Evaluating E-Cigarette Nicotine Form, Concentration, and Flavors among Youth

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Abstract

Electronic cigarette (e-cig) "vaping", while being promoted as a safer alternative to conventional cigarettes, has disproportionally attracted adolescents and young adults ("youth"). E-cigs are now the most commonly used tobacco product among youth[1], yet little research has examined youth e-cig use, addiction, health effects or strategies to help current users quit. Making matters worse, e-cigs and e-liquids continue to evolve rapidly in a minimally regulated market, resulting in a product with increasing youth appeal and addictiveness, while at the same time exposing youth to cardiovascular and pulmonary toxicants during a sensitive and critical time of development.

Over the last two years, there has been a significant surge in e-cig sales and use among youth. Regulatory and research efforts have largely focused on e-liquid flavors, that while important, do not appear to be the catalyst for the surge. Flavored e-cigs have been on the market since the late 2000s, but in a market saturated with these products, youth e-cig use declined by 29% during 2015-2016, where it remained through 2017[2]. It was not until the proliferation of nicotine-salt based (NSB) e-cigs, of which the most well-known is JUUL, that youth e-cig use increased by 135% to its current record high (28%)[1]. Unlike previous e-cigs that relied on unprotonated, or "free-base" (FB) nicotine e-liquid, NSB devices use protonated forms (i.e., nicotine salts) by lowering the pH to protonate nicotine with an acid. Because this form of nicotine is not as harsh as the free-base form, inhaling high doses of protonated nicotine is much more palatable[3]. This allows "tobacco naïve" users to inhale high levels of nicotine, increasing their odds of becoming addicted to nicotine. The tobacco industry has utilized similar methodology to alter the pH of cigarette smoke to improve palatability and nicotine delivery[4]. Making conventional cigarettes easier to inhale was associated with more aggressive and frequent puffing and increased cardiovascular and pulmonary disease risk[5]. Whether this holds true for NSB devices is suspected but not determined.

While the long-term health effects of e-cigarettes are still unclear, studies examining proximal biomarkers of health indicate that e-cig users are exposed to many cardiovascular and pulmonary toxicants, including carcinogens, such as carbonyls, furans, reactive oxygen species, and heavy metals[6, 7]. Early evidence indicates that e-cig users experience adverse health effects, including increased oxidative stress, respiratory and mucosal inflammation, arterial stiffness and altered hemodynamics and platelet activity[8]. Moreover, use of nicotine by youth can adversely affect attention, learning, and memory, as well as lead to increased impulsivity. Taken together these data suggest that sustained e-cig use will likely have notable adverse health effects[9]. Since their introduction in 2015, to our knowledge, no studies have examined the toxicological and physiological effects of NSB device use, nor the impact of nicotine form. Previous research has largely focused on free-base nicotine devices, with only a few focusing on pulmonary and cardiovascular effects.

The youth vaping epidemic in the U.S. is drawing serious concern from parents, schools, health officials and regulators. There is now a wave of support and a need to create effective e-cig policies and regulations to prevent further proliferation of youth e-cig use. One of the most effective policies may be to create a product standard for nicotine to reduce the palatability and abuse liability of e-cigs for youth;

however, strong scientific evidence is needed for the FDA to impose such regulations. The proposed project will begin to address this need.

A. Specific Aims

Utilizing a human laboratory design, we will examine the influence of nicotine form and concentration, and e-liquid flavor on youth vaping behavior, nicotine uptake, abuse liability, toxicant exposure, and acute cardiovascular and pulmonary effects. H1a: Nicotine salt (vs. free-base) and menthol flavored e- liquids (vs. tobacco flavored) will result in puffs of longer duration and higher flow rate, and a greater total inhaled aerosol volume, and H1b: demonstrate greater nicotine uptake, abuse liability, and adverse cardiovascular and pulmonary effects. H1c: High concentration, free-base nicotine (vs. salt nicotine) will result in puffs of shorter duration, lower flow rate, less volume, and lower abuse liability.

B. Significance

B.1. The Resurgence of E-cigarettes Among Youth and Adults

The risk-benefit of e-cigs continues to be debated in the public health literature. E-cigs are now the most commonly used tobacco product in the U.S. among youth and young adults, and anticipated to exceed conventional cigarette use by 2020[1]. The most recent estimates indicate that 10.8 million US adults (4.5%) have used an e-cig in the past 30 days[10]. Among high school students and young adults (18-24 years), these rates are even higher, at 4.1 million (27.5%) and 2.8 million (9.2%), respectively[1, 2]. Moreover, e-cig prevalence rates have increased significantly over the last two years, nearly doubling for youth and young adults, with the emergence of nicotine salt-based (NSB) devices[1]. Indeed, several recent studies, including those conducted by our team[11-13], suggest that NSB devices are not only seen as more appealing due to their small, sleek styling, and smooth delivery of high doses of nicotine, but that youths and adults who try NSB devices are far more likely to become regular users compared to those who try FB e-cigs, with approximately 40-60% of those who ever tried an NSB device, identifying as current users[11-13]. NSB devices have led to a resurgence in e-cig use among youth and adults alike. The combination of palatable, high doses of nicotine and small, light-weight, and reliable devices, has led to a product with high initiation and adoption potential across all age groups.

B.2. The Possible Role of E-cigarette Liquid pH: What We Have Learned From Cigarettes

In cigarette smoke, nicotine is present in protonated (i.e., less bioavailable) or unprotonated (i.e., free-base, more bioavailable) forms[14, 15]. The proportion of protonated vs. unprotonated nicotine in smoke is pH dependent; when nicotine is at a higher pH (more alkaline or basic), a larger proportion of it is in its unprotonated form, which has much greater bioavailability, becoming more easily and rapidly absorbed across biological membranes[15]. An examination of tobacco industry documents, uncovers that tobacco manufacturers focused heavily on the pH of cigarette smoke, developing numerous techniques to systematically increase the pH and desired level of FB nicotine delivery[16]. Cigarette brands with the highest smoke pH, were consistently the top sellers[16-18]. Tobacco industry research reported that “as much as 50% of the variation in [cigarette] sales could be a consequence of smoke pH[14].” Increasing the

pH of aerosolized nicotine, resulted in greater and more rapid nicotine delivery—both known to be critical in the development of addiction[14, 19]. Interestingly, tobacco industry documents also suggest that Phillip Morris in the race to increase levels of FB nicotine, had to course-correct and reduce the pH in a product that was too harsh and not acceptable to consumers [16]. As such, industry studies suggest that there is a trade-off between the level of FB nicotine and the harshness of the smoke[20, 21]. The pH breakpoint where the smoke becomes noticeably harsh and undesirable to smokers was determined to be above 7.0[17]. Consequently, tobacco manufacturers continued to refine cigarettes to balance nicotine delivery with a "smooth" smoking experience[20]. Today, cigarette smoke pH ranges from 6-7.8[22], indicating that 4.5%-29% of the total nicotine is in FB form[15, 23].

Early E-cig models relied on e-liquid with higher pH levels and consequently high levels of FB nicotine. In one study, greater than 50% of all nicotine-containing e-liquids had a pH >9.0 and only 2 samples tested had a pH of <7.0[24]. To account for this high pH level, and the harshness of FB nicotine, manufacturers had to lower the concentration of nicotine in their e-liquids (typically, 1.2-2.4% nicotine). However, these products were typically perceived to be less satisfying and palatable to smokers compared to combustible cigarettes[25]. Moreover, as manufacturers began developing e-cig devices with significantly increased power (wattage) delivered to the heating coil (5- to 10-fold greater) in an effort to improve nicotine delivery[26], users began to accommodate the increased wattage by decreasing the concentration of their high pH e-liquid (0.3-0.6%) to reduce harshness[26]. This strategy of high wattage and low nicotine concentration was successful in terms of improved nicotine delivery, but also had several unanticipated and negative effects[26]. Increased power led to increased user exposure to tobacco toxicants due to greater production of carcinogenic compounds in the e-cig aerosol, and compensatory puffing, as users needed to take larger puffs to achieve satisfactory levels of nicotine delivery[26-30].

