2016

Electronic Cigarette User Plasma Nicotine Concentration and Puff Topography: Influence of Liquid Nicotine Concentration and User Experience

Marzena M. Hiler
Virginia Commonwealth University

ELECTRONIC CIGARETTE USER PLASMA NICOTINE CONCENTRATION AND PUFF TOPOGRAPHY: INFLUENCE OF LIQUID NICOTINE CONCENTRATION AND USER EXPERIENCE

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

By: MARZENA M. HILER
B.A. University of California Irvine, 2012

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

Virginia Commonwealth University
Richmond, Virginia
September, 2016

Acknowledgements

The success of this project was contingent on the support and contributions of many. First, I would like to thank my advisor, Dr. Tom Eissenberg for his guidance, feedback, and patience during the completion of this project. I would also like to thank my committee members, Drs. Alison Breland and Imad Damaj for their thoughtful critique of this work. I would also like to acknowledge and thank VCU staff Barbara Kilgalen, Janet Austin, Kendall Pettaway, Kathleen Osei, Hannah Mayberry, and Caroline Smith for assisting with data collection. The success of this project would not have been possible without the encouragement and support of my fellow graduate student Tory Spindle who has been my closest friend and confidant during my time at VCU and Sarah Maloney whose lighthearted humor and friendship helped me during the completion of this project. Importantly, I would like to thank my parents and sister for supporting my move across the country to pursue graduate school and for their continuous support throughout my academic endeavors.

This thesis study is supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number P50DA036105 and the Center for Tobacco Products of the U.S. Food and Drug Administration. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Food and Drug Administration.

Table of Contents

List of Tables

Page

List of Figures

Page

List of Abbreviations

ANOVA	analysis of variance
BP	blood pressure
CO	carbon monoxide
CReSS	Clinical Research Support System
ECIG	electronic cigarette
HR	heart rate
IRB	Institutional Review Board
IPI	Inter-puff-interval
LOQ	limit of quantification
min	minute(s)
mg	milligram
ng	nanogram (0.0000000001 grams)
ml	milliliter
ppm	concentration in parts per million
s	seconds
VAS	visual analog scale

Abstract

ELECTRONIC CIGARETTE USER PLASMA NICOTINE CONCENTRATION AND PUFF TOPOGRAPHY: INFLUENCE OF LIQUID NICOTINE CONCENTRATION AND USER EXPERIENCE

By Marzena M. Hiler, B.A.

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

Virginia Commonwealth University, 2016

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

Electronic cigarettes (ECIGs) aerosolize an often nicotine-containing solution for user inhalation. ECIG nicotine delivery may depend on liquid nicotine concentration and user puffing behavior (topography). This study examined the relationship among liquid nicotine concentration, puff topography, and plasma nicotine concentration. Thirty-three ECIG-experienced and 31 ECIG-naïve individuals completed four laboratory sessions that differed by ECIG liquid nicotine concentration (0, 8, 18, or 36 mg/ml). A 3.3 volt "eGo" ECIG battery attached to a 1.5 Ohm dual coil "cartomizer" filled with 1 ml of 70% propylene glycol/30% vegetable glycerin nicotine liquid was used in two ECIG-bouts (10 puffs; 30 s IPI). Plasma nicotine concentration, puff topography, and HR were evaluated. Some ECIG/liquid combinations can deliver physiologically active doses of nicotine to users, and nicotine delivery depends on liquid nicotine concentration and user puffing behavior. Liquid contents, device characteristics, and user behavior should be considered when regulating ECIGs.

Electronic Cigarette User Plasma Nicotine Concentration and Puff Topography: Influence of Liquid Nicotine Concentration and User Experience

Tobacco cigarette smoking is related to a myriad of negative health consequences including cancer, cardiovascular disease, and stroke (Mathers & Loncar, 2006). Despite overall declines in consumption, tobacco use remains a pervasive public health threat. Approximately 16.8 % of U.S. adults and 9.3 % of adolescents continue to smoke combustible tobacco cigarettes (Jamal, et al., 2015; Singh et al., 2016). Because smoking remains the leading preventable cause of death in the U.S. (i.e., approximately 480,000 individuals annually; USDHHS, 2014), reducing the use of tobacco cigarettes is an important public health goal (Agaku et al., 2014). However, nicotine, a constituent of tobacco products, is dependence-producing and nicotine dependence in cigarette smokers makes smoking cessation difficult (e.g., Benowitz, 2008; Stratton, Shetty, Wallace & Bondurant, 2001). Perhaps in response to the health threat of combustible cigarettes, several alternative tobacco products have been introduced to the U.S. and global markets. Typically, these products are advertised to reduce smokers' exposure to harmful tobacco constituents, such as tobacco specific nitrosamines, polycyclic aromatic hydrocarbons, and/or carbon monoxide (CO) but often are intended to deliver nicotine. Electronic cigarettes (ECIGs) are one of the newest and most prevalent types of alternative tobacco products, but their potential to reduce tobacco-related harm is unknown. Furthermore, the health implications of long term ECIG use are unclear. Below is a description of ECIG device and liquid characteristics, prevalence and use patterns, the regulation of ECIGs, the acute effects of ECIGs (i.e., nicotine delivery) and implications for nicotine-dependent and -naïve individuals.

ECIGs are a class of products that, until recently, were unregulated in the U.S. However, in May of 2016 the Food and Drug Administration (FDA) announced that it will extend its

authority to regulate tobacco products to include ECIGs (Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016). ECIGs heat a liquid solution, often containing nicotine, and produce an inhalable aerosol. ECIGs typically contain a power source (e.g., battery), a heating element (called an "atomizer"), and store a liquid solution (composed of solvents, flavors, and sometimes nicotine) in a reservoir. While power source, heating element, and liquid solution are common features of most ECIGs, a wide variety of ECIG models exist with considerable differences in device characteristics, such as the storage and nicotine concentration of the liquid solution, the method for heater activation, and the electrical power flowing through the heater (Breland, Spindle, Weaver, & Eissenberg, 2014).

Electronic Cigarette Models

ECIG models vary considerably, and therefore are best described as a class of products (Breland et al., 2014). Over 466 different ECIG brands are available on the market with numbers continuing to increase (Zhu, Sun, Bonnevie, Cummins, Gamst, Yin, and Lee, 2014). Some of the most popular ECIG models, referred to as a "cig-alikes," resemble traditional tobacco cigarettes (see Figure 1; Breland et al., 2016). These models often contain a light-emitting-diode that glows at the non-mouth end when the heating element is activated, typically as a result of user inhalation. Cig-alike models store a liquid solution in a cartridge referred to as a "cartomizer" that is attached to or contains the internal heating element (see Figure 1). Cartridges can be depleted of liquid after some period of use and therefore require the user to replace or refill them. Other cig-alike models require disposal of the ECIG in its entirety (Breland et al., 2014; Etter, 2012). Recently released devices operate under similar principles but do not resemble a cigarette

(e.g., "JUUL", see Figure 1; Giroud, de Cesare, Berthet, Varlet, Concha-Lozano, & Favrat., 2015).

Other ECIG models do not resemble cigarettes and contain non-disposable, rechargeable batteries and either contain ECIG liquid in storage reservoirs (called "tanks") or in pre-filled cartridges (see Figure 1; Etter, 2012). Users may refill cartridges or "tanks" as needed by purchasing refill solution. By manually activating a button on the mouth-end of the device, the user can activate the heating element and ready the device for aerosol production during inhalation (Breland et al., 2014; Etter, 2012). These ECIG models allow for users to vary many characteristics including: the power (measured in watts), that can be controlled by altering the voltage (V) of the power supply and/or the resistance (Ohms or Ω) of the heating element (Etter, 2012). Manipulating product characteristics can alter the toxicant yield of the aerosol that emerges from the mouth end of the device (Kosmider, Sobczak, Knysak, Goniewicz, 2014; Talih et al., 2015).

For example, altering voltage and resistance (i.e., power) combinations in ECIG models can influence the yield of nicotine and other toxicants produced from ECIGs (e.g., Kosmider, et al., 2014; Talih et al., 2015). One analytical laboratory study demonstrated how device characteristics, puffing behavior and ECIG liquid composition may alter toxicant yields (Talih et al., 2015). In this study, aerosols were generated using machinery to simulate distinct puffing profiles of various types of users based on their puff duration in seconds (s) and puff velocity in milliliters per second (ml/s): for example, tobacco cigarette smoker puffs were 2 s duration at 33 ml/s puff velocity, slow average ECIG user puffs were 4 s at 17 ml/s, and fast extreme ECIG user puffs were 8 s at 33 ml/s. Additionally, voltage (3.3 or 5.2 V, which converts to 3.0 or 7.5 W using a 3.6 Ohm heating coil) and the nicotine concentration of the ECIG liquid (18 or 36

mg/ml) were varied. Results indicated that nicotine yield from 15 puffs varied across conditions (Talih et al., 2015). Overall, nicotine yield generated from ECIG-experienced individuals puffing profiles resulted in higher nicotine yield compared to tobacco cigarette smoker profiles. For example, when device and liquid nicotine concentration were held constant, aerosol nicotine yield after 15 puffs differed: mean (SD) for tobacco cigarette smokers puffing profiles was 0.11 mg (0.02) while for ECIG user puffing profiles nicotine yield ranged from 0.29 mg (0.08) to 0.72 mg (0.10) depending on the puffing profile (Talih et al., 2015). Nicotine yield may not necessarily represent the amount of nicotine delivered to the user's blood (i.e., nicotine delivery), but these results suggest that the longer puffs observed in ECIG-experienced individuals (relative to -naïve cigarette smokers; Hua, Yip, & Talbot, 2013) cause the device to emit more nicotine with each puff. Also, when voltage was varied and puffing behavior, resistance, and liquid nicotine concentration were held constant, aerosol nicotine yield after 15 puffs differed: mean (SD) at 3.3 V was 0.3 mg (0.01) while for 5.2 V was 1.2 mg (0.3; Talih et al., 2015). The many potential voltage and resistance (i.e., power) combinations in ECIG models highlight the need for evaluation and regulation of ECIG device characteristics.

ECIG Liquid Solutions

Similar to ECIG device characteristics, ECIG liquid solutions vary widely. These solutions are often referred to as "e-liquid" and typically are composed of solvents like propylene glycol (PG) and/or vegetable glycerin (VG) and often, but not always, flavorants and nicotine (Etter, 2012). When PG and/or VG are heated and aerosolized, they act, alone or in combination, as the vehicle for any nicotine and/or flavorants that are present (Etter, 2012).

ECIG nicotine yield can be influenced by liquid nicotine concentration or the PG/VG ratio of the liquid (Kosmider et al., 2014; Talih et al., 2015). However, few empirical

investigations have been conducted to determine how liquid nicotine concentration and the PG/VG solvent ratio influence nicotine yield during acute ECIG use. In one study, user experience, battery output, and ECIG liquid composition was analyzed to determine how these factors affect the nicotine yield of an ECIG (Kosmider et al., 2014). In this study PG/VG ratio and battery voltage were manipulated while other variables were held constant. ECIG aerosol generated and was later analyzed for nicotine yield. Nicotine yield was related directly to PG/VG ratio: higher PG levels resulted in higher nicotine yield but only when using a device under 4.8 volts. To date, this is the only study in which the influence of PG/VG ratio on nicotine yield has been investigated.

Another factor that may influence nicotine yield is the liquid nicotine concentration of ECIG liquid. In one study, user puffing behavior, ECIG liquid composition, and certain ECIG design features were manipulated in order to examine how these factors influence nicotine yield (Talih et al., 2015). ECIG aerosol was generated using machinery to simulate distinct puffing profiles of various types of users and liquid nicotine concentration was varied using either 8.5 or 15.7 mg/ml. Increases in liquid nicotine concentration were found to be associated with increases in nicotine yield. Specifically, when all other factors were held constant and liquid nicotine concentration was increased from 8.5 mg/ml to 15.7 mg/ml, mean (SD) nicotine yield increased from 3.2 (0.3) mg to 4.7 (1.0) mg after 15 puffs. Collectively, results from these studies are consistent with the notion that at least some aspects of ECIG liquid composition – PG/VG ratio and nicotine concentration – can influence ECIG nicotine yield.

Of particular concern is the inconsistency between actual and advertised nicotine concentrations observed in ECIG liquids (Bahl et al., 2012; Trehy et al., 2011). For example, some ECIG liquids advertised as containing no nicotine (0 mg/ml) contain trace levels of

nicotine (Trehy et al., 2011). In addition, the liquid nicotine concentration stored in the cartridges of six popular United Kingdom ECIG brands was analyzed to explore labeling inconsistencies (Goniewicz, Hajek, McRobbie, 2012) and a variation in liquid nicotine concentration of up to 12% was observed. Indeed, in one clinical study involving ECIG-experienced participants, two participants brought into the laboratory liquid they used regularly that was labeled "12 mg/ml" but subsequent analysis revealed that the liquid contained no measurable nicotine (Spindle, Breland, Karaoghlanian, Shihadeh, & Eissenberg, 2014). Overall, the liquids that participants brought to the laboratory in this study highlight the variety of liquid flavors, nicotine concentrations, PG/VG ratios of these products, as well as the complexity of understanding the effects of ECIG use (see Table 1; Spindle et al., 2015).

Figure 1. From the left, JUUL ECIG with disposable pod, "cig-alike" ECIG models that store ECIG liquid in a cartridge (i.e. "cartomizer"), pre-filled, or fillable cartridge, refillable and reusable "tank" system, rechargeable batteries, and modifiable or "variable voltage" batteries (Adapted from Breland et al., 2016).

Table 1.

Variability in ECIG Liquid Nicotine Concentration and PG/VG Ratio

Participant	Nicotine Concentration mg/ml	Solvents PG/VG ratio	Liquid Flavor
1	24	100/0	No flavor
2	24	50/50	Gargamel's Curse
3	24	Not available	Torque 56
4	18	70/30	Watermelon
5	24	50/50	Peach
6	24	30/70	Gold Rush
7	24	80/20	DK Blend
8	24	Not available	Menthol
9	24	30/70	Persian Winter
10	24	50/50	Vanilla Dr. Pepper
11	18	30/70	Gold Rush
12	12	30/70	Aztec
13	18	60/40	Carolina Crush

Data are from 13 individuals who participated in a study in which they used their own ECIG device and liquid. The table includes participant liquid nicotine concentrations, flavors, and propylene glycol to vegetable glycerin ratio (as indicated by product labeling; table adapted from Spindle et al., 2015).

ECIG Use Patterns and Reasons for Use

ECIGs were patented in China in 2003 (Lik, 2003) and introduced into the U.S. market in 2007 (Regan, Promoff, Dube, & Arrazola, 2013). Since their introduction ECIGs have become common in U.S. and global markets. ECIG popularity in the U.S. market can be illustrated by reports of their revenue growth, with sales having tripled from $273 to $636 million between 2012 and 2013 (Giovenco, Hammond, Corey, Ambrose, & Delnevo, 2015).

Adult ECIG Use. U.S. surveys have demonstrated steady increases in ECIG use among adults (King, Patel, Nguyen, & Dube, 2014). "Ever use" of ECIGs in U.S. adults has increased from 1.8 % in 2010 to 13 % in 2013 (McMillen, Gottlieb, Shaefer, Winickoff, and Klein, 2015). Overall, current tobacco cigarette smokers have higher ECIG use rates compared to other ECIG using groups (i.e., never smokers and former smokers; King et al., 2013). A survey among 5,939 current and former smokers in Canada, the U.S., the United Kingdom, and Australia found that 8% of current and former smokers had tried ECIGs between 2010 and 2011 (Adkison, West, Beard, Michie, Shahab, & McNeill, 2013). Heavy cigarette smokers had the highest ECIG use rates, while long term quitters had the lowest use rates (Adkison et al., 2013). A more recent national survey of 36,697 U.S. adults (≥18 years) found that 12.6% of adults reported having "ever used" ECIGs, 3.7% reported current ECIG use (defined as using ECIGs on some days) and 1.1% reported daily ECIG use (Delnevo, Giovenco, Steinberg, Villanti, Pearson, Niaura, & Abrams, 2016). A more detailed examination of the data revealed that 12.7 % of daily cigarette smokers and 11.5 % of "some-day" cigarette smokers reported current ECIG use. Among individuals who quit cigarette smoking recently (i.e., quit 1 year ago or less), 5.0% were current ECIG users relative to the 0.3 % of never smokers who were current ECIG users (Delnevo et al., 2016). Importantly, among adults, the reason reported most commonly for using ECIG use is to

reduce consumption of conventional tobacco cigarettes or to quit smoking altogether (Berg, Haardoerfer, Escoffery, Zheng, & Kegler, 2015; Richardson, Pearson, Xiao, Stalgaitis, & Vallone, 2014).

Adolescent ECIG Use. Perhaps most concerning are the ECIG use rates among youth and adolescents, especially those who have not initiated tobacco cigarette use or nicotine use. The National Youth Tobacco Survey (NYTS), a cross-sectional school based survey administered to U.S. middle and high school students, reported that, as of 2015, ECIGs were the most popular tobacco product among middle and high school students (Singh et al., 2015). Reported use rates were 5.3% for middle school and 16% for high school students (Singh et al., 2015). This same survey found statistically significant increases in current (i.e., past 30-day use) ECIG use among high school students. For example, from 2011 to 2015, current ECIG use increased from 1.5% to 16% in high school students (Singh et al., 2015). Also in 2015, of those middle and high school students who reported current tobacco use, 3 million reported also using ECIGs. Use rates have also increased among adolescents who reported never having used tobacco cigarettes. In 2011, 79,000 never-smoking adolescents reported ECIG use while 263,000 reported ECIG use in 2013 (Bunnell et al., 2014). Taken together, ECIG use is increasing among various age groups and among tobacco/nicotine experienced individuals as well as those who are tobacco/nicotine naïve.

The variability in product design/ECIG liquid features (Breland et al., 2014) paired with increasing popularity among adults and youth in the U.S. and internationally suggests a need for systematic evaluation of the effects of ECIGs. These systematic empirical investigations will be vital for effective ECIG regulation.

Regulation: Tobacco Control Act

The Family Smoking Prevention and Tobacco Control Act (or Tobacco Control Act; TCA) gave the FDA the power to regulate the manufacture, distribution and marketing of certain tobacco products (Family Smoking Prevention and Tobacco Control Act, 2009). Cigarettes, cigarette tobacco, roll-your-own tobacco, and smokeless tobacco were covered immediately by FDA's tobacco product authorities. The statute also provided FDA with the authority to issue regulations for other tobacco products not covered initially by the statute. FDA defines tobacco products as "any product made or derived from tobacco that is intended for human consumption, including any component, part or accessory of the tobacco product" (Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016). In May of 2016, under the "deeming" statute, the FDA announced that their regulatory authority would be extended to include ECIGs, which were unregulated in the U.S. for several years (Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016). ECIGs meet the statutory definition of a tobacco product as they often contain nicotine derived from tobacco. The FDA's regulatory power over ECIGs will include general controls (e.g., registration of products, listing ingredients, and provisions against adulteration and misbranding) and premarket review (Deeming Tobacco Products to Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016).

Now that FDA has asserted this new regulatory authority over ECIGs, science must help inform regulation by examining systematically what these products do, what ingredients they contain, and the extent to which they will have a positive or negative public health impact. The

information generated by tobacco scientists may guide regulatory agencies regarding the labeling, marketing, and distribution of various products. While a variety of methods and techniques are needed, and many are being applied to ECIGs, human laboratory studies already are informing regulatory agencies about the acute effects of ECIGs. Some of the acute ECIG effects that have been studied include pulmonary function, nicotine delivery, abstinence symptom suppression, and cognitive effects (Breland et al., 2014). The review below focuses on ECIG nicotine delivery, the factors that influence nicotine delivery, and the implications of ECIG nicotine delivery on various populations who use these products (i.e., nicotine-dependent and nicotine-naïve individuals).

What Do ECIGs Do: Nicotine Delivery

ECIGs are marketed to tobacco users as being capable of delivering nicotine to the user. Nicotine delivery can be characterized as the amount of nicotine detected in the user's body and is most often measured in blood plasma (i.e., plasma nicotine concentration, in ng/ml) following use. To date, various clinical laboratory studies have included measurement of plasma nicotine concentration following ECIG use, typically with within-subject study designs (e.g., Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Vansickel & Eissenberg, 2013) and sometimes including a tobacco cigarette control condition (e.g., Vansickel et al., 2010).

When assessing ECIG-associated nicotine delivery, many studies have used methodologies that are drawn from clinical laboratory methods developed to study the effects and use behavior associated with tobacco cigarettes. For example, some ECIG studies include *ad libitum* ECIG use bouts that allow the user to puff on an ECIG as often as they like during a set period of time (e.g., Farsalinos et al., 2014; Vansickel et al., 2013), as has been done for tobacco cigarettes (Breland, 2005; Blank, 2009; Gust & Pickens, 1982; Herning et al., 1983; Rose & Behm,

2003). Other ECIG studies include a combination of both *ad libitum* use and "directed ECIG use bouts" (or directed use bouts only): during directed use bouts, participants are instructed to take a specified number of puffs over the course of a fixed time period in order standardize use behavior (Vansickel et al., 2010; Vansickel & Eissenberg, 2013; Farsalinos et al., 2015), as has been done for tobacco cigarettes and other combustible tobacco products (Blank, Nasim, Hart, & Eissenberg, 2011; Griffiths, Henningfield, & Bigelow, 1982)

Standardizing participant puffing behavior has become commonplace in the evaluation of a variety of novel tobacco products (Breland, Buchhalter, Evans, & Eissenberg, 2002; Blank, Nasim, Hart, & Eissenberg, 2011). This standardization is useful to the extent that it allows for comparisons across products and the parameters used reflect actual use behavior by a population of interest (e.g., cigarette smokers). Most often the puffing behavior that is standardized includes puff count (i.e., the number of puffs taken) and inter-puff interval (IPI). IPI is often defined as the time between the onset of one puff and the onset of a subsequent puff (as seen in Vansickel et al., 2010, Farsalinos et al., 2014; Spindle et al., 2015). Data from cigarette smokers support the idea that a 10-puff bout is typical during the consumption of a single tobacco cigarette. For example, a six-condition, within-subject design study intended to compare three different techniques for measure puffing behavior (desktop, portable, or video method) involved 30 cigarette smokers using two different types of cigarettes (own brand versus ultra-light) *ad libitum* (Blank, Disharoon, & Eissenberg, 2009). Mean (SD) puff number during this ad libitum use was 9.7 puffs (3.3) with the desktop topography measurement system, 9.4 puffs (3.0) with the portable measurement system, and 9.2 puffs (3.2) with the video method recording (Blank et al., 2009). This study also included measurement of IPI in these same participants, with results suggesting that 18 s IPI was the norm. These results support the use of a 10-puff directed bout,

but do not support a 30 s IPI (See also Kleykamp, Jennings, Sams, Weaver, Eissenberg, 2008 for similar support for 10 puffs but not for 30 s IPI). However, in both studies, participants were overnight abstinent, which may have influenced user puff topography.

In one study involving 36 non-abstinent smokers, participants completed four, 5-day conditions that differed by product, and one product was the participants' own brand of tobacco cigarette. Laboratory measurement of *ad libitum* puff topography was conducted on days 1 and 5 of each condition. In the own brand condition, mean (SD) puff number was 10 puffs (3.1) on day 1 and 10 puffs (2.7) on day 5 (Breland, 2005), providing strong support for notion that 10 puffs is typical for a tobacco cigarette smoker smoking a single cigarette. Moreover, mean (SD) IPI on day 1 was 30.7 s (12.8) and on day 5 was 30.5 s (9.6; Breland 2005). This study, that included tobacco cigarette smokers who had not been abstaining from cigarettes prior to puff topography measurement, may be more representative of naturalistic puffing behavior, and results are consistent with the notion that a 30 s IPI is typical of a tobacco cigarette smoker.

Several ECIG studies now include 10-puff, 30 s IPI directed use bouts when measuring plasma nicotine concentration following acute ECIG use. One study illustrated this methodology when examining the nicotine delivery of two "cig-alike" models in 32 ECIG-naïve cigarette smokers (Vansickel et al., 2010). In this four condition, within-subject study, participants used either their own brand cigarette, an "NPRO" (18 mg/ml nicotine cartomizer) ECIG, a "Hydro" (16 mg/ml nicotine cartomizer) ECIG, or a sham (unlit cigarette). During each session, participants completed a 5 minute 10-puff use bout (with 30 s IPI) with the product assigned for that session. Mean peak changes in plasma nicotine concentration were 1.4 ng/ml for the "NPRO" and 0.5 ng/ml for the "Hydro" (Vansickel et al., 2010). However, these same users obtained mean peak changes of 18 ng/ml following tobacco cigarette use under the same

conditions. These results demonstrate the value in using 10-puff directed ECIG use bouts as they allow for standardization and comparison in nicotine delivery between tobacco cigarette and ECIG use.

Some studies indicate that ECIGs are capable of delivering nicotine, particularly when used by individuals who have previous experience using these products. For example, one within-subject study examined differences in nicotine delivery using a "cig-alike" model ("V2" ECIG with a cartomizer, device wattage not reported) and a "tank" based device ("EVIC", 9 watts) in 23 ECIG-experienced users. Both devices were filled with the same solution ("Flavourart Maxblend," 18 mg/ml nicotine concentration, 35/65 PG/VG ratio) and participants completed one 5-minute, 10-puff ECIG use bout (with 30 s IPI) and plasma nicotine concentration was measured immediately following the bout. Mean (SEM) plasma nicotine concentrations rose from 2.8 (0.4) ng/ml to 4.9 (0.5) following 10 puffs from the "cig-alike" V2 ECIG compared to an increase from 2.5 (0.3) to 7.0 (0.6) ng/ml using the "EVIC". The "cig-alike" model delivered significantly less nicotine to the user compared to the tank-based ECIG model following the 10-puff directed bout (Farsalinos et al., 2014). However, neither product delivered nicotine at levels comparable to those typically associated with combustible cigarettes (i.e., approximately 18 ng/ml after 10 puffs with a 30 s IPI; Vansickel et al., 2010), even in the hands of ECIG-experienced users.

The ability for ECIG-experienced individuals to obtain nicotine from ECIGs was also demonstrated in a study that examined nicotine delivery in eight ECIG-experienced individuals using their own devices (all of which were tank or cartridge-based models). This study included one 10-puff directed ECIG use bout (30 s IPI) and 60 minutes of *ad libitum* use (Vansickel & Eissenberg, 2013). After the directed 10-puff ECIG-use bout, average plasma nicotine

concentration (SEM) was 10.3 ng /ml (2) while their mean plasma concentration (SEM) observed after 60 minutes of ad lib use was 16.3 ng/ml (4.5; Vansickel & Eissenberg, 2013). These plasma nicotine values approach those typically observed in tobacco cigarette smokers after a 10 puff bout (i.e., approximately18 ng/ml; Vansickel et al., 2010).

Only one study to date has compared the nicotine delivery profile of ECIG-experienced and -naïve individuals while holding device features and liquid nicotine concentration constant (Farsalinos et al., 2015). In this study, 24 ECIG-experienced and 23 -naïve participants took 10 puffs (30 s IPI) from an "EVIC" ECIG (9 watts) attached to an "EVOD" atomizer, or "tank" filled with 2 ml of liquid ("Flavourart Maxblend," 18 mg/ml nicotine concentration, 35/65 PG/VG ratio; Farsalinos et al., 2015). Following 10 puffs from the ECIG mean (SEM) plasma nicotine concentrations rose from 2.1 (0.3) ng/ml to 7.9 (0.9) ng/ml in ECIG-experienced individuals and from 1.6 (0.3) ng/ml to 4.3 (0.7) ng/ml in ECIG-naïve tobacco cigarette smokers (Farsalinos et al., 2015). While there was a statistically significant between-group difference, these plasma nicotine concentrations do not approach the nicotine delivery profile of a combustible tobacco cigarette following 10 puffs (i.e., approximately18 ng/ml; Vansickel et al., 2010).

Finally, only one study has compared the nicotine delivery profile of ECIG-experienced individuals while varying liquid nicotine concentration and holding all other device features constant (Dawkins, Kimber, Feyerabend & Cocoran, 2016). In this study, 11 ECIG-experienced men were asked to use an 'eVic supreme' (3.9 V; 8.5 watts) ECIG attached to a 'Nautilus Aspire' tank filled with either low (6 mg/ml) or high (24 mg/ml) liquid nicotine in two separate study sessions. Following 60 minutes of *ad libitum* use of the study product, mean (SEM) plasma nicotine concentration boost (i.e., change in plasma nicotine concentration calculated by

subtracting baseline nicotine concentration from post ECIG use plasma nicotine concentration) in the 6 mg/ml condition was 8.6 (7.5) ng/ml after 10 minutes of use, 16.9 (11.7) ng/ml after 30 minutes, and 22.0 (16.2) ng/ml after 60 minutes. In the 24 mg/ml condition plasma nicotine concentration boost from the baseline was 33.8 (34.9) ng/ml after 10 minutes, 35.5 (28.3) ng/ml after 30 minutes, and 43.6 (34.8) ng/ml after 60 minutes. This study demonstrates larger nicotine boost when using the 24 rather than the 6 mg/ml liquid nicotine concentration. However, the 60 minute *ad lib* puffing protocol makes understanding whether a relationship between liquid and plasma nicotine concentration exists difficult to glean from these results as differences in plasma nicotine concentration may also be a function of differences in puff topography (i.e., puff number, duration or volume) across different liquid nicotine concentrations. Thus, a more controlled puffing regimen with limited puff number may have been more indicative of a direct relationship between liquid and plasma nicotine concentration.

Collectively, studies on ECIG use have shown that not all ECIG models are capable of delivering the same amount of nicotine to the user while other models may be capable of delivering nicotine profiles comparable to that of tobacco cigarette use, at least under *ad libitum* use conditions (Vansickel et al., 2013). Some studies have demonstrated that ECIGs do not deliver nicotine to inexperienced individuals (Vansickel et al., 2010). Conversely, some ECIG devices deliver nicotine to experienced individuals but only under certain conditions (Breland et al., 2014; Dawkins & Corcoran, 2014; Vansickel & Eissenberg, 2013; Farsalinos et al., 2014). Finally, only one study has demonstrated that ECIG-experienced individuals obtain higher plasma nicotine concentrations compared to -naïve individuals even when holding device, liquid nicotine concentration, and puffing constant (Farsalinos et al., 2015).

The aforementioned studies illustrate inconsistencies in nicotine delivery following ECIG use as a result of device features and user experience. The variability in nicotine delivery between ECIG-experienced and -naïve individuals may be explained by differences in smoking behavior (i.e., puff topography). For example, relative to ECIG-naïve smokers, ECIG-experienced individuals may modify their puffing behavior when using an ECIG such that their puffing differs from the puffing behavior when using a tobacco cigarette (Breland et al., 2014). Measurement of puff topography (as described below) likely is an important component of studies designed to inform ECIG regulation by providing data relevant to understanding the effects of nicotine-containing products in users.

Puff Topography

The evaluation of puff topography has been used to understand the smoking behavior of tobacco cigarette users and is now being used to characterize the puffing profile of ECIG users. Puff topography is the measurement of puffing behaviors such as puff number, volume, duration, and IPI, and is often examined using mouthpiece-based computerized devices (Blank, Disharoon, & Eissenberg, 2009). In tobacco cigarette users, puffing behavior has helped explain the relationship between nicotine intake and exposure to other harmful tobacco constituents following conventional tobacco cigarette use (Gust & Pickens, 1982; Herning, Jones, Benowitz, & Mines, 1983). For example, puff topography helped explain why "low-yield" cigarettes did not actually reduce smoking-related harm (e.g., Herning, Jones, Bachman, & Mines, 1981). One study examined the puff topography of 24 abstinent cigarette smokers using either "low," "medium," or "full flavor" cigarettes while holding tar, and carbon monoxide yields constant (Herning et al., 1981). Participants using "low-yield" cigarettes took larger and longer puffs which resulted in CO delivery comparable to that of a "full flavor" cigarette, indicating that these

"low-yield" cigarettes would do little to reduce smoking-related harm. These results demonstrate that measuring puff topography is critical when examining inhalable tobacco products.

Measurement of Puff Topography. The measurement of topography has been a valuable tool in understanding the maintenance of tobacco use and nicotine delivery of various tobacco products. The most commonly used instrument for measuring puff topography requires the placement of cigarettes into a specialized mouthpiece that is capable of detecting flow-induced pressure changes that occur as a result of user inhalation. A pressure transducer senses pressure changes and converts them to flow rate (puff velocity) using previously calibrated software. The software then calculates puff duration, volume, and IPI using these converted flow rate measurements (Blank, 2008). One such mouthpiece-based topography device known as the Clinical Research Support System (CReSS) has been validated in laboratory studies for measuring topography in cigarette smokers (Blank et al., 2009; Buchhalter & Eissenberg, 2000). Puff topography may be useful in explaining some of the variability in ECIG-associated nicotine exposure.

Puff Topography of ECIGs. To date, one published study has examined ECIG topography using a computerized measurement system specially designed to measure ECIG use (Spindle et al., 2014). The mouthpiece-based device used in this study (designed at the American University of Beirut) operated similarly to devices used to measure cigarette smoker's puff topography (e.g., CReSS) but was sensitive enough to capture accurately low flow rate puffs typically associated with ECIG use (Behar et al., 2015). Prior to this study, there was no evidence to suggest whether or not mouthpiece based topography systems would interfere with ECIG-associated nicotine delivery or subjective effects. Plasma nicotine concentration and subjective effects were measured in 13 ECIG-experienced users (with their preferred device and

liquid) during 2 sessions that differed only by the presence of the topography device (and its mouthpiece attachment). Sessions included a 5 minute 10-puff directed bout and a 90 minute *ad libitum* session. This study demonstrated that ECIG-experienced individuals are capable of obtaining nicotine when using their own devices. Specifically, mean (SEM) plasma nicotine concentration immediately following a 10-puff directed ECIG use bout 19.2 ng/ml (2.3) was significantly greater relative to baseline 2.4 ng/ml (0.2) and 10 minutes after ECIG use 10.2 ng/ml (1.1; Spindle et al., 2015). Additionally, this study further demonstrated the ability for ECIG-experienced users to obtain cigarette-like plasma nicotine concentrations (i.e., 18 ng/ml; Vansickel et al., 2010). Finally, this study demonstrated that the ECIG topography recording device did not influence nicotine delivery or most subjective responses. Mean (SEM) plasma nicotine concentration immediately following a 10-puff directed ECIG use bout was 19.9 ng/ml (1.0) in the no topography condition and 21.3 ng/ml (3.1) with the topography device present (Spindle, personal communication).

This study also demonstrated differences in puffing behavior between ECIG-experienced users and tobacco cigarette smokers by comparing puffing results (i.e., volume, duration, and flow rate) from 13 ECIG users to 123 tobacco cigarette smokers from a previous study completed in the same laboratory under similar conditions (Kleylamp, 2008). The results demonstrated that ECIG users take puffs that are, on average, larger and longer than cigarette smokers, and also have much slower flow rate-puffs. Mean (SD) of ECIG users volume were 101.4 ml (50) compared to 51.3 ml (19.2) in tobacco cigarette users. Similarly, ECIG users took longer puffs lasting 4.2 s (1.1) compared to tobacco cigarette smokers who took 1.4 s (0.4) puffs (Spindle et al., 2015). Finally, ECIG users flow rate was 24.2 ml/s (10.7) compared to cigarette smokers flow rate of 38 ml/s (9.7).

Similarly, other studies have used video observation methods to compare the puffing behavior of ECIG users to that of tobacco cigarette smokers. Some of these studies suggest that ECIG-experienced individuals take longer puffs (approximately 4 s, on average) while ECIG-naïve smokers take shorter puffs (approximately 2 s, on average; Hua et al., 2013; Farsalinos et al., 2013). The shorter puffs demonstrated by ECIG-naïve smokers are comparable to puff durations observed in cigarette smokers using their preferred brand of cigarettes (Farsalinos et al., 2013). These studies demonstrate that when using an ECIG for the first time, ECIG-naïve smokers may need to modify their puffing behavior in a manner that resembles the puffing behavior of an ECIG-experienced individual.

To date, one study has demonstrated that ECIG-naïve cigarette smokers appear to adjust their average puff duration and flow rate during the first week of a two week ECIG trial (Lee, Gawron, & Goniewicz, 2015). In this study, 20 tobacco cigarette smokers used a M201 type (Mild, Poland) ECIG containing 11.0 mg of nicotine as determined in a previous study (Goniewicz et al., 2013). Participants were asked to use the provided ECIG for two weeks as a substitute for their tobacco cigarettes. Baseline topography was measured in the laboratory prior to the 2 week ECIG trial and also 7 and 14 days after baseline. At each visit, 8-hour abstinent participants puffed on an ECIG *ad libitum* while puff topography was measured using a CressMicro monitor. During baseline, ECIG use, mean (SEM) puff duration of smokers was 2.2 s (0.1), after one week puff duration was 3.1 s (0.3), and after two weeks puff duration was 2.9 s (0.2; Lee et al., 2015). Mean puff duration after one week of ECIG use increased significantly from baseline. Puff flow rate also changed from baseline with participants having a flow rate decrease from 30.6 ml/s (2.3) to 25.1 (1.8) ml/s after one week, down to 24.8 ml/s (1.9) after week two. These data demonstrate that smokers may modify their puffing behavior after

switching from tobacco cigarettes to ECIGs. Overall, users took longer and slower puffs after one week of ECIG use and this change in puffing behavior is believed to be an adaptation to the factors that influence ECIG nicotine emissions (Lee et al., 2015). These studies highlight the importance of measuring puff topography in ECIG users in order to measure how variability in puffing behavior may alter nicotine delivery.

In summary, ECIG nicotine delivery varies considerably. The ability of ECIGs to deliver nicotine may depend on the variability in device and liquid characteristics such as liquid nicotine concentration. Also, relative to ECIG-experienced,-naïve individuals may be less effective at obtaining nicotine and this may be reflective of differences in puff topography. The nicotine delivery profile of ECIGs needs to be examined further given the potential public health implications of ECIGs delivering varying amounts of nicotine to different populations over a prolonged period of time.

Described below are the potential implications of ECIGs delivering little to no nicotine, cigarette-like levels of nicotine, or exceeding the nicotine delivery profile of a tobacco cigarette. The various populations that may be impacted by ECIG nicotine delivery include nicotine-naïve and currently nicotine-dependent individuals (i.e., adult or adolescent cigarette smokers).

ECIGs Nicotine Delivery: Implications for Various Populations

Combustible tobacco use remains the leading cause of preventable death in the U.S. and reducing tobacco related morbidity and mortality is an important public health goal (USDHHS, 2014). ECIG proponents argue that ECIGs may serve to reduce tobacco related death and disease as they do not operate via combustion and may expose their users to less harmful toxicants (e.g., tobacco specific nitrosamines, polycyclic aromatic hydrocarbons, and carbon monoxide [CO]) but purportedly continue to deliver nicotine (Etter, 2013; Glynn, 2014; Grana, Glantz, & Ling,

2011; McRobbie, Bullen, & Hajek, 2012). Other ECIG proponents surmise that ECIG use may be one approach to helping smokers quit or reduce tobacco cigarette consumption (Abrams, 2014; Etter, 2013). However, the assertion that ECIGs may be a less harmful substitute for tobacco cigarettes remains controversial (Fairchild, Bayer, Colgrove, 2014; Glynn, 2014). ECIG detractors assert that ECIGs may become widely used by nicotine-naïve individuals and may result in nicotine-dependence (Cobb, Byron, & Abrams, 2010; Grana, 2013. The potential health implications that may be associated with ECIGs delivering little to no nicotine (as in Vansickel et al., 2010), delivering some nicotine (as in Farsalinos et al., 2013) or delivering nicotine levels comparable to a tobacco cigarette (as in Spindle et al., 2015) remain unclear. Understanding how these varying levels of nicotine delivery may impact nicotine-naïve and nicotine-dependent individuals over a prolonged period remains unknown.

Nicotine-Naïve Individuals. Nicotine-naïve individuals include adults and adolescents who have never initiated nicotine/tobacco use and thus are not nicotine dependent. Nicotine-naïve youth and adolescents are particularly vulnerable to ECIG experimentation as they are often targeted by ECIG marketing (Duke et al., 2014), and may be enticed by various liquid flavors (Zhu et al., 2014). In fact, according to one national survey, the use of ECIG use among adolescents who have never used tobacco cigarettes, has tripled from 79,000 in 2011 to 263,000 in 2013 (Bunnell et al., 2014). Some speculate that previously nicotine-naïve individuals may eventually become nicotine-dependent, perhaps by initiating ECIG use by first using device/liquid nicotine combinations that deliver little nicotine and later transitioning to products that deliver more nicotine (Cobb, Hendricks, & Eissenberg, 2015). For instance, ECIGs that deliver low amounts of nicotine may serve as "starter products" for previously nicotine-naïve individuals (Blank & Eissenberg, 2015; Cobb et al., 2015). Furthermore, as dependence develops

from the use of such "starter products," ECIG users may transition to products that deliver more nicotine, such as combustible tobacco cigarettes (Wills, Knight, Sargent, Gibbons, Pagano, & Williams, 2016; Leventhal et al., 2015).

Nicotine-Dependent Individuals. While ECIGs are not marketed in the United States as cessation medications for cigarette smokers, many cigarette smokers are attempting to quit or reduce cigarette consumption through the use of these products (Grana, Popova, & Ling, 2014). In addition to the dependence-producing constituent nicotine, tobacco cigarettes contain a myriad of toxic chemicals (e.g., tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, and CO) that are implicated in various smoking related diseases and disorders (e.g., cardiovascular disease and cancers; Hecht, Carmella, Murphy, Akerkar, Brunnemann, & Hoffmann, 1993; Hoffmann & Hecht, 1985; Lakier, 1992; USDHHS, 2014). ECIGs may provide health benefits to nicotine-dependent cigarette smokers by delivering nicotine without the harmful constituents emitted from combustible tobacco cigarettes, thus presenting far fewer negative health consequences (Goniewicz et al., 2014; Polosa, Rodu, Caponnetto, Magila, & Raciti, 2013). However, in order for nicotine-dependent cigarette smokers to switch completely to using ECIGs, these devices will likely need to deliver nicotine in a reliable manner and with a delivery profile (e.g., speed, dose) that is comparable to a tobacco cigarette (Cobb et al., 2015).

The extent to which ECIGs can deliver nicotine with a profile that resembles a combustible tobacco cigarette particularly is important for nicotine-dependent smokers attempting to quit cigarette use entirely, or those seeking an alternative nicotine delivery product. For cigarette smokers trying to quit using ECIGs, products that deliver low amounts of nicotine may not facilitate complete cessation and thus may prolong tobacco cigarette use (Cobb et al., 2015). Conversely, ECIGs that deliver excessively high amounts of nicotine to the user may

promote further nicotine dependence (Blank & Eissenberg, 2015; Cobb et al., 2015), making quitting more difficult, or result in acute nicotine toxicity (Bartschat et al., 2015; Durmowicz, Rudy, & Chen, 2015). Thus, ideally, ECIGs should be designed such that they can deliver enough nicotine to substitute completely for more harmful products, like tobacco cigarettes, but not in a manner that will deliver nicotine at levels that will be toxic to any user or increase one's dependence on nicotine (Shihadeh & Eissenberg, 2015).

Summary. The rapid growth in ECIG popularity among nicotine-naïve individuals and nicotine-dependent cigarette smokers can have important individual and public health implications. Few systematic clinical evaluations of ECIGs have been conducted, leaving many questions regarding the factors that influence their nicotine delivery profile unanswered. Clinical laboratory studies of some ECIG products under very limited conditions have provided some insight on nicotine yield and delivery. However, the implications of the results of these studies are not yet understood fully. For example, the nicotine delivery from ECIGs varies considerably and may be dependent on the device and user behavior (e.g., puff topography). Additionally, there may be variability in nicotine delivery among ECIG-experienced and -naïve individuals (Farsalinos et al., 2015).

Need for Systematic ECIG Evaluation

Because ECIGs contain nicotine that is derived from the tobacco plant, they are considered tobacco products in the U.S. ECIG use is growing in popularity among various populations. ECIG nicotine delivery is of particular concern for regulatory agencies as ECIGs are being used by both nicotine-naïve individuals and by nicotine-dependent tobacco cigarette users (hoping to quit or reduce).

Systematic Evaluation of ECIGs. To date, no studies have evaluated systematically the nicotine delivery profile of ECIGs in ECIG-experienced and -naïve individuals. Systematic evaluation would require holding certain factors constant (e.g., device features) while manipulating others (e.g., liquid nicotine concentration and user experience). With systematic evaluation, the manipulated variables could be considered to be influencing outcomes of interest. The present study seeks to evaluate the nicotine delivery and puff topography of ECIGs in humans while holding device features constant and manipulating user experience and liquid nicotine concentrations.

Statement of the Problem

The rapid growth in ECIG popularity among nicotine-naïve individuals and nicotine-dependent cigarette smokers can have important individual and public health implications. Few systematic clinical evaluations of these products have been conducted, leaving many questions regarding the factors that influence their nicotine delivery profile unanswered. Clinical laboratory studies of some ECIG products under very limited conditions have provided some insight on nicotine yield and delivery. However, the implications of the results of these studies are not yet understood fully. For example, much of the ECIG research has suggested that nicotine delivery from these devices varies considerably and may be dependent on the device and user behavior (e.g., puff topography). Additionally, studies suggest that there may be variability in nicotine delivery among ECIG-experienced and -naïve individuals (Farsalinos et al., 2015). As previously mentioned, ECIG-experienced and -naïve individuals have demonstrated differences in nicotine delivery when using comparable devices (Vansickel & Eissenberg, 2013; Farsalinos et al., 2014). Puff topography analysis may explain some of these differences and computerized, mouthpiece- based puff topography (as used in previous clinical lab studies; e.g., Spindle et al.,

2015) may facilitate this understanding. Currently, no published studies have evaluated the effects of user experience, puff topography, and various liquid nicotine concentrations on a user's ability to obtain nicotine. Additionally, no studies have manipulated liquid nicotine concentration and user experience systematically, while holding device features constant.

The Present Study

This clinical laboratory study examined puff topography among ECIG-experienced and -naïve individuals with four different liquid nicotine concentrations (0, 8, 18, and 36 mg/ml) while holding all other device and liquid factors constant. Additionally, this study examined the extent to which liquid nicotine concentration and puff topography influenced nicotine delivery in ECIG-experienced versus -naïve individuals.

Statement of Hypothesis

The three main hypotheses of this study were as follows: 1) ECIG-experienced individuals will obtain higher plasma nicotine concentrations compared to ECIG-naïve tobacco cigarette smokers and 2) ECIG-experienced users will take longer and larger puffs compared to -naïve individuals 3) there will be a direct relationship between liquid nicotine concentration and plasma nicotine concentration.

Method

Selection of Participants

A total of 129 individuals met the initial study screening criteria via a telephone or online interview, and provided informed consent for the study. Sixty four of these individuals were not included in the final analyses as they were ineligible or discontinued. Of these individuals, 41 were determined to be ineligible at screening (3 ECIG-experienced; 38 -naïve individuals) and never began a session. Additionally, 22 individuals began the study (7 ECIG-experienced; 15 -

naïve individuals) but were discontinued prior to completion for the following reasons: 10 were discontinued due to failure to attend sessions, 6 were discontinued due to lack of venous access, 3 were discontinued due to non-compliance, 1 was discontinued after experiencing an adverse event (nausea), 1 exhibited elevated blood pressure, and 1 exhibited elevated heart rate. Furthermore, one participant (ECIG-naïve individual) completed all four study sessions but was not included in the final analyses when data demonstrated that the participant failed to comply with the study puffing protocol (10 puffs with 30 s IPI).

Thus, thirty-three ECIG-experienced and 31 -naïve (cigarette smokers) community volunteers completed all four sessions and were included in analyses for this between- and within-subject study. An *a priori* power analysis indicated that this number of participants per group would be sufficient to obtain a power of at least 0.80 (i.e., provide 80% chance of detecting an effect). This sample size was estimated using the means and standard deviations (SD) for two key outcome measures (plasma nicotine levels and puff duration) from two previous studies with ECIG users and tobacco cigarette smokers (Farsalinos et al., 2013; Farsalinos et al., 2015), using the SAS PROC POWER procedure (SAS Institute Inc., Cary, NC, USA).

For the outcome measure of plasma nicotine, to determine effects within groups (assuming correlations across measures of 0.6-0.8), a sample size of 16-26 participants per group were required. To determine effects for plasma nicotine between groups, data from a previous study comparing plasma nicotine in with ECIG users and tobacco cigarette smokers was used (Farsalinos et al., 2015) using the same procedure described above. From the literature, the mean (SD) plasma nicotine concentration of 7.9 ng/ml (0.9) for ECIG-experienced and 4.3 ng/ml (0.7) for -naïve individuals were used and it was determined that with a sample size of 20 participants

(10 per group) a medium effect with 80 % power could be detected (alpha < .05). From the literature, the mean (SD) puff duration of 4.2 s (0.7) for ECIG-experienced and 2.3 s (0.5) for -naïve individuals were used and with a sample size of 8 participants (4 per group), and determined that we would be able to detect a medium effect with 80% power (alpha < .05).

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, and provide their informed consent to the use of study products after having abstained from nicotine/tobacco for at least 12 hours.

Two distinct populations (ECIG-experienced and -naïve individuals) were sampled for this study and had different eligibility criteria; see Table 2 for demographic information by group. In order to be eligible for the study ECIG-experienced individuals had to report the use of ≤ 5 conventional tobacco cigarettes daily (M = 0.2; SD = 0.8), use ≥ 1 ml of ECIG solution daily (M = 3.3; SD = 3.7), use ECIG solution with a nicotine concentration ≥ 8 mg/ml (M = 17.5; SD = 5.4; see Table 3), use their ECIG for ≥ 3 months (M = 17.1; SD = 9.9), and provide an expired CO sample with a concentration ≤ 10ppm at screening (M = 3.0; SD = 2.1; suggestive of non-smoking status). To be eligible for the study, ECIG-naïve tobacco cigarette smokers had to use ≥ 10 conventional tobacco cigarettes daily (M = 16.5; SD = 9.4) have < 5 ECIG uses in their lifetime (M = 2.0; SD = 1.5), and provide a CO sample with a concentration ≥15 ppm during screening (M = 19.9; SD = 5.6; suggestive of current smoker status).

Individuals (in either group) who self-reported the following were excluded from participation: chronic disease or current, diagnosed psychiatric condition or regular use of a prescription medication (with the exception of vitamins and birth control). Individuals who weighed less than 110 pounds were also excluded as the study involves multiple blood draws and involves self-administration of potentially high nicotine concentration during certain study conditions. Individuals using marijuana > 10 days in the past 30 or using alcohol > 25 days in the past 30 were excluded. Past month use of cocaine, opioids, benzodiazepines, and methamphetamine was exclusionary and women were excluded if currently breast-feeding or if they tested positive for pregnancy at screening.

As expected, ECIG-experienced and -naïve individuals differed on several demographic characteristics pertaining to eligibility criteria (see Table 2) such that ECIG-experienced individuals smoked fewer cigarettes per day and had lower CO levels at screening relative to ECIG-naïve individuals. ECIG-experienced and-naïve individuals also differed on some demographic characteristics that did not pertain to the differing eligibility criteria. Fewer ECIG-experienced women completed this study (N = 6) relative to ECIG-naïve (N = 13). Also, ECIG-experienced individuals had significantly lower scores on the Penn State Dependence Questionnaire at screening (M = 9.9; SD = 3.4) relative to ECIG-naïve individuals (M = 12.2; SD = 4.0).

Table 2.

Results of Statistical Analyses for Demographic Data by Group.

	ECIG-experienced N = 33		ECIG-naïve N = 31			
	Mean or N	SD	Mean or N	SD	t-statistic	*p* value
Number Female	6		13		-2.1[a]	<.05
Number Caucasian	24		16		-1.8[a]	n.s.
Age (years)	30.3	8.4	30.8	9.9	-0.3[a]	n.s.
Screen CO	3.0	2.1	19.9	5.6	-16.2[a]	<.05
Cigarettes/day	0.2	0.8	16.5	9.4	-10.0[a]	<.05
Volume liquid used/day (ml)	3.3	3.7	N/A	N/A	N/A	N/A
Liquid concentration (mg/ml)	17.5	5.4	N/A	N/A	N/A	N/A
Duration cigarette use (months)	2.0	10.6	110.3	113.9	-5.4[a]	<.05
Duration ECIG use (months)	17.1	9.9	N/A	N/A	N/A	N/A
Fagerström TND[c]	4.3	2.0	4.7	1.9	-0.8[a]	n.s.
Penn State Dependence[d]	9.9	3.4	12.2	4.0	-2.0[b]	<.05
Number choosing menthol	12		19		-2.0[a]	n.s.

Note: n.s. = not significant; N/A refers to not applicable to that particular group.

[a] df = 62; [b] df = 57

[c] The Fagerström Test for Nicotine Dependence (Heatherton et al., 1986)

[d] Penn State Electronic Cigarette Dependence Index (Foulds et al., 2014)

Table 3.

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

Participant	ECIG model	Nicotine Concentration (mg/ml)	Solvent ratio: PG/VG	Battery Voltage (self-report)	Liquid Flavor
1	e-Go	24	50/50	unknown	Peach
2	Kanger	18	80/ 20	unknown	DK Blend
3	e-Go	18	N/A	4.2	Menthol
4	e-Go T	12	30/70	unknown	Persian Winter
5	i-Taste	12	50/50	unknown	Van. Dr. Pepp.
6	e-Go T	24	0/100	unknown	Gorilla Juice
7	e-Go T	12	30/70	3.3	Gold Rush
8	e-Go	18	30/70	3.3	Aztec
9	i-Taste	18	30/70	3.3	Cinnan-toast
10	iStick e-leaf	24	60/40	unknown	Carolina Crush
11	e-Go	28	50/50	3.7	Pink Tornado
12	e-Go T	12	50/50	3.7	Mnky Business
13	e-Go T	25	60/40	unknown	Carolina Cured
14	Voodoo Vape	16	30/70	3.7	White Mousee
15	e-Go T	28	30/70	unknown	Persian Winter
16	Vamo V5	18	30/70	3.7	Persian Winter
17	i-Taste	12	40/60	3.7	Spearmint
18	e-Go	18	70/30	3.7	Blueberry
19	e-Go	12	70/30	unknown	Vanilla
20	i-Taste	18	40/60	unknown	Pomegranate
21	iStick e-leaf	24	40/60	3.3	Wintergreen
22	e-Go	18	40/60	9.0	Colonel Custard
23	e-Go	12	50/50	2.0	Vanilla
24	i-Taste	18	40/60	3.3	King’s barrel
25	Vision Spinner	18	30/70	3.3	Cowboy Cut
26	Grenco G-Pro	12	30/70	4.2	Unicorn Milk
27	IPV	12	30/70	3.2	Cont. Breakfast
28	e-Go	12	30/70	3.7	Smerf Vendetta
29	e-Go	12	65/35	4.0	Jungle Juice
30	e-Go	12	65/35	4.0	Jungle Juice
31	MVP Pro	24	65/35	4.0	Jungle Juice
32	MVP Pro	24	65/35	4.0	Jungle Juice
33	e-Vic	12	65/35	4.0	Jungle Juice

Screening and Informed Consent Procedures

Interested individuals participated in a two-part screening process. First, they participated in a phone interview or online survey where they were asked about their health status, tobacco use, and ECIG use (see appendix A). Potentially eligible individuals who met the requirements for the study were asked to come to the laboratory for an in-person screening which began with an informed consent process (see appendix B). Once informed consent was obtained, individuals provided further information about their health, tobacco use, ECIG use, and demographic information and women provided urine for an immediate pregnancy test (Accutest Value hCG urine pregnancy test, Jant Pharmaceutical Corp). Lastly, participants provided a breath sample for analysis of expired air CO concentration to determine eligibility as described above. Eligible participants sampled two ECIG liquid flavors (menthol or tobacco; 0 mg/ml nicotine) and selected one of them for use in all subsequent sessions. Tobacco and menthol flavors were chosen for this study because they have been identified as two of the four most popular ECIG flavors, especially among tobacco cigarette smokers who have recently initiated ECIG use (Dawkins, Turner, Roberts, & Soar, 2013; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Spyrou, & Voudris, 2013).

Materials

In each session, participants used an "eGo" 3.3 volt, 1000 mAh ECIG battery attached to a 1.5 Ohm, dual-coil, 510-style "cartomizer". The cartomizer was pre-loaded with 1 ml of a flavored solution (tobacco or menthol), that was comprised of 70% propylene glycol/30% vegetable glycerin and contained 1 ml of one of four liquid nicotine concentrations: 0, 8, 18, or 36 mg/ml. All liquid was purchased from a local ECIG vendor, Avail (Richmond, VA) and

liquid nicotine concentration was verified prior to administration. All cartridges were produced by SmokTech (Shenzhen, China) and purchased online.

Procedures

After the completion of screening procedures, participants attended the laboratory on four days (separated by a minimum of 48 hours) for four, randomized, double-blind, independent laboratory sessions that lasted approximately 2.5 hours each. Sessions differed only by the liquid nicotine concentration placed in the cartomizer (0, 8, 18, or 36 mg/ml). Twelve-hour abstinent participants provided expired air CO concentration at the beginning of each session in order to verify abstinence from combustible tobacco (≤ 10 ppm, as in Breland et al., 2002). Participants who did not meet the expired air CO concentration for abstinence tobacco (≤ 10 ppm) were not allowed to participate in the session that day. Under normal conditions, ECIGs do not produce CO as they are not combustible like tobacco cigarettes. Therefore, abstinence from nicotine-containing products was verified retrospectively in ECIG-experienced individuals using baseline plasma nicotine concentration (the criterion used to indicate abstinence was ≤ 5 ng/ml).

At the beginning of each session expired air CO concentration was measured to ensure participants had abstained from combustible tobacco (≤ 10 ppm, as in Breland et al., 2002). Immediately after, the monitoring of physiological responses such as heart rate (HR) and blood pressure (BP) began. Then, a nurse inserted a catheter into a forearm vein. Thirty minutes following catheter insertion, 7ml of blood was sampled (-5 min) followed by the completion of computerized questionnaires intended to assess tobacco abstinence symptoms and other subjective effects (see Figure 2). After collection of baseline blood and subjective questionnaires the first of two directed ECIG-use bouts (separated by 60 minutes) began. Participants were instructed to take 10 puffs from the provided ECIG, with each puff separated by 30 s. As

mentioned previously (see introduction), puff count (i.e., the number of puffs taken) and IPI are often standardized. In the present study, IPI is defined as the time between the onset of one puff and the onset of a subsequent puff (as seen in Vansickel et al., 2010, Farsalinos et al., 2014; Spindle et al., 2014). The 10 puff bout was monitored and verified by a trained research assistant who directed the participant and ensure that puffs were taken at the correct time. Immediately following the final puff of the first bout, the second 7 ml of blood was sampled (+5 min) and subjective questionnaires were administered again. Blood samples 3 (+15 min), 4 (+30 min) 5 (+45 min), and 6 (+55 min) were collected, each followed by subjective questionnaires. Following the sixth blood sample (60 minutes after the first directed bout) the second bout began. Immediately following the final puff of the second bout, a 7th 7 ml of blood was sampled (+65 min), followed by blood samples 8 (+75 min), 9 (+90 min), and 10 (+105min). Each of these samples was also followed by administration of the subjective questionnaires (see Figure 2).

After the completion of the tenth set of subjective measures, physiological data collection was discontinued, the catheter was removed, and participants were compensated (US $75 after first session, $75 after second, $150 after the third and $200 after the fourth session).

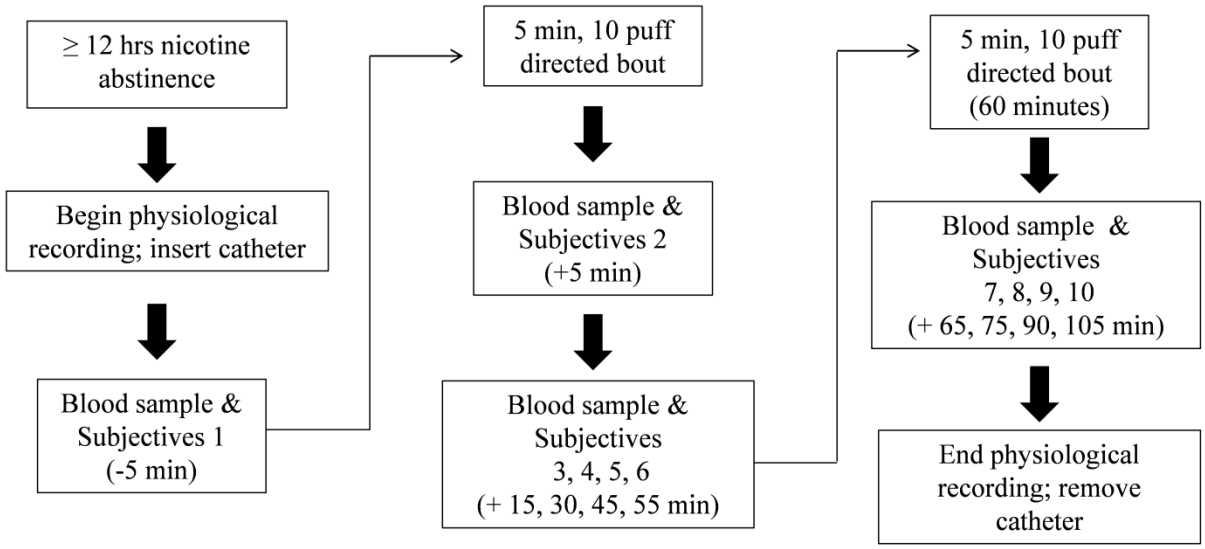


Figure 2. Session procedure involved participants visiting the laboratory for four, 2.5 hour sessions. Prior to each session, at least 12 hours of abstinence from nicotine/tobacco was required, and was verified by a pre-session CO reading of < 10 ppm. After participants met this requirement, physiological monitoring commenced, an intravenous catheter was inserted into a forearm, and the session began.

Participant Safety and Rights

The study methods and procedures described above have been used in this laboratory for over 15 years. While 12 hours of nicotine/tobacco abstinence may be uncomfortable, it is not medically dangerous and does not pose a threat to participant safety. Additionally, the blood sampling procedure that occurred via an intravenous catheter involved minimal risk of bruising or infection. This laboratory's trained nursing staff used aseptic nursing procedures and sterile, disposable equipment in order to minimize risk. The use of ECIGs/nicotine also posed minimal risk as the target population had experience with either ECIGs or conventional tobacco cigarettes.

The experienced CBPL staff is trained to ensure that participant safety and rights were maintained throughout the duration of the study. Both heart rate (HR) and blood pressure (BP) were monitored closely and sessions were discontinued if a participant's systolic BP elevated above 140 or dropped below 90 or if their HR elevated above 120 or dropped below 50. Confidentiality of data was maintained and participant data was identified using an alphanumeric code only and stored in locked rooms accessible only by CBPL staff.

Outcome Measures

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

Puff Topography. Using an ECIG topography instrument developed and manufactured at the American University of Beirut (AUB; see Spindle et al., 2015), puff topography was

measured throughout each ECIG bout. Puff topography measurements included: puff duration, volume, flow rate (a.k.a. puff velocity), number and IPI (i.e., the time between puff onset and the puff onset of the subsequent puff). This instrument is comparable to cigarette topography instruments (e.g., CReSS, see introduction and Blank, 2009) and has been tested to determine that the equipment does not interfere with nicotine delivery or abstinence symptom suppression (see Spindle et al., 2015).

This device uses mouthpieces, several of which were manufactured for this study and the device was calibrated with the mouthpiece attached prior to each session using a custom built automatic digital flow calibrator. The orifice dimensions of each mouthpiece and pressure-sensing transducer provided sensitivity sufficient to ensure valid measurements at puff velocities as low as 3 ml/sec because tobacco cigarette topography devices may not be sensitive enough to measure ECIG topography accurately (Eissenberg, 2014).

Subjective Questionnaires. Four subjective measures (Hughes-Hatsukami Scale, The Direct Effects of ECIG Use Questionnaire, Acceptability Questionnaire, and Tiffany-Drobes Questionnaire of Smoking Urges) were administered at ten separate time points. Three of these questionnaires were administered using a computerized visual analog scale (VAS) which consisted of a word or phrase centered on a horizontal line with “not at all” on the left and “extremely” on the right. Participants recorded responses by clicking a mouse at any point on a horizontal line and scores were expressed as a percentage of total line length.

Hughes-Hatsukami Withdrawal Scale. An adapted version of this VAS measure was used for this study (see Breland, Evans, et al., 2002, Buchhalter et al., 2005) intended to assess nicotine abstinence symptom suppression and was composed of 11 items: “Anxious,” “Craving and e-cigarette/nicotine,” “Depression,” “Difficulty concentrating,” “Drowsy,” “Hunger,”

"Impatient," "Irritable," "Restlessness," "Desire for sweets," and "Urge to smoke." ECIG-experienced and ECIG-naïve cigarette smokers received the exact same scale with the exception of the measure "Urge to Smoke," which was adjusted to "Urge to use an ECIG" for ECIG-experienced users (Hughes & Hatsukami, 1986, see appendix C).

Direct Effects of ECIG Use. This 10-item VAS measure, adapted from the "Direct Effects of Tobacco" scale, 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 assessed the subjective effects of ECIG use: "Did the e-cigarette make you feel more awake?," " Did the e-cigarette help 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, because topography was measured in each condition this VAS measure assessed the degree to which the topography equipment: "Alters e-cigarette use behavior," "Makes vaping less likely," "Reduces enjoyment," "Affects e-cigarette taste," "Increases awareness," and "Increases vaping difficulty" (as in Blank et al., 2009; Spindle et al., 2015).

Tiffany-Drobes QSU Brief. The QSU Brief consisted of 10 smoking-related items: "I have a desire for a cigarette/ECIG right now," "Nothing would be better than smoking a cigarette/ECIG right now," "If it were possible, I would probably smoke/use an ECIG right now," I could control things better right now if I could smoke/smoke an ECIG," "All I want right now is cigarette/ECIG," "I have an urge for a cigarette/ECIG," "Smoking/an ECIG would make

me less depressed," and " I am going to smoke/ use an ECIG as soon as possible" (Cox, Tiffany, & Christen, 2001). Smoke/smoking was substituted with ECIG/use an ECIG for ECIG-experienced participants. Participants rated each item on a 7-point scale ranging from 0 (Strongly disagree) to 7 (Strongly agree). The items form two factors: Factor 1 (intention to smoke) and Factor 2 (anticipation of relief from abstinence symptoms).

Data Analysis Plan

The outcome measures for this thesis are plasma nicotine concentration, puff topography, and heart rate. Other measures are not a focus for this thesis and are not discussed further. For plasma nicotine, values below the limit of quantification (LOQ) were replaced with the LOQ (2 ng/ml; as in Vansickel et al., 2010), as this is a more conservative approach compared to identifying each value below the LOQ as zero. For plasma nicotine concentration, missing data values were imputed by replacing the missing value with an average of the value before and after the missing plasma nicotine concentration value (of the 2,560 plasma nicotine values, only 3 were missing). Topography equipment generated values for puff velocity data to produce the topography measures puff duration, puff volume, mean puff velocity, puff number, and IPI (see Shihadeh, Azar, Antonios, & Haddad, 2004). A data cleaning procedure was performed that combined two or more puffs separated by less than 100 ms into a single puff and deleted any puffs less than 300 ms (Spindle et al., 2015). For topography data, no missing values for any of the variables were observed. Prior to analysis, HR data were averaged to produce a single value for baseline and the five minutes during each ECIG-use bout (3 values in total per session referred to as Baseline, Bout 1, and Bout 2). For HR, any missing values were replaced by the HR measurement that was recorded manually during each individual participant's session.

Statistical analyses for the three primary outcome measures (plasma nicotine, puff topography, and HR) were performed using IBM SPSS (Version 23.0). Mixed Analysis of Variance (ANOVAs) were used to examine plasma nicotine, topography, and HR data. For plasma nicotine, a mixed ANOVA with group as a between-subject factor (ECIG-experienced or -naïve individuals) and liquid nicotine concentration (hereafter referred to as "condition"; 4 levels) and time (10 levels) as within-subject factors was conducted. For puff topography, for each measure, a mixed ANOVA with group as a between-subject factor (ECIG-experienced or -naïve) condition (4 levels) and bout (2 levels) as within-subject factors was conducted. Finally, for HR, a mixed ANOVA with group as the between-subject factor (ECIG-experienced or -naïve) and condition (4 levels) and time (3 levels; baseline, HR average during bout 1 and HR average during bout 2) as within-subject factors was conducted.

In order to understand whether gender may influence key outcome measures, all data were also analyzed using mixed ANOVAs with gender as the between-subject factor (male or female) and condition (4 levels) and time (10 levels for plasma; 2 levels for topography variables; 3 for HR variables) as the within-subject factors. For ECIG-experienced individuals, 27 of 33 were men and for ECIG-naïve individuals 18 of 31 were men.

While the effects of flavor on plasma nicotine concentration following acute ECIG use have not been evaluated thoroughly, some studies suggest that flavor may influence plasma nicotine concentration (Oncken, Litt, McLaughlin, & Burki, 2015). In order to understand whether or not ECIG-liquid flavor may influence key outcome measures, all data were analyzed using mixed ANOVAs with flavor as the between-subject factor (menthol or tobacco) and condition (4 levels) and time (10 levels for plasma; 2 levels for topography variables; 3 for HR) as the within-subject factors. Prior to the start of all four sessions, participants in each group

were given the option of selecting either menthol or tobacco flavored ECIG-liquid. Ultimately, 21 ECIG-experienced individuals selected tobacco flavor and 12 selected menthol. Of the ECIG-naïve individuals, 12 selected tobacco flavor and 19 selected menthol.

ANOVAs are susceptible to violations of assumptions of sphericity. Sphericity violations occur when the variances between all combinations of related groups are unequal. Violations to sphericity can result in an increase in the Type I error rate if not corrected. The Huynh-Feldt (1976) procedure is a correction generated to adjust for sphericity violations. For all repeated measures factors, significance levels were adjusted for potential violations of sphericity using Huynh-Feldt corrections (Huynh & Feldt, 1976).

For all outcome measures, within-subject comparisons were made using Tukey's Honestly Significant Difference (HSD) test, based on the studentized range distribution, to compare all possible pairs of means (Tukey, 1949). Between-subject (i.e., ECIG-experienced versus -naïve individuals) comparisons were made using planned contrasts using independent samples t-tests. For plasma nicotine concentration, planned contrasts were conducted across groups for the time point prior to bout 1 (-5 min), immediately after bout 1 (+5 min), immediately prior to bout 2 (+55 min) and immediately after bout 2 (+65 min). Similarly, planned contrasts were used to make cross group comparisons for topography and heart rate measures. Because these planned contrasts were orthogonal at each time point, no corrections were made to type I error rate for them (Keppel, 1992).

Prior to conducting the main study analyses for plasma nicotine, topography, and HR described above, plasma nicotine data were first inspected to determine if any participants were not abstinent at prior to the onset of any session. That is, this study required $\geq$ 12 hours abstinence from all nicotine/tobacco containing products prior to each session; this level of

abstinence was verified retrospectively by examining pre-session plasma nicotine concentration for each participant and each session. Five ng/ml was selected as the cutoff for 12 hours of nicotine abstinence (i.e., individuals with a baseline plasma values of 5.0 ng/ml or higher were consider to be not abstinent) for both ECIG-experienced and -naïve individuals (see Spindle et al., 2016). Ultimately, 18 of the 33 ECIG-experienced and 21 of the 31 -naïve individuals were considered to have abstained from nicotine prior to each of the four sessions. To understand how abstinence status influenced each outcome measure within each group of participants (ECIG-experienced and -naïve individuals), and before conducting the analyses described above, all within group data first were analyzed using mixed factorial ANOVAs with abstinence status as the between-subject factor (abstinent or non-abstinent) and condition (4 levels) and time (10 levels for plasma; 2 levels for topography variables; 3 for HR) as the within-subject factors. In the results below, this analysis by abstinence status preceded and in some cases informed the overall analysis results that follow.

Results

This within and between-subject, double blind, clinical laboratory study examined the extent to which liquid nicotine concentration and puff topography influenced nicotine delivery in ECIG-experienced and -naïve individuals. Also, this study examined puff topography among ECIG-experienced and -naïve individuals with four different liquid nicotine concentrations (0, 8, 18, and 36 mg/ml) while holding all other device and liquid characteristics constant.

Plasma Nicotine

The Effect of Abstinence Status. Table 4 shows the statistical analyses for plasma nicotine using raw data to evaluate potential effects of abstinence status for ECIG-experienced and -naïve individuals. As the table shows, for ECIG-experienced individuals there was a

significant main effect of abstinence status [F (1, 31) = 9.8 p <.01] in addition to other significant main effects and a significant condition by time interaction. Among those who are ECIG-experienced, non-abstinent individuals obtained significantly higher baseline plasma nicotine concentrations in the 8 (M = 8.1; SD = 7.4), 18 (M = 9.3; SD = 7.8) and 36 mg/ml (M = 7.1; SD = 5.2) conditions relative to abstinent individuals whose baseline plasma nicotine concentrations were significantly lower in the 8 (M = 2.0; SD = 0.2), 18 (M = 2.1; SD = 0.3) and 36 mg/ml (M = 2.0; SD = 0.5) conditions, but not in the 0 mg/ml condition [ts (31) < -2.5; *p*s < .05].

For ECIG-naïve individuals Table 4 shows a significant main effect of abstinence status [F (1, 29) = 9.6, p <.01] in addition to other significant main effects and a significant condition by time interaction. Non-abstinent individuals obtained significantly higher baseline plasma nicotine concentrations in the 0 (M = 6.3; SD = 4.7), 8 (M = 4.7; SD = 3.1) and 36 mg/ml (M = 5.8; SD = 4.9) conditions relative to abstinent individuals whose baseline plasma nicotine concentrations were significantly lower in the 0 (M = 2.4; SD = 0.6), 8 (M = 2.2; SD = 0.6) and 36 mg/ml (M = 2.4; SD = 0.8) conditions, but not in the 18 mg/ml condition [ts (29) < -2.6; *p*s <.05]. The main effects of abstinence status observed for ECIG-experienced and -naïve individuals when analyzing plasma nicotine concentration using raw data appear to indicate that differences across abstinence status were due to baseline plasma nicotine differences.

To explore whether abstinence status influenced differences in plasma nicotine concentration between abstinent and non-abstinent individuals after ECIG use or whether they occurred due to baseline differences, the same analyses (i.e., mixed ANOVAs with abstinence status as the between subject factor) were conducted using plasma nicotine concentration difference scores (i.e., change scores from baseline for each individual in each condition). Using

difference scores for plasma nicotine concentration eliminates baseline plasma nicotine differences among abstainers and non-abstainers in each group (i.e., ECIG-experienced and -naïve individuals). When using difference scores for plasma nicotine concentration, no significant main effects involving abstinence status were observed for either ECIG-experienced or -naïve individuals (see Table 4). Taken together, these results suggest that increases in plasma nicotine observed post-ECIG use do not differ as a function of abstinence status and that the differences observed for plasma nicotine among abstainers and non-abstainers (using raw data) were significant due to differences in plasma nicotine concentrations at baseline. Thus, the final analyses (presented below) for plasma nicotine concentration in ECIG-experienced and -naïve individuals were conducted using difference scores and included all participants in the sample (abstinent and non-abstinent).

Table 4.

Statistical Analyses Results for Plasma Nicotine for ECIG-experienced and -naïve Individuals by Abstinence Status

Outcome measures	Condition (C) *F* value	*p* value	Time (*T*) *F* value	*p* value	Abstinence (*A*) *F* value	*p* value	*C* × *T* *F* value	*p* value
ECIG-experienced[a]								
Plasma nicotine								
Raw Data	38.0	<.001	32.7	<.001	9.8	<.01	11.8	<.001
Difference Scores	27.2	<.001	32.7	<.001	0.4	*ns*	11.8	<.001
ECIG-naïve[b]								
Plasma nicotine								
Raw Data	13.2	<.001	15.3	<.001	9.6	<.01	4.1	<.001
Difference Scores	13.6	<.001	15.5	<.001	0.01	*ns*	3.9	<.001

Note: ns = non-significant

[a]df C = (3, 93); df T = (9, 279); df A = (1, 31); df C x T = (27, 837)

[b]df C = (3, 87); df T = (9, 261); df A = (1, 29); df C x T = (27, 783)

Change from Baseline (Nicotine Boost). Among ECIG-experienced and -naïve individuals, differences observed as a function of abstinence status using raw data were no longer observed when difference score data were analyzed. Thus, the final analyses for plasma nicotine concentration in ECIG-experienced and -naïve individuals were conducted using difference score data (hereafter referred to as "nicotine boost" – the change in plasma nicotine concentration calculated by subtracting baseline nicotine concentration from post ECIG use plasma nicotine concentration). All participants (abstinent and non-abstinent) are included in this analysis.

Using nicotine boost data, a significant three-way condition by time by group interaction was observed for nicotine boost [F (27, 1674) = 2.6, p <.01]. A significant condition by time interaction [F (27, 1674) = 15.0, p < .001], a time by group (ECIG-experienced versus -naïve) interaction [F (9, 558) = 6.7, p < .01], and a significant condition by group interaction [F (3, 186) = 6.7, p < .01] were also observed. Also, significant main effects of condition [F (3, 186) = 40.3, p <.001], time [F (9, 558) = 46.4, p < .001], and group [F (1, 62) = 10.6, p < .01] were observed. Figure 3 depicts mean nicotine boost, over time, by condition (i.e., liquid nicotine concentration) for ECIG-experienced and -naïve individuals.

For ECIG-experienced individuals, immediately following bout 1, mean (SD) nicotine boost for the 0 mg/ml liquid nicotine concentration was 0.01 ng/ml (1.5), for 8 mg/ml it was 8.2 ng/ml (7.8), for 18 mg/ml it was 13.0 ng/ml (6.2), and for 36 mg/ml it was 17.9 ng/ml (17.2). Immediately following bout 2, mean nicotine boost for 0 mg/ml was -0.3 ng/ml (3.0), for 8 mg/ml it was 7.2 ng/ml (6.1), for 18 mg/ml it was 11.2 ng/ml (12.5) and for 36 mg/ml it was 14.9 ng/ml (12.4). In general, nicotine boost was significantly higher when using active liquid nicotine concentration versus placebo (0 mg/ml). Among ECIG-experienced individuals, within-

group comparisons revealed significant differences in nicotine boost between 0 mg/ml and the 8, 18, and 36 mg/ml conditions immediately following bout 1 and bout 2 (Tukey's HSD, $p < .05$). Also, significant differences in nicotine boost were observed between the 8 and 36 mg/ml conditions immediately following bouts 1 and 2. No significant differences in nicotine boost were observed between 8 and 18 mg/ml or 18 and 36 mg/ml conditions following bout 1 or 2.

For ECIG-naïve individuals, immediately following bout 1, mean (SD) nicotine boost for the 0 mg/ml liquid nicotine concentration was -0.02 ng/ml (1.5), for the 8 mg/ml it was 3.6 ng/ml (3.9), for 18 mg/ml it was 6.2 ng/ml (10.2), and for 36 mg/ml it was 6.8 ng/ml (7.1). Immediately following bout 2, mean nicotine boost for 0 mg/ml was -0.4 ng/ml (2.5), for 8 mg/ml it was 4.8 ng/ml (8.0), for 18 mg/ml it was 6.0 ng/ml (10.3) and for 36 mg/ml it was 7.4 ng/ml (9.2). In general, nicotine boost was significantly higher when using active liquid nicotine concentration versus placebo (0 mg/ml). Among ECIG-naive individuals, within-group comparisons revealed significant differences in nicotine boost between 0 and 18 mg/ml and between 0 and 36 mg/ml immediately after bout 1 and 2 (Tukey's HSD, $p < .05$). No significant differences in nicotine boost between the 0 and 8 mg/ml, 8 and 18 mg/ml or 18 and 36 mg/ml conditions were observed following bout 1 or 2 (see Figure 3).

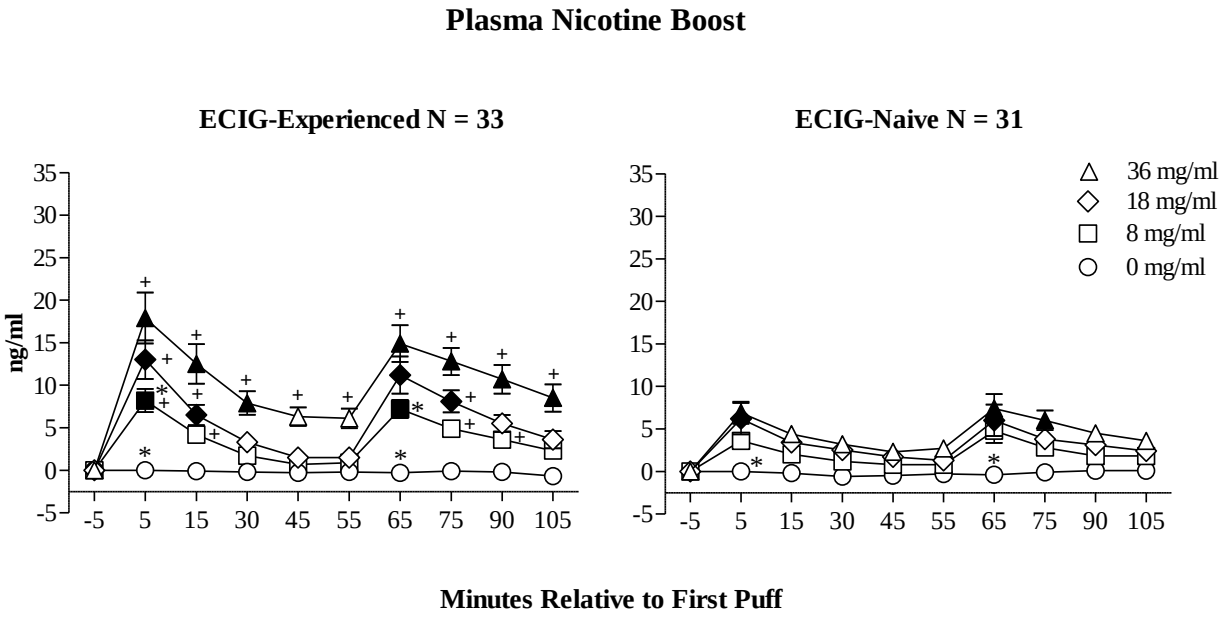


Figure 3. Mean (± SEM) nicotine boost for ECIG-experienced (N = 33) and -naïve individuals (N = 31). Arrows indicate the onset of each 10-puff ECIG use bout. Filled symbols indicate a significant difference from baseline (-5 time point), asterisks (*) indicate significant differences from the 36 mg/ml nicotine concentration at that time point (note the figure displays comparisons for the 36 mg/ml concentration for time 5 and time 65 only, see the text for more comparisons; *p*s < .05; Tukey's HSD). Plus signs (+) indicate significant between group differences at that time point for that concentration (independent t-tests; *p*s < .05).

Across groups, for nicotine boost, planned contrasts were conducted for the time point prior to bout 1 (-5 min), immediately after bout 1 (+5 min), immediately prior to bout 2 (+55 min) and immediately after bout 2 (+65 min) and revealed significant between group (ECIG-experienced versus -naïve) differences in the 8, 18, and 36 mg/ml conditions [ts (62) < -0.17; *ps* <.05] but not in the 0 mg/ml condition. ECIG-experienced individuals obtained significantly higher nicotine boost immediately following bout 1 in the 8 (M = 8.2; SD = 7.8), 18 (M = 13.0; SD = 13.2) and 36 mg/ml (M = 17.9; SD = 17.2) conditions relative to ECIG-naïve individuals who had significantly lower nicotine boost immediately following bout 1 in the 8 (M = 3.6; SD = 3.9), 18 (M = 6.2; SD = 10.2) and 36 mg/ml (M = 6.9; SD = 7.1) conditions, but not in the 0 mg/ml condition. Immediately following bout 2, ECIG-experienced individuals obtained significantly higher nicotine boost in the 18 (M = 11.2; SD = 12.5) and 36 mg/ml (M = 14.9; SD = 12.4) conditions relative to ECIG-naïve individuals whose nicotine boost was significantly lower following bout 2 in the 18 (M = 6.0; SD = 10.3) and 36 mg/ml (M = 7.4; SD = 9.2). Taken together, these results support the notion that the significant three-way interaction arises from the fact that, although both groups started at the same baseline, ECIG-experienced participants had higher nicotine boost, on average, after each bout in many active nicotine conditions.

In an additional analysis used to help clarify results, a mean peak nicotine boost value was calculated for each group (ECIG-experienced and -naïve) for each condition (0, 8, 18, and 36 mg/ml liquid nicotine concentration) for both bout 1 and bout 2. That is, for each individual participant, in each condition, a peak nicotine boost value was calculated for bouts 1 and 2. The individual peak nicotine boost were then averaged across all participants to produce a single peak nicotine boost value for each condition and group. Figure 4 depicts peak nicotine boost for each condition and bout by group. Planned contrasts were conducted to compare mean peak nicotine

boost in each condition across groups (ECIG-experienced and -naïve). For bout 1, significant between group differences were observed in the 8, 18, and 36 mg/ml conditions such that ECIG-experienced individuals obtained significantly higher mean peak nicotine boost immediately following bout 1 in the 8 (M = 8.5; SD = 7.6), 18 (M = 13.7; SD = 12.4) and 36 mg/ml (M = 20.0; SD = 16.4) conditions relative to ECIG-naïve individuals who obtained significantly lower mean peak nicotine boost immediately following bout 1 in the 8 (M = 4.0; SD = 4.0), 18 (M = 7.2; SD = 9.8) and 36 mg/ml (M = 7.6; SD = 7.2) conditions, but not in the 0 mg/ml condition [ts (62) > 2.3; *p*s <.05].

For bout 2, significant between group differences were observed such that ECIG-experienced individuals obtained significantly higher mean peak nicotine boost immediately following bout 2 in the 18 (M = 12.6; SD = 12.0) and 36 mg/ml (M = 17.0; SD = 12.7) conditions relative to ECIG-naïve individuals whose peak nicotine boost were significantly lower in the 18 (M = 6.7; SD = 10.0) and 36 mg/ml (M = 8.8; SD = 9.0) conditions, but not in the 0 or 8 mg/ml condition. [ts (62) > 2.1; *p*s <.05]

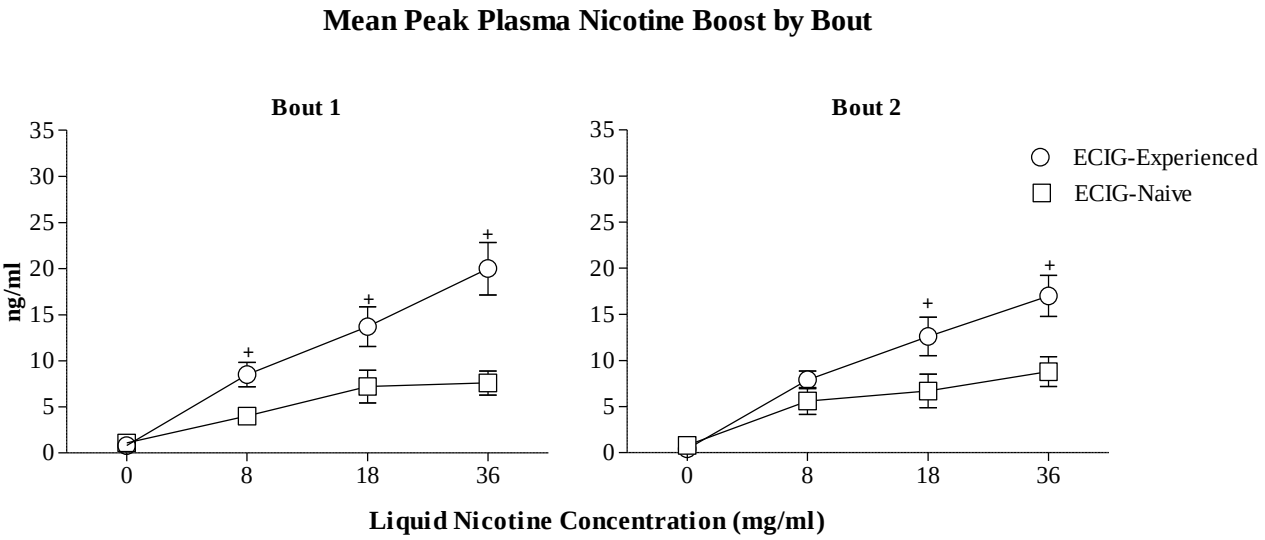


Figure 4. Mean (± SEM) peak nicotine boost derived using difference scores for ECIG-experienced (N = 33) and ECIG-naïve individuals (N = 31) by condition and bout (see text for details of how data were prepared for this analysis). Plus signs (+) indicate significant between group differences at that condition for that bout (independent t-tests; *p*s < .05).

The Effect of Gender. To explore whether gender influenced nicotine boost within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with gender as the between-subject factor) were conducted using nicotine boost data (i.e., change scores from baseline).

For ECIG-experienced individuals a significant condition by time interaction was observed [F (27, 837) = 11.8, p <.001] but no significant main effects or interactions involving gender were observed, thus, further analyses were not conducted. For ECIG-naïve individuals a significant three way condition by time by gender interaction was observed [F (27,783) = 2.1, p <.05] for nicotine boost. Also, significant condition by time [F (27,783) = 4.2, p <.001] and significant condition by gender [F (3, 87) = 6.2, p <.01] interactions were observed.

For ECIG-naïve women, immediately following bout 1, mean (SD) nicotine boost for the 0 mg/ml liquid nicotine concentration was -0.0 ng/ml (0.6), for 8 mg/ml it was 1.5 ng/ml (2.4), for 18 mg/ml it was 1.7 ng/ml (3.5), and for 36 mg/ml it was 4.1 (5.7). Immediately following bout 2, mean nicotine boost for 0 mg/ml was -0.6 ng/ml (3.5), for 8 mg/ml it was 4.7 ng/ml (11.6), for 18 mg/ml it was 1.9 ng/ml (3.0) and for 36 mg/ml it was 2.8 ng/ml. Among ECIG-naïve women, none of the mean nicotine boost for any condition differed significantly from baseline. Also, no differences across bouts 1 and 2 were detected for any condition.

For ECIG-naïve men, immediately following bout 1, mean (SD) nicotine boost for the 0 mg/ml liquid nicotine concentration was -0.01 ng/ml (1.9), for the 8 mg/ml it was 5.1 ng/ml (4.1), for 18 mg/ml it was 9.4 ng/ml (12.2), and for the 36 mg/ml it was 8.9 ng/ml (7.5). Immediately following bout 2, mean nicotine boost for 0 mg/ml was -0.3 ng/ml (1.5), for 8 mg/ml it was 4.8 ng/ml (4.1), for 18 mg/ml it was 8.9 ng/ml (12.6) and for 36 mg/ml it was 10.6 ng/ml (10.0). Significant differences in nicotine boost were observed between 0 and 18 mg/ml

and between 0 and 36 mg/ml immediately after bout 1 and bout 2 (Tukey's HSD; *p*s < .05). No significant differences in nicotine boost were observed between 0 and 8 mg/ml, 8 and 18 mg/ml or 18 and 36 mg/ml following bout 1 or 2.

Across gender for ECIG-naïve individuals, planned contrasts were conducted for nicotine boost for the time point prior to bout 1 (-5 min), immediately after bout 1 (+5 min), immediately prior to bout 2 (+55 min) and immediately after bout 2 (+65 min). Planned contrasts revealed significant gender differences such that men obtained significantly higher nicotine boost immediately following bout 1 in the 8 (M = 5.1; SD = 4.1), 18 (M = 9.4; SD = 12.2) and 36 mg/ml (M = 8.9; SD = 7.57) conditions, relative to women who obtained significantly lower nicotine boost in the 8 (M = 1.5; SD = 2.4), 18 (M = 1.7; SD = 3.5) and 36 mg/ml (M = 4.1; SD = 5.7) conditions, but not in the 0 mg/ml condition [ts (29) < -2.9; *p*s < 0.05]. Immediately following bout 2, men obtained significantly higher mean nicotine boost in the 18 (M = 8.9; SD = 12.6) and 36 mg/ml (M = 10.6; SD = 10.0) conditions relative to women who showed significantly lower mean nicotine boost in the 18 (M = 1.9; SD = 3.0) and 36 mg/ml (M = 2.8; SD = 5.6) conditions but not in the 0 and 8 mg/ml condition [ts (29) < -3.1; *p*s <.05].

The Effect of Flavor. To explore whether flavor influenced nicotine boost within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with flavor as the between-subject factor) were conducted using nicotine boost data. Significant condition by time interactions were observed for ECIG-experienced individuals [F (27, 837) = 11.8, p <.001] and ECIG-naïve individuals [F (27,783) = 4.1, p <.001], but no significant interactions or main effects involving flavor (menthol or tobacco) were observed for nicotine boost for either group. Given that the between-subject variable of interest (flavor) was not involved in any significant interactions or main effects further post hoc testing was not conducted.

Puff Topography

The Effect of Abstinence Status. Prior to conducting the main analyses, topography data were analyzed to explore whether abstinence status influenced puff topography variables of interest (i.e., puff duration, volume, flow rate, puff number, and IPI). Puff topography data were analyzed using mixed ANOVAs with abstinence status as the between-subject factor for ECIG-experienced and -naïve individuals.

For ECIG-experienced and -naïve individuals, no significant interactions or significant main effects involving abstinence status were observed for any topography variable. Importantly, the absence of significant main effects or interactions involving abstinence status among ECIG-experienced and -naïve individuals suggests that puff topography did not differ between abstinent and non-abstinent individuals in either group. Thus, the final topography analyses presented below will include abstinent and non-abstinent participants for each group.

Puff Topography. Using data from all participants (abstinent and non-abstinent), mixed ANOVAs were conducted to compare each of the puffing parameters of interest: puff duration, volume and flow rate among ECIG-experienced and -naïve individuals. Additional mixed ANOVAs were conducted to analyze the puffing variables that were experimentally controlled in this study: puff number (10) and IPI (30 s). As mentioned previously, IPI is defined here as the time between the onset of one puff and the onset of a subsequent puff (as seen in Vansickel et al., 2010, Farsalinos et al., 2014; Spindle et al., 2015).

Mean (SD) puffing parameters for ECIG-experienced and -naïve individuals are displayed in Table 5. Significant time by group interactions were observed for puff duration [F (3, 186) = 5.417, p <.05], puff volume [F (3, 186) = 1.23, p <.05] and flow rate [F (1, 62) = 4.42, p <.05]. No significant interactions for puff number or IPI were observed. Significant main

effects of group were observed for puff duration [F (1, 62) = 28.28, p < .001] and puff volume [F (1, 62) = 8.7, p <.01]. For IPI, a significant main effect of time [F (1, 62) = 8.0, p <.05] was observed. No significant main effects were observed for flow rate or puff number.

Puff Duration. For puff duration, in bout 1, ECIG-experienced individuals took significantly longer puffs in the 0 mg/ml relative to the 36 mg/ml condition (Tukey's HSD, p < .05). No other differences in puff duration were observed across conditions. For puff duration, in bout 2, ECIG-experienced individuals took significantly longer puffs in the 0 relative to the 36 mg/ml condition and in the 8 relative to the 36 mg/ml conditions. No differences in puff duration were observed across bouts in ECIG-experienced individuals.

For puff duration, in bout 1, ECIG-naive individuals took significantly longer puffs in the 0, 8 and 18 mg/ml conditions relative to the 36 mg/ml condition (Tukey's HSD, p < .05). Also, during bout 1, ECIG-naïve individuals took significantly longer puffs in the 0 relative to the 18 mg/ml condition (Tukey's HSD, p < .05). During bout 2, ECIG-naïve individuals took significantly shorter puffs in the 8, 18, and 36 mg/ml conditions relative to the 0 mg/ml condition. ECIG-naïve individuals took longer puffs in the 0, 8 and 18 mg/ml conditions relative to the 36 mg/ml condition.

Across groups, for puff duration, planned contrasts revealed significant between group (ECIG-experienced versus -naïve) differences in each condition and for each ECIG-use bout, indicating that ECIG-experienced individuals took significantly longer puffs relative to ECIG-naïve individuals when using the 0, 8, 18, and 36 mg/ml liquid nicotine concentration [ts (62) > 3.3, ps < .05].

Table 5.
Mean (SD) Puff Topography by Liquid Nicotine Concentration and Group for Bouts 1 and 2

	Liquid Nicotine Concentration (mg/ml)							
	Bout 1				Bout 2			
	0	**8**	**18**	**36**	**0**	**8**	**18**	**36**
Puff Duration (s)								
ECIG-Experienced	5.9*+ (2.4)	5.7+ (2.2)	5.0+ (1.9)	4.7+ (3.9)	6.2*+ (2.5)	6.4*+ (2.3)	5.8+ (3.4)	5.1+ (4.6)
ECIG-Naive	3.3* (1.7)	3.0* (1.5)	2.8* (1.3)	2.2 (0.8)	3.6* (2.1)	3.1* (1.6)	2.9* (1.4)	2.3 (1.0)
Volume (ml)								
ECIG-Experienced	175.7*+ (149.7)	181.0*+# (139.6)	127.0+ (80.8)	123.3 (168.1)	206.0*+ (202.9)	217.8*+ (177.3)	137.4 (87.6)	123.7 (150.0)
ECIG-Naive	100.0*# (64.8)	101.5*# (66.6)	86.5*# (59.4)	68.3 (64.1)	120.7* (76.6)	114.1* (74.1)	97.9* (67.9)	69.8 (63.9)
Flow Rate (ml/s)								
ECIG-Experienced	31.1 (24.9)	29.8 (16.7)	24.2 (11.0)	26.6 (21.3)	30.9 (20.6)	31.1 (18.2)	23.8+ (10.8)	27.4 (21.1)
ECIG-Naïve	32.5 (21.7)	34.1 (18.9)	29.8 (17.9)	31.2 (28.8)	36.4 (22.7)	36.7* (20.0)	34.6 (22.6)	30.7 (25.4)
Puff Number								
ECIG-Experienced	10.1 (0.2)	10.1 (0.4)	10.2 (0.4)	10.1 (0.3)	10.0 (0.3)	10.0 (0.2)	10.0 (0.3)	10.0 (0.2)
ECIG-Naïve	10.0 (0.2)	10.1 (0.2)	10.0 (0.0)	10.1 (0.3)	10.1 (0.3)	10.0 (0.3)	10.0 (0.2)	10.0 (0.2)
IPI (s)								
ECIG-Experienced	30.0 (0.5)	30.0 (1.2)	29.8 (1.2)	29.8 (0.8)	30.1 (1.1)	30.3 (0.7)	30.1 (1.1)	30.0 (1.0)
ECIG-Naïve	30.0 (1.1)	30.0 (0.7)	30.4 (2.7)	29.7 (1.4)	30.0 (0.9)	30.0 (1.3)	30.1 (0.6)	30.1 (0.8)

Mean (SD) puff parameters for ECIG-experienced (N = 33) and ECIG-naïve individuals (N = 31) for bouts 1 and 2. Note that puff number (10) and IPI (30 s) were controlled experimentally (see method). Asterisks (*) indicate significant differences from the 36 mg/ml condition at that bout and pound symbols (#) indicate across bout differences for that condition and group (Tukeys HSD; *p*s < .05). Plus signs (+) indicate significant differences between ECIG-experienced and -naïve individuals (using independent-samples t-tests; *p*s < .05).

Puff Volume. For puff volume, in bout 1, ECIG-experienced individuals took significantly larger puffs in the 0 relative to 18 and 36 mg/ml conditions. Also, larger puffs were taken in the 8 relative to 18 mg/ml and 36 mg/ml conditions. The same pattern was observed for bout 2. Across bouts, ECIG-experienced individuals took significantly larger puffs in bout 2 relative to bout 1 only in the 8 mg/ml condition.

For puff volume, during bout 1, ECIG-naïve individuals took significantly larger puffs in the 0 mg/ml condition relative to 18 and 36 mg/ml condition, in the 8 relative to 18 and 36 mg/ml conditions, and in the 18 mg/ml condition relative to the 36 mg/ml condition. The same pattern was observed during bout 2. Generally, larger puffs were taken when using lower liquid nicotine concentrations in both ECIG-use bouts. Also, ECIG-naïve individuals took significantly larger puffs during bout 2 relative to bout 1, in the 0, 8, and 18 mg/ml conditions but not in the 36 mg/ml condition.

Across groups, for puff volume, planned contrasts revealed significant between group differences during bout 1 in the 0, 8, and 18 mg/ml condition, such that ECIG-experienced individuals took larger puffs relative to ECIG-naïve individuals [*t*s (62) > 2.2, *p*s < .05]. For bout 2, significant between group differences for puff volume were observed in the 0 and 8 mg/ml conditions such that ECIG-experienced individuals took significantly larger puffs relative to -naïve individuals [*t*s (62) > 3.1, *p*s < .05].

Flow Rate. Among ECIG-experienced individuals, no significant differences in flow rate were observed for any condition or across ECIG-use bouts. For ECIG-naïve individuals, no significant differences in flow rate were observed for bout 1; however, for bout 2, significantly greater flow rate was observed in the 8 relative to the 36 mg/ml condition. No differences for flow rate were observed across bouts for ECIG-naïve individuals. Across groups, for flow rate,

planned contrasts revealed significant between group differences in the 18 mg/ml condition for bout 2 with ECIG-naïve individuals having greater flow rate, relative to ECIG-experienced individuals [t (62) = -2.4, p < .05]. No between group differences for flow rate were observed for any other condition or bout.

Puff Number. Across groups, for puff number, planned contrasts revealed no significant between group differences for any condition or ECIG-use bout.

IPI. Across groups, for IPI, planned contrasts revealed no significant between group differences for any condition or ECIG-use bout.

Puff Topography and the Effect of Gender. To explore whether gender influenced puff topography variables within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with gender as the between-subject factor) were conducted for puff topography. For ECIG-experienced individuals no significant main effects or interactions involving gender were observed. For ECIG-naïve individuals a significant condition by gender interaction [F (3, 87) = 3.3, p <.05] was observed for flow rate but no other significant interactions involving gender were observed for any other puff topography variables. For puff duration a significant main effect of gender [F (1, 29) = 9.1, p <.01] was observed. For puff volume, a significant main effect of gender [F (1, 29) = 10.7, p <.01] was observed. No other significant main effects of gender were observed for any other topography variables.

Mean (SD) puffing parameters for ECIG-naïve individuals are displayed in Table 6. For ECIG-naïve individuals, significant gender differences were observed for puff duration for bout 1 and 2 in every condition [ts (29) < -2.2, ps < .05]. Overall, men took significantly longer puffs relative to women during bout 1 and 2 in every condition.

Table 6.
Mean (SD) Puff Topography by Liquid Nicotine Concentration for ECIG- naïve Individuals by Gender for Bouts 1 and 2

	Liquid Nicotine Concentration (mg/ml)							
	Bout 1				**Bout 2**			
	0	**8**	**18**	**36**	**0**	**8**	**18**	**36**
Puff Duration (s)								
Men	3.9 + (1.7)	3.6 + (1.4)	3.4 + (1.3)	2.5 + (0.8)	4.3 + (2.4)	3.8 + (1.6)	3.8 + (1.4)	2.8 + (0.9)
Women	2.6 (1.3)	2.1 (1.2)	2.0 (0.9)	1.8 (0.5)	2.7 (1.4)	2.3 (1.1)	2.1 (0.9)	1.8 (0.7)
Volume (ml)								
Men	106.7 (57.1)	130.0 + (66.0)	111.6 + (60.3)	87.9 + (76.6)	133.2 (79.2)	146.2 + (73.6)	127.4 + (67.3)	89.3 + (75.9)
Women	90.9 (75.6)	62.6 (45.7)	51.9 (37.7)	41.2 (24.3)	103.3 (72.3)	69.6 (48.6)	57.1 (44.8)	42.6 (25.8)
Flow Rate (ml/s)								
Men	28.3 (10.5)	37.1 (19.0)	36.0 + (19.5)	37.0 (35.5)	31.7 (12.9)	40.1 (20.3)	39.1 (21.1)	35.1 (30.7)
Women	38.4 (30.9)	30.0 (18.9)	21.3 (11.2)	23.2 (12.8)	42.9 (31.2)	32.0 (19.5)	28.4 (24.0)	24.6 (14.6)
Puff Number								
Men	10.0 (0.0)	10.0 (0.2)	10.0 (0.0)	10.2 (0.4)	10.0 (0.2)	10.0 (0.2)	10.0 (0.0)	10.0 (0.0)
Women	10.1 (0.3)	10.1 (0.3)	10.0 (0.0)	10.0 (0.0)	10.0 (0.0)	10.0 (0.0)	10.1 (0.3)	10.0 (0.0)
IPI (s)								
Men	30.0 (0.4)	30.0 (1.2)	30.1 (1.2)	29.7 (0.5)	30.1 (0.9)	30.3 (0.6)	30.1 (1.1)	30.0 (1.0)
Women	30.0 (1.1)	30.0 (0.7)	30.0 (2.7)	29.8 (1.4)	30.0 (0.9)	30.0 (1.3)	30.1 (0.6)	30.1 (0.8)

Mean (SD) puff parameters for ECIG-naïve men (N = 18) and women (N = 15) for bouts 1 and 2. Note, IPI (30 s) and puff number (10) were controlled experimentally (see method) and data are included here to demonstrate that control. Plus signs (+) indicate significant differences between men and women (using independent-samples t-tests; *p*s < .05).

For puff volume, significant gender differences were observed for bouts 1 and 2 in the 8, 18, and 36 mg/ml conditions, such that men took significantly larger puffs relative to women in all conditions except 0 mg/ml [*t*s (29) < -2.1, *p*s < .05]. Also, significant between group differences were observed for flow rate in the 18 mg/ml condition in bout 1 such that men took significantly faster puffs relative to women in the 18 mg/ml condition only [*t*s (29) < -1.2, *p*s < .05]. No other significant between group differences were observed for gender.

Puff Topography and the Effect of Flavor. To explore whether flavor (menthol or tobacco) influenced puff topography variables within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with flavor as the between-subject factor) were conducted for puff topography. For ECIG-experienced and -naive individuals, no significant interactions or main effects involving flavor were observed.

Heart Rate

Heart Rate and the Effect of Abstinence Status. To evaluate the effects of abstinence status on HR, mixed ANOVAs with abstinence as the between-subject factor were conducted for each group (ECIG-experienced and -naïve). For ECIG-experienced individuals, a significant three way condition by time by abstinence status interaction was observed [F (6, 186) = 38.1, p <.01]. Also, significant condition by time [F (6, 186) = 11.5, p <.001] and significant time by abstinence status interactions [F (2, 62) = 4.6, p <.05] were observed. Among ECIG-experienced individuals, relative to baseline, abstinent individuals exhibited significant increases in HR during bout 1 and 2 in all active liquid nicotine conditions (8, 18, and 36 mg/ml) but not in placebo (0 mg/ml). Non-abstinent individuals had significant increases in HR during bout 1 in all active nicotine conditions but not 0 mg/ml. Non-abstinent individuals did not have significant HR increases during bout 2 in any condition. Among ECIG-naïve individuals, a significant

condition by time interaction was observed [F (6,174) = 4.8, p <.001] but no interactions or main effects involving abstinence status were observed.

To explore whether abstinence status influenced HR differences observed during ECIG use, or whether they occurred due to baseline differences (as seen above with plasma nicotine concentration), the same analyses (i.e., mixed ANOVAs) were conducted using HR difference scores (i.e., change scores from baseline for each individual in each condition). When using difference scores for HR, the interactions and main effects involving abstinence status, reported above, remained significant. Because abstinence status appears to influence HR despite the correction for baseline differences, abstinence status is believed to have influenced HR during the course of this study. Thus, final analyses were conducted using only abstinent participants and using raw HR data (rather than difference scores). For ECIG-naïve individuals, abstinence status did not appear to influence HR, however, in order to maintain consistency across groups, HR data for ECIG-naïve individuals was analyzed using only abstinent participants.

Heart Rate Analyses Using Raw Data for Abstinent ECIG-Experienced and -Naïve Individuals. Among ECIG-experienced individuals, HR differs as a function of abstinence status despite correction for baseline differences in HR. As such, raw HR data were analyzed using only data from those participants that had abstained in both groups: ECIG-experienced (N = 18) and -naïve (N = 21). Using raw HR data, a significant condition by time interaction was observed [F (6, 222) = 17.8, p < .001]. Also, a significant time by group interaction [F (2, 74) = 5.3, p < .05] was observed. Significant main effects of condition [F (3, 111) = 3.1, p < .05] and time [F (2, 74) = 73.2, p < .001] were observed. Figure 5 depicts mean HR, at baseline, during bout 1 and bout 2, by condition, for abstinent ECIG-experienced and -naïve individuals.

For ECIG-experienced individuals during bout 1, mean (SD) HR increased significantly from baseline in the 8 (M = 72.9; SD = 7.7), 18 (M = 77.7; SD = 8.1), and 36 (M = 77.4; SD = 9.2) mg/ml conditions, but not in the 0 mg/ml condition (Tukey's HSD, *p*s < .05; see Figure 5). During bout 2, mean (SD) HR increased significantly from baseline in the 8 (M = 69.5; SD = 8.1), 18 (M = 73.3; SD = 10.0), and 36 (M = 73.0; SD = 10.6) mg/ml conditions but not in the 0 mg/ml condition.

For ECIG-naïve individuals during bout 1, mean (SD) HR increased significantly from baseline in the 8 (M = 73.0; SD = 7.3), 18 (M = 74.3; SD = 7.6), and 36 (M = 76.7; SD = 8.2) mg/ml conditions, but not in the 0 mg/ml condition (Tukey's HSD, $p < .05$; see Figure 5). During bout 2, mean (SD) HR increased significantly in the 18 mg/ml (M = 70.0; SD = 7.2) and the 36mg/ml (M = 72.4; SD = 8.4) conditions, but not in the 0 or 8 mg/ml conditions. Across groups, for HR, planned contrasts revealed no significant between group (ECIG-experienced versus -naïve) differences for any condition or ECIG-use bout.

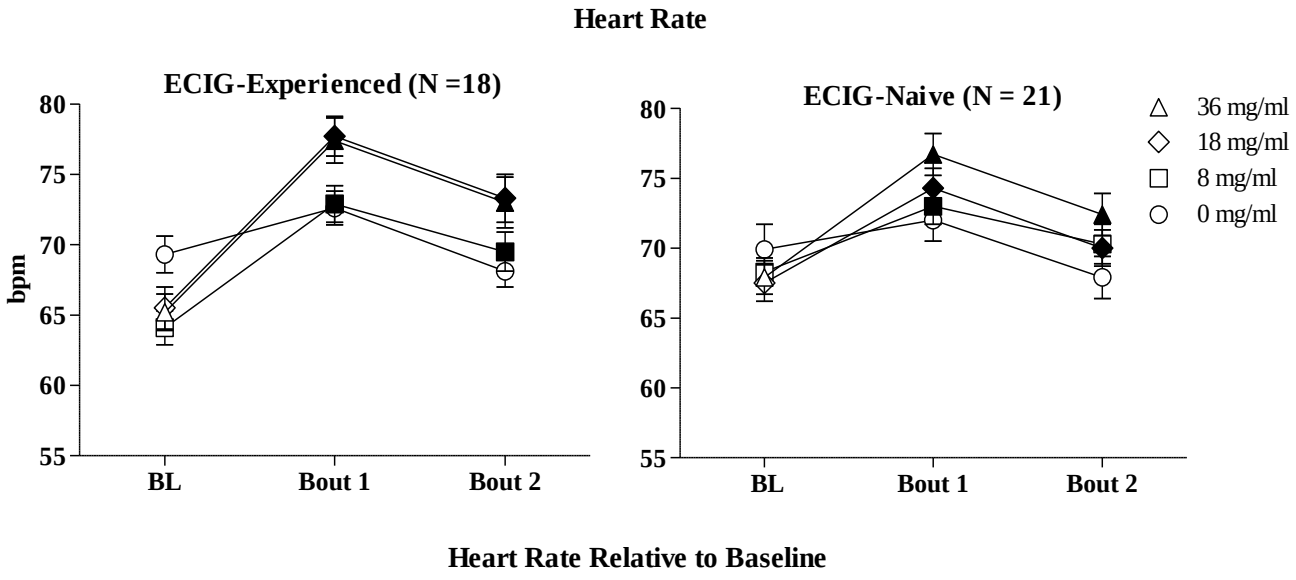


Figure 5. Mean (± SEM) for HR across conditions for abstinent ECIG-experienced (N = 18) and -naïve (N = 21) individuals. Prior to analysis, HR data were averaged to produce a single value for baseline and the five minutes during each of the two ECIG-use bouts (i.e., bout 1 and bout 2). Filled symbols indicate a significant difference from baseline; no significant between group differences were observed.

Heart Rate and the Effect of Gender. To explore whether gender influenced HR within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with gender as the between-subject factor) were conducted using raw data and only abstinent individuals. For ECIG-experienced individuals, a significant condition by time interaction was observed [F (6, 96) = 11.8, p <.01] but no significant main effects or interactions involving gender were observed. For ECIG-naïve individuals a significant condition by time interaction [F (6, 114) = 7.2, p <.01] and a main effect of gender [F (1, 19) = 5.9, p <.05] were observed.

For ECIG-naïve individuals, across gender, planned contrasts revealed significant between group differences in HR in the 36 mg/ml condition at baseline and during bout 1 [ts (19) < -2.3, ps < .05]. Mean (SD) HR was significantly higher for women at baseline (M = 72.5; SD = 5.0), during bout 1 (M = 81.7; SD = 7.1) and during bout 2 (M = 76.5; SD = 8.3) relative to men who had significantly lower HR at baseline (M = 63.6; SD = 5.5), during bout 1 (M = 72.1; SD = 6.2) and bout 2 (M = 68.7; SD = 6.9).

Heart Rate Evaluation of Flavor. To explore whether flavor influenced HR within each group (ECIG-experienced or -naïve) the same analyses (i.e., mixed ANOVAs with flavor as the between-subject factor) were conducted using HR data and abstinent individuals only. Significant condition by time interactions were observed for ECIG-experienced individuals [F (6, 96) = 11.5, p <.01] and for ECIG-naïve individuals [F (6, 114) = 7.2, p <.001] but no significant interactions or significant main effects involving flavor were observed for either group. Given that the between-subject variable of interest (flavor) was not involved in any significant interactions or main effects further post hoc testing was not conducted.

Discussion

Overview

ECIGs are a class of products that, until recently, were unregulated in the U.S. ECIG device features, liquid characteristics, and user behavior often vary considerably, making the understanding of the acute effects of ECIGs difficult. Until this report, no published studies have evaluated systematically the effects of various liquid nicotine concentrations, user experience, and puff topography on ECIG-associated nicotine delivery.

Results from this study, in which liquid nicotine concentration and user experience were varied while other factors (e.g., battery voltage, heater resistance, liquid PG:VG ratio) were held constant, indicate that liquid nicotine concentration influences nicotine delivery (as indexed by plasma nicotine concentration), that ECIG nicotine delivery is physiologically active, and that the amount of nicotine delivered depends upon user puff topography. Specifically, ECIG-experienced individuals obtained higher mean nicotine boost relative to ECIG-naïve individuals and this difference may be due to the longer and larger puffs taken by ECIG-experienced individuals. Also, puff topography differed based on liquid nicotine concentration such that longer and larger puffs are taken in the lower nicotine concentrations relative to the higher liquid nicotine concentrations. In addition to these results, the study also reveals that some participants likely did not comply with protocol-mandated nicotine abstinence, and this non-compliance has important implications for future clinical laboratory research addressing ECIG effects. Taken together, results from this study are important for ECIG regulation because they reveal how ECIG nicotine delivery might be controlled.

ECIG Nicotine Delivery

ECIG nicotine delivery is related directly to liquid nicotine concentration (when all other factors are controlled), is physiologically active, and depends upon user puff topography. The relationship between ECIG liquid nicotine concentration and user nicotine boost is illustrated in Figure 3 which demonstrates an increase in mean nicotine boost with increase of liquid nicotine concentration for each of the 2 ECIG-use bouts in ECIG-experienced and -naïve individuals. While between group differences in nicotine boost are apparent (as seen in Figures 3 and 4), the direct relationship between liquid nicotine concentration and plasma nicotine concentration can be more clearly seen when collapsed across group. Indeed, when the mean peak nicotine boost data are collapsed across groups, the effect of liquid nicotine concentration is clear: after bout 1, collapsed across group, a mean (SD) peak nicotine boost of 0.9 ng/ml (1.7) was observed in the 0 mg/ml condition, a mean nicotine boost of 6.3 ng/ml (4.8) was observed in the 8 mg/ml condition, a mean nicotine boost of 10.6 ng/ml (11.6) was observed in the 18 mg/ml condition, and a mean nicotine boost of 14.0 ng/ml (14.2) was observed in the 36 mg/ml condition. Immediately following bout 2, collapsed across group, a mean (SD) peak nicotine boost of 0.6 ng/ml (3.3) was observed in the 0 mg/ml condition, a mean peak nicotine boost of 6.8 ng/ml (7.0) was observed in the 8 mg/ml condition, a mean peak nicotine boost of 9.7 ng/ml (11.4) was observed in the 18 mg/ml condition, and a mean peak nicotine boost of 13.0 ng/ml (11.8) was observed in the 36 mg/ml condition. Thus, for both bouts and collapsed across groups, mean peak nicotine boost increased with the increase in liquid nicotine concentration using the experimental methods described here.

Results from this study indicate that the ECIG-nicotine delivery was physiologically active, as indexed by heart rate increases that were observed where nicotine was delivered

reliably (as indexed by plasma nicotine boost). To highlight this point, mean peak nicotine boost and mean HR data from abstinent ECIG-experienced individuals only, are shown together in Figure 6. Immediately after bout 1, abstinent ECIG-experienced individuals obtained mean (SD) peak nicotine boost of 0.0 ng/ml (0.2) in the 0 mg/ml condition, 6.8 ng/ml (6.2) in the 8 mg/ml condition, 11.1 ng/ml (8.3) in the 18 mg/ml condition, and 14.8 ng/ml (14.8) in the 36 mg/ml condition. A similar trend was observed in HR during bout 1 such that abstinent ECIG-experienced individuals mean (SD) HR was 72.6 beats/minute (1.2) in the 0 mg/ml condition, 72.9 beats/minute (1.3) in the 8 mg/ml condition, 77.7 (1.4) beats/minute in the 18 mg/ml condition, and 77.4 beats/minute (1.6) in the 36 mg/ml condition. Mean peak nicotine boost was significantly higher in the 8, 18, and 36 mg/ml conditions relative to 0 mg/ml (Tukey's HSD, $p < .05$). HR increases during bout 1 were significantly higher in the 18 and 36 mg/ml conditions relative to 0 mg/ml, but not in the 8 mg/ml condition. Overall, results of the present study demonstrated that ECIG-experienced and -naïve individuals were exposed to physiologically active nicotine concentrations (as indexed by observed increases in heart rate) immediately following product administration, especially at higher liquid nicotine concentrations.

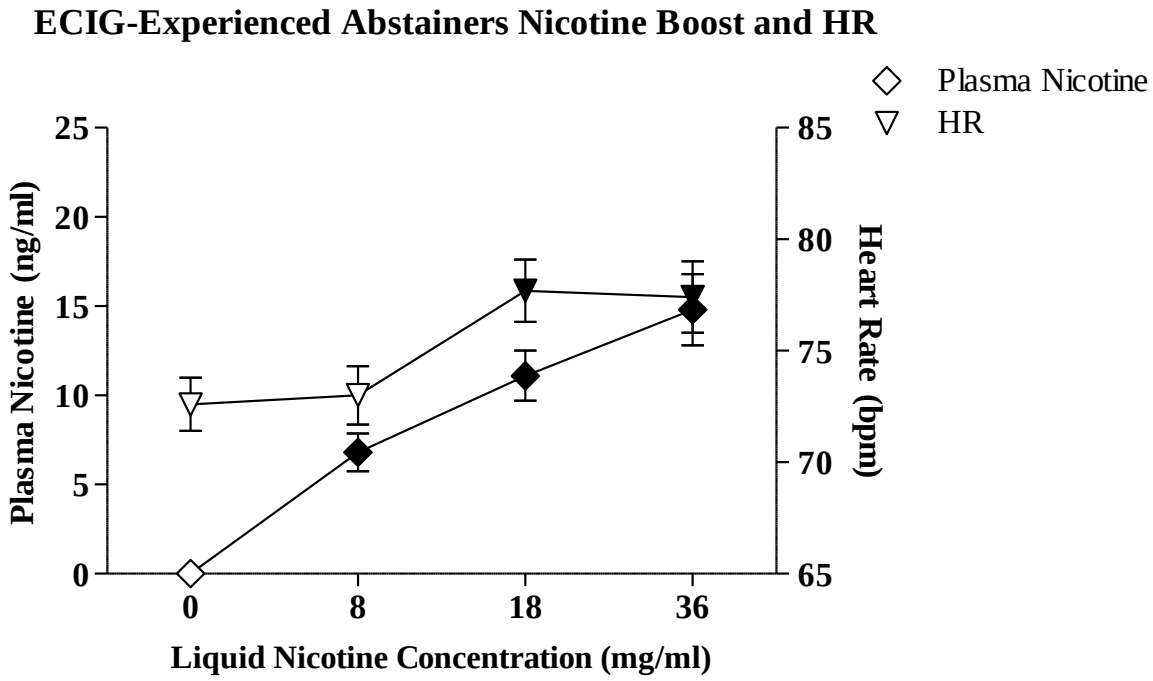


Figure 6. Mean (± SEM) peak nicotine boost and HR across conditions (liquid nicotine concentration) for abstinent ECIG-experienced individuals (N = 18). Right y-axis depicts HR during bout 1 and left y-axis depicts nicotine boost immediately following bout 1. Filled symbols indicate significant difference from 0 mg/ml at that liquid nicotine concentration (Tukey's HSD; *p*s < .05).

Similar to previous reports (Farsalinos et al., 2015) ECIG user nicotine delivery differed significantly across ECIG-experienced and -naïve individuals in this study. Despite controlling for several characteristics that may influence nicotine delivery (e.g., battery voltage, liquid PG:VG ratio, puff number) ECIG-experienced individuals obtained higher nicotine boost relative to ECIG-naïve individuals in all active nicotine conditions (see Figure 3 and Figure 4). The variability in ECIG-associated nicotine delivery across ECIG-experienced and -naïve individuals may be explained by differences in user puff topography. Analytical laboratory studies have demonstrated how differences in puff topography may influence ECIG-associated nicotine yield. For example, puff duration influences nicotine yield such that longer duration puffs result in greater nicotine yield (Talih et al., 2015). In fact, ECIG-experienced individuals take longer puffs (approximately 4 s, on average) while ECIG-naïve individuals, who are also tobacco cigarette smokers, take shorter puffs (approximately 2 s, on average; Hua et al., 2013; Farsalinos et al., 2013). Consistent with these data, the present study demonstrated that ECIG-experienced and -naïve individuals differ significantly on several puffing parameters. As displayed in Table 5, ECIG-experienced individuals took significantly longer, larger puffs relative to ECIG-naïve individuals. Thus, the significantly higher mean nicotine boost observed in ECIG-experienced individuals may be explained by the differences in puff topography across groups. For example, in the 18 mg/ml condition, immediately following bout 1, in ECIG-experienced individuals, a mean (SD) peak nicotine boost of 13.0 ng/ml (13.2) was observed while, in ECIG-naïve individuals a mean peak nicotine boost of 6.2 ng/ml (10.2) was observed. In the 18 mg/ml condition, during bout 1, ECIG-experienced individuals took mean (SD) puffs of 5.0 s (1.9) duration and ECIG-naïve individuals took mean puffs of 2.8 s (1.3) duration. Similar between group differences in mean puff duration and mean peak nicotine boost were seen across all active

liquid nicotine concentrations. As such, results from the present study suggest strongly that ECIG-nicotine delivery is related directly to user puff topography when other relevant factors are held constant.

The present study also demonstrated that ECIG user puff topography differs based on the liquid nicotine concentration used (see Table 5). For example, regardless of group (ECIG-experienced or -naïve) longer and larger puffs were taken in the lower nicotine concentrations relative to the higher liquid nicotine concentrations. During bout 1, collapsed across group, mean (SD) puff duration was 4.7 s (2.4) in the 0 mg/ml condition, 4.4 s (4.0) in the 8 mg/ml condition, 4.0 s (3.7) in the 18 mg/ml condition, and 3.5 s (2.8) in the 36 mg/ml condition. For puff volume, during bout 1, collapsed across group, mean (SD) puff volume was 140.1 ml/s (121.8) in the 0 mg/ml condition, 142.5 ml/s (116.7) in the 8 mg/ml condition, 107.4 ml/s (73.5) in the 18 mg/ml condition, and 96.7 ml/s (130.7) in the 36 mg/ml condition. These findings suggest that regardless of experience with ECIGs, puffing behavior may differ depending on liquid nicotine concentration.

The nicotine delivery findings of the present study have several implications. First, this is the first report to demonstrate systematically a direct relationship between liquid nicotine concentration and plasma nicotine concentration. As such, liquid nicotine concentration should be considered one of the several factors that influence ECIG-related nicotine delivery. Second, the mean nicotine boost observed in participants in this study were accompanied by increases in HR suggesting that the nicotine delivered from an ECIG is physiologically active, and the physiological activity of this psychomotor stimulant drug may contribute to maintenance or initiation of nicotine dependence with continued use. Third, the between group differences in puff topography observed in this study are consistent with previous reports (Farsalinos et al.,

2015; Spindle et al., 2015) and may explain the observed differences in nicotine delivery between ECIG-experienced and -naïve individuals. Understanding puff topography differences between ECIG-experienced and -naïve individuals may help in understanding why previous reports of ECIG-related nicotine delivery varied so widely. For example, early evaluations of ECIG-associated nicotine delivery demonstrated the following: after 10 puffs, ECIG-naïve cigarette smokers were unable to obtain measureable amounts of nicotine from an ECIG (Vansickel et al., 2010), after 10 puffs of a tank-based ECIG, ECIG-experienced individuals were able to obtain some nicotine (M = 6.6 ng/ml; Farsalinos et al., 2014), and that when using their own device/liquid *ad libitum*, some ECIG-experienced individuals obtained plasma nicotine concentrations that exceeded those observed in tobacco cigarette smokers (M = 35 ng/ml; Spindle, 2015). The variability in ECIG nicotine delivery across these reports may be explained by the differences in ECIG experience of the population sampled (i.e., ECIG-naïve in Vansickel et al., 2010; ECIG-experienced in Spindle et al., 2015) and the related differences in puff topography of those populations. However, the variability in the devices and liquids used in previous studies makes the examination of the specific factors that may influence nicotine delivery (i.e., device characteristics, liquid characteristics, puff topography) difficult. Overall, generalizations regarding ECIG nicotine delivery across devices that vary in power and other design features may be challenging, especially if puff topography and liquid nicotine concentration are not taken into account.

Finally, ECIG-experienced and -naïve participants in this study may have altered the duration and volume of their puffs when using certain liquid nicotine concentrations and this behavioral alteration may be a result of two different mechanisms. First, perhaps the larger and longer puffs observed in the lower liquid nicotine concentrations relative to the higher liquid

nicotine concentrations may be an attempt, by users, to obtain higher nicotine levels by adjusting puff topography. Second, the higher liquid nicotine concentrations may be perceived as more "harsh" and thus more difficult to inhale. However, further examinations are required in order to understand the mechanism behind the larger and longer puffs observed at lower liquid nicotine concentrations.

Puff Topography

In the present study, mean ECIG nicotine boost varied significantly across ECIG-experienced and -naïve individuals, perhaps due to the longer and larger puffs taken by ECIG-experienced individuals. Consistent with the findings of the present study, previous reports indicate that when using an ECIG, ECIG-experienced individuals take longer duration puffs (approximately 4 s, on average) while ECIG-naïve individuals, who are also tobacco cigarette smokers, take shorter puffs (approximately 2 s, on average; Hua et al., 2013; Farsalinos et al., 2013). Taken together, the between group differences observed in nicotine boost and puff topography indicate that the ability to obtain nicotine from an ECIG may be a learned behavior that requires practice. One possible explanation for the differences observed in puffing behavior between ECIG-experienced and -naïve individuals is that ECIG-experienced individuals, over time, have learned that longer duration puffs result in greater nicotine delivery. Indeed, data from analytical laboratory studies corroborate that longer-duration puffs result in greater nicotine yield (Talih et al., 2015). Specifically, during a longer duration puff, the heater coil of an ECIG is activated for a longer period of time resulting in a larger proportion of the puffing time spent in a higher-temperature phase (Talih et al., 2015). When puffing occurs in a higher temperature phase the result is higher nicotine evaporation and greater nicotine yield. Thus, longer duration puffs lead to greater nicotine yield and potentially greater nicotine delivery to the user. Perhaps, over

time, those ECIG users that took longer duration puffs from an ECIG were reinforced by subsequent nicotine boost that may have resulted in even longer duration puffs in the future. ECIG-naïve tobacco cigarette smokers are one group of individuals who may be motivated to switch completely from combustible cigarettes to ECIGs and these cigarette smokers may seek an ECIG nicotine delivery profile comparable to a tobacco cigarette (e.g., nicotine boost of 16.8 ng/ml immediately after the 10^{th} puff of a 10-puff bout; Vansickel et al., 2010). For those ECIG-naïve smokers attempting to obtain cigarette-like nicotine boost from an ECIG, altering their puffing behavior in a manner that is associated with higher nicotine delivery (i.e., longer duration puffs) may assist in achieving higher nicotine delivery. As such, if ECIGs are to be used in place of tobacco cigarettes, and if that replacement depends upon the ECIG matching the nicotine delivery of a tobacco cigarette, then there is a potential need for more detailed instructions regarding proper puffing techniques when using ECIGs in order for ECIG-naïve cigarette smokers to obtain cigarette-like nicotine boosts from an ECIG from their very first 10 puffs.

To date, one study has demonstrated that ECIG-naïve cigarette smokers appear to adjust their puffing parameters during the first week of a two-week ECIG use period (Lee, Gawron, & Goniewicz, 2015). Specifically, ECIG-naïve cigarette smokers modified their puffing behavior after switching from tobacco cigarettes to ECIGs by taking longer and slower puffs. For example, during baseline ECIG use, mean (SEM) puff duration of smokers was 2.2 s (0.1), after one week puff duration was 3.1 s (0.3), and after two weeks puff duration was 2.9 s (0.2; Lee et al., 2015). Puff flow rate also changed from baseline with participants flow rate decreasing from 30.6 ml/s (2.3) to 25.1 (1.8) ml/s after one week, down to 24.8 ml/s (1.9) after week two. The observed changes in puffing behavior may be evidence that perhaps engaging in certain puffing behaviors is more rewarding, such as when more nicotine is delivered to the user when taking

certain puffs. Changes in puffing behavior after a two week ECIG use period indicate that after two weeks of *ad libitum* ECIG use, ECIG-naïve tobacco cigarette smokers alter their puffing behavior significantly such that puff durations are longer. These results support the idea that, over time, ECIG-naïve individuals may learn how to puff an ECIG in a manner that is consistent with increases nicotine yield and nicotine delivery (i.e., longer duration puffs; Talih et al., 2015).

In sum, the variability in mean nicotine boost among ECIG-experienced and -naïve individuals may be explained by differences in puff topography. In general, ECIG-experienced individuals take longer and larger puffs relative to ECIG-naïve individuals, which may explain their ability to obtain more nicotine from an ECIG. Perhaps ECIG-experienced individuals have learned, over time that longer duration puffs result in greater nicotine delivery. Perhaps the puffing behavior required to obtain nicotine from an ECIG is a learned behavior that requires practice. Thus, for ECIG-naïve tobacco cigarette smokers looking to achieve cigarette-like nicotine delivery from an ECIG (e.g., nicotine boost of 16.8 ng/ml; Vansickel et al., 2010), altering puffing behavior in a manner that is consistent with higher nicotine yield and delivery (i.e., longer duration puffs; Talih et al., 2015) may be one way to increase ECIG-associated nicotine delivery.

ECIG Nicotine Delivery Profile Can Exceed That of Combustible Cigarettes

As in previous reports (e.g., Spindle et al., 2016), several ECIG-experienced individuals across each of the active liquid nicotine concentrations in the present study were able to obtain a nicotine boost that exceeds what is typically observed in tobacco cigarette smokers (e.g.,16.8 ng/ml mean nicotine boost; Vansickel et al., 2010) under similar puffing conditions (i.e., 10 puffs; 30 s IPI). Specifically, following the first 10-puff ECIG use bout, three ECIG-experienced individuals exceeded a cigarette-like nicotine boost when using the 8 mg/ml liquid (M = 25.2

ng/ml; SD = 10.1), nine exceeded cigarette-like nicotine boost when using the 18 mg/ml liquid (M = 30.3 ng/ml; SD = 11.1) and 13 exceeded cigarette-like boost when using the 36 mg/ml liquid (M = 35.5 ng/ml; SD = 14.7). Conversely, 30 obtained below cigarette-like nicotine boost when using the 8 mg/ml liquid (M = 6.5 ng/ml; SD = 5.3), 24 obtained below cigarette-like nicotine boost when using the 18 mg/ml liquid (M = 6.4 ng/ml; SD = 6.0), and 20 obtained below cigarette-like nicotine boost when using the 36 mg/ml liquid (M = 6.6 ng/ml; SD = 4.7).

Among ECIG-naïve individuals, three obtained cigarette-like nicotine boost when using the 18 mg/ml liquid (M = 32.2 ng/ml; SD = 14.3) and three obtained cigarette-like boost when using the 36 mg/ml liquid (M = 22.3 ng/ml; SD = 3.7). Conversely, 31 obtained below cigarette-like nicotine boost when using 0 mg/ml liquid (M = 0.0 ng/ml; SD = 1.5), 31 obtained below cigarette-like nicotine boost when using the 8 mg/ml liquid (M = 3.6 ng/ml; SD = 3.8), 28 obtained below cigarette-like nicotine boost when using the 18 mg/ml liquid (M = 3.4 ng/ml; SD = 4.6), and 28 obtained below cigarette-like nicotine boost when using the 36 mg/ml liquid (M = 5.2 ng/ml; SD = 5.1).

These findings have several important implications. First, under certain conditions, ECIG-experienced and -naïve individuals are able to achieve and sometimes exceed the nicotine boost observed after 10 puffs of a tobacco cigarette (i.e., 16.8 ng/ml; Vansickel et al., 2010). Second, as seen in previous studies (Dawkins & Corcoran, 2013; Farsalinos et al., 2014; Spindle et al., 2015; Vansickel & Eissenberg, 2013), nicotine delivery varied considerably among ECIG-experienced individuals in this study. For example, some ECIG-experienced individuals, in the present study, were able to achieve much higher nicotine boost relative to the nicotine boost seen after 10 puffs of a tobacco cigarette (e.g., 66.18 ng/ml after 10 puffs of a 3.3 V “eGo” battery using 36 mg/ml liquid nicotine concentration), while others only were able to obtain minimal

nicotine boost. Given that several factors such as device characteristics (i.e., battery voltage, heater resistance) and liquid characteristics (i.e., liquid solvents) were held constant in this study, they likely did not contribute to the variability in ECIG-associated nicotine delivery. Instead, one possible explanation for the variability in nicotine delivery is individual variability in puff topography. As such, puff topography should be considered a highly important variable in ECIG-associated nicotine delivery.

Measurement of Abstinence

The present study also reveals that some participants likely did not comply with protocol-mandated nicotine abstinence, and this non-compliance has important implications for future clinical laboratory research addressing the acute effects of ECIGs and other non-combustible tobacco products. As in previous studies of tobacco and nicotine containing products (Dawkins et al., 2013; Spindle et al., 2015; Vansickel et al., 2010; Kotlyar, et al., 2007; Perkins, Grobe, Weiss, Fonte, & Caggiula, 1996), ≥ 12 hours nicotine/ tobacco abstinence was required prior to each laboratory session for ECIG-experienced individuals and ECIG-naïve smokers. Nicotine/tobacco abstinence is required to assess nicotine delivery and the examination of abstinence symptom suppression associated with using nicotine/tobacco (e.g., Dawkins et al., 2016; Spindle et al., 2015; Dawkins & Corcoran, 2014; Kotlyar, et al., 2007; Perkins et al., 1996).Consistent with previous reports, in this study, short-term abstinence from combustible products, such as tobacco cigarettes, was evaluated with a test of expired air CO concentration. However, under normal conditions, ECIGs are not combustible and do not produce CO. Therefore, ECIG-experienced individuals had abstinence verified retrospectively (using a criterion of plasma nicotine concentration < 5 ng/ml; as in Spindle et al., 2016) and ultimately, 18 of the 33 ECIG-experienced individuals and 21 of the 31 ECIG-naïve tobacco cigarette smokers were considered

to have abstained from nicotine prior to each of the four sessions. Thus, this study highlights a challenge with studying nicotine/tobacco use when short-term abstinence from non-combustible tobacco products (i.e., ECIGs) cannot be verified immediately (i.e., prior to the start of the study session). Given that some outcome measures such as HR in this study and, potentially, subjective effect measures of nicotine/tobacco abstinence may be affected by nicotine abstinence, measuring short-term ECIG abstinence will continue to be challenging in future research until an immediate, reliable, and cost-effective method for verifying abstinence from non-combustible tobacco products is discovered.

Among those in the study who were ECIG-experienced, almost half were considered to be non-abstinent during the study. Non-abstinence among study participants who were required to abstain is informative for several reasons. First, perhaps ECIG-experienced individuals are aware that ECIGs do not operate via combustion and that CO measurement is not a reliable measure for ECIG abstinence. If this speculation is correct, measurement of ECIG abstinence increasingly may be problematic in the clinical laboratory. Second, another possible explanation for failure to comply with protocol-required nicotine/tobacco abstinence was that participants, including non-smoking, ECIG-experienced individuals, experienced difficulty when trying to abstain. This difficulty abstaining is reminiscent of the difficulty cigarette smokers report when attempting to abstain from nicotine/tobacco (e.g., Hughes & Hatsukami, 1986; Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005) and may indicate that ECIG-experienced individuals in this study were experiencing a similar aversive abstinence syndrome (e.g., Hughes & Hatsukami, 1986). Because ECIGs have been shown, under some conditions, to deliver physiologically active nicotine to the user (Spindle et al., 2015; Vansickel & Eissenberg, 2013), perhaps the same aversive syndrome that is experienced by abstinent tobacco cigarette smokers,

and that is considered a hallmark of tobacco/nicotine dependence, also may occur in ECIG users, and may also be an indicator of dependence in this population. Some reports suggest that ECIG-associated nicotine dependence may be less severe than dependence on tobacco cigarettes (Etter & Eissenberg, 2015; Foulds et al., 2014). Importantly, these reports may have been based on ECIG-experienced individuals who were using ECIGs that were less effective at delivering nicotine than those on the market today (e.g., Vansickel et al., 2012; Farsalinos et al., 2015). More research regarding ECIG-associated nicotine dependence is required to understand why some ECIG users did not abstain during the course of this study, and to what extent this failure to abstain is an indicator of nicotine dependence.

Prior to this report, several clinical laboratory studies verified tobacco/nicotine abstinence in tobacco cigarette smokers using a test of expired air CO (e.g., ≤ 10 ppm; Dawkins et al., 2016; Vansickel et al., 2010; Mendelson et al., 2008; Breland et al., 2002). However, in the present study, despite verifying tobacco abstinence (using expired air CO ≤ 10 ppm), 10 of the 31 ECIG-naïve tobacco cigarette smokers were considered to be not abstinent when plasma nicotine concentration was analyzed retrospectively (using a criterion of plasma nicotine concentration < 5 ng/ml at baseline; as in Spindle et al., 2016). There are two potential explanations for why combustible tobacco cigarette smokers appeared abstinent (as indexed by having expired air CO of ≤ 10 ppm) prior to the start of the session, but had baseline plasma nicotine concentrations that exceeded 5 ng/ml. First, with several non-combustible nicotine delivery products on the market (i.e., ECIGs, nicotine replacement therapies, smokeless tobacco products), tobacco cigarette smokers may have used a non-combustible form of nicotine delivery prior to the session in order to avoid the aversive abstinence syndrome associated with tobacco abstinence (e.g., Hughes & Hatsukami, 1986). Second, perhaps the expired CO criterion of ≤ 10 ppm may lead some

individuals who have smoked a combustible tobacco product within 12 hours to be misclassified as abstinent (Cropsey, Eldridge, Weaver, Villalobos, & Stitzer, 2006). Indeed, recent reports have demonstrated that perhaps the optimal CO criterion cut off for 24 hour tobacco cigarette abstinence is either between 3-6 ppm (Cropsey, Elridge, Weaver, Villalobos, & Stitzer, 2006) or below 5 ppm (Perkins et al., 2012). However, these cut off recommendations are made to classify between smokers and non-smokers and may be more valuable for cessation studies rather than studies requiring acute abstinence from tobacco cigarettes. Nonetheless, future studies may benefit from enforcing a stricter CO criterion cut off for abstinent tobacco cigarette smokers (e.g., < 5ppm; Perkins et al., 2012). Additionally, for non-combustible tobacco products, such as ECIGs, no immediate biochemical measures for verifying acute nicotine abstinence have been discovered. Measuring short-term nicotine abstinence will continue to be challenging in future nicotine and tobacco research until a fast, reliable, and cost-effective method for verifying abstinence from non-combustible tobacco products is discovered.

Regulatory Implications

Under the "deeming" statute of The Family Smoking Prevention and Tobacco Control Act, FDA has begun to regulate the labeling, marketing, and distribution of ECIGs. Regulation of ECIGs will require an understanding of what these products do, what ingredients they contain, and the extent to which they will have a positive or negative public health impact. Information from the present study was intended to evaluate systematically several aspects of ECIG-associated nicotine delivery (i.e., liquid and device characteristics) to inform effective ECIG regulation. Despite not having the necessary empirical information regarding ECIG-associated nicotine delivery, some countries are already attempting to regulate ECIG liquids and components.

For example, in an attempt to protect individual and public health in Europe, European Union Directive 2014/40/EU recently limited the liquid nicotine concentration of ECIG liquids to 20 mg/ml. The rationale behind this regulation was to limit the nicotine delivery from an ECIG to what is comparable when using a tobacco cigarette (i.e., approximately 16 ng/ml, on average; Vansickel et al., 2010). However, this regulation failed to account for several variables that also influence ECIG-associated nicotine delivery. Failure to consider variables other than liquid nicotine concentration (i.e., device characteristics, liquid solvents such as PG:VG, and user puff topography) can lead to regulatory decisions that fail to serve their intended purpose. Indeed, doubling device power can triple the nicotine yield when liquid nicotine concentration is held constant (Talih et al., 2015). Given that the devices used in the current study were powered at approximately 7 Watts (i.e., $3.3\text{Volts}^2/1.5$ Ohms = 7.26 Watts), and that devices that can be powered to 60 Watts are now marketed (e.g., myvaporstore.com), attempts to control nicotine yield and/or delivery by limiting liquid nicotine concentration alone are unlikely to be effective.

Results from the present study may inform regulators that ECIG nicotine delivery is directly related to liquid nicotine concentration (when all other factors are controlled). However, ECIG nicotine delivery is also dependent upon user puff topography and the present study demonstrated significant between group differences in nicotine delivery based on user experience (i.e., puff topography). The variability in ECIG-associated nicotine delivery should serve to inform regulators to be cautious about making regulatory decisions that isolate certain variables, such as liquid nicotine concentration, when several other important variables also influence ECIG nicotine delivery. For example, if regulation were to limit ECIGs to high-Watt/low nicotine concentration combinations, the nicotine delivery from these device/liquid combinations may be similar to the nicotine delivery from a low Watt/high nicotine

concentration combination due to variability in puff topography (Shihadeh & Eissenberg, 2014). Thus, regulators should consider not only device characteristics and liquid nicotine concentration but also user behavior when making regulatory decisions regarding ECIGs. One way to consider user behavior in regulation may be to design ECIG devices that limit user puff duration or puff number in order to limit the nicotine delivery profile of some device/liquid nicotine combinations.

Limitations

Several important limitations of the present study should be considered. First, the results obtained from this study's directed puffing protocol (10 puffs with 30 s IPI) may differ from those that might be seen after *ad libitum* puffing in ECIG-experienced or -naïve individuals. While there are advantages to controlling some puff topography parameters, such as the ability to compare across studies and products, future studies seeking to evaluate ECIG nicotine delivery and puff topography in a more naturalistic manner may use an *ad libitum* puffing protocol and vary liquid nicotine concentration.

Another potential limitation of this study involves the absence of a combustible tobacco cigarette control condition that would have allowed for more direct comparison of "own brand" topography and nicotine delivery across the puffing parameters used in this study (i.e., 10 puffs with a 30 s IPI). However, several previous evaluations of nicotine delivery and puff topography when using a tobacco cigarette have been conducted and data from those studies can be used to make comparisons with the results from this study (Vansickel et al., 2010, Kleykamp et al., 2008). Also, because many of the ECIG-experienced individuals in the present study were former cigarette smokers, ethical concerns arise when planning a study that involves asking

former cigarette smokers to use a product that is known to be dependence-inducing and lethal when those individuals no longer use that product.

The use of a single cartomizer type in the present study may be considered a limitation as the cartomizer used may not be representative of the cartomizers or tanks typically used by experienced ECIG users. Perhaps the puff topography observed when using the study cartomizer may not be indicative of the puff topography that may be exhibited if participants (especially ECIG-experienced individuals) used their preferred tank or cartomizer. However, ECIG device features and parts (cartomizers and tanks) vary markedly. Selecting a cartomizer that is more "representative" of what is typically used by those individuals who participated in this study would be difficult and may have compromised internal validity. Because device features may influence ECIG-associated nicotine delivery, standardizing the device was intended to eliminate the potential influence of device characteristics on the outcome measures. Also, the use of a single cartomizer type ensured accurate topography measurement in this study. Future studies seeking to evaluate more naturalistic puffing behavior may benefit from using a mouthpiece-based device that can accurately measure puff topography in tank-based ECIGs.

Finally, because this study was not designed to measure the effects of gender or flavor preference, it may have lacked sensitivity to detect differences related to these factors. Because puff topography differences between men and women have been observed among tobacco cigarette smokers (Melikian et al., 2006), future studies may benefit from using a larger sample sizes to explore potential gender differences in ECIG user puff topography.

Conclusions

This within and between-subject clinical laboratory study evaluated the extent to which liquid nicotine concentration and puff topography influence plasma nicotine concentration in

ECIG-experienced and -naïve individuals. Results demonstrated that liquid nicotine concentration is directly related to plasma nicotine concentration and that ECIGs can deliver physiologically active nicotine concentrations to ECIG-experienced and ECIG-naïve smokers following 10 puffs. Generally, ECIG-experienced individuals obtained significantly higher mean plasma nicotine boost relative to ECIG-naïve smokers and this difference depends upon differences in user puff topography. Under some conditions, some ECIG-experienced and -naïve individuals obtained a nicotine boost that was greater than the mean nicotine boost typically observed after 10 puffs from a tobacco cigarette under similar laboratory conditions. ECIG-experienced individuals took longer and larger puffs relative to ECIG-naïve individuals which may explain the variability in nicotine boost across groups. Taken together, the results of this study support that ECIG nicotine delivery can vary based on liquid nicotine concentration and user experience (i.e., puff topography). Finally, regulators should consider device characteristics and user behavior as well as liquid nicotine concentration when making regulatory decisions intended to control ECIG nicotine delivery.

List of References

Abrams, D. B., (2014). Promise and peril of e-cigarettes: can disruptive technology make cigarettes obsolete?. *JAMA, 311*(2), 135-136.

Adkison, S. E., O'Connor, R. J., Bansal-Travers, M., Hyland, A., Borland, R., Yong, H. H., ... & Fong, G. T. (2013). Electronic nicotine delivery systems: international tobacco control four-country survey. *American journal of preventive medicine, 44* (3), 207-215.

Arrazola, R. A., Singh, T., Corey, C. G., Husten, C. G., Neff, L. J., Apelberg, B. J., ... & McAfee, T. (2015). Tobacco use among middle and high school students—United States, 2011-2014. *MMWR Morb Mortal Wkly Rep, 64* (14), 381-5.

Bahl, V., Lin, S., Xu, N., Davis, B., Wang, Y. H., & Talbot, P. (2012). Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models. *Reproductive Toxicology, 34* (4), 529-537.

Behar, R. Z., Hua, M., & Talbot, P. (2015). Puffing Topography and Nicotine Intake of Electronic Cigarette Users. *PloS one*, *10* (2), e0117222.

Berg, C. J., Haardoerfer, R., Escoffery, C., Zheng, P., & Kegler, M. (2015). Cigarette users' interest in using or switching to electronic nicotine delivery systems for smokeless tobacco for harm reduction, cessation, or novelty: a cross-sectional survey of US adults. *Nicotine & Tobacco Research*, *17*, 245-255.

Benowitz, N.L. (2008). Neurobiology of nicotine addiction: implications for smoking cessation treatment. *The American Journal of Medicine, 121*, S3-S10.

Blank, M.D. (2008). *Comparison for Three Methods for Measuring Smoking Behavior.* (Doctoral Dissertation). Retrieved from ProQuest, UMI Dissertations Publishing.

Blank, M. D., Disharoon, S., & Eissenberg, T. (2009). Comparison of methods for measurement of smoking behavior: mouthpiece-based computerized devices versus direct observation. *Nicotine & Tobacco Research*, *11*, 896-903.

Blank, M.D., Nasim, A., Hart, A. Jr., Eissenberg, T. (2011) Acute effects of cigarillo smoking. *Nicotine & Tobacco Research, 3*(9), 874-879.

Breland, A.B., Buchhalter, A.R., Evans, S.E., & Eissenberg, T. (2002). Evaluating acute effects of potential reduced-exposure products for smokers: Clinical laboratory methodology. *Nicotine & Tobacco Research, 4*, 131-140.

Breland, A.B. (2005). *Evaluating Potential Reduced Exposure Products for Smokers.* (Doctoral Dissertation). Retrieved from ProQuest, UMI Dissertations Publishing.

Breland, A.B., Kleykamp, B.A., & Eissenberg, T. (2006). Clinical laboratory evaluation of potential reduced exposure products for smokers. *Nicotine & Tobacco Research, 8,* 727-38.

Breland, A.B., Spindle, T.R., Weaver, M., & Eissenberg, T.E. (2014). Science and electronic cigarettes: Current data, future needs. *Journal of Addiction Medicine, 8,* 223-233.

Breland, A., Soule, E., Lopez, A., Ramôa, C., El-Hellani, A., & Eissenberg, T. (2016). Electronic cigarettes: what are they and what do they do?. *Annals of the New York Academy of Sciences.* Doi: 10.1111/nyas.12977.

Buchhalter, A. R., Acosta, M. C., Evans, S. E., Breland, A. B., & Eissenberg, T. (2005). Tobacco abstinence symptom suppression: the role played by the smoking-related stimuli that are delivered by denicotinized cigarettes. *Addiction, 100,* 550-559.

Buchhalter, A.R., & Eissenberg, T. (2000). Preliminary evaluation of a novel smoking system: Effects on subjective and physiological measures and on smoking behavior. *Nicotine & Tobacco Research, 2,* 39-43.

Bunnell, R. E., Agaku, I. T., Arrazola, R., Apelberg, B. J., Caraballo, R. S., Corey, C. G., ... & King, B. A. (2014). Intentions to smoke cigarettes among never-smoking US middle and high school electronic cigarette users, National Youth Tobacco Survey, 2011-2013. *Nicotine & Tobacco Research, ntu166.*

Cobb, C. O., Hendricks, P. S., & Eissenberg, T. (2015). Electronic cigarettes and nicotine dependence: evolving products, evolving problems. *BMC medicine, 13*(1), 1.

Cox, L. S., Tiffany, S. T., & Christen, A. G. (2001). Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine & Tobacco Research, 3*(1), 7-16.

Dawkins, L., Turner, J., Roberts, A., & Soar, K. (2013). 'Vaping' profiles and preferences: an online survey of electronic cigarette users. *Addiction, 108* (6), 1115-1125.

Dawkins, L., & Corcoran, O. (2014). Acute electronic cigarette use: nicotine delivery and subjective effects in regular users. *Psychopharmacology, 231*(2), 401-407.

Dawkins, L. E., Kimber, C. F., Doig, M., Feyerabend, C., & Corcoran, O. (2016). Self-titration by experienced e-cigarette users: blood nicotine delivery and subjective effects. *Psychopharmacology*, 1-9.

Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016. 81 FR 28974 (May 10, 2016). Docket No. FDA-2014-N-0189.

Delnevo, C. D., Giovenco, D. P., Steinberg, M. B., Villanti, A. C., Pearson, J. L., Niaura, R. S., & Abrams, D. B. (2016). Patterns of electronic cigarette use among adults in the United States. Nicotine & Tobacco Research, *18*(5), 715-719.

Duke, J. C., Lee, Y. O., Kim, A. E., Watson, K. A., Arnold, K. Y., Nonnemaker, J. M., & Porter, L. (2014). Exposure to electronic cigarette television advertisements among youth and young adults. *Pediatrics*, *134* (1), e29-e36.

Eissenberg, T. (2014, February). Acute effects of electronic cigarettes in adults: nicotine delivery and abuse liability. Oral presentation in the Symposium entitled "Electronic Cigarettes: current state of the knowledge and future research" Chaired by Dr. Suchitra Krishnan-Sarin. 20th Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT). Seattle, WA.

Etter, J. F. (2012). *The electronic cigarette: An alternative to tobacco?* Geneva, Switzerland: Jean-François Etter.

Etter, J. F. (2013). Should electronic cigarettes be as freely available as tobacco? Yes, *British Medical Journal*, 346: f3845.

Etter, J. F., & Eissenberg, T. (2015). Dependence levels in users of electronic cigarettes, nicotine gums and tobacco cigarettes. *Drug and alcohol dependence*, *147*, 68-75.

Family Smoking Prevention and Tobacco Control Act, 2009. 123 STAT.1776 (June 22, 2009).

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., Spyrou, A., & Voudris, V. (2013). Impact of flavour variability on electronic cigarette use experience: an internet survey. *International journal of environmental research and public health*, *10* (12), 7272-7282.

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2013). Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: implications for research protocol standards definition and for public health authorities' regulation. *International Journal of Environmental Research and Public Health*, *10*, 2500-2514.

Farsalinos, K. E., Spyrou, A., Tsimopoulou, K., Stefopoulos, C., Romagna, G., & Voudris, V. (2014). Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Scientific Reports*, *4*, 1-71.

Farsalinos, K. E., Spyrou, A., Stefopoulos, C., Tsimopoulou, K., Kourkoveli, P., Tsiapras, D., ... & Voudris, V. (2015). Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naïve users (smokers). *Scientific reports*, *5*.

Giroud, C., de Cesare, M., Berthet, A., Varlet, V., Concha-Lozano, N., & Favrat, B. (2015). E-Cigarettes: A Review of New Trends in Cannabis Use. *International Journal of Environmental Research and Public Health*, *12*(8), 9988–10008. http://doi.org/10.3390/ijerph120809988

Giovenco, D.P., Hammong, D., Corey, C.G., Ambrose, B.K., & Delnevo, C.D. (2015). E-cigarette market trends in traditional U.S. retail channels, 2012-2013. *Nicotine & Tobacco Research*, *17* (10), 1279-1283.

Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., ... & Jacob, P. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. To*bacco control*, *23* (2), 133-139.

Grana R. A., Popova L., Ling P. M. (2014). A Longitudinal analysis of electronic cigarette use and smoking cessation. *JAMA Internal Medicine*, *174*, 812–13.

Griffiths, R. R., Henningfield, J. E., & Bigelow, G. E. (1982). Human cigarette smoking: manipulation of number of puffs per bout, inter-bout interval and nicotine dose. *Journal of Pharmacology and Experimental Therapeutics*, *220* (2), 256-265.

Gust, S.W. and Pickens, R.W. (1982). Does cigarette nicotine yield affect puff volume? *Clinical Pharmacology and Therapeutics, 32*(4), 418-422.

Hajek, P. (2014). Electronic cigarettes have a potential for huge public health benefit. *BMC medicine*, *12* (1), 225.

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C. & Fagerstrom, K. O. (1986) The Fagerstrom Test for Nicotine Dependence: a review of the Fagerstrom Tolerance Questionnaire. British Journal of Addiction, *86*, 1119–1127.

Hecht, S. S., Carmella, S. G., Murphy, S. E., Akerkar, S., Brunnemann, K. D., & Hoffmann, D. (1993). A tobacco-specific lung carcinogen in the urine of men exposed to cigarette smoke. *New England journal of medicine*, *329* (21), 1543-1546.

Herning, R.I., Jones, R.T., Bachman, J., and Mines, A.H. (1981). Puff volume increases when low-nicotine cigarettes are smoked. *British Medical Journal, 283*(6285), 187-189.

Herning, R. I., Jones, R. T., Benowitz, N. L., & Mines, A. H. (1983). How a cigarette is smoked determines blood nicotine levels. *Clinical Pharmacology & Therapeutics, 33*(1), 84-90.

Hoffman, D. & Hecht, S.S. (1985). Nicotine-derived N-nitrosamines and tobacco-related cancer: current status and future directions. *Cancer Research, 45*, 935-944.

Hua, M., Yip, H., & Talbot, P. (2013). Mining data on usage of electronic nicotine delivery systems (ENDS) from YouTube videos. *Tobacco control*, *22*, 103-106.

Hughes, J.R. & Hatsukami, D. (1986). Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry, 43*, 289-294.

Huynh, H., & Feldt, L. S. (1976). Estimation of the Box correction for degrees of freedom from sample data in randomised block and split-plot designs. *Journal of Educational Statistics, 1*, 69-82.

Jamal, A., Homa, D. M., O'Connor, E., Babb, S. D., Caraballo, R. S., Singh, T., ... & King, B. A. (2015). Current cigarette smoking among adults—United States, 2005–2014. *MMWR Morbidity and Mortality Weekly Report*, *64* (44), 1233-1240.

King, B. A., Alam, S., Promoff, G., Arrazola, R., & Dube, S. R. (2013). Awareness and ever-use of electronic cigarettes among US adults, 2010–2011.*Nicotine & tobacco research*, *15*, 1623-1627.

Kleykamp, B.A., Jennings, J.M., Sams, C., Weaver, M.F., & Eissenberg, T. (2008). The influence of transdermal nicotine on tobacco/nicotine abstinence and the effects of a concurrently administered cigarette in women and men. *Experimental and Clinical Psychopharmacology*, *16*, 99-112.

Kotlyar, M., Mendoza-Baumgart, M. I., Li, Z. Z., Pentel, P. R., Barnett, B. C., Feuer, R. M., ... & Hatsukami, D. K. (2007). Nicotine pharmacokinetics and subjective effects of three potential reduced exposure products, moist snuff and nicotine lozenge. *Tobacco Control*, *16* (2), 138-142.

Kosmider, L., Sobczak, A., Fik, M., Knysak, J., Zaciera, M., Kurek, J., & Goniewicz, M. L. (2014). Carbonyl compounds in electronic cigarette vapors—effects of nicotine solvent and battery output voltage. *Nicotine & Tobacco Research*, ntu078.

Kosmider, L., Sobczak, A., Knysak, J., Goniewicz, M.L. (2014, February). Effects of solvent and battery output voltage on nicotine levels released from electronic cigarette. *Paper presented at the 20th Annual Meeting of the Society for Research on Nicotine and Tobacco*, Seattle, WA.

Lakier, J.B. (1992). Smoking and cardiovascular disease. *The American Journal of Medicine*, *93*, 1A-8S.

Lee, Y. H., Gawron, M., & Goniewicz, M. L. (2015). Changes in puffing behavior among smokers who switched from tobacco to electronic cigarettes. *Addictive behaviors*, *48*, 1-4.

Li, J., Bullen, C., Newcombe, R., Walker, N., & Walton, D. (2013). The use and acceptability of electronic cigarettes among New Zealand smokers. *The New Zealand Medical Journal*, *126*, 48-57.

McMillen, R. C., Gottlieb, M. A., Shaefer, R. M. W., Winickoff, J. P., & Klein, J. D.(2014) Trends In Electronic Cigarette Use Among US Adults. *Nicotine and Tobacco Research* E-pub.

Mendelson, J. H., Goletiani, N., Sholar, M. B., Siegel, A. J., & Mello, N. K. (2008). Effects of smoking successive low-and high-nicotine cigarettes on hypothalamic–pituitary–adrenal axis hormones and mood in men. *Neuropsychopharmacology*, *33*(4), 749-760.

My Vapor Store Inc [Myvaporstore's website for e-cigarettes and e-liquids]. Available at: http:// myvaporstore.com/. Accessed August 25, 2016.

Perkins, K. A., Grobe, J. E., Weiss, D., Fonte, C., & Caggiula, A. (1996). Nicotine preference in smokers as a function of smoking abstinence.*Pharmacology Biochemistry and Behavior*, *55*(2), 257-263.

Pisinger, C., & Godtfredsen, N. S. (2007). Is there a health benefit of reduced tobacco consumption? A systematic review. *Nicotine & Tobacco Research, 9* (6), 631-646.

Polosa, R., Rodu, B., Caponnetto, P., Maglia, M., & Raciti, C. (2013). A fresh look at tobacco harm reduction: the case for the electronic cigarette. *Harm Reduction Journal*, *10* (1), 1.

Regan, A. K., Promoff, G., Dube, S. R., & Arrazola, R. (2013). Electronic nicotine delivery systems: adult use and awareness of the 'e-cigarette' in the USA. *Tobacco Control*, *22*, 19-23.

Richardson, A., Pearson, J., Xiao, H., Stalgaitis, C., & Vallone, D. (2014). Prevalence, harm perceptions, and reasons for using noncombustible tobacco products among current and former smokers. *American journal of public health*, *104*, 1437-1444.

Rose, J.E., & Behm, F.M. (2003). Effects of low nicotine content cigarettes on smoker intake, *Nicotine & Tobacco Research, 6* (2), 309-319.

Singh, T., Arrazola, R.A., Corey, C.G., et al., (2016). Tobacco use among middle and high school students—United States, 2011–2015. *MMWR. Morbidity and mortality weekly report*, *65*, 361-367.

Shihadeh A., Azar S., Antonius C., & Haddad H. Towards a topographical model of narghile water-pipe café smoking. (2004). *Biochemistry, Pharmacology, and Behavior, 79,* 75-82.

Spindle, T., Breland, A., Karaoghlanian, N., Shihadeh, A., & Eissenberg, T. (2015). Examination of electronic cigarette user puff topography: The effect of a mouthpiece based topography measurement device on plasma nicotine and subjective effects. *Nicotine and Tobacco Research, 17* (2), 142-149.

Stratton, K., Shetty, P., Wallace, R., & