nicotine include headache, dizziness, shakiness, nausea, vomiting or diarrhea, weakness, and fast heartbeat. You will be observed for any of these side effects and if they occur, we may stop the session.
7) Smoking Withdrawal: The participant may experience some discomfort when abstaining from smoking on the days of the lab visits. Symptoms can include irritability, frustration, anxiety, depressed mood or sadness, desire or craving to smoke, difficulty concentrating, and increased appetite or hunger. These feelings can be uncomfortable but they are normal, temporary, and usually mild.

Avoiding Risks during Pregnancy

Smoking during pregnancy can lead to miscarriage, preterm delivery, stillbirth, birth defects, and other problems. To avoid risks to the participant and fetus, female participants will be tested for pregnancy at every visit. If a participant becomes pregnant during the study, she will be withdrawn from the study.

Expected benefits of participation

There are no immediate benefits from participating in the study. Participants will have the chance to learn more about the effects of smoking, and to help us learn more about how nicotine in cigarettes affects

teen smoking. The information obtained from this study may ultimately help the Food and Drug Administration (FDA) decide how best to regulate tobacco products with the goal of improving public health.

Baseline Procedures for Eligible Participants ONLY

The following physiological measures will be collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) Weight and height**,** will be measured to determine the participant's Body Mass Index. Weight will be measured in kilograms and height will be measured in centimeters.

The following assessments will be administered using Qualtrics or REDCap:

1) Fagerstrom Test for Nicotine Dependence, (FTND; Heatherton et al., 1991)
2) Cigarette Dependence Questionnaire, a measure of nicotine dependence that combines questions from the FTND, NDSS, WISMD and PATH dependence questionnaires.
3) Smoking/Vaping Identity Questionnaire (van den Putte, et al., 2009), a two item measure of how well smoking or vaping represents a person's lifestyle or personal viewpoint.
4) American Thoracic Society Questionnaire (Comstock et al., 1979; Cassidy et al., 2015), a measure of cough, shortness of breath and other respiratory symptoms
5) Environmental and Social Influences on Tobacco Use Questionnaire (adapted from Nondahl, Cruickshanks, & Schubert, 2005), which measures tobacco smoke exposure at home, work and socially
6) Perceived Health Risks Scale (PHRS; Hatsukami et al., 2015), a measure of the perceived addictive potential and other health risks associated with cigarettes
7) Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegan, 1988), which measures symptoms of positive and negative affect
8) Minnesota Nicotine Withdrawal Scale (MNWS; Hughes & Hatsukami, 1986), a measure of nicotine withdrawal
9) Questionnaire of Smoking Urges-Brief – Usual Brand Cigarette (QSU; Cox, Tiffany, & Christen, 2001; Tiffany & Drobes, 1991), which measures the urge to smoke
10) Cigarette Evaluation Scale (CES; Westman, Levin, & Rose, 1992; Cappelleri, et al., 2007), which measures responses to cigarettes (e.g., reward, satisfaction)
11) Smoking Expectancies Questionnaire, which will be used to understand factors that motivate adolescents to smoke cigarettes.
12) Vaping Expectancies Questionnaire (VEQ), which assesses participant's expectancies regarding the use of vaping devices
13) Vaping Device Utility Questionnaire, an assessment of the reasons for using vaping devices
14) Cigarette Purchase Task – Usual Brand Version (Jacobs & Bickel, 1999; MacKillop et al., 2008) which will be used to generate cigarette demand curves. Participants will be asked to report the number of cigarettes that they would consume in a day at various costs. Several indices of demand are generated from the raw values, including demand intensity (consumption at zero price), Omax (maximum amount of money allocated to cigarettes), breakpoint (the first price at which a subject reports zero consumption) and Pmax (the price at which Omax occurs). This task will indicate whether prolonged VLNC cigarette use reduces cigarette demand and increases sensitivity to increases in cigarette costs.

Biological specimens collected, stored, and entered into CENIC Biosample Collection Platform:

1) Saliva sample for NMR assessment: Participants will be asked to provide two saliva samples during Baseline for assessment of nicotine metabolite ratio (NMR), an indicator of CYP2A6 enzyme activity (Benowitz, et al., 2003). Participants must wait 30 minutes after arrival to the lab before collecting the two saliva samples. During this time, participants cannot eat, drink, chew gum or smoke cigarettes. Samples will be stored at temperatures no more than -20°C. Saliva samples will be sent regularly to be analyzed and stored at the University of Minnesota.

2) Spot urine sample for smoking biomarker assessment**:** Participants will be asked to provide a urine sample while in the lab to assess baseline total nicotine equivalents (TNEs). Samples will be stored at temperatures no more than -20°C. Urine samples will be sent regularly to be analyzed and stored at the University of Minnesota.

Researchers will complete the End of Visit Evaluation Form, which will be entered into REDCap.

Lab Session 1 Procedures

Participants will return for Lab Session 1 approximately one week after their screening visit. Session 1 scheduling window is a minimum of three days and maximum of 21 days later. Outside of this window, study staff should seek approval from the PI or Project Coordinator. Participants will arrive at each session having abstained from smoking sufficiently to achieve a breath CO level that is ≤ 5 ppm or ≤ 50% below the CO level obtained at screening/baseline.

During the Lab Session 1, participants (N=120) will be randomized equally into one of two experimental conditions (VLNC or NNC cigarettes). Participants will be assigned a cigarette that matches their menthol preference.

Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO (must be ≤5 ppm or ≤ 50% below the CO level obtained at screening/baseline to begin session)
2) Blood Pressure
3) Heart Rate
4) Urinary pregnancy test, if applicable

The following assessments will be administered as an interview and entered into REDCap by the interviewer:

1) Concomitant Medications Form
2) Health Changes Questionnaire, which will assess any health changes since the previous session
3) Adverse Event Form, if applicable, will assess the nature, severity, duration, action taken, and outcome of an adverse event.
4) TLFB Questionnaire

PRE-TASK assessments will be administered using Qualtrics or REDCap:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS

Sampling Phase Procedures

In the *Sampling Phase* (~20 min), participants will be oriented to task procedures and shown the products available for that session. In Lab Session 1, only one product - NNC or VLNC cigarettes - will be available. Once the task begins, all instructions will be delivered via computer; sessions will be continuously observed by a research assistant through a one-way mirror. Participants will be prompted via computerized instructions to take four timed puffs of the cigarette and then complete the Product Rating forms (see description below). There will be a 15-min delay prior to the *Preference Phase*. During protocol breaks, participants will be allowed to watch TV or read magazines.

Product Rating Forms will be administered using Qualtrics or REDCap immediately after sampling the product:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS
4) CES- Study Cigarette Version
5) PHRS- Study Cigarette Version
6) Cigarette Purchase Task –Study Cigarette Version

Preference Phase Procedures

After the *Sampling Phase* has been completed, participants will complete the *Preference Phase* (30 min), which is designed to determine the relative reinforcement value of each product. During this phase, participants undergo a series of 10 choice trials, each lasting 1 minute, with a 2-min inter-trial interval. During each trial, participants will be presented (via computer) with a choice between taking 2 puffs of the cigarette or to abstain from puffing. Indices derived from this task include: total number of cigarette puffs; and total number of choices to abstain from product use. The maximum puffing rate for this task is 20 puffs in 30 minutes.

POST-TASK Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO

Vaping Device Practice

At the end of Lab Session 1, participants be presented with the vaping device and oriented on how it will be used during Lab Sessions 2-5. The participant will watch an instructional video on how to use the device and then the RA will show the participant how to operate the device. Next, participants will be instructed how to puff (start and stop) when signaled by the computer. For this vaping practice, participants will use a flavorless e-liquid with 0 mg/ml nicotine. Participants will not be further instructed regarding puffing unless their initial puffs fail to activate the device, in which case they will be instructed to puff harder (Vansickel et al., 2012).

Sampling e-cigarette flavors

After completing the vaping practice, participants will sample the e-liquid flavors. Vaping devices containing e-liquids with 0 mg/ml nicotine in the cigarette and non-cigarette flavors will be sampled. Participants will take a few puffs from each e-liquid flavor and then will select one flavor from the cigarette-flavor category and one flavor the non-cigarette flavor category to use in subsequent sessions. The nicotine concentration of the e-liquid used in this session (0 mg/ml) differs from those used in future sessions to avoid affecting choices during future session.

Researchers will complete the End of Visit Evaluation Form, which will be entered into REDCap.

Lab Sessions 2-4 Procedures

Lab Sessions 2-4 should be scheduled at a minimum interval of 48 hours apart and ideally no more than one week later. Every attempt will be made to schedule participants at approximately the same time of day as Lab Session 1 (± 2 hours). If participants are not able to attend within the ideal scheduling window, the session will be scheduled on the next available day outside the ideal scheduling window. If a longer interval is needed between sessions, the RA will contact the PI or Project Coordinator for approval. All sessions will be scheduled consecutively and no session will be skipped unless otherwise informed by the PI or project coordinator. Participants will arrive at each session having abstained from smoking sufficiently to achieve a breath CO level that is ≤ 5 ppm or ≤ 50% below the CO level obtained at screening/baseline.

Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO
2) Blood Pressure
3) Heart Rate
4) Urine pregnancy test, if applicable

The following assessments will be administered as an interview and entered into REDCap by the interviewer:

1) Concomitant Medications Form
2) Health Changes Questionnaire
3) Adverse Event Form, if applicable
4) TLFB Questionnaire

PRE-TASK assessments will be administered using Qualtrics or REDCap:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS

<u>Sampling Phase Procedures</u>

In the *Sampling Phase* (35 min), participants will be oriented to task procedures and shown the products available for that session. In Lab Sessions 2 through 5, two products will be available: (a) cigarettes (NNC or VLNC) and (b) vaping devices containing e-liquids with the assigned nicotine concentration and flavor characteristics for that session. Participants will have selected their e-liquid flavors during Lab Session 1.

Once the task begins, all instructions will be delivered via computer; sessions will be continuously observed by a research assistant through a one-way mirror. Participants will be prompted via computerized instructions to take four timed puffs of the cigarette and then complete the Product Rating Forms. Following a 15-min delay to allow the effects of the cigarette to dissipate, participants will be prompted to take four timed puffs of the vaping device and then complete the Product Rating Forms, followed by another 15-min delay prior to the *Preference Phase*. During protocol breaks, participants will be allowed to watch TV or read magazines.

Product Rating Forms will be administered using Qualtrics or REDCap immediately after sampling the product:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS
4) CES – Study Cigarette and Vaping Device Versions
5) PHRS – Study Cigarette and Vaping Device Versions
6) CPT – Study Cigarette and Vaping Device Versions

Preference Phase Procedures

After the *Sampling Phase* has been completed, participants will complete the Preference Phase (30 min), which is designed to determine the relative reinforcement value of each product. During this phase, participants undergo a series of 10 choice trials, each lasting 1 minute, with a 2-min inter-trial interval. During each trial, participants will be presented (via computer) with a choice between taking 2 puffs of the cigarette, 2 puffs of the e-cig, or to abstain from puffing. Indices derived from this task include: total number of cigarette puffs; total number of e-cig puffs; and total number of choices to abstain from product use. The maximum puffing rate for this task is 20 puffs in 30 minutes.

