EXAMINATION OF ELECTRONIC CIGARETTE USER PUFF TOPOGRAPHY: THE EFFECT OF A MOUTHPIECE-BASED TOPOGRAPHY MEASUREMENT DEVICE ON PLASMA NICOTINE AND SUBJECTIVE EFFECTS

Tory Spindle
Virginia Commonwealth University

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By: TORY R. SPINDLE
B.S. Virginia Commonwealth University, Spring, 2013

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Virginia Commonwealth University
Richmond, Virginia
September, 2015
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<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
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<tr>
<td>CO</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>CReSS</td>
<td>Clinical Research Support System</td>
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<tr>
<td>ECIG</td>
<td>electronic cigarette</td>
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<tr>
<td>FTC</td>
<td>Federal Trade Commission</td>
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<tr>
<td>HSD</td>
<td>honestly significant difference</td>
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<td>HR</td>
<td>heart rate</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>IPI</td>
<td>Inter-puff-interval</td>
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<tr>
<td>LOQ</td>
<td>limit of quantification</td>
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<tr>
<td>MRTP</td>
<td>modified-risk tobacco product</td>
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<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram (0.000000001 grams)</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>NRT</td>
<td>nicotine replacement therapy</td>
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<tr>
<td>PAHs</td>
<td>polycyclic aromatic hydrocarbons</td>
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<tr>
<td>ppm</td>
<td>concentration in parts per million</td>
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Abstract

EXAMINATION OF ELECTRONIC CIGARETTE USER PUFF TOPOGRAPHY: THE EFFECT OF A MOUTHPIECE-BASED TOPOGRAPHY MEASUREMENT DEVICE ON PLASMA NICOTINE AND SUBJECTIVE EFFECTS.

By Tory R. Spindle, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

Virginia Commonwealth University, 2015

Major Director: Thomas Eissenberg, Ph.D.
Professor of Psychology
Department of Psychology and Center for the Study of Tobacco Products

Electronic cigarettes (ECIGs) operate by heating a nicotine-containing solution resulting in an inhalable aerosol. Nicotine delivery may be affected by users’ puffing behavior (puff topography), and little is known about the puff topography of ECIG users. The purpose of this study was to examine the extent to which a mouthpiece-based topography measurement device influences the acute effects associated with ECIG use.

Twenty-nine experienced ECIG users completed two sessions differing only by the presence of a mouthpiece-based topography recording device. In both sessions, participants completed one 10 puff, 30 sec inter-puff interval (IPI) ECIG-use bout and
another 90 minute *ad libitum* bout. Acute ECIG effects (plasma nicotine concentration, heart rate [HR], and subjective effects) were largely unaffected by the presence of the topography recording device. Evaluating ECIG puff topography through clinical laboratory methodology is necessary to understand the effects of these products (including toxicant exposure) and to inform their regulation.
Examination of Electronic Cigarette User Puff Topography: The Effect of a Mouthpiece-based Topography Measurement Device on Plasma Nicotine and Subjective Effects.

The health risks of smoking cigarettes such as lung cancer, stroke, and cardiovascular disorders are well documented (Mathers & Loncar, 2006). However, cigarette smoking remains the leading preventable cause of death resulting globally in an estimated 6 million, and nationally approximately 480,000 deaths per year (Agaku, King, & Dube, 2014). In addition, roughly $133 billion is spent annually on health care related expenses associated with smoking (Campaign for Tobacco-Free Kids, 2014). Furthermore, 18% of adults in the U.S. are current smokers (Agaku et al., 2014), and 23% of high school and 6.7% of middle school students currently use some form of tobacco (CDC, 2014). Reducing tobacco consumption and increasing tobacco cessation therefore is a public health necessity.

There are a variety of health benefits observed following abstinence from combustible tobacco. Smoking-related diseases such as cardiovascular disorders and cancers are primarily the result of exposure to harmful constituents of tobacco smoke (e.g., carbon monoxide [CO], tobacco-specific nitrosamines [TSNAs], and polycyclic aromatic hydrocarbons [PAHs]; USDHHS, 2014). Cessation from tobacco can greatly reduce smokers’ exposure to these harmful constituents, resulting in immediate (e.g. reduction in CO levels) and long-term health benefits (e.g., reduction in risk of certain lung, mouth, and esophageal cancers: Fagerström, 2002; USDHHS, 2014).

Despite the numerous health benefits associated with smoking cessation, few smokers are successful in quitting. Each year, 69% of cigarette smokers in the U.S. say
they want to quit (CDC, 2011), and 52% make a quit attempt (Agaku et al., 2014). However, relapse rates are very high, with approximately 6% of smokers remaining abstinent (CDC, 2011), despite the availability of numerous pharmacological (e.g., nicotine replacement therapy [NRT]) and behavioral (e.g., cognitive behavioral therapy) smoking cessation treatments (Fiore et al., 2008). Smoking cessation is difficult to achieve primarily because another constituent of tobacco (nicotine), is a drug of abuse with chemically addicting properties that can promote physiological dependence (USDHHS, 2014).

**Influence of Nicotine on Tobacco Consumption and Abstinence Symptom Suppression**

Initial episodes of nicotine self-administration are thought to be reinforced positively (similar to other stimulant drugs such as cocaine and amphetamine). Positive reinforcement occurs when the addition of a stimulus (e.g., nicotine) following a behavior (e.g., smoking) increases the likelihood of that behavior occurring again (Glautier, 2004). More specifically, initial episodes of nicotine consumption stimulate the nicotinic acetylcholine receptors (Benowitz, 2008; Henningfield & Keenan, 1993), inducing the release of dopamine and other neurotransmitters, thereby increasing the likelihood that successive episodes of nicotine consumption will occur (Benowitz, 2008). The acute, positive reinforcing effects of nicotine include: mild euphoria and heightened arousal (Watkins, Koob, & Markou, 2000). In addition to these positive reinforcing effects, chronic users may continue to self-administer nicotine due to a different mechanism: negative reinforcement. Negative reinforcement occurs when the removal of an aversive
stimulus following a particular behavior increases the likelihood of that behavior occurring again (Eissenberg, 2004). Prolonged use of tobacco products can lead to physiological dependence and adverse symptoms (e.g., irritability, difficulty concentrating, and anxiety) following abstinence (Hughes & Hatsukami, 1986; Watkins et al., 2000). Thus, cigarette users may continue to smoke because smoking can suppress these unpleasant abstinence symptoms (Eissenberg, 2004).

Numerous studies have demonstrated that tobacco use is often maintained in smokers by negative reinforcement. Cigarette smokers often cite abstinence symptoms as a principal reason for continuing to smoke and/or preventing them from quitting smoking (Cummings, Jaen, & Giovino, 1985; Gilbert, Sharpe, Ramanaiah, Detwiler, & Anderson, 2000). In addition, several studies have revealed that smokers who experience more severe abstinence symptoms are more likely to continue using tobacco (Piasecki et al., 2000; Piasecki, Jorenby, Smith, Fiore, & Baker, 2003a; Piasecki, Jorenby, Smith, Fiore, & Baker, 2003b; Piper et al., 2011). For example, secondary analysis of a large scale clinical trial that evaluated the efficacy of the 21 mg nicotine patch and bupropion for smoking cessation revealed that across all conditions of the study, abstinence symptom severity was a significant predictor of smoking relapse, suggesting that the more intense these smokers’ abstinence symptoms were, the more likely they were to relapse to combustible tobacco (Piasecki et al., 2003a).

Several human laboratory studies have shown that various abstinence symptoms can be suppressed in tobacco-abstinent smokers following consumption of their own brand of cigarettes (Brelan, Evans, Buchhalter, & Eissenberg, 2002; Brelan,
Kleykamp, & Eissenberg, 2006; Buchhalter & Eissenberg, 2000, Vansickel, Cobb, Weaver, & Eissenberg, 2010). For example, an acute evaluation of the effects of own brand cigarettes relative to two different types of electronic cigarettes (ECIGs) and a sham (unlit cigarette) in 12-hour-abstinent smokers revealed that own brand cigarettes significantly reduced subjective measures of “urge to smoke a cigarette,” “impatient,” “anxious,” “irritability/frustration/anger,” “craving a cigarette,” and “restlessness” (Vansickel et al., 2010). In addition, non-nicotine stimuli that accompany smoking can suppress abstinence symptoms in the absence of nicotine. Specifically, one double blind study revealed that over a five-day period, participants using de-nicotinized cigarettes reported reductions in the subjective items “urge to smoke a cigarette,” “desire for sweets,” and “hunger” (Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005).

Tobacco abstinence symptoms can also be suppressed by pharmacologically pure nicotine (Evans, Blank, Sams, Weaver, & Eissenberg, 2006; Kleykamp, Jennings, Sams, Weaver, & Eissenberg, 2008; Teneggi et al., 2002). In one clinical examination of the acute effects of the nicotine patch in 8-hour-abstinent smokers, abstinence symptoms were suppressed following patch administration in a dose-dependent manner (Kleykamp et al., 2008). The results of these and related studies concerning the relationship between pharmacologically pure nicotine and abstinence symptom suppression in smokers have led to the utilization of NRT as a tobacco cessation aid. NRTs can increase short-term cessation rates by 50 - 70%, and can be administered via a nicotine patch, nicotine lozenge, nicotine gum, nicotine nasal spray, and nicotine inhaler (Stead et al., 2012). As
described below, NRTs are a few of the numerous products designed to reduce tobacco-related health risks.

**Harm Reduction**

The extremely low rates of tobacco cessation and the observation that pharmacologically pure nicotine can alleviate tobacco abstinence symptoms have prompted many public health officials to endorse harm reduction strategies to decrease tobacco-related morbidity and mortality and promote tobacco cessation. Harm reduction advocates accept that a drug will continue to be used, often noting that total abstinence within a population is impractical, and thus work toward reducing the adverse outcomes for those who continue to use the drug (Stratton, Shetty, Wallace, & Bondurant, 2001). In the case of tobacco, harm reduction has been attempted by altering characteristics of cigarettes to reduce the toxicant content of smoke. For example, Advance cigarettes purported to reduce users’ exposure to TSNAs (Breland, Evans, Buchhalter, & Eissenberg, 2002).

The concept of harm reduction has also led to the development of alternative tobacco products that purportedly reduce harm associated the use of conventional tobacco products. These alternative products are sometimes referred to as “Potential Reduced Exposure Products” (PREPs) and in complying with recent legislation, the Food and Drug Administration has introduced the term “Modified Risk Tobacco Products” (MRTPs) to describe products that are modified and will be marketed with the intent to reduce tobacco-related morbidity and mortality (Deyton, Sharfstein, & Hamburg, 2010). Typically, these products are designed to reduce smokers’ exposure to harmful tobacco
constituents (e.g. TSNAs, PAHs, CO) but continue to deliver the addicting component of tobacco (nicotine). The realization that the combustion of tobacco is responsible for the formation of several harmful constituents in tobacco smoke (e.g. CO and PAHs) has led to the emergence of numerous non-combustible potential MRTPs (USDHHS, 2014). Examples of non-combustible potential MRTPs include: tobacco pouches (e.g. Swedish “snus”: Cobb, Weaver, & Eissenberg, 2010), low-nitrosamine tobacco pellets (e.g. Ariva and Stonewall: Blank & Eissenberg, 2010; Hatsukami et al., 2011), and nicotine-impregnated rubber disks (e.g. Verve: Zeller, 2012). Other previous products (no longer on the market) that purportedly reduced toxicant exposure by heating, rather than combusting tobacco were Philip Morris’ Accord and R.J. Reynolds’ Eclipse (Brelan et al., 2006; Buchhalter & Eissenberg, 2000). ECIGs are one of the newest and most popular types of alternative tobacco products, but their ability to reduce tobacco-related harm remains unclear.

**Background on ECIGS**

ECIGs were first patented in China in 2003 (Lik, 2003) and introduced into the U.S. market in 2007 (Regan, Promoff, Dube, & Arrazola, 2013). Since their inception, ECIGs have increased in popularity with use rates increasing among youth and adults steadily (CDC, 2013; King, Alam, Promoff, Arrazola, & Dube, 2013). In addition, ECIGs are featured in advertising, television, and movies (Grana, Glantz, & Ling, 2011), and ECIG sales in the U.S. are expected to exceed $2 billion in 2014 (Herzog & Gerberi, 2013). Despite their rapid growth in popularity, many questions remain about ECIGs, and limited research has been conducted.
ECIGs are a class of products that typically contain some common components including: a power source (usually a battery) and a heating element (commonly referred to as an atomizer). The element heats a solution that contains solvents (e.g. propylene glycol and/or vegetable glycerin) and sometimes, but not always, flavorings and nicotine. The user inhales the resulting aerosol. Despite these common features, ECIGs vary in their design features and appearance considerably. For example, some ECIGs are designed to resemble the shape, size and color of tobacco cigarettes. These ECIG models are sometimes referred to as “cigalikes,” and often store the liquid solution in a cartridge. The cartridges often contain the heating element, and are sometimes referred to as “cartomizers.” Users activate the heating element of these devices simply by inhaling through the mouth-end of the cartridge, and a light-emitting-diode (LED) glows at the non-mouth end when the heating element is activated. After the solution is depleted, some “cigalike” models require the user to replace or refill the cartridge, while others require the user to dispose of the entire ECIG (Breland, Spindle, Weaver, & Eissenberg, 2014; Etter 2012).