By lowering the pH and protonating the nicotine in e-liquid, inhaling high concentrations (3-6%) of protonated nicotine (i.e., nicotine salts) is much more palatable, enabling low-powered e-cig devices (<10 watts) to provide nicotine delivery comparable to a cigarette[31-33]. This combination of high concentrations of protonated nicotine but low power may be beneficial in terms of reduced exposures to tobacco-related toxicants[32], because lower coil temperatures reduce the amount of toxicants produced, and still give high levels of nicotine per puff[32], reducing the number and size of puffs (smaller puff volume) needed to achieve satisfying nicotine levels[34]. On the other hand, with smaller, easily hidden devices (nicotine salt devices are often smaller since their low power requirement allows for smaller batteries), improved sensory experience, and higher concentrations of nicotine, use of nicotine salt e-liquid may result in: 1) more frequent dosing; 2) more intense puffing (faster flow rate, longer puff duration), with potential for greater deposition and absorption of nicotine deeper in the respiratory tract influencing lung cancer risk; and 3) greater nicotine delivery, abuse liability, and appeal[34, 35]. Indeed, support for the possibility of more frequent dosing and/or more intense puffing is the finding from a recent study demonstrating that adolescent users of nicotine salt devices exhibited higher mean urinary levels of the nicotine metabolite cotinine (244.8 ng/mL) than what is typically seen among adolescent smokers (155.2 ng/mL)[36, 37.]

Lastly, the tobacco industry, especially in the manufacturing of smokeless tobacco products, manipulated pH to develop "starter products"—the industry's term for products that are highly flavored, with a combination of low pH and low nicotine[19]. These products were developed to facilitate tobacco product initiation and to increase uptake and addiction[19]. Over time, as users developed tolerance to nicotine, they were able tolerate products with higher levels of biologically available nicotine[19]. While JUUL, the e-cig market leader with approximately 50% of unit sales and nearly 80% of the estimated market dollar share[36], originally only contained e-liquid with a nicotine concentration of 5%, the company now offers a lower nicotine concentration pod (3% nicotine), which is strangely higher than 20 mg/mL permitted for e-liquids sold in the European Union[36]. JUUL has provided no rationale for the lower nicotine concentration pod. Given that the 5% nicotine pod has demonstrated nicotine delivery and subjective effects that almost completely mimic a combustible cigarette[36], the potential for compensation (i.e., greater consumption rate for the 3% nicotine pod) is high. Additionally, it is likely that with reduced nicotine concentration, these devices will be even more palatable, especially to nicotine naïve users. In summary, controlled studies examining the interaction between e-liquid pH and nicotine concentration are critical to help public health officials determine how manipulation of these levels influence users' device power settings, subjective effects and puffing topography, as they are critical in the determination of abuse liability and exposure to harmful tobacco toxicants and disease risk.

C. Approach and Preliminary Studies

Our team has conducted five studies examining NSB e-cig devices[11-13], including two recently completed human laboratory studies[11, 12]. Results of these studies demonstrated that NSB e-cigs can deliver cigarette-like levels of nicotine[28, 38], have the potential for significant levels of addiction[11-13], and that lower e-liquid pH was associated with reduced perceived harshness of the aerosol ($p<.05$), increased nicotine uptake ($p<.05$), and increased puffing flow rates and average puff volumes ($ps<.05$).

D. Research Design and Methods

i. Design Overview

This study (Figures 1 and 3) will include Option A consisting of 9 lab visits each approximately 2 hours long, except visit 1, which will last up to 3 hours ***OR*** Option B consisting of 5 lab visits each containing 2 vaping sessions separated by a 3-hour washout period and lasting approximately 6 hours. Figure 2 illustrates the study e-cig and e-liquid variations. Figure 3 depicts the sequence of the proposed study. We will conduct an experimental study examining how the manipulation of nicotine form (nicotine salt (NSB) vs. free-base (FB)) influences vaping behavior, abuse liability, toxicant exposure and heart and lung health.

Figure 1. Vaping Session

Vaping Session	
12 hour nicotine abstinence	
Start of Session:	Surveys Respiratory Measures Cardiovascular Measures Blood Draw (0 min)
STD Puffing (5 min):	Blood Draw (5 min) Surveys
Ad-lib Puffing (30 min):	Blood Draw (10 min) Blood Draw (35 min) Surveys
3-hour washout (Visits 2-5 between vape sessions) (Option B Only)	
3 hrs. post exposure:	Respiratory Measures Cardiovascular Measures (Option B Only)
1 vaping session will occur at each visit for 9 visits (Option A Only)	
1 vaping session will occur at the first visit / 2 vaping sessions will occur at the final 4 visits (Option B Only)	

Figure 2. E-cig and e-liquid for Vaping Sessions

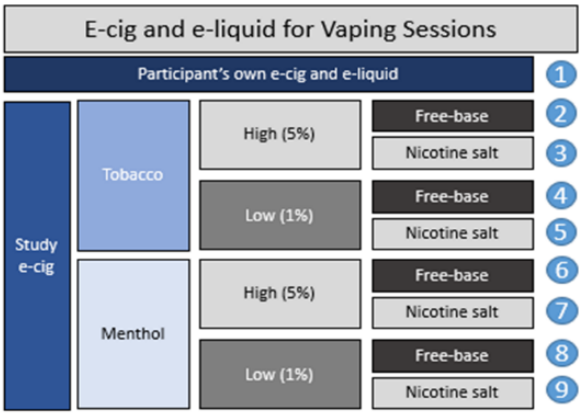
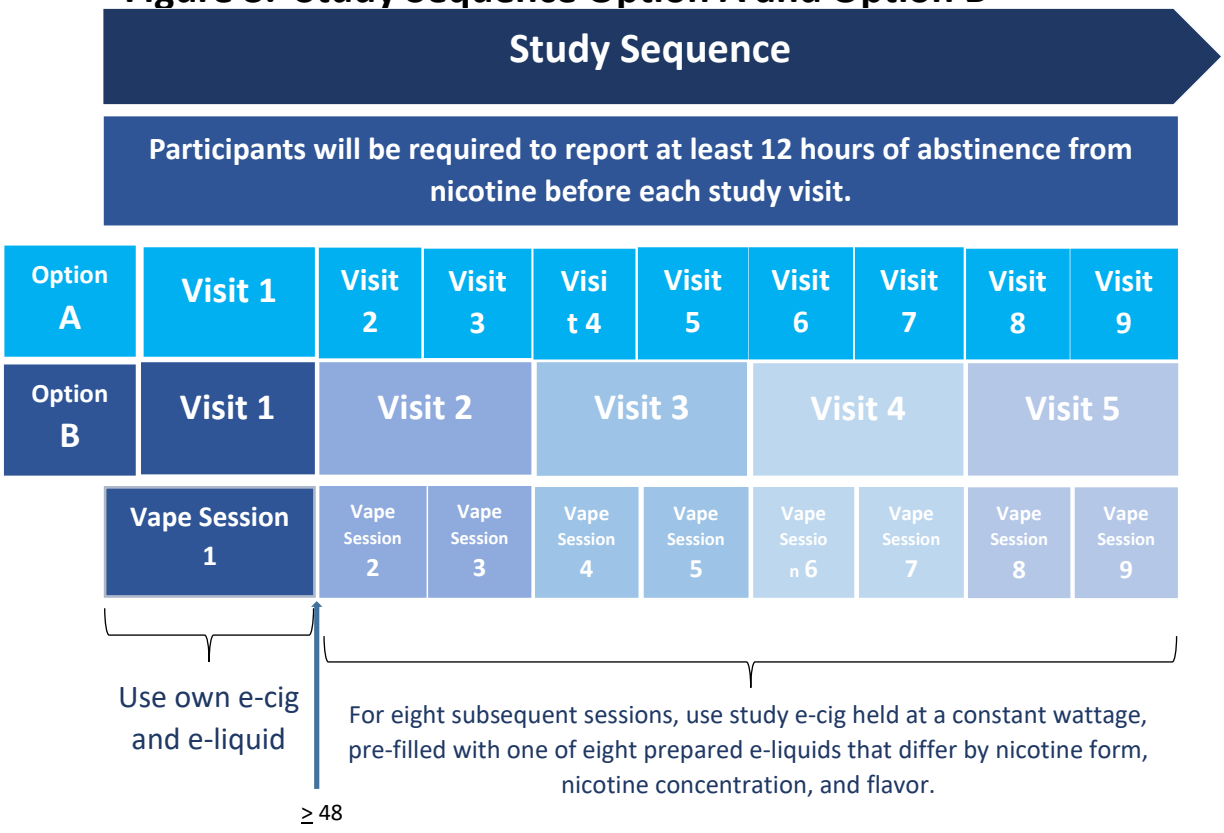