POST-TASK Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO

Researchers will complete the End of Visit Evaluation Form, which will be entered into REDCap.

Lab Session 5 Procedures

Lab Session 5 should be scheduled at a minimum interval of 48 hours after Lab Session 4.

Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO
2) Blood Pressure
3) Heart Rate
4) Urine pregnancy test, if applicable

The following assessments will be administered as an interview and entered into REDCap by the interviewer:

1) Concomitant Medications Form
2) Health Changes Questionnaire
3) Adverse Event Form, if applicable
4) TLFB Questionnaire

PRE-TASK assessments will be administered using Qualtrics or REDCap:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS

Complete Sampling Phase

Procedures are the same as previous lab sessions.

Product Rating Forms will be administer using Qualtrics or REDCap immediately after sampling the product:

1) MNWS
2) QSU-10 Usual Brand Version
3) PANAS
4) CES – Study Cigarette and Vaping Device Versions
5) PHRS – Study Cigarette and Vaping Device Versions
6) CPT – Study Cigarette and Vaping Device Versions

Complete Preference Phase

Procedures are the same as previous lab sessions.

POST-TASK Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

1) CO

End of Study Procedures

After a participant has completed all study procedures and has been paid for participation the research assistant should read the following script and give the participant the *CDC Tobacco Facts Sheet* and *Kick the Habit Manual.*

> *"Before you go, we want to encourage you to quit smoking cigarettes and stop using vaping devices. Quitting use of all tobacco products, including vaping devices, is best for your health. The risks associated with vaping devices are largely unknown. However, most public health experts agree that use of a vaping device instead of combustible cigarettes likely results in reduced health risks. We would like to provide you with some resources should you decide to try to abstain from smoking (give "Kick the Habit" and hotline information), and some online resources about vaping device risk (give list of online e-cigarette risk websites). Please also feel free to consult with your physician and use any medications he/she deems appropriate."*

The following assessment will be administered as an interview:

1) **Predicted Behavior Interview,** will assess hypothetical actions adolescents and/or adults may engage in to obtain conventional cigarettes in the event a low nicotine product standard for cigarettes is implemented.

The following assessment will be administered using Qualtrics or REDCap:

1) End of Study Questionnaire

Researchers will complete the End of Visit Evaluation Form, which will be entered into REDCap.

Debrief Information

If a participant withdraws before data collection and the final session are complete, researchers will attempt to speak with the participant to encourage them to quit smoking cigarettes and to stop using vaping devices. If unable to speak with the participant directly, we will mail debriefing information to the address on file.

30 Day Follow-Up Phone Call

Participants will receive a follow up phone call between 25 and 35 days after Lab Session 5 to assess their smoking patterns and tobacco product use since the study ended. If participants indicate increases in tobacco product use, then information on the potential harms will be reviewed and followed with a strong recommendation to quit using all tobacco products. Smoking cessation resources and referrals will be provided.

Additionally, any Adverse Event Forms that remain open from the last session will be discussed. If the participant became pregnant during the study, this would have been recorded as an adverse event. During this visit, the research assistant will confirm her due date. This event will remain open until delivery. At that time the licensed medical professional will contact the participant to ask a few questions about the baby's health and will update the Adverse Event Form.

Follow-Up Qualitative Interview

A follow-up qualitative interview will be conducted remotely via Zoom or telephone with participants that previously completed the CENIC-2, P3 study, prior to the COVID-19 shutdown, and who agreed to be re-contacted with future research opportunities. This interview should take between 20-30 minutes and will be audio-recorded. After completion of the interview, participants will be sent via email a $25 Amazon e-gift card for their time and participation. Participants will be provided the link to the Coping with Stress CDC website, https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/managing-stress-anxiety.html.

In the case of minors who are interested in participation, research staff will call the minor's parent/guardian either by telephone or Zoom. The study information will be provided and any questions that the parent/guardian has will be answered by research staff. If the parent/guardian is willing to consent to their child's participation, the research assistant will complete the verbal consent process with the parent/guardian. If the parent/guardian is unable to complete the consent process at the time, staff may offer to email a copy of the consent form addendum to the parent/guardian and the verbal consent process will be scheduled at a later time. Minor participants will only be scheduled for an interview following verbal consent of their parent/guardian. Minor participants will provide verbal assent to participate. Participants and/or parents can request an emailed copy of the consent/assent form addendum to be saved or printed for their records.

Tobacco Product Information

Spectrum Cigarettes to be used in this project

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
NNC	NRC600	CN	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
NNC	NRC601	CN-Men	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
VLNC	NRC102	RN	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
VLNC	NRC103	RN-Men	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04

*Legend:	
RN	Reduced Nicotine
RN-Men	Reduced Nicotine-Menthol

CN	Conventional Nicotine
CN-Men	Conventional Nicotine-Menthol

Vaping Device to be used in this study

All participants will use the same device type. The device will be an ego vaping device with a 650 mah battery with a 2ml tank clearomizer. These clearomizers are disposable and will be pre-filled by a staff member. E-liquid will differ across Sessions 2-5. All e-liquid will be purchased from a United States manufacturer and wholesale distributor of e-liquid, and will contain 70% Propylene Glycol and 30% Vegetable Glycerin. Nicotine concentration will be either 0 mg nicotine/ml (used during e-liquid flavor sampling), 3 mg nicotine/ml, or 18 mg nicotine/ml. Two cigarette flavor options will be available (e.g. "light" tobacco, and tobacco/menthol) and four non-cigarette flavor options will be available (e.g. fruit, candy, and vanilla). A flavorless e-liquid option will be used while participants are familiarizing themselves with the vaping devices.

