“THE IMPACT OF E-CIGARETTE LIQUID NICOTINE FORM ON PUFFING BEHAVIOR, ABUSE LIABILITY, AND CANCER RISK” (NCT05455086)

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The impact of E-cigarette liquid nicotine form on puffing behavior, abuse liability, and cancer risk (PeloPET)

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A. Abstract

Since their market emergence, electronic cigarettes (ECs) have garnered both criticism and praise regarding their potential promise to reduce the risks associated with conventional cigarette use. While researchers have attempted to keep pace, ECs and e-liquids have rapidly evolved. Over the last two years there has been a significant surge in EC sales and use among youth and adults alike, drawing significant concern from health officials and regulators. The surge appears to be largely driven by a new category of EC—nicotine salt-based (NSB) devices, of which the most well-known is JUUL, garnering nearly 80% of the e-cigarette market. Unlike previous ECs that relied on unprotonated, or "free-base" nicotine e-liquid, NSB devices use protonated forms (i.e., nicotine salts) by combining nicotine with an acid. With a shift to protonated forms of nicotine in e-liquid, inhaling high concentrations of nicotine (3-6%) is much more palatable. With improved sensory experience and higher concentrations of nicotine, use of protonated forms may result in 1) more intense puffing, with potential for greater deposition and absorption of nicotine deeper into the respiratory tract influencing lung cancer risk, and 2) greater nicotine delivery, abuse liability, and appeal to non-users, a concerning finding since ECs contain numerous carcinogens and many EC users go on to smoke conventional cigarettes. To begin to examine the risk-benefit of e-liquid nicotine form, the overall aim of the proposed study is to evaluate the effect of free-base nicotine vs. nicotine salt e-liquids on EC puffing behavior, abuse liability, deposition and absorption of nicotine in the respiratory tract. Using a randomized crossover-design, up to 30 adult EC users will complete four standardized EC puffing sessions inside the Positron Emission Tomography (PET) scanner, with two e-liquids that differ primarily by the dominant nicotine form. E-liquid will be prepared with unlabeled and radiolabeled ^{11}C-nicotine for PET imaging to determine the impact of nicotine form on: 1) changes in EC puffing behavior; 2) EC abuse liability; and 3) nicotine deposition and absorption in the respiratory tract. The study has the potential for high public health impact as it will provide the scientific foundation the FDA and other public health agencies need to establish effective regulatory strategies for the manufacture, distribution, and marketing of ECs.

B. Specific Aims

Since their market emergence, electronic cigarettes (ECs) have garnered both criticism and praise regarding their potential promise to reduce the risks associated with conventional cigarette use. While researchers have attempted to keep pace, ECs and e-liquids have rapidly evolved. Over the last two years, there has been a significant surge in EC sales and use among youth and adults alike[1,2], drawing significant concern from health officials and regulators, as e-cigarettes expose users to addictive nicotine, and toxic and carcinogenic carbonyls, furans, reactive oxygen species, and heavy metals[3,4]. The surge appears to be largely driven by a new EC category—nicotine salt-based (NSB) devices, of which the most well-known is JUUL, garnering nearly 80% of the e-cigarette market[5]. Unlike previous ECs that relied on unprotonated, or "free-base" nicotine e-liquid, NSB devices use protonated forms (i.e., nicotine salts) by combining nicotine with an acid. While past research indicates that protonated nicotine is significantly less harsh to inhale[6], it is not yet clear what impact this will have on EC puffing patterns, abuse liability, and downstream health effects. Making conventional cigarettes easier to inhale was associated with more aggressive and frequent puffing and increased lung cancer disease risk[7]. Whether this holds true for NSB devices is yet to be seen.

Consistently utilizing free-base nicotine in their e-liquids, the evolution of early EC models centered on increasing device power (wattage) delivered to the heating coil. This strategy was successful in terms of improved nicotine delivery, but also had several unanticipated and negative effects[8]. Increased power

led to increased user exposure to tobacco toxicants due to greater production of carcinogenic compounds in the EC aerosol. However, with increased heating came compensatory puffing as users began reducing the concentration of nicotine in their e-liquid (<1.2%) to improve their sensory experience (high doses of free-base nicotine at high wattage are very harsh)[8-11].

With protonated forms of nicotine in e-liquid, inhaling high concentrations of nicotine salts (3-6%) is much more palatable, allowing smaller, inconspicuous low-powered EC devices to provide effective nicotine delivery[12,13]. This combination of high nicotine but low power may be beneficial in terms of reduced exposures to tobacco-related toxicants, including lower heating of the coil, reducing the amount of toxicants produced and higher levels of nicotine per puff, reducing the number of puffs needed to achieve satisfying nicotine levels[14]. On the other hand, with smaller, easily hidden devices, improved sensory experience, and higher concentrations of nicotine, use of nicotine salts may result in: 1) more frequent dosing; 2) more intense puffing, with potential for greater deposition and absorption of nicotine deeper into the respiratory tract influencing lung cancer risk; and 3) greater nicotine delivery, abuse liability, and appeal to non-users, a concerning finding since ECs contain numerous carcinogens and many adolescent EC users go on to smoke conventional cigarettes[14,15].

To begin to examine the risk-benefit of e-liquid nicotine form, the overall aim of the proposed study is to evaluate the effect of free-base vs. nicotine salt based e-liquids on EC puffing behavior, abuse liability, and deposition and absorption of nicotine in the respiratory tract. Using a randomized crossover-design, up to 30 adult EC users will complete three study visits that include four 1-puff standardized EC sessions, with two e-liquids that differ only by the dominant nicotine form. For visits that include PET scanning, each e-liquid will be prepared with [^{11}C]-nicotine for PET imaging to determine amount and location of deposition and absorption of nicotine in the brain and respiratory tract. Imaging visits will be preceded by 12 hours of nicotine abstinence, and sessions will be separated by approximately 2 hours to allow an estimated 6 half-lives of [^{11}C]-nicotine decay. Outcome measures will include: 1) puffing topography captured by the study EC device; 2) abuse liability including reported drug liking/satisfaction and measured amount and rate of uptake of [^{11}C]-nicotine in the brain during the puffing sessions; and 3) respiratory tract deposition and absorption of [^{11}C]-nicotine.

Aim 1: To examine the influence of nicotine form on puffing behavior and abuse liability. *H1a: NSB e-liquid will result in puffs of longer duration and higher flow rate, and H1b: demonstrate greater abuse liability. H1c: FB nicotine will result in puffs of shorter duration, lower flow rate, lower inhaled volume, and lower abuse liability.*

Aim 2: To evaluate the influence of nicotine form and concentration on nicotine distribution in the brain and respiratory tract. *H2. Compared to FB nicotine, nicotine salts will result in greater nicotine distribution in the lower (vs. the upper) respiratory tract and greater amount and rate of uptake in the brain.*

C. Significance

The cost-benefit of ECs continues to be debated in the public health literature. ECs are now the most commonly used tobacco product in the U.S. among youth and young adults and anticipated to exceed total conventional cigarette use by 2020[1]. The most recent estimates indicate that 10.8 million US adults (4.5%) have used an EC in the past 30 days[2]. Among high school students and young adults (18-24 years), these rates are even higher, at 3.1 million (20.8%) and 2.8 million (9.2%), respectively[1].

EC 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, 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 free-base ECs, with approximately 40-60% of those who ever tried an NSB device, identifying as current users.

While the long-term health effects are still unclear, studies examining proximal biomarkers of health indicate that EC users are exposed to toxic and carcinogenic carbonyls, furans, reactive oxygen species, and heavy metals and experience adverse health effects, including increased oxidative stress, respiratory and mucosal inflammation, and adverse cardiovascular effects. These data suggest that sustained EC use will likely have notable adverse health effects[4], yet to our knowledge, no studies have examined the toxicological and physiological effects of NSB use. All previous toxicological EC studies have focused on free-base nicotine devices, and only two studies, to our knowledge, both conducted by our team and currently in preparation for publication, have examined the pharmacological profile of NSB devices.

Consequently, we do not have sufficient data to determine what impact the use of protonated nicotine in ECs will have on smoking behaviors, abuse liability, and health, nor what will be the most effective regulatory strategies to protect public health. The proposed study will begin to address the current knowledge gap by directly examining the effect of free-base vs. nicotine salt e-liquids on EC puffing behavior, abuse liability, and deposition and absorption of nicotine in the respiratory tract and rate of uptake in the brain. The results from this study will provide much needed scientific information to public health officials and regulators.

Innovation

There is a dearth of research examining behavioral effects of NSB devices, the most widely used ECs on the market, and no published studies examining their pharmacological, toxicological, and physiological effects. The proposed study will be the first to systematically examine the impact of the nicotine form (free-base vs. protonated) in e-liquids on EC puffing behavior, abuse liability, and deposition and absorption of nicotine in the respiratory tract. Moreover, the use of [^{11}C]-nicotine for PET imaging will provide clear resolution and definitive conclusions as to the rate of nicotine delivery to the brain (critical for the initiation and maintenance of nicotine addiction) and the amount and location of nicotine deposition to the respiratory tract (critical for understanding uptake of nicotine in the body).

D. Approach and Preliminary Studies

Our team brings combined expertise in all areas necessary for the successful implementation of a human laboratory study examining the impact of nicotine form (free-base vs. protonated) in EC liquid on puffing behavior, nicotine delivery, abuse liability, deposition, and resulting harm from vaping ECs. Specifically, we have conducted nearly 20 human laboratory studies examining the behavioral, pharmacological, toxicological, and/or physiological effects of non-cigarette tobacco products including 5 ongoing NIH studies, 3 examining ECs. We have also recently conducted 4 studies examining NSB EC devices[16,17], including two recently completed human laboratory studies (manuscripts in prep; see submitted abstracts[10,11]). Results of these studies demonstrated that NSB ECs achieved cigarette-like levels of nicotine delivery, have the potential for significant levels of addiction, and that higher levels of

protonated nicotine (i.e., lower e-liquid pH) was associated with reduced perceived harshness of the aerosol, increased nicotine uptake, and higher abuse liability. We have an established toolkit of validated methodologies and standard operating procedures for use in this study. Our team has also conducted several short-term as well as long-term randomized trials assessing tobacco use behavior. Additionally, the radiochemistry and imaging teams at the Wake Forest Clinical and Translational Science Institute (CTSI) have extensive experience in radiosynthesis and analysis of [^{11}C]-nicotine and with a variety of imaging approaches, including the dynamic perfusion imaging that will be utilized in this study. A methodology for imaging usage of a radiolabeled EC e-liquid in real time has been fully developed with the assistance and approval of University Radiation Safety staff and the process is ready for final optimization and immediate imaging sessions with use of [^{11}C]-nicotine.

E. Research Design and Methods

i. Design Overview

Utilizing a 4-session, within-subjects, factorial design, up to 30 e-cigarette users (10 NSB EC users; 10 FB EC users) will attend 3 laboratory visits at Wake Forest Baptist Medical Center's PET Research Facility. At the first visit, they will select the flavor they can tolerate and vape in both the free-base and nicotine salt form without coughing (see **Figure 1** for e-liquid combinations). For the remaining two visits, they will complete four standardized, single-puff vaping sessions (2 sessions/visit) using the same EC device, containing unflavored or flavored e-liquid and radiolabeled [^{11}C]-nicotine and native nicotine (3%) either predominantly in the free-base or protonated form. The initial orientation visit will include informed consent, a health screening/physical exam, and a vaping session in which they will learn to use the study EC (Reflex, Evolv LLC, or similar) and sample 4 e-liquids to determine whether they can vape the unflavored e-liquid without coughing. The subsequent two visits will include PET scanning of the head and chest (two separate scanning sessions), each after a single-puff vaping session. The nicotine form (free-base or protonated) will be randomly selected for the visit in the flavor (flavored vs. unflavored) selected at the first visit, as will the order of the focal area of scanning (e.g., first head, then chest). At the final visit, the participant will vape the other nicotine form in the flavor selected in visit 1, and the order of the focal area of scanning will be randomly selected.