Other ECIG models do not resemble cigarettes, contain rechargeable batteries, and either store solution in reservoirs known as “tanks” or in cartridges. Users of these devices can buy the refill solutions separately and refill their tank or cartridge as needed or can buy cartridges pre-loaded with their preferred solution. The heating element in these models is usually activated manually by pressing a button near the mouth-end of the device (Breland et al., 2014; Etter 2012). In some of these models, users can alter the power flowing through the heater by changing the battery’s voltage in an effort to modify
characteristics of the subsequent aerosol (Etter, 2012; Shihadeh, Salman, Balhas, Karaoghlanean, Melvani, & Eissenberg, 2013).

In addition to varying in design features and appearance, the solutions intended for ECIGs also vary considerably. Nicotine concentrations of these solutions generally range from 0 to 36 mg/ml, and the solutions come in a variety of flavors (e.g. tobacco, menthol, dessert, and fruit: Breland et al., 2014; Vaperzone Inc., 2014). One recent study determined that there were at least 466 unique ECIG brands and 7,764 solution flavors (in terms of unique linguistic labels) available for purchase over the internet, and that these figures were increasing steadily (Zhu et al., 2014). To add to the complexity, discrepancies between actual and advertised nicotine concentrations have been observed in numerous ECIG liquids (Trehy et al., 2011).

The viability of ECIGs as an effective harm reduction strategy remains uncertain. Overall, features that would likely indicate that a potential MRTP would be an effective harm reduction strategy at the individual level would include: (1) reduce the users’ exposure to harmful smoke constituents, ideally by helping smokers to quit using combustible tobacco completely (2) suppress abstinence symptoms as effectively as tobacco cigarettes, thereby facilitating tobacco cessation and (3) not be susceptible to compensatory behavioral changes that may undermine their harm reduction potential (Hatsukami et al., 2007). From a public health standpoint, ECIGs could not be considered a viable harm reduction option if they: (1) encourage former cigarette smokers to relapse, (2) act as a gateway to conventional cigarettes for nicotine-naïve individuals, (3) re-normalize smoking, by undermining smoke-free laws or as a result of celebrity
endorsements for example, (4) expose non-users to harmful levels of ambient aerosol, (5) promote dual use among smokers (i.e. allowing smokers to use ECIGs in locations where cigarettes are banned), thereby decreasing the likelihood of tobacco cessation, and/or (6) result in nicotine addiction in those who are non-smokers (Fairchild, Bayer, & Colgrove, 2014; Glynn, 2014; Maziak, 2014). Many of the public health concerns associated with ECIGs are difficult to assess, and may remain unanswered for several years. Therefore, these public health concerns are beyond the scope of this study. However, careful and systematic empirical evaluations of toxicant exposure (including nicotine), abstinence suppression, and smoking behaviors associated with ECIG-use in clinical laboratory settings (such as the present study) can provide insight into the viability of ECIGs as a harm reduction strategy for individual users. As described below, similar clinical evaluations of other potential MRTPs has revealed their overall impact on smokers’ health and harm reduction utility for individual users.

**Evaluation of Potential MRTP’s: Toxicant Exposure and Abstinence Symptom Suppression**

Toxicant exposure associated with the use of potential MRTPs is often examined to assess their utility for harm reduction. For example, exposure to nicotine and nicotine’s metabolite cotinine can be measured in blood plasma (e.g. Cobb et al., 2010; Evans et al., 2006; Kleykamp et al., 2008; Vansickel et al., 2010). As previously mentioned, repeated administrations of nicotine can result in physiological dependence, and nicotine self-administration can suppress the aversive symptoms smokers experience during periods of tobacco abstinence (Fiore et al., 2008; Stead et al., 2012). Therefore, examining nicotine
delivery of potential MRTPs can provide insight into their viability as harm reduction strategies and/or cessation aids. More precisely, potential MRTPs likely will be most successful at replacing combustible tobacco cigarettes completely if they deliver nicotine at levels that will attenuate to abstinence symptoms and maintain use via negative reinforcement, thereby decreasing the likelihood of relapse to combustible tobacco. However, these products also should not deliver nicotine at levels that will be toxic to any user and ideally, the nicotine dose that they deliver will not produce positive reinforcement in previously nicotine-naïve users.

Acute evaluations of nicotine delivery from NRTs have revealed that, unlike cigarettes, nicotine is delivered from NRTs gradually and in most instances slowly. Specifically, nicotine from NRTs is absorbed through the oral mucosa, nasal mucosa, or skin, while nicotine from cigarette smoke is absorbed by the alveoli of the lungs (Le Houezec, 2003; Stead et al., 2012). Absorption of tobacco smoke by the alveoli results in more rapid and efficient delivery of nicotine into the bloodstream relative to mucosal or transdermal absorption associated with NRT use (Le Houezec, 2003). This relatively slow delivery of nicotine, in addition to the lack of non-nicotine stimuli associated with smoking, may explain why NRTs are less effective at abstinence symptom suppression compared to cigarettes, why they can be ineffective long-term cessation aids (Fiore et al., 2008) and also suggests that they can be improved upon from a harm reduction perspective.

In addition to measuring exposure to nicotine, assessing users’ exposure to harmful carcinogenic constituents found in tobacco smoke (e.g. TSNAs, PAHs, CO) is
often of interest when examining potential MRTPs. Exposure to metabolites of TSNAs can be assessed using urinalysis, but only after relatively long-term exposure due to a relatively long-term half-life (e.g., Benowitz et al., 2005; Breland et al., 2006). Exposure to CO can be analyzed in the user’s expired breath (e.g. Benowitz et al., 2005; Breland et al., 2006). One study examined TSNA, PAH, and CO exposure associated with the use of two potential MRTPs (Advance and Eclipse) that were marketed as products that could reduce smokers’ risk of tobacco-related disease. Advance was a combustible product purported to reduce users’ exposure to TSNAs while Eclipse heated two tobacco plugs at the tip of the device using a carbon heating element and also purported to decrease users’ toxicant exposure relative to conventional cigarettes. Thirty-five participants completed four Latin-square-ordered 5-day conditions in which they smoked either own brand, Eclipse, Advance, or no product. Urine was collected on days 1, 3, and 5 of each condition and analyzed for the TSNA metabolite 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL), and the PAH metabolite 1-hydroxypyrene (1-HOP), and expired air CO was measured each day. In addition, on days 1 and 5, participants used the products assigned to that condition in the laboratory. Over the course of five days, Advance reduced participants’ exposure to NNAL and CO relative to participants’ own brand of cigarettes. In the Eclipse condition, participants’ expired CO was increased while their NNAL levels and 1-HOP levels did not differ relative to own brand (Breland et al., 2006). These results demonstrated that Advance, but not Eclipse, reduced toxicant exposure relative to participants’ own brand of cigarettes.
Several other studies have demonstrated the feasibility of simultaneously examining acute nicotine exposure and abstinence symptom suppression associated with using potential MRTPs (Breland, Buchhalter, Evans, & Eissenberg, 2002; Cobb et al., 2010; Evans et al., 2006; Kleykamp et al., 2008; Vansickel et al., 2010). These studies have provided insight into the viability of various potential MRTPs as harm reduction strategies. One study examined the acute effects of several non-combustible oral tobacco products (Ariva, Marlboro Snus, Camel Snus, and the Commit nicotine lozenge) in 28 abstinent smokers relative to their own brand of cigarettes and denicotinized cigarettes. Although the oral, non-combustible products did not expose users to CO, they did not suppress abstinence symptoms or deliver nicotine as effectively compared to own brand cigarettes (Cobb et al., 2010). Another study examined the acute effects of two potential MRTPs (Accord and Eclipse; both claimed to reduce tobacco-related health risks by heating rather than burning tobacco) relative to own brand and denicotinized cigarettes. Twenty 12-hour abstinent smokers participated in this four condition, Latin-squared design study. Results indicated that relative to own brand cigarettes, Accord was less effective at reducing abstinence symptoms and increasing plasma nicotine concentrations. Conversely, Eclipse suppressed abstinence symptoms similarly to own brand cigarettes and delivered significantly more nicotine relative to Accord. However, Eclipse exposed users to greater amounts of CO relative to own brand cigarettes (Breland et al., 2002). Taken together, the results of these studies demonstrate that numerous potential MRTPs, some still in existence (oral, non-combustibles) and others not (Accord and Eclipse), may not be effective harm reduction options for smokers either due to ineffective abstinence
symptom suppression (e.g., oral, non-combustibles) or failure to reduce exposure to harmful toxicants (e.g., Eclipse).

ECIG proponents argue that ECIGs are a viable harm reduction strategy that can decrease tobacco-related morbidity and mortality considerably (Hajek, 2012; Nitzkin, 2014; Phillips, 2009). However, given the novelty of ECIGs and the substantial variability in their design features, much research is needed concerning their acute effects. The limited studies that have examined the acute effects of ECIGs in clinical laboratory settings are described below.

**Toxicant Exposure and Tobacco Abstinence Symptom Suppression Associated with ECIG Use**

The few studies that have examined the acute effects associated with ECIG use in clinical laboratory settings have examined nicotine and CO exposure and abstinence symptom suppression primarily (Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Nides, Leischow, Bhatter, & Simmons, 2014; Vansickel et al., 2010; Vansickel & Eissenberg, 2013; Yan & D’Ruiz, 2015).

Some of these studies have revealed that ECIGs deliver nicotine with different effectiveness, depending on the device. For example, one within-subjects design study examined nicotine delivery in 23 experienced ECIG users using a “cigalike” ECIG (“V2” ECIG with a cartomizer, battery wattage not reported) in one condition and a tank-based device (“ECIV”, 9 watt battery) in another. In both conditions, the devices were filled with the same solution (“Flavourart Maxblend,” 18 mg/ml nicotine concentration) and participants underwent two smoking bouts [one in which participants were instructed to
take 10 puffs of their ECIG with each puff separated by 30 seconds (i.e., a directed smoking bout), and one in which participants used the device *ad libitum* for 1 hour]. Plasma nicotine concentrations were recorded immediately after the 5 minute smoking bout and at 15 minute intervals in the *ad libitum* bout. The “cigalike” model delivered significantly less nicotine (ng/ml) to the user than the tank-based ECIG model at each time point including after the 5 min bout (cigalike condition, $M = 4.9$, SEM = 0.5; tank condition, $M = 6.6$, SEM = 0.6) at the end of the 60 min *ad libitum* bout: cigalike condition, $M = 15.8$, SEM = 1.2; tank condition, $M = 23.5$, SEM = 1.9 (Farsalinos et al., 2014).

Nicotine delivery associated with ECIG use also appears to be related to users’ smoking behaviors. Specifically, experienced ECIG users may be able to extract nicotine from ECIGs more effectively than ECIG naïve cigarette smokers. One study examined nicotine delivery in eight experienced ECIG users using their own devices (all of which were tank or cartridge-based models) using two smoking bouts (identical to Farsalinos et al., 2014). After the 5-minute directed bout users average plasma nicotine concentration (SEM) was 10.3ng/ml (0) while their mean plasma concentration (SEM) observed after 60 minutes of *ad libitum* use was 16.3 ng/ml (5.5; Vansickle & Eissenberg, 2013). While the plasma concentrations observed in these experienced ECIG users were smaller than those seen in other reports (Farsalinos et al., 2014), these values still approached those typically observed in tobacco cigarette smokers. Additionally, experienced ECIG users are also able to obtain nicotine using “cigalike” devices (Dawkins & Corcoran, 2014; Farsalinos et al., 2014) whereas inexperienced users using similar devices may be unable
to obtain nicotine (Vansickel et al., 2010). For example, experienced users instructed to use a “SKYCIG” “cigalike” device with a cartridge containing a nicotine concentration of 18 mg/ml were able to increase their plasma nicotine concentrations (ng/ml) significantly relative to baseline after a 10-puff, 5-minute bout (M = 6.8; SEM = 1.2) and a 60-minute *ad libitum* bout (M = 13.9; SEM = 2.1; Dawkins & Corcoran, 2014). Conversely, ECIG naïve cigarette smokers failed to extract nicotine from two “cigalike” devices (“NPRO,” 18 mg cartomizer and “Hydro,” 16 mg cartomizer) after two consecutive 10-puff directed smoking bouts (Vansickel et al., 2010). Further complicating the matter, nicotine delivery varies substantially among experienced users using the same devices (Dawkins & Corcoran, 2014; Vansickel & Eissenberg, 2013). For example, 10 minutes after 10 puffs from identical “cigalike” ECIGs, participants’ plasma nicotine concentrations ranged from 2.5 to 13.4 ng/ml (Dawkins & Corcoran, 2014).