Product Distribution

The Administrative Core will be responsible for removing all identifying information from cigarettes, blinding the cigarettes for the research staff, and shipping cigarettes to our site as needed based on recruitment. The participants, investigators, and study staff will not have knowledge of which cigarette is given to a participant or whether different participants received the same or different cigarettes.

The Administrative Core will ship the e-liquid products to our site as needed based on recruitment. Because participants will be selecting their e-liquid flavors during Session 1, a staff member who is not involved in participant enrollment will create e-liquid kits that include the appropriate flavors and nicotine concentrations based on the randomization schedule generated by the Administrative Core. The participants, investigators, statisticians, and study staff working with participants will not have knowledge of which e-liquid nicotine concentration is given to a participant during each laboratory session.

The site will be responsible for tracking all tobacco products received and used by participants during the lab sessions.

Participant Compensation

Participants are free to discontinue at any time and will receive compensation for the sessions completed at the same rate listed below. They will receive payment on the date when they would have completed the study. However, they will not receive the fixed bonus for attending all sessions. Study compensation will be administered to participants using the ClinCard payment system utilized by Brown University.

Screening- Eligibility Only	**$ 35.00**
Baseline	$ 35.00
Sessions 01-05	$ 375.00
Completion Bonus	$ 125.00
Total	**$ 570.00**

Quit Attempts During the Study

At each lab session, we will assess cigarette smoking use via the TLFB. If the participant does not report

smoking any cigarettes on the TLFB, then the research assistant will ask the participant if she/he is currently abstaining with the intention of quitting for good.

If a Participant is Currently Abstaining from Smoking with the Intention to Quit:

- Encourage participant to continue abstaining from smoking
- Provide the participant with the '*Kick the Habit*' manual and local smoking cessation resources
- Pay participant for visit attendance and end the visit.
- Participant will be withdrawn from the study.

Adverse Events and Withdrawal or Monitoring of Participants

Identifying Adverse Events

While participating in the study, adverse events and concomitant medications will be assessed at every study visit and vital signs and carbon monoxide will be obtained. Adverse events will typically be identified during the administration of the Health Changes Questionnaire. Other events may be identified from physiological study measures or by spontaneous reports during non-scheduled assessments.

Questionnaire items that will be reviewed:

1) ***Have you had any negative changes in your physical or mental health since your last visit? If yes, briefly describe.***
2) ***Since your last visit, have you received any form of medical care? If yes, briefly describe.***

No new AE required if one or more conditions below are met and the description does not otherwise meet the definition of an AE.

a. Existing AE already open for reported symptom
b. Pre-existing condition without increase in severity or frequency of symptoms (brief medical history will be updated if not previously reported).
c. Received preventative or follow-up medical care.
d. Other (explain).

Physiological data that will be reviewed:

- CO level: The 'Adverse Event Form' should be completed if the average CO within a visit is:
  - CO is greater than 50 ppm if CO at Screening/Baseline Visit is < 20 ppm.
  - CO is greater than 60 ppm if CO at Screening/Baseline Visit is 20 – 34 ppm.
  - CO is greater than 70 ppm if CO at Screening/Baseline Visit is 35 – 49 ppm.

- Blood Pressure:
  - The 'Adverse Event Form' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual blood pressure measurement during the same visit is **at or above 160/100**
  - The 'Adverse Event Form' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual blood pressure measurement during the same visit **is below 90/50 and the participant is experiencing symptoms** listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'
- Heart Rate:
  - The 'Adverse Event Form' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual heart rate measurement during the same visit is **at or above 105 bpm.**

- The 'Adverse Event Form' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual heart rate measurement during the same visit is **below 45 bpm and the participant is experiencing symptoms** listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'

Management of SAEs and Other Study Risks

The site medical professional will review all AEs. A study participant may be discontinued from the study if the medical professional and/or PI determine it is the best decision in order to protect the safety of a participant. In the event that a participant either withdraws from the study or the investigator decides to discontinue a participant due to an AE/SAE, the participant will have appropriate follow-up medical monitoring. The participant experiencing an AE/SAE will be followed until the problem resolves, stabilizes, or is clearly unrelated to the study cigarettes. Any AE that remains open will be reviewed and closed at the 30 day follow-up interview.

Reporting of SAEs to the IRB, FDA, and NIDA

Serious adverse events (SAEs) as defined in 21 CFR 312.32 (death, life-threatening, hospitalization (initial or prolonged), disability or permanent damage, congenital anomaly/birth defect, required intervention to prevent permanent impairment or damage (devices), other serious (important medical outcomes)) that are related or possibly related to study participation will be reported to the Administrative Core, all site IRBs, the NIDA Scientific Officer, the NIDA Project Officer, FDA, and the Data Safety and Monitoring Board within 72 hours. All Site IRBs require that fatalities related to the study be reported within 24 hours, that all other SAEs be reported within 5 business days. Reports of all SAEs will also be documented within NIDA's SAE data monitoring system, or SAETRS, within 72 hours.