Fig 1. Study E-Liquid Combinations

		Nicotine Form	
		NSB (>85% Protonated)	**FB (<50% Protonated)**
Nicotine Flavor	**Tobacco Flavored**	Tobacco Flavored-NSB	Tobacco Flavored-FB
	Unflavored	Unflavored-NSB	Unflavored-FB

ii. Study Procedures

All study visits will take place at in North Carolina, specifically the WAKE Health Research PET Center, where participants will be given consent form, complete screening procedures, vaping sessions, PET/CT scans, and complete online questionnaires and surveys on a tablet. Physical materials will be stored in a locked file cabinet, locked office or suite at the WAKE Health Research PET Center, 2036 Queen Street, Winston Salem, NC 27157 only. Electronic data will be entered, stored, and secured through REDCap and managed by Ohio State University. Tablets will be supplied by Ohio State.

Visit #1:
Participants will complete an online screening questionnaire determining eligibility prior to the visit. Once deemed eligible, participants will come into the laboratory to give informed consent, and participate in health screening/physical exam and a vaping session in which they will learn to use the study device (Reflex, Evolv LLC, or similar) and sample 4 e-liquids at 3% nicotine concentration to determine whether they can vape both nicotine forms (free-base and protonated). The 4 e-liquids will vary based on flavor (unflavored and flavored) and protonation level (0-100%). The unflavored set of two e-liquids (0% and 100% protonation) are the preferred test articles, but if the participant cannot vape four puffs from each of them without coughing, then they will sample the flavored set of two e-liquids (0% and 50% protonated) to determine if they can vape four puffs from each of them without coughing. Puffing topography captured by the study EC will be collected to determine the participant's typical puff volume for each e-liquid tested. If the participant cannot vape the test e-liquids without coughing, they will be deemed ineligible for further participation. Eligible participants will be given a study device and 1 pod of each test e-liquid to take home and practice using at least 3 days (20 puffs per day with the study device and until they feel comfortable using the device) so that they can become familiar with using the study device and the test e-liquids. After review of the topography data stored on the device, participants that vape small volumes (<20 mL per puff) may be deemed ineligible from further participation. Participants will also be provided instructions that they must remain nicotine abstinent for 12 hours before Visit #2 and Visit #3.

Visit #2:
Following confirmation of 12-hour nicotine abstinence from combustible tobacco products via exhaled CO (<10ppm), and after confirmation that participants practiced at least 3 days with the device and pods using the test e-liquids, participants will be randomized to nicotine form (free-base or protonated) using either unflavored or flavored e-liquid (determined from Visit #1) and order of the focal area of scanning (head/neck or chest). Participants will also complete a questionnaire assessing several measures of evaluating the perceived abuse liability of the study e-liquids. Participants will complete a standardized 1-puff vaping bout using the same device, with the same e-liquid, flavor, and nicotine, propylene glycol, and glycerol concentration. Specifically, participants will be randomized to undergo a dynamic scanning sequence (either 0-15 min head and 5 min chest scan, or 0-15 min chest and 5 min head scan). The imaging agent, [^{11}C]-nicotine, will be delivered to the participant by the participant taking a single puff of the e-liquid (protonated or unprotonated), in prone position inside the PET scanner; puff duration and volume will be determined by the participant (ad libitum). After the first scanning session is complete, the participant will rest outside the scanner for a >1-hour washout period so that the [^{11}C]-nicotine can decay (6 half-lives). Participants will not be permitted to leave the PET center waiting lobby and cannot leave the facility (except for restroom breaks).

The participant will repeat the single puff vaping bout with the same e-liquid, but will be scanned according to the other dynamic sequence. At least a 1-week period should elapse between visits 2 and 3.

Visit #3:
Visit #3 is essentially identical to Visit #2, but the participant will conduct the two single puff vaping bouts using the e-liquid containing the other form of nicotine.

Visit	**PET**	**CT - Anatomic**
Visit #1: Informed consent; select test article; puffing topography	None	None
Visit #2a:	1 puff (ad lib) of [^{11}C]-Nicotine with protonated nicotine 0-15 min head scan; 16-20 min chest scan	Head Chest
	WASHOUT PERIOD of >1 h	
Visit #2b:	1 puff (ad lib) of [^{11}C]-Nicotine with protonated nicotine 0-15 min chest scan; 16-20 min head scan	Chest Head
	≥1 WEEK	
Visit #3a:	1 puff (ad lib) of [^{11}C]-Nicotine with unprotonated nicotine 0-15 min head scan; 16-20 min chest scan	Head Chest
	WASHOUT PERIOD of >1 h	
Visit #3b:	1 puff (ad lib) of [^{11}C]-Nicotine with unprotonated nicotine 0-15 min chest scan; 16-20 min head scan	Chest Head
	DEBRIEFING	

Table 1. Activities conducted during participant laboratory visits.