Importantly, several of these studies have also demonstrated that ECIGs do not expose users to detectable levels of CO after use (Farsalinos et al., 2014; Nides et al., 2014; Vansickel et al., 2010). For example, ECIG-naïve cigarette smokers using a “cigalike” “NJOY” ECIG were not exposed to CO after undergoing two consecutive 10-puff, 5 minute directed bouts (Nides et al., 2014). Furthermore, CO levels did not increase in experienced ECIG users using a “V2” “cigalike” ECIG or an “ECIV” tank-based model after undergoing a 5-minute, 10-puff bout and 60-minute *ad libitum* bout consecutively (Farsalinos et al., 2014).

In each of the aforementioned ECIG studies (i.e., Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Nides et al., 2014; Vansickel et al., 2010; Vansickel &
Eissenberg, 2013), at least partial abstinence symptom suppression after ECIG use was observed, even in instances when no nicotine was delivered to the users (Vansickel et al., 2010). These results suggest that ECIGs may suppress tobacco abstinence symptoms partially by delivering non-nicotine behavioral stimuli associated with smoking (e.g. hand-to-mouth movements, feeling at the back of the throat, and sight of smoke-like aerosol).

These results partially support the idea that ECIGs may be a viable harm reduction strategy, in that they can suppress tobacco abstinence symptoms and do not expose users to CO. However, the observed inconsistencies regarding ECIG-associated nicotine delivery are troublesome, to the extent that nicotine delivery to the user is a key feature in predicting long-term use (and preventing relapse to combustible tobacco cigarettes). To understand the discrepancies observed in these clinical laboratory studies in terms of nicotine delivery better, a detailed analysis of ECIG users’ smoking behaviors (i.e. puff topography) is necessary, as there may be a specific set of behaviors required to obtain nicotine. As described below, the measurement of puff topography has been critical to understanding the effects of other products marketed with claims of harm reduction.

**Evaluation of Potential MRTPs: Puff Topography**

Puff topography is the quantitative measurement of individuals’ puff behaviors, including puff number, puff duration, puff volume, puff velocity (flow rate), and interpuff interval (IPI; Blank, 2008). The measurement of puff topography is critical when examining the effects of conventional cigarette and potential MRTP use because nicotine
intake, as well as the intake of other harmful smoke constituents, is largely determined by
the manner in which products are used (Gust & Pickens, 1982; Herning, Jones, Benowitz,

The importance of puff topography analysis is perhaps best illustrated by the
example of so-called “low yield” cigarettes. Despite being marketed to reduce smoking-
related health risks and exposure to harmful smoke constituents, “low-yield” cigarettes
were not evaluated initially, contributing to the widespread misconception that these
products are healthier, when in fact few, if any, positive health benefits are associated
with these products. Historically, machine-based smoking techniques that failed to
consider variability in users’ smoking behaviors have been used to assess toxicant
exposure. For example, the Federal Trade Commission (FTC) method for estimating
cigarette toxicant exposure consists of machine-smoking a cigarette in a standardized
procedure (i.e., 2 second, 35 ml puffs are performed until the cigarette reaches a length of
23 mm) and then analyzing the toxicant content of the resulting smoke (Hoffmann,
Djordjevic, & Hoffman, 1997; FTC, 2000). Based on the content of the smoke, cigarettes
can be classified into one of several categories: “full flavor,” “low-yield,” etc. Puff
topography analysis in several studies has revealed that the FTC method of measurement
is not predictive of actual toxicant exposure (including nicotine), because it does not
account for the high variability in human puffing behaviors and/or compensatory
smoking behaviors observed in smokers that switch from “full flavor” to “low-yield”
cigarettes (Baldinger, Hasenfratz, & Battig, 1995; Gust & Pickens, 1982; Herning, Jones,
Bachman, & Mines, 1981; Zacny & Stitzer, 1988). For example, one study examined puff
topography in 24 abstinent cigarette smokers using three cigarettes which differed by nicotine yield ("low," "medium," and "full flavor") but remained constant in terms of tar and CO yields and taste. Puff topography analysis showed that when using the "low-yield" cigarettes, users took puffs of greater volume, thus resulting in CO delivery similar to cigarettes that were not "low-yield" (Herning et al., 1981). Another such study examined puff topography in six smokers using cigarettes that differed again only by FTC-predicted nicotine yield. Smokers in this study underwent a two-day period of ad libitum use followed by three experimental sessions. Within each session, participants smoked two cigarettes ad libitum with nicotine yields of 0.3 ("low-yield") 1.3 ("medium-yield") or 2.5 ("full flavor"). Results revealed that as FTC-predicted nicotine yield decreased puff volume, puff duration, puff number, and CO exposure increased (Gust & Pickens, 1982). The results of these studies indicate that smokers may alter their smoking behaviors when switching to "low-yield" cigarettes, possibly in an attempt to titrate their nicotine intake. The compensatory smoking behaviors observed when users switch to "low-yield" cigarettes results in toxicant exposure that can approximate that of conventional "full flavor" cigarettes, undermining their harm reduction potential.

Topography data obtained from smokers in laboratory settings can be used to estimate toxicant exposure more accurately than the FTC method. For example, one study examined nicotine and tar yields produced from "low-yield," "medium-yield," and "full flavor" cigarettes by programming a smoking machine to either puff according to:

topography parameters normally used by the FTC method or topography data obtained from actual smokers. Results demonstrated that the FTC method underestimated toxicant
exposure and individual variability in topography considerably. Indeed, nicotine and tar yields based on topography data from actual smokers were 2-3 times higher than predicted by the FTC method (Djordjevic, Hoffmann, & Hoffman, 1997).

These studies highlight the importance of puff topography analysis in understanding the use and toxicant exposure associated with combustible tobacco products. However, the methodologies used to measure topography can differ substantially.

**Measurement of Puff Topography**

Initial research regarding the analysis of smoking behaviors relied on macro-level aspects of cigarette consumption (e.g., cigarettes smoked per day). However, as revealed by more detailed analysis of puff topography (e.g., Gust & Pickens, 1982; Herning et al., 1983), such macro-level measures do not account for the variability in smoking behavior and therefore are poor predictors of toxicant exposure (Guyatt, Kirkham, Mariner, Baldry, & Cumming, 1989). The two most common methodologies for measuring puff topography (i.e. observational and mouthpiece-based desktop devices) are described below.

Puff topography can be measured via observational methods. Specifically, smoking bouts can be video recorded in a laboratory setting enabling trained video scorers to measure several topography variables (i.e., puff number, puff duration, and IPI). Several studies that have examined puff topography via observational methods have used multiple video scorers and demonstrated high inter-rater reliability, indicating the reliability of direct observation as a topography measurement tool (Frederiksen, Miller, &
Peterson, 1977; Lichtenstein & Antonuccio, 1981; Blank, Disharoon, & Eissenberg, 2009). However, observational methods are labor intensive and time consuming. The authors of one study noted that the video scoring process lasted approximately 240 hours (Blank et al. 2009). Additionally, observational methods cannot be used to examine the topography measures of puff volume or puff velocity.

Because of the shortcomings of observational topography analysis, researchers have developed more precise and efficient instruments for measuring puff topography. The most commonly used versions of these instruments require cigarettes to be placed in a specialized mouthpiece that can detect flow-induced pressure changes across an orifice in the mouthpiece as a result of an inhalation. The pressure changes are sensed by a pressure transducer and converted to flow rate (puff velocity) via previously calibrated software. The converted flow rate measurements subsequently are used by the software to calculate other puff topography variables (puff duration, IPI, volume, and number: Blank, 2008). Portable mouthpiece-based topography recording devices that operate via similar mechanisms (i.e., calculating topography variables based on changes in flow rate) have also been used and validated (see: Blank, 2008). Numerous laboratory studies have demonstrated the reliability of mouthpiece-based devices such as the Clinical Research Support System for Laboratories (CReSS) for measuring topography in cigarette smokers (Blank et al., 2009; Buchhalter & Eissenberg, 2000). Furthermore, at least one study has demonstrated high reliability in measurement of topography recorded in cigarette smokers’ between mouthpiece-based and observational methods (Blank et al., 2009).
However, as described below, the ability of mouthpiece-based devices to measure ECIG topography accurately has not been reported previously and may pose several challenges.

**Puff Topography of ECIGs**

Measuring ECIG topography using extant mouthpiece-based systems such as CReSS may be challenging for several reasons. First, the mouthpieces used by computerized topography measurement systems may alter ECIG-associated nicotine delivery. The aerosol produced by ECIGs may condense inside the mouthpiece, inhibiting nicotine delivery and/or altering user behavior and effects.

Second, the results of the few studies that have examined puff topography in ECIG users using observational methods suggest that the topography of experienced ECIG users may differ from that of tobacco cigarette smokers (Farsalinos, Romangna, Tsiapras, Kyrzopoulos, & Voudris, 2013; Hua, Yip, & Talbot, 2013) and these differences may challenge the capabilities of topography measurement systems used for combustible tobacco products. One of these studies examined puff duration in 80 participants (35 smokers, 45 ECIG users) using a second generation ECIG (“eGo-T;” solution nicotine concentration: 9 mg/ml). ECIG users completed one 20 minute *ad libitum* ECIG-use bout, while cigarette smokers completed one 10 minute *ad libitum* ECIG-use bout and another bout in which they smoked two identical cigarettes provided by the researchers *ad libitum*. Cigarette smokers took significantly shorter puffs when using an ECIG (M = 2.4 sec) and when smoking cigarettes (M = 2.1 sec) relative to experienced ECIG users using an ECIG (M = 4.2 sec: Farsalinos et al., 2013). Another study utilized YouTube videos to quantify puff topography of individuals using
conventional cigarettes (N = 9) and ECIGs (N = 64). Again, ECIG users took significantly longer puffs (M = 4.3 sec) compared to cigarette smokers (M = 2.4 sec). ECIG users also exhibited large variability in puff durations (range: 1.9 – 8.3 sec: Hua et al., 2013).

In addition to the longer puff durations observed in ECIG users relative to cigarette smokers, some pilot research using the mouthpiece-based system CReSS suggests that ECIG users may also exhibit lower flow rates. The mean peak flow (or maximum flow rate) of a small sample of ECIG users (N = 4) using their preferred device/liquid combination was 18.4 ml/sec, whereas peak flow rates observed in cigarette smokers typically fall into the 50 ml/sec range (Eissenberg, 2014). Low flow rate puffs may reduce the accuracy of puff duration and volume measured by a mouthpiece-based system, as these systems rely on flow rate to detect the start and end of a puff. More precisely, mouthpiece-based systems are only capable of sensing a flow rate above a certain threshold (for CReSS, 15 ml/sec; Stewart, Vinci, Adams, Cohen, & Copeland, 2013) meaning any portion of a puff below that threshold will not be recorded (Blank, 2008). Some portion of a puff from an ECIG user likely will not be recorded using an extant mouthpiece-based system such as CReSS, given the close proximity of CReSS’s flow-sensing threshold to the peak flow rates observed in ECIG users (Eissenberg, 2014). Collectively, previous ECIG topography research suggests current computerized topography devices that were designed to measure cigarette topography may need to be altered to measure ECIG topography adequately.
The importance of measuring topography accurately and reliably when assessing potential MRTPs such as ECIGs cannot be overstated, as other studies have demonstrated that toxicant exposure observed in a laboratory setting (e.g., CO and nicotine exposure) from other tobacco products is highly correlated with toxicant yields produced by smoking machines programmed to puff in a way that mimics those participants’ puff topography (Shihadeh & Eissenberg, 2011). If this same correlation holds true for ECIGs, topography data recorded from ECIG users in a laboratory setting can be used to predict ECIG user toxicant exposure precisely. Furthermore, understanding the relationship between ECIG puff topography and toxicant exposure can inform ECIG users, regulatory agencies, and researchers of specific puffing behaviors necessary to minimize harm associated with the novel product while maximizing its ability to substitute completely for more harmful products, like tobacco cigarettes.

**Statement of the Problem**

ECIGs are rapidly growing in popularity, but little is known about them and few clinical evaluations have been conducted. Previous clinical laboratory studies have been crucial in understanding toxicant exposure (including nicotine), tobacco abstinence symptoms, and behavioral changes associated with the use of numerous other potential MRTPs. Some research has demonstrated that nicotine delivery from ECIGs varies, depending on the device and user behavior, and that ECIGs are capable of suppressing tobacco abstinence symptoms. For example, ECIG naïve cigarette smokers may be unable to obtain nicotine from certain ECIGs (Vansickel et al., 2010), whereas experienced ECIG users are capable of obtaining nicotine using their preferred devices.
(Vansickel & Eissenberg, 2013). Furthermore, nicotine delivery varies among experienced ECIG users substantially. Analysis of users’ puffing behaviors (i.e., puff topography) may increase our understanding of the variability in ECIG-associated nicotine delivery, as it has been critical to understanding the use of other novel tobacco products (Gust & Pickens, 1982; Herning et al., 1983). Puff topography of cigarette smokers can be analyzed by computerized measurement systems that require a specialized mouthpiece. While there have been a limited number of studies that have attempted to measure ECIG topography using computerized measurement systems designed for measuring cigarette smokers’ puff topography (e.g., Behar, Hua, & Talbot, 2015; Lee, Gawron, & Goniewicz, 2015), it is currently unknown if the mouthpiece required by these systems will interfere with ECIG-associated nicotine delivery, physiological effects, or subjective effects (including withdrawal suppression).