Reporting of IRB Actions to NIDA

Actions taken by the Brown University IRB in response to AEs/SAEs will be reported to NIDA in the annual noncompetitive continuation application, as will reports of changes or amendments to the protocol as a result of an AE/SAE. Recommendation for trial discontinuation, for significant changes or amendments to the protocol, or other significant findings as a result of an SAE will be reported immediately to the NIDA Scientific Officer and Project Officer by the Project PI.

Reporting Changes or Amendments to the Protocol

Any changes or amendments to the protocol made in response to adverse events/SAEs will be discussed with Eric Donny, PhD and Dorothy Hatsukami, PhD, and then requested in writing to the IRB, which will then grant or deny permission to make the requested change in protocol. Changes that significantly alter the scope of the research or the ability of the research to achieve its specific aims will be submitted to Eric Donny, PhD, the DSMB, FDA, and NIDA for approval prior to implementation. NIDA will be informed of any change that is significant enough for IRB review and changes the protocol in substantive ways (e.g., changes to procedures, recruitment strategy, inclusions/exclusion criteria), before such changes are implemented (except an urgent safety measure). Minor changes that do not alter the protocol in substantive ways (e.g., change in staff; new advertising venues) will not be reported to NIDA. Changes in protocol will also be documented in the noncompetitive continuation application.

Withdrawal or Monitoring of Participants

For the participant's protection, participants will be withdrawn immediately from the study if any of the following occur:

1) Cardiovascular disease (CVD) event: Typically includes MI (heart attack), PTCA (angioplasty/stenting), bypass surgery, stroke, peripheral vascular disease (arterial blockages in arms or legs leading to procedure or surgery). Less common CVD problems would be new cardiac arrhythmias (e.g., new atrial fibrillation) or new valvular disease (e.g., mitral or aortic regurgitation).
2) DVT/PE (deep vein thrombosis/pulmonary embolism, i.e., blood clots in the venous system).
3) Suicide Attempt: A participant will be withdrawn if he/she attempts suicide at any time during participation in the study.
4) Psychiatric Hospitalization: A participant will be withdrawn if he/she is hospitalized for psychiatric reasons at any time during participation in the study.
5) Pregnancy: If participant indicates she is pregnant or has a positive pregnancy test at any session, she will be withdrawn from the study, and this event will remain open until delivery. At that time the LMP will contact the participant to ask a few questions about the baby's health and will update the open 'Adverse Event Form'.

The following will be monitored and can lead to the participant being withdrawn by the PI or Licensed Medical Professional:

1) Blood pressure (BP) or heart rate (HR) changes: Systolic BP outside the range of 90-159, diastolic BP outside the range of 50-99, or heart rate outside the range of 45-104.
2) Expired breath Carbon Monoxide >80 ppm
3) Any hospitalization or debilitation in which participation in the study could be detrimental to the recovery process. This will be self-reported by the participant and will be reviewed by the site PI and medical professional to determine whether continued participation in the study is appropriate.
4) If a participant is behaving in an inappropriate or threatening manner, admits to lying about eligibility criteria, including omitting previous medical diagnoses and medications, is participating in other smoking research studies that could affect the primary outcome measures, does not follow study instructions, etc., then the PI can withdraw him/her from the study at the PI's discretion.
5) If a participant fails to attend her/his Lab Session 1 within the 21-day allowable visit window, she/he will not be eligible to reschedule the visit or continue participation in the study.

Investigational Tobacco Product

The Co-PIs of this project, Drs. Colby and Tidey, will apply for an Investigational Tobacco Product (ITP) application to the FDA to cover the experimental cigarettes being used in this study.

Certificate of Confidentiality

To help protect the participant's privacy, Drs. Colby and Tidey have obtained for a Certificate of Confidentiality from the National Institutes of Health. With this certificate, the researchers cannot be forced to disclose information that may identify the participants, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the participants, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the Federal Food and Drug Administration (FDA).

The Certificate of Confidentiality does not prevent the participant or a member of their family from voluntarily releasing information about themselves and their involvement in the research. If an insurer, employer or other person obtains the participant's written consent to receive research information, then the researcher may not use the Certificate to withhold that information. The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without consent, information that would identify the individual as a participant of the research project in instances such as evidence of child abuse or a participant's threatened violence to self or others.

Outcome Variables

Primary Outcome Variables:

- Number of cigarette puffs

Secondary Outcome Variables:

- Number of vaping device puffs
- CO boost

Exploratory Outcome Variables:

- Number of choices for cigarette puffs
- Number of choices for e-cig puffs
- Number of choices to abstain from puffing
- Measures of compliance: drop-out rate
- Measures of discomfort/dysfunction: MNWS, QSU, PANAS
- Measures of cigarette characteristics: CES, Cigarette Purchase Task
- Measures of perceived risk: Perceived Health Risk Scale
- Measures of cardiovascular function: heart rate, blood pressure
- Biomarkers: TNE, NMR, Cotinine, CO

Statistical Approach

Demographics and baseline data will be compared between the groups randomized to NNC or VLNC cigarettes using t-tests and chi-square tests. The endpoints of interest are the number of puffs from cigarettes, the number of puffs from e-cigs, and the number of choices to abstain from both cigarettes and e-cigs. Descriptive statistics will summarize the data by cigarette group and by session. Results will be presented in tabular and graphical format.

The **primary aim** is to examine the effects of cigarette nicotine content, e-cig nicotine concentration and e-cig flavor on choices to smoke. The primary outcome measure is the number of cigarette puffs, and the secondary outcomes are the number of e-cig puffs, and CO boost.