Figure 3. Study Sequence Option A and Option B



ii. Study Procedures

Utilizing a within-subjects, factorial design, 60 e-cig users (aged 21-25 years) will complete vaping sessions which will include a standardized, 5-minute, 10-puff vaping bout (30 seconds between each puff) followed by 30 minutes of ad libitum vaping. Prior to session one, participants will be asked to bring their own e-cig device and a full tank/pod to the first visit. In session one, participants will use their own e-cig and e-liquid. After visit 1, participants will take home a study e-cig device and 1 weighed pod pre-filled with study e-liquid (the participant was randomized to at the end of visit 1) to practice (20 puffs per day with the study device and until the participant feels comfortable) using the device before visit 2. Practice use with the study e-cig device and pre-filled pod will be verified based on data downloaded (via eScribe) from the device during visit 2. In the subsequent vape sessions, participants will use a study e-cig held at a constant wattage, and pre-filled with e-liquid of different nicotine form (free-base vs. nicotine salt), concentration (low-1% vs. high-5%), and flavoring (tobacco vs. menthol). There is no known commercial pair of nicotine liquids that differ only by nicotine form. For this study, we will prepare 8 e-liquids that differ by nicotine form, nicotine concentration, and flavor, simulating characteristics of popularly used nicotine liquids[32, 39]. E-liquids will be prepared from pure (≥99%) S-(-)-nicotine; protonation and dilution of nicotine will be done using USP grade benzoic acid, and glycerol and propylene glycol, respectively. We will submit an Investigational Tobacco Product application to the U.S. FDA regarding the clinical use of the e-liquids. All sessions will be completed over nine visits for schedule Option A and 5 visits for schedule Option B. Study Schedule A consist of 9 separate visits and 9 separate vaping sessions. Study schedule B will consist of five separate visits (each visit will include up to 2 vaping sessions) each separated by a 3-hour washout period to allow nicotine levels to return to baseline. Each visit will be at least 48 hours apart. Participants will be required to report at least 12 hours abstinent from nicotine before each study visit. Participants will be told that their abstinence will be confirmed at the time of visit via blood plasma nicotine analysis. This is a partial bogus pipeline[40]; 3mL venous blood sample will be collected for later analysis.; those with plasma nicotine levels >3ng/mL at the time of their visit will be replaced post hoc with another participant. We conservatively estimate replacing 10% of participants. Measures of topography, nicotine uptake, abuse liability, subjective effects, and cardiovascular and pulmonary effects will be collected (see Table 1). Exposure to select toxicants, including nicotine and menthol in total particulate matter, and gas phase carbonyls and volatile organic compounds, will also be estimated post hoc using puff playback machine smoking.

1. Recruitment Feasibility and Retention

a) Recruitment

We intend to recruit 130 e-cig users from the community over an 18-month period and have them complete nine study visits (Option A) or five study visits (Option B); therefore, we need 3-4 participants to be recruited and complete study procedures per month.

We are confident our recruitment approach, laboratory facilities, and equipment redundancy will result in a sufficient participant processing rate, given our successful completion of other tobacco-related research of similar design[41]. E-cig users will be recruited from advertisements through a variety of media outlets and the internet, including Study Search, as well as community events. Participants from

other studies who have agreed to be contacted regarding other study opportunities will also be contacted. Staff from those studies will prepare contact letters/emails and call participants on behalf of this study. Interested participants will be referred to this study for screening. Participants will access the screening questionnaire using a public survey link generated by REDCap. Based on our team's previous studies, we conservatively assume a 20% attrition rate. Thus, we need to recruit 130 participants to have 60 complete the study. Participants who meet the following eligibility criteria will be asked to take part in the study.

Inclusion criteria: 1) a current e-cigarette user (≥1 vaping bout per day) for at least the past 3 months , 2) 21-25 years old, 3) willing to abstain from all tobacco and nicotine for at least 12 hours prior to lab sessions, 4) willing to complete nine lab visits/vaping session lasting up to 2 hours each or five lab visits lasting up to 6 hours each (except Visit 1) , 5) able to read and speak English, 6) willing to provide informed consent

Exclusion criteria: 1) self-reported diagnosis of lung disease including asthma, cystic fibrosis, or chronic obstructive pulmonary disease, 2) history of cardiac event or distress within the past 3 months, 3) currently pregnant (determined using urine pregnancy test), planning to become pregnant, or breastfeeding, 4) use of other tobacco products >10 days in the past month, 5) current marijuana use >10 times per month, 6) currently engaging in a vaping cessation attempt.

Participants' eligibility will be determined over the phone or via REDCap's online screener. Those who are eligible and willing to participate will be invited to sign an informed consent and complete their baseline visit in a private participant room at the Ohio State University. All participants will be given adequate time to review the informed consent with a trained research staff to help answer any questions that may arise during the consent process. Additionally, a copy of the informed consent will be given to all participants. A pregnancy test will be completed at the initial visit as well as before starting all the in-lab visits to ensure that the participant is not pregnant.

b) Retention and Compensation

If participants choose Option A, they will receive $50, $50, $50, $50, $75, $75, $75, $75, and $100 for completing visit 1, 2, 3, 4, 5, 6, 7, 8, and 9, respectively, for a total of $600. Participants who choose Option B will receive $50, $100, $125, $150, and $175 for completing visit 1, 2, 3, 4 and 5, respectively, for a total of $600. Consistent with our previous studies, payments will be made using the Greenphire ClinCard to increase accountability and facilitate ease of payment. We will facilitate visits by offering weekend appointments and using additional retention strategies (e.g., reminder calls/texts/emails). Participants will receive reminder calls in addition to email or text reminders. Reminders will be sent by text or email based on a participant's preferred method of contact.