**The Present Study**

This study used clinical laboratory methods to compare nicotine delivery and subjective effects of experienced ECIG users when they use their preferred ECIG with and without a mouthpiece-based topography recording device. Additionally, ECIG topography was measured in the session in which the ECIG was attached to the mouthpiece-based device and compared to cigarette topography data from previous studies (e.g., Kleykamp et al., 2008). The topography recording device used consisted of modified hardware and software designed to record low flow rates puffs associated with ECIG use.

**Statement of Hypothesis**
Based on the results of previous research, it was hypothesized that participants in the present study would take longer puff durations relative to those observed in cigarette smokers in other studies. Additionally, participants were hypothesized to obtain significantly less nicotine, and report less favorable subjective responses (including less abstinence symptom suppression) after ECIG use in the session in which a topography mouthpiece is attached to the ECIG relative to when no mouthpiece is present.

**Method**

**Selection of Participants**

A total of 58 participants provided informed consent. Twenty-nine of these participants were ultimately not included in the final analyses. Of these non-completers, 11 began the study but withdrew prior to completion and 16 were determined to be ineligible at screening and thus never began a session: seven did not meet the study criteria for an experienced user (e.g., used < 1 ml of ECIG liquid per day), five exhibited elevated blood pressure, two were discontinued due to lack of venous access, one was discontinued for self-reporting prescription drug use, and one was discontinued due to elevated HR. Additionally, two participants (who used the same ECIG solution) completed the study but their data were not included in the final data set due to analyses (as described in Breland et al., 2006) detecting no nicotine in their ECIG liquid, despite the liquid being advertised as containing 12 mg/ml nicotine.

Thus, 29 ECIG-using community volunteers (22 males, 24 white) completed this within-subjects study and were included in the final analyses. A power analysis completed before the study began revealed that 30 participants should be sufficient to
obtain power of at least 0.80 (i.e. provide 80% chance of detecting an effect), given the within-subjects design and the assumption of a medium effect size and repeated measures correlation greater than 0.80 for the outcome plasma nicotine (Barcikowski & Robey, 1985). Indeed using these same assumptions, the 29 participants included in the final analyses were still sufficient to obtain power of at least 0.80 for the outcomes plasma nicotine, HR, and puff duration.

Participants were recruited by Institutional Review Board (IRB)-approved advertisements and/or word-of-mouth. All experimental sessions took place at the Clinical Behavioral Pharmacology Laboratory (CBPL) located on Virginia Commonwealth University’s (VCU) medical campus. The CBPL is part of VCU’s Center for the Study of Tobacco Products (CSTP). In order to be eligible for the study, participants had to be healthy, aged 18-55 (M = 29.6; SD = 7.7), use ≤ 5 conventional tobacco cigarettes daily (M = 0.1; SD = 0.3), use ≥ 1 ml of ECIG solution daily (M = 2.7; SD = 1.4), use ECIG solution with a nicotine concentration ≥ 12 mg/ml (M = 15.0; SD = 5.0; see Table 1), and must have used their ECIG for ≥ 3 months (M = 10.2; SD = 9.2; all according to self-report).

Participants were excluded for self-reported history of chronic disease or psychiatric condition, regular use of a prescription medication, marijuana use >10 and alcohol use >25 days in the past 30, and use of other illicit drugs (e.g. cocaine, opioids, benzodiazepines, and methamphetamine) in the past 30 days. Women were excluded if they tested positive for pregnancy (by urinalysis) at screening.
**Screening and Informed Consent Procedures**

All interested participants took part in a two-part screening process. The first part consisted of a phone interview where participants were asked about their health status and tobacco use (see appendix A). Participants who met the requirements for the study were asked to come to the laboratory for an in-person screening, in which they provided information about their health, tobacco use, and demographic information. Additionally, during the in-person screening, participants provided their informed consent to participate in the study (see appendix B) and women provided urine for a pregnancy test.

**Participant Safety and Rights**

The study methods and procedures involve minimal risk, and similar methods and procedures have been conducted numerous times at the CBPL over the course of 15 years without a serious adverse event. Twelve hours of nicotine abstinence may result in mild discomfort that is not medically dangerous. In addition, the blood drawing procedure involves minimal risk of bruising and/or infection at the catheter site, but these risks are minimized by the trained nursing staff and sterile nursing procedures. The risks and side effects of using ECIGs/nicotine are routine for the target population.

The trained CBPL staff ensured protection of participants’ safety and rights throughout the study. HR and blood pressure (BP) were monitored continuously and sessions were ended prematurely if a participant’s systolic BP dropped below 90 or above 140 or if their HR dropped below 50 or above 120. Data were treated with professional standards regarding confidentiality and were identified by an alphanumeric code only and stored in locked rooms only available to CBPL staff.
Materials

For each session, participants used their preferred ECIG device and solution. Participants provided their preferred ECIG and laboratory staff purchased the ECIG solution from each participant’s usual source (e.g. either internet or local ECIG shop). Table 1 lists the devices, liquid nicotine concentrations, and flavors (based on product labeling) that were used. The solvents used in ECIG liquids and battery voltage may influence nicotine yield (Kosmider, Sobczak, Knysak, & Goniewicz, 2014), so, when available, Table 1 also includes the advertised ratio of propylene glycol (PG) to vegetable glycerin (VG) and battery voltage. Because the sizes of tank and cartridge systems abound and therefore may not have been compatible with the mouthpiece-based topography system that was used, all participants were required to use a 510 cartridge with 1.5 ohms resistance and dual heating-coils. All cartridges were produced by SmokTech (Shenzhen, China) and purchased locally in Richmond, Virginia.

Procedures

After completion of the screening procedures (including informed consent), participants attended VCU’s CBPL on two days (separated by a minimum of 48 hours) to complete two approximately 3-hour sessions: one in which a mouthpiece-based topography recording device was attached to their ECIG and one in which it was not. Session order was counter-balanced across participants. Prior to each session, participants were asked to abstain from nicotine/tobacco for ≥ 12 hours. At the beginning of each session, participants’ expired air CO concentration was measured to verify abstinence from combustible tobacco (≤ 10 ppm, as in Breland et al., 2002). Because ECIGs are
non-combustible and therefore do not produce CO under normal conditions; retrospective examination of baseline plasma nicotine concentration was used to ensure abstinence. Ultimately, five of the 29 participants included in the final analyses were suspected to have not abstained from nicotine, but were included in the analyses because none of the results changed upon excluding them from the dataset and the higher N resulted in greater statistical power. Mean (SD) baseline plasma concentrations for the five participants suspected to have not abstained was 11.3 ng/ml (2.8) while the mean value for the 24 who remained abstinent was 2.4 ng/ml (0.9). An independent samples t-test revealed that these two baseline plasma nicotine means were significantly different: $t(27) = -14.6, p < .01$. After expired air CO was measured, physiological monitoring of HR and BP commenced. Then, an intravenous catheter was inserted into a forearm vein, and 7 ml blood was sampled. Participants then completed computerized questionnaires to assess their nicotine withdrawal and other effects (see below). Thirty minutes after session onset, participants were instructed to take 10 puffs from their ECIG, with each puff separated by 30 seconds; an observer instructed participants when to puff and verified compliance. Other than the puff number and IPI, puffs were ad libitum in this initial bout. Immediately following the tenth puff, an additional 7 ml blood was sampled, and participants completed the same questionnaires again. After 10 and 20 additional minutes, two more 7 ml blood samples were collected. Following this fourth blood sample, participants completed a 1 hour and 30 minute ad libitum ECIG-use session in which they were instructed to take as many puffs as they liked, whenever they liked. During the ad libitum bout, three additional 7 ml blood samples were taken, one every 30
minutes. Immediately after the *ad libitum bout*, participants completed the same subjective questionnaires for a third time. Fifteen minutes after the *ad libitum* bout was completed, the eighth and final blood sample occurred, the catheter was removed, and participants were compensated (US $100 after first session, US $150 after second).

**Outcome Measures**

**Physiological measures.** All blood samples were centrifuged, stored at -70°C, and sent to VCU’s Bioanalytical Analysis Core Laboratories for analysis of nicotine concentration (limit of quantitation (LOQ) = 2 ng/ml; see Breland et al., 2006). HR was monitored every 20 seconds using Criticare Systems model 507, fitted with pulse oximeter. Participants’ expired air CO was measured via a BreathCO monitor (Vitalograph, Lenexa, KS).

**Subjective questionnaires.** Four questionnaires were administered using a computerized visual analog scale (VAS), consisting of a word or phrase centered on a horizontal line with “not at all” on the left and “extremely” on the right. Responses were recorded by participants by moving a mouse cursor and clicking at any point on the horizontal line, with scores being expressed as a percentage of total line length. Where necessary, questionnaires were modified such that when the words “cigarette” or “smoking” appeared in the original, they were replaced by “e-cigarette” or “vaping” (a colloquial term for ECIG use; Hajek, Etter, Benowitz, Eissenberg, & McRobbie, 2014) for this study. These questionnaires were administered on three occasions during the study sessions: prior to ECIG use, after the directed bout, and after the *ad libitum* ECIG-use bout.
Table 1.

ECIG Device and Solution Characteristics (Based on Product Labeling and Manufacturer Information).

<table>
<thead>
<tr>
<th>Participant</th>
<th>ECIG model</th>
<th>Nicotine Concentration (mg/ml)</th>
<th>Solvent ratio: PG/VG</th>
<th>Battery Voltage</th>
<th>Liquid Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i-Taste</td>
<td>24</td>
<td>50/50</td>
<td>N/A</td>
<td>Peach</td>
</tr>
<tr>
<td>2</td>
<td>Smoke Tech</td>
<td>24</td>
<td>80/20</td>
<td>N/A</td>
<td>DK Blend</td>
</tr>
<tr>
<td>3</td>
<td>V2 Cigs</td>
<td>24</td>
<td>N/A</td>
<td>4.2</td>
<td>Menthol</td>
</tr>
<tr>
<td>4</td>
<td>Tsunami</td>
<td>24</td>
<td>30/70</td>
<td>N/A</td>
<td>Persian Winter</td>
</tr>
<tr>
<td>5</td>
<td>iTaste</td>
<td>24</td>
<td>50/50</td>
<td>N/A</td>
<td>Van. Dr. Pepp.</td>
</tr>
<tr>
<td>6</td>
<td>Vamo V2</td>
<td>24</td>
<td>0/100</td>
<td>N/A</td>
<td>Gorilla Juice</td>
</tr>
<tr>
<td>7</td>
<td>e-Go</td>
<td>24</td>
<td>30/70</td>
<td>3.3</td>
<td>Gold Rush</td>
</tr>
<tr>
<td>8</td>
<td>e-Go T</td>
<td>12</td>
<td>30/70</td>
<td>3.3</td>
<td>Aztec</td>
</tr>
<tr>
<td>9</td>
<td>e-Go</td>
<td>12</td>
<td>30/70</td>
<td>3.3</td>
<td>Cinnan-toast</td>
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<td>10</td>
<td>iTaste</td>
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<td>60/40</td>
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<tr>
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<td>Stingray</td>
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<td>50/50</td>
<td>3.7</td>
<td>Pink Tornado</td>
</tr>
<tr>
<td>12</td>
<td>Stingray</td>
<td>18</td>
<td>50/50</td>
<td>3.7</td>
<td>Mnky Business</td>
</tr>
<tr>
<td>13</td>
<td>iTaste MVP</td>
<td>24</td>
<td>60/40</td>
<td>N/A</td>
<td>Carolina Cured</td>
</tr>
<tr>
<td>14</td>
<td>eGo</td>
<td>12</td>
<td>30/70</td>
<td>3.7</td>
<td>White Mousee</td>
</tr>
<tr>
<td>15</td>
<td>iTaste MVP</td>
<td>12</td>
<td>30/70</td>
<td>N/A</td>
<td>Persian Winter</td>
</tr>
<tr>
<td>16</td>
<td>e-Go T</td>
<td>12</td>
<td>30/70</td>
<td>3.7</td>
<td>Persian Winter</td>
</tr>
<tr>
<td>17</td>
<td>e-Go T</td>
<td>28</td>
<td>40/60</td>
<td>3.7</td>
<td>Spearmint</td>
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<td>e-Go T</td>
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<td>70/30</td>
<td>3.7</td>
<td>Blueberry</td>
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<td>70/30</td>
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<td>Vanilla</td>
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<td>28</td>
<td>40/60</td>
<td>3.3</td>
<td>Wintergreen</td>
</tr>
<tr>
<td>22</td>
<td>i-Taste</td>
<td>18</td>
<td>40/60</td>
<td>9.0</td>
<td>Colonel Custard</td>
</tr>
<tr>
<td>23</td>
<td>i-Taste</td>
<td>12</td>
<td>50/50</td>
<td>2</td>
<td>Vanilla</td>
</tr>
<tr>
<td>24</td>
<td>i-Taste</td>
<td>12</td>
<td>40/60</td>
<td>3.3</td>
<td>King’s barrel</td>
</tr>
<tr>
<td>25</td>
<td>eGo</td>
<td>18</td>
<td>30/70</td>
<td>3.3</td>
<td>Cowboy Cut</td>
</tr>
<tr>
<td>26</td>
<td>Aspire</td>
<td>12</td>
<td>30/70</td>
<td>4.2</td>
<td>Unicorn Milk</td>
</tr>
<tr>
<td>27</td>
<td>iStick e-leaf</td>
<td>12</td>
<td>30/70</td>
<td>3.2</td>
<td>Cont. Breakfast</td>
</tr>
<tr>
<td>28</td>
<td>iStick e-leaf</td>
<td>24</td>
<td>30/70</td>
<td>3.7</td>
<td>Smerf Vendetta</td>
</tr>
<tr>
<td>29</td>
<td>Vamo V2</td>
<td>18</td>
<td>65/35</td>
<td>4.0</td>
<td>Jungle Juice</td>
</tr>
</tbody>
</table>

Direct Effects of Nicotine. The direct effects of nicotine scale, which assessed the direct effects of ECIG-associated nicotine delivery and nicotine–related side effects, consists of 10 items: “Confused,” “Dizzy,” “Headache,” “Heart Pound,” “Lightheaded,” “Nauseous,” “Nervous,” “Salivation,” “Sweaty,” and “Weak” (Evans et al., 2006).

Direct Effects of ECIG Use. This scale, adapted from the “Direct Effects of Tobacco” scale (Brelant et al., 2006), was developed with items reported in studies assessing the subjective effects of smoking (e.g. Foulds et al., 1992; Pickworth, Bunker, & Henningfield, 1994). This scale consists of 10 items: “Did the e-cigarette make you feel more awake?,” “Did the e-cigarette calm you down?,” “Did the e-cigarette help with concentration?,” “Did the e-cigarette make you dizzy?,” “Was the e-cigarette pleasant?,” “Did the e-cigarette reduce hunger?,” “Would you like another e-cigarette right now?,” “Was the e-cigarette satisfying?,” “Did the e-cigarette make you sick?,” and “Did the e-cigarette taste good?”

Acceptability Questionnaire. Finally, in the topography mouthpiece condition, this questionnaire used six VAS items to assess the degree to which the topography equipment: “Altered e-cigarette use behavior,” “Made vaping less likely,” “Reduced
enjoyment,” “Affected e-cigarette taste,” “Increased awareness,” and “Increased vaping difficulty” (as in Blank et al., 2009).

**Puff topography.** Puff topography measurements including puff volume, duration, number, IPI, and flow rate (a.k.a. puff velocity) were made using an ECIG topography instrument developed and manufactured at the American University of Beirut (AUB). This instrument operated similarly to commercially available cigarette topography instruments (e.g., CReSS, see introduction and Blank, 2009). Importantly, the mouthpiece’s orifice dimensions and pressure-sensing transducer provided sensitivity sufficient to ensure valid measurements at puff velocities as low as 3 ml/sec because topography devices used to study tobacco cigarette smoking behavior may not be sensitive enough to measure ECIG topography accurately (Eissenberg, 2014). For example, CReSS, the most commonly used mouthpiece-based device for measuring cigarette smokers’ puff topography, has a flow-detecting threshold of 15 ml/sec (Stewart et al., 2013).

Several mouthpieces were manufactured for the device that fit the cartomizer type used in the current study. Each mouthpiece was calibrated separately prior to each session using a custom built automatic digital flow calibrator.

**Data Analysis Plan**

For plasma nicotine data, any instances where the measurement was lower than the assay’s LOQ was replaced with the LOQ (2 ng/ml) as in previous work (e.g., Vansickel et al., 2010), as this method was a more conservative approach than identifying each value below the LOQ as zero. Prior to analysis, HR data were averaged to produce a
single value for the five minutes prior to each ECIG-use bout and prior to each blood draw (10 values in total). One participants’ HR data was incomplete due to an equipment malfunction and were thus excluded from the HR analysis. At the end of each ECIG use session with the mouthpiece, the topography instrument software integrated puff velocity data to produce the topography measures puff number, puff duration, puff volume, IPI, and mean puff velocity (see Shihadeh, Azar, Antonios, & Haddad, 2004 for details). Prior to analysis, the software performed a data cleaning procedure to correct for transducer noise. Data cleaning consisted of combining into a single puff any two puffs that were separated by less than 100 ms and deleting any puffs with a duration of less than 300 ms. Remaining data for each measure were averaged for each participant.

Repeated measures analysis of variance (ANOVAs) were used to examine subjective questionnaires, plasma nicotine, and HR data. For subjective measures that were administered in both conditions (i.e., Hughes-Hatsukami, direct effects of ECIG use, and direct effects of nicotine) two within-subject factors were included: session (with and without mouthpiece) and time (baseline, post-directed bout, and post- ad libitum bout). Data from each questionnaire item was analyzed individually. A two (session) by eight (time) repeated measures ANOVA was used to examine plasma nicotine, as there were five additional time points for this measure. A two (session) by nine (time) repeated measures ANOVA was used to examine HR data, as there were nine time points for this measure. Violations of sphericity were adjusted using Huynh-Feldt corrections, and Tukey’s Honestly Significant Difference (HSD) was used to analyze any significant main effects and interactions.
The six items assessing the influence of topography equipment, administered in the mouthpiece condition only, were analyzed using a paired t-test with two levels of time as the within-subject factor. Again, each questionnaire item was examined individually. Pre-ECIG use scores were not relevant for this questionnaire, as participants could not judge the influence of the topography equipment prior to using it.

Independent samples t-tests were conducted to compare puff topography data from the mouthpiece condition in the present study to puff topography data from 123 tobacco cigarette smokers, as described in Kleykamp et al., (2008). Paired samples t-tests were also conducted to compare the puff topography from the directed and *ad libitum* bouts in the mouthpiece condition. Lastly, Pearson correlation coefficients (*r*) were used to explore relationships among peak plasma concentrations in the directed and *ad libitum* bouts and participants’ liquid nicotine concentration, PG:VG ratio, and puff count (in the *ad libitum* bout only). All statistical analyses were performed using IBM SPSS (Version 22.0).

**Results**

Statistical analyses (main effects and interactions) for all measures are displayed in Table 2. The main effect of time and the interaction between time and condition were of greatest interest, as the main effect shows the influence of ECIG use and the interaction shows the extent to which the effects of ECIG use over time were affected by the presence of a topography mouthpiece. Importantly, of all 33 measures described in Table 2, only two showed a significant time by condition interaction, suggesting minimal influence of the mouthpiece condition overall.
Physiological Measures

As Table 2 indicates, a significant main effect of time (and no significant interaction with condition) was observed for plasma nicotine \[ F(7, 196) = 36.5, p < .001 \]. Figure 1 shows the mean data for each condition and time point. Collapsed across condition, mean plasma nicotine concentration immediately after the directed ECIG-use bout (20.6 ng/ml, SEM = 2.8) was significantly greater relative to baseline (4.0 ng/ml, SEM = 0.7) and 10 minutes-post directed bout (11.7 ng/ml, SEM = 1.4). Collapsed across condition, mean plasma nicotine concentration at 30 (25.7 ng/ml, SEM = 3.5), 60 (31.2 ng/ml, SEM = 3.7), and 90 (35.0 ng/ml, SEM = 4.3) minutes of the ad libitum bout were also significantly greater relative to baseline (4.0 ng/ml, SEM = 0.7) and 5 minutes-pre ad libitum bout (9.6 ng/ml, SEM = 1.3). Indeed, with the exception of time point 4 (5 min pre-ad libitum bout), all time points were significantly different relative to baseline, (Tukey’s HSD, \( p < .05 \)).

For HR, Table 2 shows that there was also a significant main effect of time \[ F(8, 216) = 19.8, p < .001 \] and no significant interaction with condition. Collapsed across condition, mean HR at baseline was significantly lower (66.3 bpm, SEM = 1.3) relative to the post-directed bout value (73.3 bpm, SEM = 1.3), and the values at 30 (73.9 bpm, SEM = 1.5), 60 (73.6 bpm, SEM = 1.6), and 90 (74.4 bpm, SEM = 1.7), minutes after the onset of the ad libitum bout (Tukey’s HSD, \( p < .05 \)). With the exception of time points 4 (5 min-pre-ad libitum) and 5 (start of ad libitum bout), all time points were significantly greater than baseline (Figure 2; Tukey’s HSD, \( p < .05 \)).
Subjective Measures

Hughes-Hatsukami Withdrawal Scale. As Table 2 indicates, significant main effects of time (and no significant interaction with condition) were observed for the items “Anxious,” “Craving,” “Depression,” “Hunger,” “Irritable,” “Restless,” and “Urge to Vape” \( F_s > 3.3, ps < .05 \). Figure 3 shows the results for “Craving,” and “Irritable” (two of the items with the largest \( F \) values).

A significant condition by time interaction was observed for the item “Impatient” \( F (2, 56) = 4.0, p < .05 \). Mean values for the three time points in the no mouthpiece condition were: baseline: \( (7.3, \text{SEM} = 3.0) \), post-directed \( (5.5, \text{SEM} = 2.4) \), post- *ad libitum* \( (4.8, \text{SEM} = 2.3) \) while mean VAS scores in the mouthpiece condition were: baseline: \( (10.6, \text{SEM} = 3.0) \), post-directed \( (3.0, \text{SEM} = 1.6) \), post- *ad libitum* \( (3.9, \text{SEM} = 2.0) \). Post-hoc testing (Tukey’s HSD) revealed no differences between conditions at any time point.

Direct effects of nicotine. Significant main effects of time were observed for the items “Dizzy,” and “Lightheaded” \( F_s > 6.3, ps < .01 \). Post-hoc tests (Tukey’s HSD) revealed that collapsed across condition, the mean post-directed bout VAS score \( (12.2, \text{SEM} = 2.9) \) was significantly greater relative to baseline \( (2.7, \text{SEM} = 1.2) \). A significant main effect of condition was observed for the item “Confused” \( F (1, 28) = 4.4, p < .05 \). Collapsed across time, higher mean ratings were observed in the no mouthpiece condition \( (1.7, \text{SEM} = 0.7) \) relative to the mouthpiece condition \( (0.6, \text{SEM} = 0.2) \) for this item.
Table 2.
Statistical Analyses Results for Plasma Nicotine and Subjective Measures (Directed Bout + Ad libitum bouts).