F. Recruitment Feasibility and Retention

i. Recruitment

A total of up to 30 current e-cigarette users (aged 21-50 years) will be recruited from the general community via targeted internet advertisements i.e., local newspaper, advertisements, craigslist, Facebook, Instagram, and Twitter as well as flyers and word-of-mouth advertising in the North Carolina Triad Area (Greensboro, Winston-Salem, and High Point). Based on our team's previous studies, we conservatively assume a 20% attrition rate. Participants will complete an online screener questionnaire, and those 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 6 months (confirmed by NicAlert saliva testing strip or similar test) 2) Between 21-50 years old 3) Willing to provide informed consent and abstain from all tobacco, nicotine, and marijuana use for at least 12 hours prior to visits 2 and 3 4) Willing to participate in 3 laboratory visits 5) Read, write, and speak English 6) If utilizing study provided rideshare services, will need to live within 20 miles from Wake Forest University
Exclusion criteria: 1) Self-reported diagnosis of lung disease including asthma, cystic fibrosis, or chronic obstructive pulmonary disease 2) Unstable or significant psychiatric conditions (past and stable conditions will be allowed) 3) History of cardiac event or distress within the past 3 months 4) Currently pregnant (as indicated by urine pregnancy test at the start of each laboratory visit), planning to become pregnant, or breastfeeding (women only) 5) Use of other tobacco products (e.g., cigarette, cigar, etc.) >5 days in the past month 6) Currently engaging in a tobacco product cessation attempt

Assessment of Eligibility

Interested potential participants will respond to online advertisements and complete an online screening questionnaire to determine eligibility. Eligible participants interested in participating will be scheduled for the physical examination and then scanning at the PET center. After obtaining their informed consent, potential participants will be asked about their medical history, including smoking history, and will have their pulse, weight, height, and temperature measured. A urine pregnancy test (dipstick) will also be performed for women of childbearing potential at each visit. Potential participants who do not have a self-reported diagnosis of the exclusion conditions may be excluded if the study physician determines that the history, physical findings, or laboratory studies reveal information that may jeopardize the participant's safe study participation.

ii. Informed Consent

Informed consent (including a description of the nature, purpose, risks, and benefits of the study) will take place through both oral and written explanation of the study. A legally valid signature will be obtained via REDCap. 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 (even if they sign electronically in REDCap). Informed consent will be collected by IRB-approved study personnel. The Ohio State University Institutional Review Board will approve recruitment scripts and materials, consent forms, and all study procedures . All participants will provide written consent before any study data are collected.

iii. Compensation and Retention

Participants will be compensated $50 for completing Visit #1, and an additional $20 if they bring the device back to the laboratory for their Visit #2. Participants will be compensated $400 for completing Visit #2 and $500 for completing Visit #3. Total participant remuneration, if all visits are completed and the study EC device, is returned is $970. To recruit and retain participants, as well as decrease attrition, rideshare services (i.e., Uber and Lyft) will be made available to participants (within 20 miles or less of the study location) as a transportation option to and from study visits.

Additionally, to ensure that the utilization of the PET scanner is maximized, participants will be given the opportunity to volunteer as an alternate for a no-show participant on a visit 2 that is scheduled prior to their regularly scheduled study visit 2. They will be compensated an additional $25 to be an alternate whether the regularly scheduled participant shows for their study visit or not. If the regularly scheduled study participant shows (within 15 minutes of their regularly scheduled appointment time), the alternate will be scheduled at their regular visit 2 time, receive $25 compensation for serving as an alternate, and return home. If the regularly scheduled participant does not show, the alternate will take the place of the regularly scheduled participant attending visit 2.

If participants attend a visit and there are technological issues, error, or chemistry fails (i.e. – cyclotron/scanner/radiochemistry module/hot e-liquid production) preventing the visit from occurring, participants will be compensated $50 for their time and inconvenience. If participants attend Visit #2 and/or #3 and their first scan is successful, but the second scan is not, due to technological issues, error, or chemistry fails, they will receive partial compensation (half of the visit compensation) for the designated amount of that visit (i.e. - $200 and $250 for Visits #2 and #3, respectively).

Payments will be made using the Greenphire ClinCard to increase accountability and facilitate ease of payment. We will also facilitate study visits by offering evening and weekend appointments and additional retention strategies (e.g., multiple sources of contact, reminder calls/texts/emails). These methods are consistent with our team's previous and current studies and have resulted in strong retention rates.

iv. Study Products

EC Device: All participants will complete puffing sessions using an Evolv Reflex device (**Figure 2)**, which is able to vape both FB and NSB e-liquid. Device specifications include: 750mAh battery, 3-25 watts output range, 2ml pod capacity, 37.5 grams weight, 1.0 amps of charging current, and 84mm x 300mm x 11mm dimensions with the pod installed. We will use the eScribe software available to retrieve information about the participant's use of the product in the field (and during the standardized puff session) including the number of puffs, puff duration, puff volume, flow rate, time of day puff was taken, wattage, coil temperature for each puff, and inter-puff-interval, which we view as a great strength. A new e-liquid pod will be used for each participant and each puffing session. Participants will be provided pre-filled and weighed e-liquid pods.

Figure 2. Study EC Device

E-liquid: There is no known commercial pair of nicotine liquids that differ only by nicotine form. For this study, we will prepare four [^{11}C]-nicotine liquids that differ by nicotine form (due to presence/absence of benzoic acid) and nicotine concentration, simulating characteristics of popularly used nicotine liquids[13,18]. Radiolabeled [^{11}C]-nicotine will be prepared according to the method previously developed by our team[19]. (Solingapuram et al, JNM 2019, 13:36) **Table 2** summarizes the key ingredients of the test liquids, which will be prepared from pure, "cold" (≥99%) (S)-nicotine; protonation and dilution of the radiolabeled nicotine will be done using USP grade benzoic acid (Low pH/protonated only), and glycerol and propylene glycol, respectively. [^{11}C]-labeled (S)-nicotine will be produced by our team according to applicable production guidelines for inhalants[19](Solingapuram et al, JNM 2019, 13:36). In short, purified [^{11}C]-labeled (S)-nicotine will be formulated with the corresponding e-liquid. The [^{11}C]-labeled (S)-nicotine concentration, radiochemical purity, bacterial endotoxin levels, and specific activity will be determined post-synthesis following all the approved SOPs. After formulation, appropriate amount of the e-liquid containing [^{11}C]-nicotine will be loaded in the e-cigarette cartridge in the PET scanner room. The total radioactivity in the cartridge of the e-cigarette will not exceed a 5.0 mCi dose delivered to the patient. The release of radioactivity is expected to be 15 +/- 5% during the inhalation phase[20]. The total delivered dose to the participant will be determined and recorded. We will submit an Investigational Tobacco Product application to the U.S. FDA to receive approval for the e-liquids.