2. Detailed Study Procedures

a) Study Sessions

Prior to the start of the first laboratory visit, twelve-hour tobacco abstinence will be assessed via self-report and confirmed with exhaled carbon monoxide testing (eCO≤ 10ppm). A research assistant will

explain the study to the participant and answer any questions they may have. The participant will give an exhaled breath sample into the handheld CO monitor to determine if they are eligible to participate in the laboratory vaping session. If the participant's exhaled CO concentration $\leq$10 ppm, they are deemed eligible to participate further, and will be asked to read the informed consent form. Once all participant questions have been answered, the participant will be asked to sign their consent to participate in the study. Pregnancy exclusion will also be confirmed with a urine test. Participants will be asked to avoid eating (anything), drinking coffee, tea, sodas, juice, alcohol, and smoking (including any cigarette/nicotine replacements such as electronic cigarettes) at least 3 hours prior to their visit since that will affect the results of certain measures. Participants will not be allowed to eat or drink (other than water) during the vaping sessions, but will be provided a light snack prior to IV placement and the start of vaping sessions. Participants will be allowed to eat and drink during the 3-hour washout periods for Option B visits 2-5.

First, participants will complete background measures (Sociodemographic Measures, Tobacco Use History (including the Timeline Followback (TLFB) interview– week, month, lifetime and the Sensory E-Cigarette Expectancies Scale (SEES)[42]), and the E-Cig Dependence (modified Cigarette Dependence Scale)[43]. To measure nicotine uptake, a forearm venous catheter (placed by the research nurse) will be used to sample blood (~5 mL) as participants complete an initial 30-minute ad libitum vaping session using their own e-cig device and e-liquid. Blood will be collected at 4 time points (t=0, 5, 10, and 35 minutes) during each vaping session.

Markers of endothelial dependent and independent function will be measured by B-mode ultrasound[44]. Arterial stiffness, blood pressure, and heart rate will be measured at baseline. Arterial stiffness and vascular reactivity will be measured with pulse wave velocity (Vicorder or similar) and pulsewave-analysis (AiX75, SphygmoCor, or similar)[45]. Inflammation will be measured by plasma levels of IFN- β, IL-1β, IL-17, TNF-α as well as markers of oxidative stress, including OxLDL and Malondialdehyde[46]. Laboratory spirometry, airway inflammation, and airway reactivity[47-49] will be measured. Laboratory Spirometry will be conducted in a standing position and according to the recommendations of ATS Clinical Guidelines[47]. Forced expiratory volume (FEV1), forced vital capacity (FVC), and FEV1/FVC. Airway Inflammation will be assessed using exhaled nitric oxide via the NIOX VERO. The current literature shows immediate changes in airway inflammation immediately follow e-cig use[48]. Airway Reactivity will be measured via TremoFlo, which is a well validated measurement of central obstruction, peripheral obstruction, and dynamic collapse[49] and provides curves of Resistance (R) and Reactance (X) as well as parameters reflecting large and small airway function. The current literature show an increase in impedance, respiratory resistance and overall peripheral airway resistance following e-cig use[48].

In visit 1, which will last up to 3 hours, participants will use their own e-cig and e-liquid and participate in a single vaping session. After visit 1, participants will take home a study e-cig device and 1 weighed pod pre-filled with tobacco flavored e-liquid (the participant is randomized to at the end of visit 1) to practice (20 puffs per day with the study device and until the participant feels comfortable) using the device before visit 2. Practice use with the study e-cig device and pre-filled pod will be verified based on data downloaded (via eScribe) from the device during visit 2. After visit 1, for the subsequent visits

participants will use a study e-cig held at a constant wattage, and pre-filled with e-liquid of different nicotine form (free-base vs. nicotine salt), concentration (low-1% vs. high-5%), and flavoring (tobacco vs. menthol). There is no known commercial pair of nicotine liquids that differ only by nicotine form. For this study, we will prepare 8 e-liquids that differ by nicotine form, nicotine concentration, and flavor, simulating characteristics of popularly used nicotine liquids[32, 39]. Prior to each visit, e-liquids will be prepared from pure (≥99%) S-(-)-nicotine; protonation and dilution of nicotine will be done using USP grade benzoic acid, and glycerol and propylene glycol, respectively.

E-cig abuse liability will be measured across several domains during and after the vaping session. During the vaping session, e-cig puff topography will be measured to produce measures of puff count, puff duration, inter-puff-interval, puff flow rate, average puff volume, and total puff volume[50]. Post vaping session, subjective effects including the Drug Effects/Liking Questionnaire and modified Cigarette Evaluation Questionnaire (mCEQ)[51, 52] will be conducted.

Next, behavioral economic demand will be assessed using the E-Cigarette Purchase Task[53] and E-Cig craving/suppression of craving and withdrawal will be assessed using the Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form[54], and the Minnesota Nicotine Withdrawal Scale[55]..

E-liquid consumption will be determined by weighing (mg) the participant e-liquid pod before and after the vaping session. To estimate toxicant exposure, the e-cig device, e-liquid, and participants' average puffing topography data for that e-liquid (across all participants) will be used to control a vaping machine. The e-cig aerosol produced will be collected on filters or in impingers for later analysis using high performance liquid chromatography, gas chromatography/mass spectrometry or similar methods of instrumental analysis.

E-cig device characteristics, including device voltage, coil resistance, and pressure drop; and e-liquid characteristics including pH, nicotine concentration, and PG/VG ratio will be determined. Since participants will primarily use the study e-cig and e-liquid for this study, to determine exposures associated with the least and most intense puffing behaviors, topographies in the upper tertile will be averaged to produce a single topography, and likewise for the lowest tertile. Machine vaping will be conducted for each e-liquid type, using the associated human-derived puffing regimens, to estimate the range of tobacco toxicant exposure.

For the 8-test e-liquids, these measures will be repeated during each of the visits. Study schedule A consists of 9 separate visits and 9 separate vaping sessions. Study schedule B consists of 5 visits. The first lasting about 2 hours and the subsequent visits 2-5 lasting about 6 hours each, 2 vaping sessions each visit, and each session separated by a 3-hour washout period to allow nicotine levels to return to baseline.

After each visit has been completed, participants will sign to confirm they have received $50, $50, $50, $50, $75, $75, $75, $75, and $100 for completing visit 1, 2, 3, 4, 5, 6, 7, 8, and 9, respectively, for a total of $600 if they chose Option A or received $50, $100, $125, $150, and $175 for completing visit 1, 2, 3, 4

and 5, respectively, if they chose Option B. Participant compensation will be loaded on a Greenphire ClinCard.

b) Study Products

For the first study session, participants will vape using their own e-cig and e-liquid. After visit 1, participants will take home a study e-cig device and 1 weighed pod pre-filled with study e-liquid (the participant was randomized to at the end of visit 1) to practice (20 puffs per day with the study device and until the participant feels comfortable) using the device before visit 2. Practice use with the study e-cig device and pre-filled pod will be verified based on data downloaded (via eScribe) from the device during visit 2. In the subsequent sessions, participants will use a study e-cig held at a constant wattage, and pre-filled with e-liquid of different nicotine form (free-base vs. nicotine salt), concentration (low-1% vs. high-5%), and flavoring (tobacco vs. menthol). There is no known commercial pair of nicotine liquids that differ only by nicotine form. We will prepare 8 e-liquids that differ by nicotine form, nicotine concentration, and flavor, simulating characteristics of popularly used nicotine liquids[53, 54]. E-liquids will be prepared from pure (≥ 99%) S-(-)-nicotine; protonation and dilution of nicotine will be done using USP grade benzoic acid, and glycerol and propylene glycol, respectively.

c) Authentication of Key Biologicals and/or Chemical Resources

E-liquids

E-liquids are prepared using USP-grade ingredients, when available, in an onsite clean room at Flagship Vapor Company, or similar clean-room facility. We will conduct independent chemical analysis and testing of these liquids via proton nuclear magnetic resonance spectroscopy (HNMR) to confirm appropriate quantities of nicotine, propylene glycol, glycerol, benzoic acid and menthol. We will submit an Investigational Tobacco Product application to the U.S. FDA for the e-liquids. Both MPIs Wagener and Shields and Co-I Brinkman have submitted ITP applications to FDA resulting in an FDA letter of no further concerns regarding their use in clinical trials (U01DA045537; U01DA045530; R01CA209961; R01CA229306).

Chemicals and other molecular reagents

All reagents necessary for the completion of chemical and molecular assays in the proposed study will be procured and stored by shared resources at OSU (NPASR and PhASR) and VCU Bioanalytic Corp under established standards and protocols. Certificates of analysis will be maintained, and reagents will be stored as specified, and used prior to expiration.

3. Protocol Adherence and Quality Control

Data Management

All data collection will follow HIPAA guidelines. Data will be collected directly from the participant by a research assistant and/or research nurse. Data will include participant responses to computer-based survey questionnaires, exhaled breath CO, blood sample collections, e-liquid pod container weights, e-

liquid characteristics, e-cig device characteristics, physiological (pulmonary) effects, cardiovascular measures, markers of endothelial dependent and independent function, and puff topography.

Quality Assurance

All research staff will have completed Human Subjects and HIPAA training. Standard operating procedures (SOP) will be developed, and all staff will be trained to ensure adherence to the SOP. As is standard practice for our team's current studies, each visit will have its own checklist of specific measures to be completed and the order in which they are to be administered. To reduce data entry errors, participants will enter data into secured computer-based questionnaires. All specimens collected for biomarker analysis will be given individualized bar codes. All key on-site personnel will meet face-to-face weekly throughout the entire study. During these meetings, recruitment, enrollment, data collection, data monitoring results, and any concerns/issues will be discussed.

iii. Measures

All measures (see Table 1) are validated and have been used previously by our team. Sociodemographic measures and tobacco use history (including the Timeline Followback (TLFB) interview– week, month, lifetime and the Sensory E-Cigarette Expectancies Scale (SEES)[42]) will be collected in addition to e-cig dependence using the modified Cigarette Dependence Scale[43]. E-cig Abuse liability will be measured across several domains, including 1) E-cig puff topography to produce measures of puff count, puff duration, inter-puff-interval, puff flow rate, average puff volume, and total puff volume[50], 2) Subjective effects, including the Drug Effects/Liking Questionnaire and modified Cigarette Evaluation Questionnaire (mCEQ)[51, 52], 3) Behavioral economic demand using the E-Cigarette Purchase Task[53], and 4) E-cig craving/suppression of craving and withdrawal using the Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form[54] and Minnesota Nicotine Withdrawal Scale[55]. Nicotine uptake: a forearm venous catheter will be used to sample blood (~5 mL). Blood will be collected at 4 time points (t=0, 5, 10, 35 min) during each vaping session. E-liquid consumption: e-liquid containers for pods will be weighed (mg) before and after each vaping session. Toxicant exposure: to estimate toxicant exposure, the e-cig device, e-liquid, and participants' average puffing topography data for that e-liquid (across all participants) will be used to control a vaping machine. The e-cig aerosol produced will be collected on filters or in impingers for later analysis using high performance liquid chromatography, gas chromatography/mass spectrometry or similar methods of instrumental analysis. E-cig device characteristics, including device voltage, coil resistance, and pressure drop; and e-liquid characteristics including pH, nicotine concentration, and PG/VG ratio will be determined. Since participants will primarily use the study e-cig and e-liquid to determine exposures associated with the least and most intense puffing behaviors, topographies in the upper tertile will be averaged to produce a single topography, and likewise for the lowest tertile. Machine vaping will be conducted for each e-liquid type, using the associated human-derived puffing regimens, to estimate the range of tobacco toxicant exposure. Laboratory spirometry, airway inflammation, and airway reactivity[47-49] will be measured. Laboratory spirometry will be conducted in a standing position and according to the recommendations of ATS Clinical Guidelines[47]. Forced expiratory volume (FEV1), forced vital capacity (FVC), and FEV1/FVC. Airway inflammation will be assessed using exhaled nitric oxide via the NIOX VERO. The current literature shows immediate changes

in airway inflammation immediately follow e-cig use[48]. Airway reactivity will be measured via TremoFlo, which is a well validated measurement of central obstruction, peripheral obstruction, and dynamic collapse[49] and provides curves of Resistance (R) and Reactance (X) as well as parameters reflecting large and small airway function. The current literature shows an increase in impedance, respiratory resistance and overall peripheral airway resistance following e-cig use[48]. Markers of endothelial dependent and independent function will be measured by B-mode ultrasound[44]. Arterial stiffness, blood pressure, and heart rate will be measured at baseline. Arterial stiffness and vascular reactivity will be measured with pulse wave velocity (Vicorder or similar) and pulsewave-analysis (AiX75, SphygmoCor or similar)[45]. Inflammation will be measured by plasma levels of IFN- β, IL-1β, IL-17, TNF-α as well as markers of oxidative stress, including OxLDL and Malondialdehyde[46]

Evaluating E-Cigarette Nicotine Form, Concentration, and Flavors among Youth

Table 1: Project 3 Sub-study 2 Study Measures		
Project/Study	P3S2	
Visit	1	2-9 (Option A) or 2-5 (Option B)
Background Measures		
Sociodemographic Measures	X	
Tobacco Use History & Sensory E-Cigarette Expectancies Scale (SEES)	X	
E-Cig Dependence (modified Cigarette Dependence Scale)	X	
Timeline Followback (week, month, lifetime)	X	
E-cig Abuse Liability		
EC Puff Topography (puff count, puff duration, inter-puff interval, puff flow rate, average puff volume, total puff volume)	X	X
Subjective Effects		
Drug Effects/Liking Questionnaire	X	X
modified Cigarette Evaluation Questionnaire (mCEQ)	X	X
Sensory E-Cigarette Expectancies Scale	X	X
Behavioral Economic Demand		
E-Cigarette Purchase Task	X	X
E-cig Craving/Suppression of Craving and Withdrawal		
Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form	X	X
Minnesota Nicotine Withdrawal Scale Self-Report	X	X
Nicotine Uptake		
Forearm Venous Catheter	X	X
E-liquid Consumption		
e-liquid containers for pods will be weighed	X	X
Toxicant Exposure		
Average topography “puff playback,” and e-cig aerosol produced and collected on filters	X	X
Liquid and Gas Chromatography/Mass Spectrometry	X	X
E-cig Device Characteristics		
Device voltage, coil resistance, pressure drop	X	X
E-liquid Characteristics		
pH, nicotine concentration, and PG/VG ratio	X	X
Physiological Effects (Pulmonary)		
Laboratory Spirometry (SpiroLab)	X	X
Airway Inflammation (NIOX VERO)	X	X
Airway Reactivity (TremoFlo)	X	X
Cardiovascular Measures		
Vascular Reactivity (Vicorder or similar)	X	X
Blood Pressure	X	X
Heart Rate	X	X
Markers of endothelial dependent and independent function		
B-mode ultrasound (Vicorder or similar)	X	X
Arterial stiffness		
Pulse wave velocity and analysis (Vicorder or similar)	X	X