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Condition (C)</th>
<th>p value</th>
<th>Time (T)</th>
<th>p value</th>
<th>C x T</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>F value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma nicotine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5</td>
<td>ns</td>
<td>36.5</td>
<td>&lt;.001*</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Heart Rate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1</td>
<td>ns</td>
<td>19.8</td>
<td>&lt;.001*</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Hughes-Hatsukami&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxious</td>
<td>1.2</td>
<td>ns</td>
<td>9.0</td>
<td>&lt;.001*</td>
<td>1.1</td>
<td>ns</td>
</tr>
<tr>
<td>Craving</td>
<td>2.24</td>
<td>ns</td>
<td>51.7</td>
<td>&lt;.001*</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Depression</td>
<td>0.4</td>
<td>ns</td>
<td>5.4</td>
<td>&lt;.05*</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Difficulty Concentrating</td>
<td>1.1</td>
<td>ns</td>
<td>3.1</td>
<td>ns</td>
<td>1.8</td>
<td>ns</td>
</tr>
<tr>
<td>Drowsy</td>
<td>0.3</td>
<td>ns</td>
<td>0.7</td>
<td>ns</td>
<td>1.4</td>
<td>ns</td>
</tr>
<tr>
<td>Hunger</td>
<td>0.2</td>
<td>ns</td>
<td>9.9</td>
<td>&lt;.001*</td>
<td>0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Impatient</td>
<td>0.0</td>
<td>ns</td>
<td>5.0</td>
<td>&lt;.05*</td>
<td>4.0</td>
<td>&lt;.05*</td>
</tr>
<tr>
<td>Irritable</td>
<td>1.1</td>
<td>ns</td>
<td>13.4</td>
<td>&lt;.001*</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Restless</td>
<td>2.3</td>
<td>ns</td>
<td>3.3</td>
<td>&lt;.05*</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Sweets</td>
<td>0.3</td>
<td>ns</td>
<td>0.5</td>
<td>ns</td>
<td>0.0</td>
<td>ns</td>
</tr>
<tr>
<td>Urge to Vape</td>
<td>1.4</td>
<td>ns</td>
<td>47.6</td>
<td>&lt;.001*</td>
<td>0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Direct Effects of Nicotine&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confused</td>
<td>4.4</td>
<td>&lt;.05*</td>
<td>1.4</td>
<td>ns</td>
<td>0.5</td>
<td>ns</td>
</tr>
<tr>
<td>Dizzy</td>
<td>0.1</td>
<td>ns</td>
<td>6.3</td>
<td>&lt;.01*</td>
<td>0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Headache</td>
<td>0.6</td>
<td>ns</td>
<td>0.1</td>
<td>ns</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Heart Pound</td>
<td>0.5</td>
<td>ns</td>
<td>2.3</td>
<td>ns</td>
<td>1.6</td>
<td>ns</td>
</tr>
<tr>
<td>Lightheaded</td>
<td>0.2</td>
<td>ns</td>
<td>6.8</td>
<td>&lt;.01*</td>
<td>1.2</td>
<td>ns</td>
</tr>
<tr>
<td>Nauseous</td>
<td>0.3</td>
<td>ns</td>
<td>0.4</td>
<td>ns</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Nervous</td>
<td>0.1</td>
<td>ns</td>
<td>1.4</td>
<td>ns</td>
<td>1.3</td>
<td>ns</td>
</tr>
<tr>
<td>Salivation</td>
<td>0.5</td>
<td>ns</td>
<td>2.0</td>
<td>ns</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Sweaty</td>
<td>0.7</td>
<td>ns</td>
<td>3.0</td>
<td>ns</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Weak</td>
<td>0.0</td>
<td>ns</td>
<td>0.4</td>
<td>ns</td>
<td>0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Direct Effects of Vaping&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>1.0</td>
<td>ns</td>
<td>10.6</td>
<td>&lt;.001*</td>
<td>1.4</td>
<td>ns</td>
</tr>
<tr>
<td>Calm</td>
<td>1.6</td>
<td>ns</td>
<td>25.2</td>
<td>&lt;.001*</td>
<td>0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.0</td>
<td>ns</td>
<td>8.1</td>
<td>&lt;.01*</td>
<td>0.0</td>
<td>ns</td>
</tr>
<tr>
<td>Dizzy</td>
<td>0.0</td>
<td>ns</td>
<td>6.0</td>
<td>&lt;.01*</td>
<td>0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Pleasant</td>
<td>0.8</td>
<td>ns</td>
<td>139.8</td>
<td>&lt;.001*</td>
<td>2.8</td>
<td>ns</td>
</tr>
<tr>
<td>Reduce hunger</td>
<td>0.3</td>
<td>ns</td>
<td>8.2</td>
<td>&lt;.01*</td>
<td>0.3</td>
<td>ns</td>
</tr>
<tr>
<td>Right Now</td>
<td>0.4</td>
<td>ns</td>
<td>22.7</td>
<td>&lt;.001*</td>
<td>0.1</td>
<td>ns</td>
</tr>
<tr>
<td>Satisfying</td>
<td>0.2</td>
<td>ns</td>
<td>127.6</td>
<td>&lt;.001*</td>
<td>2.4</td>
<td>ns</td>
</tr>
<tr>
<td>Sick</td>
<td>0.0</td>
<td>ns</td>
<td>0.3</td>
<td>ns</td>
<td>1.3</td>
<td>ns</td>
</tr>
<tr>
<td>Taste Good</td>
<td>3.6</td>
<td>ns</td>
<td>130.4</td>
<td>&lt;.001*</td>
<td>3.9</td>
<td>&lt;.05*</td>
</tr>
</tbody>
</table>

Note: ns = non-significant.

<sup>a</sup>df C = (1, 28); df T = (7, 196); df C x T (7, 196).

<sup>b</sup>df C = (1,27); df T = (8, 216); df C x T (8, 216).

<sup>c</sup> df C = (1,28); df T = (2, 56); df C x T (2, 56).
Figure 1. Mean data (± SEM) for plasma nicotine across conditions (N = 29). ECIG-experienced participants completed a 10-puff ECIG use bout (30 sec IPI) and a 90 min ad libitum bout in two conditions: with (circles) and without (triangles) a topography mouthpiece attached to their preferred ECIG battery. Black bars beneath the X axis indicate when ECIG use could occur. Filled symbols indicate a significant difference from baseline (-30; the first time point). All p’s < .05; Tukey’s HSD.
Figure 2. Mean data (± SEM) for HR across conditions (N = 28). A malfunction of the equipment resulted in incomplete data collection for one participant out of the 29 completers who were used for all other analyses. ECIG-experienced participants completed a 10-puff ECIG use bout (30 sec IPI) and a 90 min ad libitum bout in two conditions: with (circles) and without (triangles) a topography mouthpiece attached to their preferred ECIG battery. Black bars beneath the X axis indicate when ECIG use could occur. Filled symbols indicate a significant difference from baseline (-0; the first time point). All p’s < .05; Tukey’s HSD.
**Direct effects of ECIG use.** As Table 2 indicates, significant main effects of time were observed for the items “Awake,” “Calm,” “Concentrate,” “Dizzy,” “Pleasant,” “Reduce Hunger,” “Would like another ECIG Right Now,” “Satisfying,” and “Taste Good.” The items with two of the largest F values (“Taste Good” and “Satisfying”) are depicted in Figure 4. A significant condition by time interaction was also observed for the item “Taste Good” \( F(2, 56) = 3.9, p < .05; \) see Figure 4. The interaction between condition and time is explained by a significantly greater post-directed bout mean VAS score in the no mouthpiece condition (83.2, SEM = 3.5) relative to the condition with the topography mouthpiece present (72.7, SEM = 5.0; Tukey’s HSD, \( p < .05 \), see Figure 4).

A significant condition by time interaction was also observed for the item “Taste Good” \( F(2, 56) = 3.9, p < .05; \) see Figure 4. The interaction between condition and time is explained by a significantly greater post-directed bout mean VAS score in the no mouthpiece condition (83.2, SEM = 3.5) relative to the condition with the topography mouthpiece present (72.7, SEM = 5.0; Tukey’s HSD, \( p < .05 \), see Figure 4).

**Acceptability questionnaire.** Within the mouthpiece condition, none of the six topography equipment items administered differed between the post-directed and post- *ad libitum* bouts: “Altered e-cigarette use behavior,” \( t(28) = 1.4, p = ns \), “Made vaping less likely,” \( t(28) = 0.7, p = ns \), “Reduced enjoyment,” \( t(28) = 0.18, p = ns \), “Affected e-cigarette taste,” \( t(28) = 1.4, p = ns \), “Increased awareness,” \( t(28) = -0.7, p = ns \), and “Increased vaping difficulty” \( t(28) = 0.6, p = ns \).
**Puff Topography**

Paired samples t-tests were conducted to compare all puff topography measures between the directed and *ad libitum* bouts within the mouthpiece condition. A significant difference was observed for puff duration [$t(28) = 3.55, p < .01$], indicating that participants took longer puffs on average during the *ad libitum* bout (5.3, SEM = 0.4) relative to the directed bout (4.5, SEM = 0.3; Table 3). In addition, a significant difference was found for puff volume [$t(28) = 2.46, p < .05$], demonstrating that participants also exhibited larger mean puff values in the *ad libitum* (148.5, SEM = 22.2) compared to the directed bout (124.6, SEM = 16.6). No differences were observed between bouts for flow rate. Additionally, a paired samples t-test revealed no difference between the two conditions (i.e., no mouthpiece and mouthpiece) for puff number observed in the *ad libitum* bouts [$t(28) = 0.65, p = \text{ns}$; see Table 3]. As shown in Table 4, independent-samples t-tests were conducted to compare puff duration, volume, and flow rate values from the directed bout in the present study to the same variables recorded in a previous study in which tobacco cigarette smokers smoked their preferred brand *ad libitum* (Kleykamp et al., 2008). Results revealed significant differences for each of the three variables: puff duration: [$t(150) = 20.29, p < .001$]; puff volume: [$t(150) = 8.4, p < .001$]; and flow rate: [$t(150) = -4.08, p < .001$].

In order to examine the relationship between nicotine delivery during the *ad libitum* bout, puff topography, and various ECIG solution characteristics, exploratory Pearson correlations were conducted. Specifically, peak plasma concentrations during the *ad libitum* bout in the two conditions were correlated with the number of puffs taken
during the *ad libitum* bout in the corresponding condition. Correlations were also
performed between peak plasma concentrations for each condition and PG:VG ratio and
liquid nicotine concentration. In addition, puff volume and duration were correlated with
peak plasma concentrations in the mouthpiece condition. No significant correlations were
found between peak plasma concentrations in either condition and puff number, PG:VG
ratio, or liquid nicotine concentration nor were any significant correlations found between
puff volume or duration and peak plasma concentrations in the mouthpiece condition.
Figure 3. Mean ratings (+/- SEM) for two visual analog scale items from 29 experienced ECIG users using their preferred device and strength/flavor in two sessions that differed by whether a mouthpiece-based topography system was attached to the ECIG. The no mouthpiece condition is represented by triangles while the mouthpiece condition is represented by circles. Black bars beneath the X axis indicate when ECIG use could occur. “Craving an e-cigarette” (left) and “Irritable” (right) were from the Hughes-Hatsukami withdrawal scale. Filled symbols indicate a significant difference from baseline (i.e., -30). All ps < .05; Tukey’s HSD.

Figure 4. Mean ratings (+/- SEM) for two visual analog scale items from 29 experienced ECIG users using their preferred device and strength/flavor in two sessions that differed
by whether a mouthpiece-based topography system was attached to the ECIG. The no mouthpiece condition is represented by triangles while the mouthpiece condition is represented by circles. Black bars beneath the X axis indicate when ECIG use could occur. “Did the ECIG Taste Good” (left) and “Was the ECIG Satisfying” (right) were from the Direct Effects of ECIG-use scale. Filled symbols indicate a significant difference from before ECIG use (i.e., -30). An asterisk (*) denotes a significant difference between conditions at that time point. All ps < .05; Tukey’s HSD.

Table 3.

*Mean (SD) puff parameters for Directed and Ad libitum ECIG-use Bouts.*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Duration (s)</th>
<th>Volume (ml)</th>
<th>Flow Rate (ml/s)</th>
<th>IPI (seconds)</th>
<th>Puff number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouthpiece</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Directed</td>
<td>4.51*</td>
<td>124.56*</td>
<td>27.78</td>
<td>25.19 (1.55)</td>
<td>9.97 (0.12)</td>
</tr>
<tr>
<td></td>
<td>(1.55)</td>
<td>(89.13)</td>
<td>(19.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad lib</td>
<td>5.29</td>
<td>148.52</td>
<td>27.47</td>
<td>102.77 (63.07)</td>
<td>62.55 (32.34)</td>
</tr>
<tr>
<td></td>
<td>(2.08)</td>
<td>(119.6)</td>
<td>(22.63)</td>
<td>Range: 10-161</td>
<td></td>
</tr>
<tr>
<td><strong>No Mouthpiece</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

45
Note, for the directed bouts, IPI and puff number are kept at 10 puffs and 30 seconds apart. In the no mouthpiece condition, puff number was counted manually by trained staff. Asterisks (*) indicate a significance difference for puff duration and volume between the directed and *ad libitum* bouts in the topography condition and N/A indicates values that were not measured for that particular topography variable and condition. No differences were observed between conditions for puff number.

Table 4.

**Mean (SD) puff parameters for ECIGs and Tobacco Cigarettes.**

<table>
<thead>
<tr>
<th></th>
<th>Duration (s)</th>
<th>Volume (ml)</th>
<th>Flow Rate (ml/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECIGs (directed bout, mouthpiece condition only)</td>
<td>4.51 (1.55)*</td>
<td>124.56 (89.13)*</td>
<td>27.78 (19.48)*</td>
</tr>
<tr>
<td>Tobacco Cigarettes</td>
<td>1.36 (0.38)</td>
<td>51.29 (19.22)</td>
<td>37.97 (9.66)</td>
</tr>
</tbody>
</table>

(Kleykamp et al., 2007, N = 123; see also Kleykamp et al., 2008). Data from cigarette smokers were taken from the first cigarette of a session in which no other sources of nicotine were available. Independent-samples T-tests (equal variances not assumed) were
used to compare means for puff parameters between the present study and Kleykamp, (2007). Asterisks (*) indicate a significance difference between ECIGs and tobacco cigarettes on that measure ($p < .01$).

**Discussion**

Puff topography is a useful measure for understanding the extent to which tobacco products, such as combustible cigarettes, expose users to nicotine and other harmful smoke constituents. ECIGs are one of the newest alternative tobacco products to emerge in the U.S. and global market, but little is known about the acute effects of ECIGs or the puff topography of ECIG users. Further understanding of the puff topography associated with ECIG use will likely result in a better understanding of the toxicant exposure (including nicotine) of these products and may also inform ECIG regulatory decisions.
This study demonstrated the extent to which the most common method for measuring tobacco cigarette puff topography (mouthpiece-based computerized devices) was suitable for measuring ECIG puff topography while also providing valuable information concerning the acute effects of ECIGs.

**Measurement of ECIG User Puff Topography**

The measurement of puff topography has been critical to understanding the toxicant yield as well as user toxicant exposure associated with tobacco cigarettes. For example, puff topography measurement was paramount to understanding that the FTC method for assessing cigarette toxicant exposure (consisting of using a standardized puffing regimen to produce smoke for subsequent toxicant analyses) largely was not useful because it did not use topography parameters that were similar to actual human behavior and it also did not account for the great deal of individual variability in smoking behaviors and thus often underestimated actual toxicant exposure (Djordjevic et al., 1997). Furthermore, puff topography analyses have been critical to understanding that smokers often compensate by taking longer and/or larger puffs from “low yield” cigarettes, thus resulting in toxicant exposure (e.g., CO) comparable to that observed when using “full flavor” cigarettes (Gust & Pickens, 1982; Herning et al., 1981). Puff topography measurement has also proven to be critical to understanding the toxicant yield associated with ECIGs. For example, one study has demonstrated that puff duration, but not puff velocity, is an important predictor of ECIG nicotine yield (i.e., greater puff durations result in greater nicotine yields, Talih et al., 2015). However, before a comprehensive understanding of ECIG puff topography can be achieved, the suitability
of using mouthpiece-based topography measurement devices for measuring ECIG topography must be determined, as these devices have been critical for providing accurate and reliable assessments of cigarette smoker’s puff topography (Blank et al., 2009; Buchhalter & Eissenberg, 2000).

**Suitability of Mouthpiece-Based Device**

The present study was the first to examine whether a mouthpiece-based topography measurement device is applicable for the measurement of ECIG puff topography. ECIG-experienced participants completed two sessions that differed only by the presence of a mouthpiece-based topography device. Puff topography was recorded in the session that included the topography mouthpiece using specialized hardware and software developed specifically for measuring ECIG puff topography. In addition, puff topography variables (i.e., puff duration, volume, and flow rate) recorded in the directed bout of the mouthpiece condition in the present study were compared to cigarette smokers puff topography from a previous study (i.e., Kleykamp et al., 2008) and to puff topography recorded in the *ad libitum* bout of the mouthpiece condition. Participants’ puff count during *ad libitum* use was also recorded and compared across conditions (puff count was measured observationally in the no mouthpiece condition). Lastly, the extent to which the presence of the device influenced the acute effects of ECIG use including nicotine delivery, subjective effects, and physiological effects (i.e., HR) was assessed.

Several puff topography variables were recorded in the condition in which the topography mouthpiece was attached to the participants’ ECIG. Importantly, the mean puff duration in the directed bout of this study (4.5 s) is largely consistent with previous
reports (e.g., 4.2 s, Farsalinos et al., 2013; 4.3 s, Hua et al., 2013). In one previous study (Behar, Hua, & Talbot, 2015), ECIG-experienced users exhibited shorter mean puff durations (2.7 s) and smaller mean puff volumes (51 ml) relative to those observed in the present study’s directed bout (4.5 s and 124.6 ml, respectively). However, these relatively shorter and smaller puffs may have been the result of the topography recording device used in that study. That is, the CReSS device that was used to record puff topography may not have provided sufficient sensitivity to accommodate the low flow-rate puffs often observed in ECIG users, as it was designed to accommodate higher flow rates consistent with cigarette smoking. Indeed, the relatively lower flow rates that were detected in the present study and in a previous report (Behar et al., 2015) highlight the need for the use of specialized topography measurement equipment to measure ECIG topography accurately.

Because previous studies have demonstrated that ECIG-naïve cigarette smokers may exhibit different puff topography relative to experienced ECIG users (e.g., Farsalinos et al., 2013). Comparisons were made between puff topography recorded in the directed bout of the present study among experienced ECIG users to puff topography recorded from experienced cigarette smokers who smoked a cigarette ad libitum in a previous study (Kleykamp et al., 2008). As Table 4 shows, ECIG users in this study took longer, larger, and slower puffs relative to the comparison group of cigarette smokers.

Participants’ puff count during ad libitum use was also recorded and compared across conditions (puff count was measured observationally in the no mouthpiece condition). Results provided no evidence that the topography mouthpiece interfered with
puff count. Within the mouthpiece condition, puff topography values recorded from the directed bout were compared to those recorded from the *ad libitum* bout. Differences were observed between bouts for puff duration, volume, and IPI. Specifically, participants exhibited longer puff durations/IPIs and larger puff volumes in the *ad libitum* bout relative to the directed.

The puff topography results from this study have several important implications. First, the observed differences in puff topography between experienced ECIG users in this study and tobacco cigarette smokers from previous reports (Kleykamp et al., 2008) suggest that ECIG users who were previous smokers may learn to adjust their puff topography, possibly in order to extract the level of nicotine they were accustomed to getting from conventional cigarettes. Importantly, all participants in the present study were former cigarette smokers. At least one study has demonstrated that ECIG-naïve cigarette smokers when given an ECIG for a period of one week, appear to adjust their average puff duration and flow rate in a manner consistent with the results reported here (Lee et al., 2015). This apparent learning curve could be problematic for smoking cessation purposes, given that ECIGs do not typically provide users with instructions regarding proper puffing techniques. Furthermore, future clinical trials testing the efficacy of ECIGs for smoking cessation may want to provide participants with puffing instructions to maximize their likelihood of obtaining nicotine and/or suppressing nicotine withdrawal symptoms, thus potentially limiting attrition, or may need to provide participants with a device that will deliver comparable levels of nicotine without requiring a change in behavior.
Raising additional implications, participants in the present study exhibited significantly larger and longer puffs with greater IPIs when in the ad libitum relative to the directed bout. These findings may suggest that controlled ECIG puffing parameters used in this study and numerous others (e.g., Vansickel et al., 2010) may inhibit users from exhibiting their natural puff topography, even for measures such as puff duration that are not intentionally kept consistent. In other words, although controlling certain puffing parameters during ECIG use can make comparisons to cigarette smoking easier for several outcomes and also increase the internal validity of a study, this controlled puff topography may not be indicative of real-world ECIG use. Importantly, previous studies have demonstrated that when user puff topography is mimicked precisely and used to produce aerosols from other tobacco products (e.g., waterpipe), excellent correlations between toxicant yield and user toxicant exposure are observed (Shihadeh & Eissenberg, 2011). In order to determine accurately the toxicant content of aerosols produced from ECIGs and thereby inform ECIG regulation, future studies likely will need to examine ECIG use under more naturalistic conditions which may only be possible with an ambulatory puff measurement device as has been developed for cigarette smokers (Blank et al., 2009).

In addition to the topography results mentioned above, this study also demonstrated that for virtually all of the measures collected in this study, significant main effects of time were observed with very few (2/33 measures) significant interactions between time and condition. This lack of significant time by condition interactions suggests that there is little evidence from this study that the presence of the mouthpiece-
based topography measurement device influenced any of the observed acute effects of ECIG use including nicotine delivery and subjective and physiological effects. Therefore, the section below describing these acute effects will focus on the numerous significant main effects of time that were observed.

**Assessment of Acute ECIG Effects**

**Physiological measures.** The physiological measures assessed in the present study provide evidence regarding acute nicotine exposure and cardiovascular effects associated with controlled (10-puff directed bout, 30 sec IPI) and *ad libitum* ECIG use (90 min). Increases in plasma nicotine and HR were seen after each product administration.

After the 10-puff directed bout, collapsed across condition, a mean increase of 16.6 ng/ml was observed for plasma nicotine while a mean increase of 7 bpm was observed for HR. This observed increase in plasma nicotine after 10 puffs from participants’ preferred ECIG battery/solution is consistent with plasma nicotine increases observed in cigarette smokers smoking their own brand of cigarettes in previous studies (e.g., mean = 16.7 ng/ml; Vansickel et al., 2010). Furthermore, the plasma nicotine increases exhibited by these participants were accompanied by increases in HR. Previous research has similarly demonstrated that delivery of nicotine from pharmaceutical products (Evans et al., 2006) or other tobacco products such as cigarettes (Buchhalter & Eissenberg, 2000; Vansickel et al., 2010) is typically associated with increases in HR.

During the *ad libitum* bout, collapsed across condition, a mean peak plasma concentration of 35 ng/ml (SD = 23.36) was observed after 90 minutes. Interestingly, the
mean peak plasma concentrations detected here are comparable to plasma nicotine levels achieved by cigarette smokers during *ad libitum* cigarette use periods in previous studies (e.g., Foulds et al., 1992: mean = 27.0 ng/ml, SD = 9.9, N = 30; Yan & D’Ruiz, 2015: mean = 29.2 ng/ml, SD = 10.8; N = 24). Thus, this study demonstrated that in terms of nicotine exposure and cardiovascular effects, participants’ preferred ECIG and solution resulted in similar physiological effects relative to those observed in cigarette smokers in previous studies.

Importantly, similar to previous studies (Dawkins & Corcoran, 2013; Farsalinos et al., 2014; Vansickle & Eissenberg, 2013), nicotine delivery varied considerably among the experienced users in this study. For example, some participants were able to achieve much higher plasma nicotine concentrations relative to those seen after smoking a single tobacco cigarette (e.g., > 100 ng/ml using 18 mg/ml, PG:VG: 30:70 ECIG liquid with 3.3V “eGo” battery), while others were only able to obtain minimal doses of nicotine. One possible explanation for this variability in nicotine delivery is individual variability in puff topography, but given that the devices and liquids used in this study were not standardized this relationship was difficult to examine. However, the lack of a significant correlation between puff number in the *ad libitum* bout of each condition and mean peak plasma concentration suggest that at least this particular aspect of puff topography does not influence nicotine delivery. This variability in nicotine delivery may also be explained by the variability in device and solutions used. More specifically, recent research examining factors that influence ECIG aerosol nicotine yield (Talih et al., 2015) have demonstrated that device characteristics such as battery voltage and heater
resistance and solution characteristics such as nicotine concentration can all affect the amount of nicotine that emerges from the mouth-end of an ECIG. That is, higher battery voltage, lower heater resistance, and higher liquid nicotine concentrations all increase nicotine yield. Given that none of these factors were held constant in this study, they all likely contributed to the observed variability in nicotine delivery.

**Subjective effects.** Abstinence symptom suppression, direct effects of ECIG use, and the direct effects of nicotine were examined using various questionnaires in this study. Additionally, in the mouthpiece condition, the extent to which the presence of the topography mouthpiece influenced overall acceptability of ECIG use was assessed.

Participants’ preferred ECIG and liquid produced reliable abstinence symptom suppression for several important VAS items. For example, collapsed across condition, mean ratings of “Urge to Vape” decreased by approximately 32 points after the directed bout, and an additional 11 points after the *ad libitum* bout (VAS scale, 0-100). Similar pronounced post-ECIG use reductions were observed for the items “Anxious,” “Craving an ECIG,” and “Irritable.” The reductions in these withdrawal suppression-indicative VAS items are comparable to those observed after smoking a single cigarette in previous studies (e.g., Buchhalter, Schrinel, & Eissenberg, 2001; Vansickle et al., 2010). Other important subjective effects to examine when assessing a potential MRTP are the direct effects of nicotine and the product itself. Numerous items on both the Direct Effects of Nicotine Scale and the Direct Effects of ECIG use scale changed significantly following ECIG use. That these participants appeared to experience abstinence symptoms as a result of abstaining from ECIG use for at least 12 hours and subsequently were able to
alleviate these abstinence symptoms after using their device may be an indication of nicotine dependence. However, given that all participants in this study were former cigarette smokers, it is unclear the extent to which this apparent nicotine dependence was already present prior to becoming an ECIG user.

Participants also answered several questions in the mouthpiece condition that assessed the extent to which they perceived the topography mouthpiece as influencing their normal ECIG-use behaviors. No differences were observed between the post-directed and post- *ad libitum* VAS scores for any of these items. Taken together, results from the subjective questionnaires administered in this study demonstrated that participants’ subjective perceptions of their preferred ECIG device following directed and *ad libitum* use were not influenced by the presence of a mouthpiece-based topography recording device.