The **secondary aim** to is to examine the effects of these factors on product appeal and sensory effects (Cigarette/E-Cig Evaluation Scale), perceived health risks (Perceived Health Risks Assessment), and pre- to post-use changes in craving (QSU), withdrawal symptoms (MNWS), and affect (PANAS). The primary statistical model to test both aims will be a linear mixed model carried out according to the 2 x 2 x 2 design, where the between-subject factor is NNC or VLNC cigarettes and the two within-subject factors relate to e-cig flavor (cigarette or non-cigarette) and nicotine concentration (very low or moderate). The session where no e-cigs are included will be analyzed separately by comparing choices for NNC vs. VLNC cigarettes using a two sample t-test with unequal variances. The mixed model for the balanced factorial design will allow for the evaluation of the three main effects and their interactions. The major concern for this

approach is the occurrence of non-constant variance for the number of puffs of each product or the number of times abstaining as the dependent variables. Therefore, sensitivity analyses will be performed to evaluate the robustness of our assumptions. A generalized estimating equation (GEE) model uses sandwich estimators and thus is less affected by unequal variances, however, it is more negatively impacted by the occurrence of missing observations. Additionally, a negative binomial regression approach will check if modeling the number of puffs as a count variable provides a better fit to the data. The first mixed model will assume that participant characteristics are balanced between groups due to randomization and include only the three main effects and their interactions.

Subsequent analyses will add in participant factors including gender, baseline CPD, and nicotine dependence as potential moderators of study outcomes (**Exploratory Aim**). Possible interactions between the main effects and these covariates will be investigated. To test whether the use of e-cigs mediates the decrease in cigarette smoking and CO exposure, a causal model such as the marginal structural model (MSM) will be considered.

The primary analysis of all endpoints will adhere to the intent-to-treat principle where all participants will be included in the analysis according to the group to which they were randomly assigned. A secondary per-protocol analysis will include only participants who complete all 5 sessions.

Power Analysis

Very little has been reported in the literature on the effect of VLNC cigarettes or the dual use of e-cigs on adolescent smokers that could guide sample sizes for the proposed study. To determine a reasonable effect size (ES=mean difference/common SD) for sample size calculations, we considered several recent studies investigating alternatives to usual brand cigarettes in adolescents and young adults. In the first phase on CENIC (Project 1), CPD at weeks 2 and 6 were recorded for adult smokers using cigarettes with the same level of nicotine planned for our current project (15.8 mg/g vs 0.4 mg/g). Selecting out the youngest participants (18-24 years of age), the comparison between these two nicotine groups resulted in an ES of 0.76 at 2 weeks and 0.85 at 6 weeks. A recent study of adolescent smokers (Cassidy et al., 2015) used a very similar design as the current proposal. Participants made a series of 20 choices between puffs of their usual brand cigarettes or money, where the amount of money varied over three sessions ($0.00, $0.10, $0.50). The ES for the comparison between 10 and 50 cents was .73. Therefore, we determined that n=60 in each cigarette group would allow for the detection of an ES of 0.60 or higher with statistical power of at least 90% for a two-sided, two-sample test where the significance level is 0.05. For the within-subject comparisons defined by the 2 e-cig factors, an ES of 0.5 or more for the two main effects would have at least 97% power in a repeated measures analysis if n=60 per cigarette group with a significance level of 0.05. Study enrollment will continue until 60 subjects completing all 6 sessions in each group. Sample size and power calculations were carried out using PASS 13 (NCSS, LLC. Kaysville, Utah, 2014).

Subject Identifier

The subject identifier is an alpha-numeric combination. Example: G-C001 would be Brown University's first subject.

Project Identifier:
H = Project 3

Site Identifier:
C = Brown University

Subject ID:
001-899

Data Collection Time Points Identification Numbers:
92= Screening/Baseline Session
01= Lab Session 1
02= Lab Session 2
03= Lab Session 3
04= Lab Session 4
05= Lab Session 5
30= 30 day follow-up phone call
99= Unscheduled visit

Data Storage
Data will be stored locally at Brown University and at the University of Minnesota Masonic Cancer Center's Bioinformatics Core for at least 7 years after study completion.

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Statistical Analysis Plan

CENIC 2, Project 3

3/1/2018

Draft 4 (8/31/2018)

Finalized 9/28/2022

Table of Contents

1. Introduction

This document will serve as the Statistical Analysis Plan for CENIC 2 Project 3, entitled "Effects of Very Low Nicotine Content Cigarettes and E-cigarette Characteristics on Smoking in Adolescents. This document describes the planned statistical analysis for evaluating the effects of cigarette nicotine content, electronic cigarette (e-cig) nicotine concentration and e-cig flavor on combustible cigarette smoking in adolescents within a laboratory setting. Details for the proposed analysis of the primary and secondary endpoints are provided.

2. Trial Objectives

This study of adolescent smokers will examine the effects of cigarette nicotine content and e-cig availability, nicotine content and flavoring using a laboratory-based preference task in which the nicotine content of cigarettes, the concurrent availability of e-cigs, and the characteristics of e-cigs (nicotine concentration and flavors), are manipulated. The **primary aim** is to test the effects of nicotine content in cigarettes, and nicotine concentration and flavors in e-cigs on choices to smoke a combustible cigarette, vape an e-cig, or abstain from both, in a sample of adolescent smokers. The **secondary aim** will test the effects of nicotine content in cigarettes, nicotine concentration in e-cigs, and e-cig flavors on subjective effects. Lastly, the **exploratory aim** explores the extent to which participant factors (gender, CPD and dependence) influence the effects of nicotine content in cigarettes, nicotine concentration in e-cigs, and flavors in e-cigs.