Table 2. Identity and mass of main ingredients used to make the study e-liquids.

ITP Name	(–)-Nicotine (g)	Benzoic acid (g)	Vegetable Glycerol (g)	Propylene Glycol (g)	e-Liquid Flavoring (g)	Total Mass (g)
T3FB	22.50	none	(a)	(a)	727.50 Ghost	750.00
T3NS	22.50	16.94	(a)	(a)	710.56 Ghost	750.00
U3FB	22.50	none	509.25	218.25	none	750.00
U3NS	22.50	16.94	497.39	213.17	none	750.00

(a) The mass ratio of propylene glycol to vegetable glycerol (PG:VG) is 30:70.

G. Protocol Adherence and Quality Control

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, we will use secure computer-based questionnaires for participants to complete, including daily diaries. All key on-site personnel will meet face-to-face weekly throughout the entire study. Off-site investigators will also participate in these weekly meetings during the first year for project start-up; however, this will be reduced to every other week as the study progresses. During these meetings, recruitment, enrollment, data collection, data monitoring results, and any concerns or issues that may arise will be discussed. We will also plan for all key personnel to meet (virtually) at the Ohio State University and Wake Forest University sites in the first year for start-up and then in the final year to discuss analyses, manuscripts, and future research based off of this study.

H. Measures

All measures, summarized in **Table 3**, have been used and validated previously by our team. ***Questionnaire data*** will be collected electronically into a secure, encrypted database (REDCap) by a trained research assistant. Sociodemographic measures will assess participant age, sex, marital status, ethnicity, employment status, occupation, years of education, and socioeconomic status. Tobacco use history will assess years of tobacco use, age of first use, tobacco type, brand, frequency, quantity, and duration of use all of nicotine/tobacco products including cigars, cigarillos, little cigars, pipe tobacco, chewing tobacco, snuff, snus, EC/vape/mod/APV/e-hookah, and hookah tobacco. EC Dependence will be measured with a modified 12-item Cigarette Dependence Scale[21]. Abuse liability of products will be measured by self-report of subjective effects, including an adapted version of the Drug Effects/Liking Questionnaire will assess the desire and liking of both study products, positive and negative effects (i.e., side effects), and perceived strength and effectiveness. The modified Cigarette Evaluation Questionnaire (mCEQ)[22] will also assess subjective responses to e-cigarettes (e.g., reward, satisfaction)[23,24]. EC puff topography will be measured with the study EC device, including measures of puff count, puff duration, inter-puff-interval, puff flow rate, average puff volume, and total puff volume. Puff topography is a validated and sensitive behavioral measure of abuse liability, is highly stable and associated with level of dependence, and predicts level of exposure to harmful tobacco-related toxicants[25–27]. To corroborate 12-hour nicotine abstinence prior to visits 2 and 3, e-cigarette urges/craving will be measured using the 10-item Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form [28]. A modified version (replacing the word "cigarette" with "e-cigarette") for e-cigarette users and use conditions. Additionally, nicotine withdrawal will be assessed using the empirically validated 15-item version of the Minnesota Nicotine Withdrawal Scale[29].

[^{11}C]-nicotine formulated with a specific eLiq (protonated or free-base) will be produced at the Wake Forest PET Research Center. Subjects will be inhaling a single puff of vapor containing [^{11}C]-nicotine in either protonated or free-base e-Liquid (10 µL). Dynamic 0-15 min PET imaging will be obtained in the head-neck regions followed by a 5 min chest (oral cavity-lungs) scan. The same scan will be repeated with both the e-liquid formulations with 0-15 min chest and 5 min head scans. The subject will be scanned for 15 min in a sequence of 245 frames of 1-4 sec each. Followed by it, a 5 min scan is obtained from regions below the head i.e., oral cavity, trachea, esophagus, bronchi and lungs. All the images will be conducted using GE PET/CT Discovery MI DR scanner. Whole brain radioactivity will be calculated as a percentage of total absorbed dose

per kilogram of brain tissue. Standard uptake values (SUVs), Time activity curves (TACs), and percent variance in total radioactivity will be determined and compared at each time point.

Table 3: Study Measures and Outcomes			
Visit	1	2	3
Background Measures			
Additional Screening	X		
Sociodemographic Measures	X		
Tobacco Use History/Dependence	X		
NicAlert Test Strip	X		
Medical History	X		
Abuse Liability			
EC Topography		X	X
Drug Effects/Liking Questionnaire		X	
Modified Cigarette Evaluation Questionnaire (mCEQ)		X	
Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form (modified)		X	X
Minnesota Nicotine Withdrawal Scale		X	X
Biomarkers of Exposure/Effect			
Exhaled Carbon Monoxide	X	X	X
Procedures			
Pregnancy Test	X	X	X
PET/CT Imaging		X	X

I. Statistical Methods

i. Power Analysis and Data Analytic Plan

The present study is a pilot study that will be used for preliminary data for a larger R01 submission. Assuming an alpha value of 0.05 and a power of 0.8, a sample size of at least 8 participants is required to observe significant differences in the dose released. As a result, larger populations are likely required in order to evaluate a significant difference between the groups. If the power is set to 0.95 to account for this, then the number of participants required per group is 14. In order to ensure significance and to account for any participants that do not comply, a total of 30 participants is required.

All PET images will be reconstructed to generate quantitative images for absolute determination of radioactivity distribution. Corrections for the decay of [^{11}C] will be applied. The deposition will be shown in 3D and co-localized relative to the anatomy (as provided by the CT images). The total radioactivity will be determined in the head/neck and thorax (e.g., brain, oral cavity, trachea, esophagus, bronchi, lungs, and other relevant soft tissues like muscle). Analysis of other tissues within the imaging field of view may be performed if [^{11}C] activity is observed. The biodistribution, uptake, and clearance will be evaluated in the specified tissues. The results obtained using conventional PET/CT will be compared to the results obtained using the new digital technology. The percent variance in the total radioactivity will be determined at each time point for each tissue. We are not performing intra-

individual comparator examinations. We are focusing on assessing the new generation digital technology and have comparable historical benchmark data available for comparison.

Each participant will serve as his/her own control. We will plot time-activity curves for the [^{11}C] in the lung and brain over the 60-minute scanning period. For both participant groups, we will compare:

- The lung and brain concentration of [^{11}C] at peak uptake (Cmax for lung and brain).
- The time to peak uptake for the upper and lower lung and whole brain regions (Tmax for upper and lower lung and brain).
- The area under the nicotine concentration curve (AUC) for lung and brain regions.
- Puffing topography (puff duration, volume, and flow rate (average and peak) and interpuff interval.
- Demand indices, liking/satisfaction, craving, withdrawal suppression.

For imaging outcomes, topography and subjective effects, all relevant outcome variables will first be examined with descriptive statistical analyses that focus on describing and understanding patterns and distributions. We will identify any potential outliers, assess distributional assumptions, and apply appropriate transformations if the normality assumption is violated. To test the impact of pH of e-liquid vaped on rate and concentration of [^{11}C]-nicotine uptake in the brain and lung regions, and subjective effects, the primary comparison arms will be free-base vs. nicotine salt-based nicotine e-liquid. For each hypothesis we will compare the difference in mean outcomes (post – pre-vaping bout, where appropriate) per laboratory session using the mixed effects modeling approach for repeated measures analysis of variance that uses a random effects model to account for multiple observations from the same study subject's outcome measures.

Conceptually, the analysis approach reduces to a simple paired t-test for parametric analysis or Wilcoxon signed-rank test for non-parametric analysis... This approach allows for proper treatment of the multiple observations per study subject's outcome data by accounting for the correlation of data observations within the same study subject's outcome. Mixed effects modeling also allows for the dataset to have some missing values, unequal time points between subjects, and flexible covariance structures.. Pairwise comparisons will be adjusted for multiple testing procedures. All statistical tests will be two-tailed, and no assumptions are made *a priori* about the direction of potential differences in primary outcome distributions.

J. Gender/Minority/Pediatric Inclusion for Research

i. Inclusion of Women and Minorities

According to U.S. Census Data, 53% of Winston-Salem residents are female. In our previous studies with smokers, 55-62% of participants were female. In Winston-Salem, racial composition is 56% White, 35% Black or African American, 2% Asian, 0.2% American Indian/Alaska Native, 0.1% Native Hawaiian/Other Pacific Islander, and 3% two or more races. The ethnic composition of individuals living in Winston-Salem is 6% and 15% Hispanic/Latino, respectively. 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 team's 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 and community centers.

ii. Inclusion of Children

Participation in the proposed study will be restricted to individuals 21 years of age and older. This exclusion is because the use of tobacco products by minors is illegal and the concern of introducing and potentially addicting children and adolescents to another tobacco product (i.e., electronic cigarettes).

K. Human Participants

i. Potential Risks

The research protocol calls for current e-cigarette users to use a study e-cigarette. The participants are already using e-cigarettes and will only be asked to use what will be a fully characterized e-cigarette device and e-liquids for which an FDA ITP will be submitted and "approved" (i.e., receive an FDA letter of "no concern"). Questionnaires, saliva, and exhaled breath procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) risk of using e-cigarettes, b) risk associated with radioactivity exposure, c) loss of confidentiality or privacy, and d) lack of appropriate storage of nicotine-containing products in a house with children and pets.

ii. Protections Against Risk

a) 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) will take place through oral 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. The Ohio State University Institutional Review Board will approve recruitment script and materials, consent forms, and all study procedures. All participants will provide consent before any study data is collected.

Efforts to reduce risk are as follows:

a) Risk of using e-cigarettes: The risk of side effects and adverse events are very low. These products are sold online, and at e-cigarette specialty stores and convenience stores nationwide, without a prescription. 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, then to the Ohio State University 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 event (potential for nicotine overdose) 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 EC use). Lab studies of toxin exposure suggest that ECs 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 and they will be provided the number to the North Carolina Tobacco Quitline (QuitlineNC).

b) **Risk of radiation exposure**: The risks of radiation exposure are minimized with the use of a CT scan at a very low dose and the lowest dose of [^{11}C]-nicotine (3 mCi) used in a nicotine PET imaging study. However, participants' level of risk from this level of exposure will be explained by giving them a comparison to an everyday situation to help them make an informed decision. Participants will be told, "To give you an idea about how much radiation you will get, we will make a comparison with an every-day situation. Everyone receives a small amount of unavoidable radiation each year. Some of this radiation comes from space, and some comes from naturally-occurring radioactive forms of water and minerals. This dosage would give your body the equivalent of about 5 extra months' worth of this natural radiation. A possible health problem seen with radiation exposure is an increased cancer risk. This extra cancer risk is higher at younger ages and for girls and women. The extra lifetime risk of dying of a fatal cancer due to the radiation exposure from this research may range from about one in 49,000 to about one in 20,000. At such low radiation exposures, scientists disagree about the amount of risk. These estimates are very uncertain, and there may be no extra risk at all."

c) **Loss of Confidentiality and Privacy**: Study subjects' confidentiality will be maintained at all times. Subjects will be assigned a unique study identification number (study ID). All study data will be identified by study ID only. Any electronic information (e.g., questionnaire data, laboratory data, tracking systems, etc.) will only be accessible to authorized study personnel who have the necessary password(s). All computer systems will be password protected against intrusion; all network-based inter-site communications of confidential information will be encrypted. Access to computer-stored information will require simultaneous knowledge of the data format, computer language, file name and passwords. 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.