E. Statistical Methods

Generalized linear mixed models will be employed to identify associations of nicotine form, nicotine concentration, and flavoring with various outcome measures (e.g., plasma nicotine, spirometry, flow-mediated dilation). Models will include relevant covariates (e.g., gender), a random effect for participant, main effects for nicotine form and concentration, and the interaction between nicotine form and concentration and nicotine form and flavor. Because of multiple measurements tested, we will assume a significance threshold corresponding to one expected false discovery per 100 tests (p=0.01).

i. Power Analysis

This study, for each of the main effects and alpha = 0.01, achieves 80% power to detect an effect size of 0.5 standard deviations of the paired differences. For interaction effects, we will estimate 80% power (effect size 0.5 SD) to distinguish between any two of the interaction groups (e.g., free base/low nic, free base/high nic), while correcting for the pairwise tests (p=0.01/6=0.0017). Carryover and period effects will also be examined.

ii. Data Analytic Plan

Because product sampling will be randomized using a Latin Square, a fixed effect for session and a random effect for sequence will be included in all analyses.

iii. Missing Data

While we will make every effort to minimize the missing data for this study, missing data can arise due to various reasons. Violation of missing complete at random (MCAR) will be checked by evaluating whether any covariates are associated with missing data. If so, these covariates will be subsequently included and controlled for in the GLMM model. Our primary analysis will use mixed models which assumes Missing At Random (MAR) to deal with the missing data problem. In addition, sensitivity analyses will also be conducted using results from multiple imputation and complete cases strategies.

F. Gender/Minority/Pediatric Inclusion for Research

i. Inclusion of Women and Minorities

Inclusion of Women and Minorities: According to U.S. Census Data, 51.3% of Columbus, OH residents are female. In our previous studies with smokers, 55-62% of participants were female. According to U.S. Census data, the racial composition of individuals living in Columbus is 61% White, 28% Black or African American, 5% Asian, 0.2% American Indian/Alaska Native, 0% Native Hawaiian/Other Pacific Islander, and 4% two or more races. The ethnic composition of individuals living in Columbus is 6% Hispanic/Latino. We expect that our distributions will be similar to these but we may potentially have a larger distribution of ethnic and racial minorities, based on our previous studies. However, we will continuously monitor enrollment in order to ensure that we are meeting recruitment goals to avoid under-recruiting minorities. If the targeted enrollment for minorities is not met because they do not

respond to the advertisements, we will make special efforts to solicit their participation by advertising in community newspapers, local church organizations, and community centers.

ii. Inclusion of Children

This study will be restricted to individuals 21 years of age and older, which is the legal age to purchase tobacco products.

G. Human Participants

i. Recruitment and Informed Consent

At first contact, all participants will be screened according to the study's inclusion/exclusion criteria. Those who are eligible will be given a brief verbal overview of the study and invited to participate. Informed consent (including a description of the nature, purpose, risks, and benefits of the study) of the participant will take place through both oral and written explanation of the study. The voluntary nature of the study and the participant's right to withdraw at any time will be stressed during the consent process. A copy of the informed consent will be provided to the participant in written form at the time of consent for them to keep. Informed consent will be collected by IRB approved study personnel. Recruitment script and materials, consent forms, and all study procedures will be approved by the OSU Institutional Review Board. All participants will provide written consent before any study data is collected.

ii. Potential Risks and Protections against Risk

There are minimal risks associated with this protocol. The protocol requires e-cig users 21 to 25 years of age to undergo 12 hours of tobacco/nicotine abstinence on nine occasions. These e-cig users will complete 9 vaping sessions – one session in which participants will use their own e-cig and e-liquid and subsequent sessions in which participants will use a study e-cig held at a constant wattage, and pre-filled with e-liquid of different nicotine form (free-base vs. nicotine salt), concentration (low-1% vs. high-5%), and flavoring (tobacco vs. menthol).

The participants are already using e-cigarettes and will only be asked to use or switch to what will be a fully characterized e-cigarette device and ITPs that have been fully described to FDA Center for tobacco products. Questionnaires, exhaled breath, and urine collection procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) risk of using e-cigarettes, b) loss of confidentiality or privacy, and c) risk of infection from blood draws.

1. Risk of Using ECs:

Risks

The risk of side effects and adverse events are very low. The commercial device we are using is sold online, and at e-cigarette specialty stores and convenience stores nationwide, without a prescription.

Protections

Nevertheless, all participants will be screened for general medical precautions (pregnancy, cardiovascular disease) and monitored for adverse events during the study period. Study personnel will assess for adverse events via self-report at all follow-up visits. Any serious adverse events will be reported to the study's DSMB, and then to the OSU IRB and to the NIH. We will withdraw participants who have a serious adverse event, or become pregnant, begin to breastfeed, or have a cardiovascular or pulmonary event during the study. The most likely adverse (potential for nicotine overdose) event is anticipated to be rare (<5% in our team's previous studies) and mild (nausea, headache, disrupted sleep), and will be handled quickly (i.e., advice to participant to reduce or stop e-cig use). Lab studies of toxin exposure suggest that e-cigs incur no greater risk to health than do conventional cigarettes. Indeed, e-cigarettes generally show lower levels of harmful and potentially harmful constituents. To date, e-cigarette studies discussing adverse events report mild and tolerable side effects that generally resolved completely over time with continued use; the most predominant of which were mouth/throat irritation, cough, and headache. In four randomized clinical trials, no serious adverse events were reported, and the e-cigarette group and the nicotine patch group had comparable levels of adverse events in two studies. The most common were mouth irritation, throat irritation, dry cough and headache. Following the completion of the study, we will encourage all participants to quit their use of e-cigarettes.

2. Loss of Confidentiality and Privacy:

Risks

There is a risk of breach of confidentiality or a loss of privacy associated with taking part in the study.

Protections

Protection against loss of confidentiality and privacy will be maintained by numerically coding all data, disguising identifying information, and keeping data locked in file drawers or in a secure, password-protected database. Only study research assistants and the PI will have the information that connects participant's name and ID number. All electronic data will be numerically coded and stored in a password protected database, on a password protected computer in a secure research space. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication.

3. Venous Draws:

Risks

The risks involved in drawing blood from a vein may include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling of dizziness or lightheadedness when the blood is drawn, nausea, and rarely, an infection at the site of the blood draw.

Protections

Blood will be drawn by trained research staff/research nurse. IVs will be placed by an RN or LPN. Sterile instruments will be used, and for blood draws, the participant's skin will be cleaned with an alcohol wipe at the site of the needle stick.

iii. Potential Benefits of the Proposed Research

Whereas no assurance can be made to an individual participant that s/he will personally benefit from this research, the experience should be beneficial. The immediate benefits of this research are scientific in nature, which in the long-term should benefit society as a whole. The study will also benefit e-cig users, as a group, by providing information as to the abuse liability of other e-cig/e-liquid products; and serve as evidence to inform regulatory action that improves public health. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

H. Data and Safety Monitoring Plan and Board

i. Plan

Data will be analyzed initially after 20 participants are accrued, to ensure electronic data capture systems employed (i.e., REDCap) are accurately capturing data and to ensure the format and completeness of all data collected.

ii. Data and Safety Monitoring Board

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly). The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report (biannually for Phase II and quarterly for Phase I) that will be reviewed by the committee per the IRB of record as per the policies of the IRB. All Serious Adverse Events are to be submitted to the DSMC for their review. Submissions are made via OnCore.

Adverse Events

Adverse events will be assessed by study staff at each visit via participant self-report and managed immediately. All adverse events will be reported to the OSU IRB. We will monitor for risk of smoking/vaping by screening participants for general medical precautions (pregnancy, cardiovascular disease). Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and Dr. Wagener. Dr. Wagener will be responsible for completing an Adverse Events Form should an event occur. Dr. Wagener will report Serious Adverse

Events to the OSU IRB within 24 hours of having received notice of the event. Dr. Wagener will gather any information needed to investigate the event and to determine subsequent action. Any subsequent action will be documented and reported to the OSU IRB and the Program Officer at NIH. Adverse event reports will be reviewed annually with the OSU IRB to ensure participant safety.

Collection of Adverse Events

The collection of adverse events will be on a self-report basis and logged within an electronic data capture system (REDCap) or collected using standardized paper forms and will only be identified with the study ID of the participant.

I. Addendum

i. COVID-19 Related Procedures

Due to the COVID-19 pandemic, processes and procedures have been implemented to help protect participants and research staff. These processes and procedures are to be followed as long as social distancing requirements are necessary for conducting study visits.

Only one study participant per study coordinator will attend study visits at the CTR at any given time. All study participants will be provided with a face mask upon entry. Only one coordinator will meet the participant at their car for a temperature check**,** direct the participant into the building, and the two of them will ride the elevator to the 4th floor physically distanced at least 6 feet apart, both wearing masks. No more than 2 persons may ride the elevator at any given time. The participant will be immediately escorted to a private exam/draw room. Therefore, there will be no waiting in open lobby/waiting areas.

When in the exam room, the study coordinator will stand at least 6 feet away from the study participant to give instructions. Afterwards, the study coordinator will leave the exam room to allow the study participant to conduct the instructed procedures. The study coordinator and study participant will be at least 6 feet away from one another and wearing protective masks at all times during each visit.

Each study coordinator will have a designated exam/draw room and smoking room in which to conduct their designated research study. Each smoking room is separated from the staff control station in the hallway by its own door and contains a large window for the study coordinator to be able to see in and monitor study participant activity within the room. There is also a speaker and microphone system within each smoking room along with the Genetec software system on the outside of each room at the smoking room computer stations. Therefore, the study coordinator and study participant can communicate without being in the room together.

For study measures which cannot be physically distanced, appropriate PPE will be worn at all times by research staff during these procedures including goggles, face masks, gloves, and isolation gowns or lab coats.

After each participant visit is complete, there will be at least a 45-minute period for cleaning and air exchanges in the negative pressure rooms and for cleaning exam rooms and equipment before the next

participant visit. All smoking rooms are under negative pressure with a ventilation rate of 36.8 – 44.1 air changes per hour (ACH).

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The Ohio State University Combined Consent to Participate in Research and HIPAA Research Authorization

Study Title: **Evaluating E-Cigarette Nicotine Form, Concentration, and Flavors among Youth**

Principal Investigator: **Theodore L. Wagener, PhD**

Sponsor: **American Heart Association (AHA)**

- **This is a consent form for research participation.** It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.
- **Your participation is voluntary.** You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.
- **You may or may not benefit as a result of participating in this study.** Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
- **You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate.** If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

Key Information About This Study

The following is a short summary to help you decide whether or not to be a part of this study. More detailed information is listed later in this form.

You are being asked to participate in this research study to examine and learn more about the influence of nicotine on youth vaping behavior as well as the heart and lung effects associated with this behavior. If you choose to participate, you can do 5 or 9 visits, which involve vaping sessions, the details of each schedule option will be discussed below. You will be asked to complete regular surveys, heart and lung measures, and blood and urine samples will be collected. There is no direct benefit to you by participating; however, using this research, we hope the information learned will help us understand how e-cigarettes affect smoking behavior and health as well as assist in creating effective e-cig

policies and regulations to prevent further increase of youth e-cig use. The risks of this study include discomfort in answering questions asked of you, risks of nicotine to pregnant or breastfeeding females, and the risks of e-cigarette exposure. More information about these risks can be found below. You will be compensated for your time and effort in this study.

1. Why is this study being done?

The purpose of this study is to examine the influence of nicotine form, concentration, and e-liquid flavor on youth vaping behavior. It is also to examine nicotine uptake (the dose), abuse liability (the tendency of use), toxicant exposure (the harmful tobacco-related substances a user may be exposed to), and the heart and lung effects of vaping behavior.

2. How many people will take part in this study?

Up to 130 people will take part in this study.

3. What will happen if I take part in this study?

The following provides a summary of what will take place at each visit.

Option A: Over the course of 9 study visits, there would be 9 vaping sessions. There is one vaping session during today's visit and 8 subsequent vaping sessions during the subsequent visits.

Option B: Over the course of 5 study visits, there will be 9 vaping sessions. There is one vaping session during today's visit and 2 vaping sessions separated by 3 hours of downtime during each of the 4 remaining visits.

After initially choosing Option A or B for your scheduled visits, you will be required to stay in that option's visit structure for the entirety of the study. So, please take time to consider the best option for you.

At each visit, you will complete regular surveys and physical measures such as blood pressure, weight, and breath tests. If you are female, we will perform a pregnancy test to ensure you are not pregnant at the beginning of each visit. During each visit, we will perform a lung function test and collect biosamples including blood (a venous catheter will be placed to repeatedly collect samples at different time periods during the vaping sessions) and urine.

The lung function test consists of breathing hard into a machine several times. It is painless and only takes a few minutes to complete. The blood sample is approximately

15mL or one tablespoon of blood. The blood will be used to look at the different amounts of nicotine-related chemicals present in the blood.

You will be required to be nicotine-free (abstinent) at least 12 hours before each study visit. We will confirm you are nicotine-free at the beginning of each visit via blood plasma nicotine analysis. During the first visit, you will use your own e-cig and e-liquid. After visit 1, you will take home a study e-cig device and 1 pod with pre-filled tobacco flavored e-liquid to practice (20 puffs per day with the study device and until you feel comfortable using the device) using the device before visit 2. In the subsequent vaping sessions, you will use a study e-cig pre-filled with e-liquid. Each vaping session will include a standardized, 5-minute, 10-puff vaping bout (30 seconds between each puff) followed by 30 minutes of ad libitum (as desired) vaping.

During the vaping sessions, measures of vaping behavior (topography) will be assessed.

You will not be allowed to eat or drink (other than water) during the vaping sessions, but you will be allowed to have a light snack prior to placement of the IV and vape session starting. If you choose Option B you can eat and drink during the 3-hour washout/downtime periods.

4. How long will I be in the study?

You will be in this study up to 6 months depending on what study schedule you choose, A or B, how your visits are scheduled and your ability to remain abstinent prior to each visit.

Option A: Over this period, your first visit will last up to 3 hours and then 8 subsequent visits that will last up to 2 hours each.

Option B: Over this period, your first visit will last up to 3 hours and there will be 4 subsequent visits that will last up to 6 hours each.

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent. Examples of when this may happen are, if you are not following the study requirements, become pregnant during the study, or for any other reason we feel your participation is not in your best interest or the study's best interest.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher first.

5. Can I stop being in the study?

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

16. What risks, side effects or discomforts can I expect from being in the study?

Survey Risks

Some people feel uncomfortable answering questions about smoking or their health. You may choose to select "Refuse to answer" as your response. Since these surveys will be administered through the University approved Research Electronic Data Capture (REDCap) system, any information collected will be in a secure database accessible only to authorized study staff.

Reproductive Risks for Women

If you are a female, you must not be and should not become pregnant nor breastfeed an infant while on this study. Using cigarettes or e-cigarettes while pregnant or breastfeeding may involve risks to an embryo, fetus or infant, including birth defects which are currently unforeseeable. In order to reduce your risk of pregnancy, you or your partner should use one or more acceptable methods of birth control regularly and consistently while you are on this study.

If you become pregnant or suspect that you are pregnant during this study, you should immediately inform the study personnel. We will conduct a pregnancy test at each visit to ensure that you are not currently pregnant. The study product may be discontinued until the result of the pregnancy test is known. If pregnancy is confirmed, you may be withdrawn from the study. Payment for all aspects of obstetrical, child, or related care will be your responsibility.

Blood Draws

There is a slight risk of discomfort, bruising, or infection with a blood draw. Your blood will be drawn by trained research staff.

Lung/Carbon Monoxide Tests

The breath and lung carbon monoxide tests are not associated with risks, but repetitive testing within a short time frame may cause dizziness or shortness of breath.

E-cigarette Devices

All of the products in our study are currently available for purchase in stores; however, there was no FDA evaluation of these devices prior to their being commercially available. **As a result, we do not know the long-term safety, risks, or if these devices contain ingredients that are known to be toxic to humans.**

16. What benefits can I expect from being in the study?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope that the information learned from this study will help us to understand how nicotine form, concentration, and flavor affects people's vaping behaviors and health.

8. What other choices do I have if I do not take part in the study?

You may choose not to participate without penalty or loss of benefits to which you are otherwise entitled.

9. Will my study-related information be kept confidential?

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law.

Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- U.S. Food and Drug Administration;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor supporting the study, their agents or study monitors; and
- Your insurance company (if charges are billed to insurance).

We will work to make sure that no one sees your survey responses without approval. But, because we are using the Internet, there is a chance that someone could access your online responses without permission. In some cases, this information could be used to identify you. Your data will be protected with a code to reduce the risk that other people can view the responses. The Ohio State University will keep your information in password protected databases and locked research files in a secure environment and will protect it to the full extent of the law.

The NIH has issued a Certificate of Confidentiality for this study. This Certificate provides extra protection for you and your study information, documents, or samples (blood, tissue, etc.). The Certificates are issued so that we cannot be required to disclose any identifiable, sensitive information collected about you as a part of this study in a lawsuit or legal proceeding. We are also prevented from releasing your study information

without your consent. This is a layer of protection over and above the already existing protections in place for you and your information, documents, or samples.

However, these protections do not apply in some situations. For example, we may have to release your information if a law requires us to do so, the Agency that is funding this study requests the information, or if the FDA tells us to release this information. We may also use your information to conduct other scientific research as allowed by federal regulations.

Study information that has health implications may be placed in your medical record where authorized employees may see the information. Further, authorized requests for your records (medical record release for continuity of care) may result in research-related information being released.

Please talk to your study team, or contact the Office of Responsible Research Practices at 614-688-8641, if you have questions.

If this study is related to your medical care, your study-related information may be placed in your permanent hospital, clinic, or physician's office records. Authorized Ohio State University staff not involved in the study may be aware that you are participating in a research study and have access to your information.

If we find information that significantly impacts your health, we will share it with you. You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search the website at any time.

10. Will my de-identified information and bio-specimens be used or shared for future research?

Yes, it/they may be used or shared with other researchers without your additional informed consent. De-identified information does not include any details that could make it possible for others to recognize you.

11. What are the costs of taking part in this study?

There is no cost to you if you participate in this study.

12. Will I be paid for taking part in this study?

By law, payments to participants are considered taxable income.

You will receive the following compensation for your time:

Option A	**Option B**
Visit 1: $50	Visit 1: $50
Visit 2: $50	Visit 2: $100
Visit 3: $50	Visit 3: $125
Visit 4: $50	Visit 4: $150
Visit 5: $75	Visit 5: $175
Visit 6: $75	
Visit 7: $75	
Visit 8: $75	
Visit 9: $100	

The maximum amount you can potentially earn by end of study if you follow study instructions is $600.

Payments will be made using the Greenphire ClinCard to increase accountability and facilitate ease of payment.

13. What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Wexner Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

14. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of research participants.

15. HIPAA AUTHORIZATION TO USE AND DISCLOSE INFORMATION FOR RESEARCH PURPOSES

I. What information may be used and given to others?

- Past and present medical records;
- Research records;
- Records about phone calls made as part of this research;
- Records about your study visits;
- Information that includes personal identifiers, such as your name, or a number associated with you as an individual;
- Laboratory and other test results
- Diaries and questionnaires
- Records about any study drug you received; and
- Records about the study device.

II. Who may use and give out information about you?

Researchers and study staff.

III. Who might get this information?

- The sponsor of this research. "Sponsor" means any persons or companies that are:
  - working for or with the sponsor; or
  - owned by the sponsor.
- Authorized Ohio State University staff not involved in the study may be aware that you are participating in a research study and have access to your information;
- If this study is related to your medical care, your study-related information may be placed in your permanent hospital, clinic, or physician's office record;
- Others: National Cancer Institute

IV. Your information <u>may</u> be given to:

- The U.S. Food and Drug Administration (FDA), Department of Health and Human Services (DHHS) agencies, and other federal and state entities;
- Governmental agencies in other countries;
- Governmental agencies to whom certain diseases (reportable diseases) must be reported; and
- The Ohio State University units involved in managing and approving the research study including the Office of Research and the Office of Responsible Research Practices.
- Researchers and laboratories outside of the Ohio State University.

V. Why will this information be used and/or given to others?

- To do the research;
- To study the results; and
- To make sure that the research was done right.

VI. When will my permission end?

There is no date at which your permission ends. Your information will be used indefinitely. This is because the information used and created during the study may be analyzed for many years, and it is not possible to know when this will be complete.

VII. May I withdraw or revoke (cancel) my permission?

Yes. Your authorization will be good for the time period indicated above unless you change your mind and revoke it in writing. You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the researchers. If you withdraw your permission, you will not be able to stay in this study. When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others.

VIII. What if I decide not to give permission to use and give out my health information?

Then you will not be able to be in this research study and receive research-related treatment. However, if you are being treated as a patient here, you will still be able to receive care.

IX. Is my health information protected after it has been given to others?

There is a risk that your information will be given to others without your permission. Any information that is shared may no longer be protected by federal privacy rules.

X. May I review or copy my information?

Signing this authorization also means that you may not be able to see or copy your study-related information until the study is completed.

16. Who can answer my questions about the study?

For questions, concerns, or complaints about the study, or if you feel you have been harmed as a result of study participation, you may contact Profile Study staff at 1-844-744-2447.

For questions related to your privacy rights under HIPAA or related to this research authorization, please contact a privacy officer at 614-293-4477.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact the Office of Responsible Research Practices at 1-800-678-6251.

If you change your mind about taking part in this study, you can contact Profile Study staff by phone at 1-844-744-2447. Staff may ask if you want to withdraw from the entire study or parts of it.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I choose study schedule Option A _____ I choose study schedule Option B _____

I am not giving up any legal rights by signing this form. I will be given a copy of this form**.**

Printed name of participant	**Signature of participant**
	AM/PM
	Date and time
Printed name of person authorized to consent for participant (when applicable)	**Signature of person authorized to consent for participant (when applicable)**
	AM/PM
Relationship to the participant	**Date and time**

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent	**Signature of person obtaining consent**
	AM/PM
	Date and time

Witness(es) - *May be left blank if not required by the IRB*

Printed name of witness	**Signature of witness**
	AM/PM
	Date and time
Printed name of witness	**Signature of witness**
	AM/PM
	Date and time