3. Trial Design

This is a randomized, single site, double-blind, 2 x 2 x 2 laboratory–based study conducted on 120 adolescent daily smokers. These smokers will be assigned to receive either normal nicotine content (NNC) or very low nicotine content (VLNC) cigarettes. The first laboratory session will be a control session with only cigarettes, and no e-cigs, available. This will be followed by the 4 e-cig laboratory sessions with cigarette (tobacco; tobacco menthol) flavored e-liquid or non-cigarette (e.g., fruit; mint; vanilla; candy) flavored e-liquid and two levels of nicotine concentration as shown in the table below.

NNC cigarettes: 5 sessions (*n*=60)			**VLNC cigarettes:** 5 sessions (*n*=60)		
	e-liquid flavors			**e-liquid flavors**	
e-liquid nic concentration	tobacco	non-tobacco	**e-liquid nic concentration**	tobacco	non-tobacco
3 mg/ml	x	x	3 mg/ml	x	x
18 mg/ml	x	x	18 mg/ml	x	x
No e-cig control	x		No e-cig control	x	

3.1. Randomization

Randomization will be done in two stages. In the first stage, each individual will be randomized in a 1:1 ratio to either the normal nicotine content (NNC) or the very low nicotine content (VLNC) cigarettes in a double blinded fashion. The randomization will be stratified by gender, menthol status and cigarettes per day (<9, 9+). The goal is 60 subjects per cigarette group. The second randomization stage will be done within the subjects. Individuals in the two cigarette groups will be assigned to all four combinations of sessions involving e-cigs (flavor and nicotine content each at 2 levels) in counter balanced order, which provides 24 possible orders for the four e-cig sessions. The no e-cig session will always be first followed by the e-cig sessions, in an order randomly chosen from one of the 24 possible orders without replacement. Once all 24 session orders have been exhausted, the process will start over with another set of 24 orders. Each cigarette group (n=60) will end up with the same arrangement of the four sessions occurring 2 to 3 times. This will not allow for the evaluation of an order effect in the data analysis, but it will prevent a systematic bias due to the arrangement of the sessions.

3.2. Sample Size

Very little has been reported in the literature on the effect of VLNC cigarettes or the dual use of e-cigarettes on adolescent smokers. To determine a reasonable effect size (ES=mean diff/common SD) for sample size calculations, we considered several recent studies investigating alternatives to usual brand cigarettes in adolescents and young adults. In the first project of the CENIC 1 center grant, cigarettes per day (CPD) at weeks 2 and 6 were recorded for adult smokers using cigarettes with the same level of nicotine planned for our current project (15.8 mg/g vs 0.4 mg/g). (Donny et al., 2015) Selecting out the youngest participants (18-24 years of age), the comparison between these two nicotine groups resulted in an ES of 0.76 at 2 weeks and 0.85 at 6 weeks. A recent study of adolescent smokers (Cassidy et al., 2015) used a very similar design as the current proposal. Participants made a series of 20 choices between puffs of their usual brand cigarettes or money, where the amount of money varied over three sessions assigned to each subject in random order ($0.00, $0.10, $0.50). The ES for the comparison between 10 and 50 cents was 0.73. Therefore, based on these previous studies, we determined that 60 subjects per cigarette group would allow for the detection of an ES of 0.60 or higher with minimum statistical power of 90% for a two-sided, two-sample test where the significance level is 0.05. For the within subject comparisons defined by the 2 factors for the e-cig characteristics, an ES of 0.5 or more for the two main effects would have at least 97% power in a repeated measures analysis if the sample size is 60 per cigarette group with a significance level of 0.05. Study enrollment will continue until the number of subjects completing all 5 sessions reaches 60 in each group (120 total). Sample size and power calculations were carried out using PASS 13 (NCSS, LLC. Kaysville, Utah, 2014).

4. Study Populations

4.1. Intent-to-treat

The primary analysis of the between subject endpoints will adhere to the intent-to-treat principle. Under this principle, all subjects randomized to either the VLNC or NNC arm will be included in the analysis in the group to which they were randomized regardless of protocol violations and compliance to treatment assignment.

4.2. Per-Protocol

A secondary per-protocol analysis will include only participants who complete all 5 sessions.

5. Trial Endpoints

5.1. Primary Endpoint

- Number of cigarette puffs taken: Among the 5 sessions, the first session is always cigarettes only, no e-cigs, and each of the 10 choices is between taking 2 cigarette puffs or abstaining from smoking. At each session where e-cigs are available (i.e., Sessions 2-5), each of the 10 choices is between taking 2 cigarette puffs, 2 e-cig puffs, or abstaining from both. The maximum number of cigarette puffs taken will be 20 for each of the five sessions.