Several implications can be drawn from the acute physiological and subjective effects associated with ECIG use seen in this study. First, the levels of nicotine these ECIG users were able to obtain from their devices were comparable to those previously seen in cigarette smokers using their own brand of cigarettes under similar conditions. Specifically, on average, cigarette smokers take 10 puffs from a single cigarette while exhibiting approximately 30 second IPIs. Under these same puffing parameters, ECIG users in the present study were able to obtain commensurate increases in plasma nicotine, suggesting that ECIGs are capable of delivering nicotine rapidly and efficiently to the user unlike NRTs (Le Houezec, 2003) or other alternative tobacco products (e.g., oral, non-combustibles; Cobb et al., 2010). In addition, when ECIG users were free to puff on
their preferred device and ECIG liquid *ad libitum*, similar peak plasma concentrations were detected compared to those seen previously in cigarette smokers left to use their own brand of cigarettes in a similar manner. Second, results of the subjective measures administered in this study demonstrated that participants’ abstinence symptoms were significantly reduced following acute ECIG use, suggesting that these ECIG users may have been dependent on nicotine. Additionally, in some instances plasma nicotine concentrations observed after ECIG use were much higher than expected from a tobacco cigarette. There is not clear rationale for a device that can deliver more nicotine than a tobacco cigarette, as higher doses of nicotine could lead to increased risk of nicotine dependence (potentially promoting the use of multiple products and/or making cessation more difficult) and may also be toxic to a user.

**Limitations**

Despite the numerous strengths of the present study and useful implications that can be drawn from the results, several limitations are also apparent. First, participants were permitted to use their preferred ECIG battery and liquid, both of which varied considerably. Assessing the relationship between puff topography and nicotine delivery was challenging, given that many of the device/solution characteristics that varied between participants (e.g., liquid nicotine concentration, liquid PG:VG ratio, battery voltage, heater resistance) have all been shown to alter the amount of nicotine ultimately found in the aerosol produced by an ECIG (Talih et al., 2015). Future research would benefit by attempting to control some of these factors, while manipulating systematically another, in order to elucidate that particular factor’s influence on ECIG acute effects. The
standardization of the cartomizer used in this study, while necessary to ensure accurate
topography recording, was another limitation. Participants may have exhibited different
puff topography or extracted nicotine differently had they used their preferred tank or
cartomizer. The creation of a mouthpiece-based device that can accommodate more tank-
based and cartomizer-based ECIGs may be necessary for future studies. Additionally, a
placebo-controlled study would need to be conducted to determine unequivocally that the
increases in HR and abstinence symptom suppression that were observed were the result
of nicotine being delivered to the user. Lastly, the laboratory setting is a limitation of this
study. As stated previously, if ECIG users were able to use their device outside of the lab
and an ambulatory topography-recording device were used to record their puff
topography, more naturalistic puff topography data would likely be generated relative to
the data collected in the present study.

Conclusions

This study used clinical laboratory methods to examine ECIG topography and the
acute effects of ECIG use including nicotine delivery, physiological effects (HR), and
subjective effects. Furthermore, the extent to which the presence of a mouthpiece-based
topography recording device influenced these acute effects was also assessed. Results
demonstrated that ECIG users in the present study exhibited significantly different puff
topography from that observed previously in cigarette smokers. Additionally, ECIG-
associated nicotine delivery, HR, most subjective measures, and puff number (during ad
libitum ECIG-use) were not affected by the presence of the topography mouthpiece.
Taken together, results from this study support the continued use of mouthpiece-based
recording devices for measuring ECIG topography in clinical laboratory research. Nonetheless, future research may also benefit from the use of a portable ECIG topography-recording device that is capable of recording ECIG users’ topography in a more naturalistic setting. Furthermore, the increases in plasma nicotine and reductions in abstinence symptoms seen after ECIG use in this study lends further support for the argument that ECIGs may be a viable nicotine replacement alternative to tobacco cigarettes.

List of References
List of References


Shihadeh A., Azar S., Antonius C., & Haddad H. Towards a topographical model of


Appendix A

Telephone Screening Form

**Introduction:** This is a research study about how to best measure how people use e-cigarettes

**Purpose:** To compare two methods to measure e-cigarette use behavior

**Study Details:** If you are eligible for this study, you will be asked to visit our lab on the MCV campus for two sessions. These sessions will begin at approximately the same time each day, will take approximately 3 hours each, and will be separated by at least 48 hours. We will ask you to abstain from all tobacco products and e-cigarettes, and all nicotine containing products (like the gum or patch) for at least 12 hours before each session. When you arrive to the lab for session, we will ask you to take a simple breath test to make sure that you have complied with these restrictions. Side effects from tobacco/nicotine abstinence can include irritability, anxiety and restlessness, excessive hunger, difficulty concentrating, and sleep disturbance. Though uncomfortable, these feelings are not medically dangerous.

At the beginning of each session, a nurse will insert an IV catheter into your arm that will stay there for the entire session. This catheter will be used to draw blood periodically (less than 1 tablespoon per sample, 8 samples). We will also monitor your heart rate and blood pressure and ask you to respond to several questionnaires to measure how you feel before and after vaping. There is some risk of bruising at the catheter site, and there is a minimal risk of infection associated with any blood draw.

For the session, we will ask you to provide your own e-cig with a fully charged battery. We will provide to you a new cartridge or cartomizer of the brand, flavor, and nicotine dose that you choose. During the session we will ask you to vape using your e-cig at two separate times.

When you smoke your e-cigarette, you may notice that it is connected to a computer and that there are pieces of equipment attached to the e-cigarette. The computer and this equipment are measuring how you vape (the size and number of the puffs that you take). Also, we will record on video your smoking behavior in both sessions. These video recordings will also be used to measure how you vape (for example, the number of puffs that you take).

**Confidentiality:** We will not tell anyone the answers that you give us; however,
information from the study and the consent form signed by you may be looked at or copied for research or legal purposes by the sponsor of the research, or by Virginia Commonwealth University.

**Payment:** You will receive $100 after completing the first session and $150 after completing the second session. Thus, the total amount earned for finishing the entire study is $250.

“Does this sound like something you want to participate in?”

Document caller’s response by circling either:  
Yes  or  No  
*If yes, continue with the following questions.*

**Telephone Screening Questionnaire**

**Interviewer:** “I would like to ask you some questions about yourself and your health status as well as your use of e-cigarettes, tobacco, alcohol, and other drugs. Completion of these questions will take approximately 10 minutes of your time. The purpose of these questions is to determine whether or not you are eligible to participate in the study I just described, in addition to other studies currently ongoing in our laboratory. All of your responses are confidential. You are not required to answer any question and you may stop this interview at any time. May I begin the questions?”

Document caller’s response by circling either:  
Yes  or  No  
*If Yes: begin form. If No: thank caller for calling.*

How did you hear about us/our studies?  
________________________

**Personal Information:**  
1. “What is your first name?”  
________________________

2. “What is a phone number at which you can be contacted?”  
________________________

4. “If we call and you are not available, may we leave a message?”  
   Circle   Yes  
   or  No
5. “What is your date of birth?”
________________________________________

6. “What is your height?”

7. “What is your weight?”

8. “Which identifier best describes you?”

9. “Did you graduate high school?”

10. “Did you obtain your GED?”:

11. “Do you have any chronic health concerns or problems?”

12. “Are you under a doctor’s care for a medical condition?”

13. “Are you taking any prescription or over-the-counter medications?”

14. Do you have any psychiatric conditions like depression or anxiety?

15. “Have you ever been diagnosed with high or low blood pressure?”

Cigarette use:
16. Have you smoked tobacco cigarettes in the past year? ○ Circle Yes or No

If Yes: “When was the most recent occasion you smoked tobacco cigarettes?

Circle: Within the past 30 days or 2 to 3 months ago or 4 to 6 months ago or More than 6 months ago

If No: Go to Question 19

17. “How many cigarettes/day do you smoke?” Write in exact number and also circle appropriate category: ______ (num of cigs)

10 or less 11-20 21-30 31 or more

18. “For how long have you smoked this number?”

19. “Have you ever used an electronic cigarette?” ○ Circle Yes or No

If Yes: ask the following questions

“What is your preferred e-cig brand?”

“Do you ever use other brands of e-cig?”

“What is your preferred cartridge or e-liquid strength?”

“Do you ever use other strengths?”

“On average, how many cartridges or ml of nicotine solution do you vape per day? (Please indicate liquid or cartridge)”

“For how long have you vaped this amount?”

“Where do you purchase your e-cig cartridges and/or nicotine solution?”

Interviewer: “I am now going to ask questions about alcohol and drug use. Please remember that you are not required to answer any question and you may stop this interview at any time.”

Alcohol use:
20. “Have you ever been treated for alcohol abuse/dependence?”
   Circle Yes or No
   If Yes: “When was your treatment completed?”: mnth/year

21. “Do you use (drink) alcoholic beverages?”
   Circle Yes or No
   If No: Skip the remainder of this section.

22. “How many alcoholic drinks (by alcohol I mean beer, wine, or liquor) do you have on a typical day? (num of drinks)

23. “How many days out of the last 30 have you used alcohol? (num of days)

Marijuana use:
24. Have you ever, in your lifetime, smoked marijuana or hashish? Circle Yes or No
   If No: Skip the next question.

25. “How many days out of the last 30 have you smoked marijuana?” (number of days)

Other drug use:
26. “Have you used any other illegal drugs within the past month?” Circle Yes or No
   If Yes: “Please identify which drug or drugs.”

For women only:
27. “Are you currently pregnant?” Circle Yes or No
28. “Are you currently breast-feeding a child?” Circle Yes or No

Interviewer: “Thank you for responding to these questions. I need to pass on your responses to the principal investigator who will then determine whether or not you are eligible to participate in a study; someone will contact you within approximately one week if you are eligible. If you are not eligible for any of our current studies, then you will not be contacted.”
[If respondent does not have a phone, they can call us back in a few days]
Appendix B

Informed Consent form

Title. Measurement of electronic cigarette (ECIG) use topography

VCU IRB Number: HM 15411

Investigator. Dr. Thomas Eissenberg

Sponsor. National Institutes of Health

This consent form may contain words that you do not understand. Please ask the study staff to explain any words that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

Purpose of the study. The purpose of this research study is to learn about how you use electronic cigarettes and what effects they produce.

Description of the study and procedures. If you agree to join the study, you will be asked questions about your general health, smoking history, and marijuana and alcohol use. If you are a woman you will need to provide a urine sample that will be tested immediately for pregnancy. If you are pregnant you cannot participate in this study. Your responses will be confidential.

If the urine tests and your answers to our questions indicate that you fulfill the entry criteria, we will ask you to participate in two, approximately 3-hour sessions here at the Clinical Behavioral Pharmacology Laboratory located on VCU’s medical campus. The two sessions will begin at approximately the same time each day, and will be separated by at least 48 hours. Before each session, we will ask you to abstain from all electronic cigarette and other tobacco products for at least 12 hours. We will also ask you to abstain from all food and caffeinated beverages for 1 hour before each session. In addition, the use of any nicotine-containing products (like the gum or patch) is prohibited. We will ask you to take a simple breath test to make sure that you have complied with these restrictions. Our tests are not perfect, but they are the only measures that we can accept to make certain that you have complied with the no tobacco/no nicotine restrictions.

At the beginning of the session, a nurse will insert an IV catheter into your arm that will stay there for the entire session. This catheter will be used to draw blood periodically (less than 1 tablespoon per sample, 8 samples). We use this method because participants tell us that it is more comfortable than repeated “sticks” with a needle. During this session we will take much less blood than the amount you would give in a single
donation at a blood drive. We will also monitor your heart rate and blood pressure and ask you to respond to several questionnaires to measure how you feel before and after you use an electronic cigarette.

For the session, we will ask you to provide your own electronic cigarette with a fully charged battery. If you think you need additional batteries, please bring them with you. We will provide to you a new cartridge or cartomizer of the brand, flavor, and nicotine dose that you choose. During the session we will ask you to use your electronic cigarette at two separate times. The first time, we will ask you to take only 10 puffs, and we will tell you when to take each of these puffs. The second time we will ask you to use the electronic cigarette however you’d like. If you need another cartridge or cartomizer during this time, please let us know and we will provide it to you. At each of these two times we need you to remain seated in a comfortable chair while you are using the electronic cigarette.
**Appendix C**

Hughes-Hatsukami Withdrawal VAS Scale (Hughes & Hatsukami, 1986).

These phrases may or may not describe how you feel right now. Please respond to each word of phrase with how you feel RIGHT NOW.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Urges to use an e-cigarette</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Irritability/frustration/anger</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Anxious</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Difficulty Concentrating</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Restlessness</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Hunger</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Impatient</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>CRAVING an e-cigarette</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Drowsiness</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Depression/ feeling blue</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Desire for Sweets</td>
<td></td>
</tr>
</tbody>
</table>
Vita

Tory Richard Spindle was born in Fredericksburg, VA on May 29th 1990 and is a current U.S. citizen. He is a graduate of Stafford high school in Fredericksburg, VA and has a B.S. in psychology from Virginia Commonwealth University (VCU) in Richmond, VA, which he received in 2013. He began the Health psychology doctoral program at VCU in August, 2013.