d) **Lack of Appropriate Storage of EC Products**: Participants will be instructed to keep their EC cartridges up and away from their children and pets to protect against unintentional poisoning. Only child-proof e-liquid cartridges will be provided to participants. Although overdose or accidental ingestion is very unlikely and has not occurred in Dr. Wagener's previous studies, all participants will be provided the state and national poison control telephone line as well as a "tip sheet" on recognizing signs of nicotine overdose.

iii. COVID-19 Considerations

Assuming COVID-19 is still active in the population at the start of the study, all subjects will follow best practices for clinical screening of COVID-19 prior to each visit and on the day of the visit will be in effect at Wake Forest. Participants will be screened prior to arriving on campus for COVID-19 symptoms and exposures. On campus, they will have their temperature taken and required to wear a mask. Staff will wear masks, face shields, gloves and gowns during the study procedures. If antibody testing is validated at any time during the study, this will be incorporated into the screening. Research staff will follow OSU/WF policies by wearing face masks and eye protection, using frequent hand hygiene, and gloves

when touching a participant. Disinfectant policies after each visit also will be followed. At the start of study, and at any time clinical protocols may change, these will be adopted.

iv. Potential Benefits of the Proposed Research

Whereas no assurance can be made to an individual participant that he/she will personally benefit from this research, the experience should be beneficial. In general, this study will provide a benefit to future e-cig users as the findings will be reported to public health officials and the FDA to inform policy, both for reducing toxicity (if any) and to identify harmful effects. All participants will be encouraged to quit using e-cigarettes at the completion of the study. Adequate protections are in place in the event of unlikely and mild risks for study participation. Research results will not be returned to the subject. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

v. Importance of Knowledge to be Gained

This study is an innovative investigation that will have important public health implications given the rapid proliferation of e-cigarettes. The study has the potential for high public health impact as it will provide the scientific foundation, the FDA, and other public health agencies need to establish effective regulatory strategies for the manufacture, distribution, and marketing of nicotine salt-based ECs.

L. Data and Safety Monitoring Plan

i. Plan

Collection of Adverse Events: The collection of adverse events will be on a self-report basis by the participant and logged by research staff within an electronic data capture system (REDCap) or collected using standardized paper forms and will only be identified with the study's ID of the participant. All Serious Adverse Events will also be reported in OnCore database by Ohio State research staff.

Adverse Event Reporting Timelines:

- All intervention staff are required to notify the PIs of any Serious Adverse Events immediately.
- All Serious Adverse Events will be reported immediately to the medical monitor who will determine their severity and appropriate action or response.
- In accordance with current standard procedure, the PIs will notify the Ohio State University IRB of all Serious Adverse Events within 24 hours.
- In accordance with new policy and procedure, the PIs will notify the National Institutes of Drug Abuse of all Serious Adverse Events within 72 hours.
- All Non-serious AEs will be reviewed weekly by the medical monitor or PI, for categorization and possible action.

Data Safety Management Plan Administration

Adverse Events: All observed or volunteered adverse events regardless of suspected causal relationship to study product will be assessed by study staff and recorded on the adverse event page(s) of the Adverse Event Form or within an electronic data capture system (REDCap). For the purpose of this study, hereafter, Study Product will be used to refer to e-cigarettes. Events involving adverse Study Product reactions or illnesses with onset during the study should be recorded. Exacerbation of pre-existing illness is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the trial. It may include worsening or increase in severity of signs or symptoms of the illness, increase in frequency of signs and symptoms of an intermittent illness, or the appearance of a new manifestation/complication. Exacerbation of a pre-existing illness should be considered when a patient/subject requires new or additional concomitant medication or non-medication therapy for the treatment of that illness during the trial. Lack of or insufficient clinical response, benefit, efficacy, therapeutic effect, or pharmacologic action should not be recorded as an adverse event. The medical monitor at Wake Forest University, will be responsible for distinguishing between exacerbation of pre-existing illness and lack of therapeutic efficacy. For all adverse events, the PIs will pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a Serious Adverse Event requiring immediate notification to the medical monitor. For all adverse events, sufficient information should be obtained by the PIs to determine the causality of the adverse event (i.e., Study Product or other illness). The PIs are required to assess causality and indicate that assessment on the Case Report Form. Follow-up of the adverse event, after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator or his/her designated representative.

Serious Adverse Events: All Serious Adverse Events regardless of study product group or suspected relationship to Study Medication must be reported immediately to the medical monitor, then to IRB, then to NIDA, and in OnCore. A Serious Adverse Event is any adverse study product experience occurring at any dose that: (1) results in death; (2) is life-threatening; (3) results in inpatient hospitalization or prolongation of existing hospitalization; (4) results in a persistent or significant disability/incapacity; or (5) results in congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered Serious Adverse Events when, based upon appropriate medical judgment, they may jeopardize the patient/subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Any Serious Adverse Event or death must be reported immediately independent of the circumstances or suspected cause if it occurs or comes to the attention of the principal investigator at any time during the study through the last follow-up visit required by the protocol or 30 days after the last administration of Study Product, whichever comes later. Any Serious Adverse Event occurring at any other time after completion of the study must be promptly reported if a causal relationship to Study Product is suspected. The only exception to these reporting requirements is Serious Adverse Events that occur during a period in which no study product is administered. For all Serious Adverse Events, the investigator is obligated to pursue and provide information as requested by the Ohio State University IRB in addition to that on the Adverse Event Form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event,

including concomitant medications and illnesses, must be provided. The PIs will ensure that information is reported immediately and information entered in the Adverse Event Form are accurate and consistent.

Preventing and Limiting Adverse Events: We will monitor for risk of using e-cigarettes by screening participants for general medical precautions. The most likely adverse event (potential for nicotine overdose) is anticipated to be rare and mild based on the PI's previous studies, and will be handled quickly (i.e., advice to participant to reduce or eliminate nicotine use). Lab studies of toxin exposure suggest that e-cigarettes incur no greater risk to health than do combustible cigarettes. The PIs and study personnel will be available for any questions that participants may have about e-cigarettes. Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and the PI.

M. Literature Cited

1. Wang T, Gentzke A, Sharapova S, Cullen K, Ambrose B, Jamal A. Tobacco product use among middle and high school students – United Sates, 2011-2017. *MMWR Morb Mortal Wkly Rep.* 2018;67(22):629-633.

2. Mirbolouk M, Charkhchi P, Kianoush S, et al. Prevalence and distribution of e-cigarette use among US adults: behavioral risk factor surveillance system, 2016. *Annals of internal medicine*. 2018;169(7):429-438.

3. Statement from FDA Commissioner Scott Gottlieb, M.D., on new steps to address epidemic of youth e-cigarette use [press release]. Washington, DC2018.

4. National Academies of Sciences E, Medicine. *Public health consequences of e-cigarettes*. National Academies Press; 2018.

5. LaVito A. Popular e-cigarette Juul's sales have surged almost 800 percent over the past year. CNBC: Health and Science. https://www.cnbc.com/2018/07/02/juul-e-cigarette-sales-have-surged-over-the-past-year.html. Published 2018. Accessed August 25 2019.

6. Brunnemann K, Hoffmann D. The pH of tobacco smoke. *Food and Cosmetics Toxicology*. 1974;12(1):115-124.

7. National Cancer Institute. Risks Associated with Smoking Cigarettes with Low Machine-Measured Yields of Tar and Nicotine. *Monograph* 13. 2001.

8. Wagener TL, Floyd EL, Stepanov I, et al. Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users. *Tobacco control*. 2017;26(e1): e23-e28.

9. Tackett AP, Lechner WV, Meier E, et al. Biochemically verified smoking cessation and vaping beliefs among vape store customers. *Addiction*. 2015;110(5):868-874.

10. Leavens ELS, Mehta T, Hale JJ, Shihadeh A, Halquist MS, Wagener TL. Nicotine delivery and abuse liability of pod-mod electronic cigarettes compared to combustible cigarettes. Submitted abstract.

11. Brinkman M, Keller-Hamilton B, Carney-Knisely G, et al. Effect of pH of the nicotine liquid on JUUL puffing behavior and nicotine uptake. Submitted abstract.

12. Ramamurthi D, Chau C, Jackler RK. JUUL and other stealth vaporisers: hiding the habit from parents and teachers. *Tobacco control*. 2018: tobaccocontrol-2018-054455.

13. Talih S, Salman R, El-Hage R, et al. Characteristics and toxicant emissions of JUUL electronic cigarettes. *Tobacco control*. 2019: tobaccocontrol-2018-054616.

14. Wagener TL. Will the Debate Over e-Cigarettes Start Cooling Down? *JAMA network open*. 2018;1(8):e185945-e185945.

15. Leventhal AM, Strong DR, Kirkpatrick MG, et al. Association of electronic cigarette use with initiation of combustible tobacco product smoking in early adolescence. *JAMA*. 2015;314(7):700-707.

16. Leavens ELS, Stevens EM, Brett EL, et al. JUUL electronic cigarette use patterns, other tobacco product use, and reasons for use among ever users: Results from a convenience sample. *Addict Behav.* 2019;95:178-183.

17. Brett El, Stevens EM, Wagener TL, et al. A content analysis of JUUL discussions on social media: Using Reddit to understand patterns and perceptions of JUUL use. *Drug Alcohol Depend*. 2019;194:358-362.

18. Herzok B, Nielsen KP. Tobacco 'All Channel' Data 1/27. *Wells Fargo Equity Research Reports*. 2018.

19. Ghosh A, Woolum K, Knopp MV, Kumar K. Development and optimization of a novel automated loop method for production of [^{11}C]-nicotine. *Applied Radiation and Isotopes*. 2018;140:76-82.

20. Bergsrom M, Nordberg A, Lunell E, Antoni G, Langstrom B. Regional deposition of inhaled 11C-nicotine vapor in the human airway as visualized by positron emission tomography. *Clin Pharmacol Ther*. 1995;57(3):309-317.

21. Etter JF, Le Houezec J, Perneger TV. A self-administered questionnaire to measure dependence on cigarettes: the cigarette dependence scale. *Neuropsychopharmacology*. 2003;28(2):359.

22. Zacny JP, Conley K, Marks S. Comparing the subjective, psychomotor and physiological effects of intravenous nalbuphine and morphine in healthy volunteers. *Journal of Pharmacology and Experimental Therapeutics*. 1997;280(3):1159-1169.

23. Cappelleri JC, Bushmakin AG, Baker CL, Merikle E, Olufade AO, Gilbert DG. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. *Addict Behav*. 2007;32(5):912-923.

24. Westman E, Levin E, Rose J. Smoking while wearing the nicotine patch-is smoking satisfying or harmful. *Clinical Research*. 1992;40(4):A871-A871.

25. Gass JC, Germeroth LJ, Wray JM, Tiffany ST. The reliability and stability of puff topography variables in non-daily smokers assessed in the laboratory. *Nicotine & Tobacco Research*. 2015;18(4):484-490.

26. Perkins KA, Karelitz JL, Giedgowd GE, Conklin CA. The reliability of puff topography and subjective responses during ad lib smoking of a single cigarette. *Nicotine & Tobacco Research*. 2011;14(4):490-494.

27. Lee EM, Malson JL, Waters AJ, Moolchan ET, Pickworth WB. Smoking topography: reliability and validity in dependent smokers. *Nicotine & Tobacco Research*. 2003;5(5):673-679.

28. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine & Tobacco Research*. 2001;3(1):7-16.

29. Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. *Archives of general psychiatry*. 1986;43(3):289-294.