5.2. Secondary Endpoints

- Number of e-cig puffs taken (0 to 20)
- CO boost

5.3. Exploratory Endpoints

- Number of choices for cigarette puffs (0 to 10)
- Number of choices for e-cig puffs (0 to 10)
- Number of choices to abstain from puffing (0 to 10)
- Measures of compliance: drop-out rate
- Measures of discomfort/dysfunction: MNWS, QSU, PANAS
- Measures of cigarette characteristics: CES, Cigarette Purchase Task indices
- Measures of perceived risk: Perceived Health Risk Scale
- Measures of cardiovascular function: heart rate, blood pressure
- Biomarkers: TNE, NMR, Cotinine, CO

5.4. Safety Endpoints

- Adverse Events (AEs)
- Serious adverse events (SAEs)

6. Statistical Analysis

6.1. General Approach

The primary objective of this trial is to compare the effects of VLNC and NNC cigarettes, and of the availability and characteristics of e-cigs, on preference for cigarette puffs, e-cig puffs, or puffing abstinence. The within subject factors (between sessions) will allow for the comparison of two levels of nicotine concentration in e-cigs and two categories of e-cig flavors in a 2 x 2 factorial design. Statistical analyses will be performed using SAS or R. All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below. All statistical tests will be two-tailed and a p-value less than 0.05 will be considered significant for all endpoints.

6.2. Describing the Study Population

Demographics and baseline data will be compared between the groups randomized to NNC or VLNC cigarettes. This will include the following: demographic characteristics (age, sex, race, ethnicity, education), tobacco use history (e.g. cigarettes per day, menthol status, vaping frequency, other tobacco product use), TLFB and nicotine dependence (PATH, total score on FTND), etc. Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by two-sample tests (t-test or Wilcoxon rank sum test). Categorical covariates will be summarized by frequencies and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate.

6.3. Primary Endpoint Analysis

The primary endpoint is the total number of puffs of their study cigarette taken at each of the 5 laboratory sessions. The participant can have up to 20 puffs on the cigarette and, therefore, the result is an integer that ranges from 0 to 20.

6.3.1. Primary Analysis

Descriptive statistics will summarize the number of cigarette puffs by cigarette group and by session. Results will be presented in tabular and graphical format. The primary statistical model will be a linear mixed model (2) carried out according to the 2 x 2 x 2 design, where the between-subject factor is NNC or VLNC cigarettes and the two within-subject factors relate to e-cig flavor (cigarette or non-cigarette flavors) and nicotine content (very low or moderate). The session where no e-cigs are included will be analyzed separately by comparing the normal and reduced nicotine cigarettes groups using linear regression adjusting for baseline CPD from TLFB to improve precision. Sandwich standard

errors will be used to account for potential heteroscedasticity. Data from the four sessions where e-cigs are available will be analyzed using a linear mixed model to account for potential correlation between repeated measures on an individual. The mixed model for the balanced factorial design will allow for the evaluation of the three main effects and their interactions. The major concern for this approach is the occurrence of non-constant variance for the number of cigarette puffs. Therefore, sensitivity analysis will be performed to evaluate the robustness of our assumptions. A generalized estimating equation (GEE) model uses sandwich estimators and thus is less affected by unequal variances, however, is more negatively impacted by the occurrence of missing observations. Additionally, a negative binomial regression approach will check if modeling the number of puffs as count variables provides a better fit to the data. The first mixed model will assume that participant characteristics are balanced between groups due to randomization and include only the three main effects and their interactions along with the stratification variables (gender, menthol status, and CPD [continuous, from the BL TLFB]) and the number of cigarette puffs from session 1 to increase precision.

6.3.2. Secondary Analysis

As a secondary analysis, the above analysis will be repeated accounting for any baseline imbalances. This will include covariates provided in Section 6.2 that differ across cigarette groups (VLNC, NNC) at baseline with a p-value less than 0.20.

6.4. Secondary Endpoint Analysis

Secondary endpoints will be summarized by cigarette group and lab session using the mean and standard deviation. Number of puffs of e-cigs taken are measured in sessions 2 to 5, where e-cigs are available. CO boost (i.e., post-choice CO minus pre-choice CO) is measured at every session (1 to 5). The analysis of the secondary endpoints will be a linear mixed model following the same 2 x 2 x 2 factorial design outlines in section 6.3.1. First with only main effects and interaction terms along with the stratification and the baseline values to increase precision, and then a second model adding in any important baseline characteristics as potential moderators of study outcomes.

6.5. Exploratory Endpoint Analysis

The exploratory endpoints include the number of choices for cigarette puffs and e-cig puffs, number of choices to abstain from puffing, CES, Perceived Health risks, QSU, MNWS, PANAS, measures of cardiovascular function and biomarkers. We expect that biomarkers of exposure, and possibly other endpoints, will be skewed and will be log-transformed for analysis. These variables will be summarized using the geometric mean and differences between groups will be summarized by ratios of geometric means and 95% confidence intervals (CI). Any measurement that is obtained multiple times across lab sessions will be analyzed using a linear mixed model to

account for the within subject effect. Drop-out rates will be compared between the two cigarette groups with a chi-squared test.

6.6. Safety

AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared between the two cigarette groups. We expect AEs and SAEs to be extremely rare in this trial and, therefore, no formal statistical comparison of the rate of AEs and SAEs across treatment groups is planned for this trial.

6.7. Missing Data and Outlier Analysis

Every effort will be made to limit the amount of missing data in this trial. Study participants will be incentivized to attend the study sessions. That said, some level of missing data is inevitable in a study of this kind. We will compare subjects who do and do not complete the study sessions in order to identify baseline covariates associated with study completion. We will also complete a sensitivity analysis of primary and secondary endpoints using multiple imputation with Markov Chain Monte Carlo (MCMC) method (4) carried out in PROC MI in SAS. If the cigarette group is associated with missing data, we will conduct multiple imputation for each cigarette group separately. Values can be imputed for both continuous and categorical variables. Following imputation, standard statistical methods can be applied. A final single assessment of treatment arm difference will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS. These analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

6.8. Interim Analyses

No interim analysis is planned.

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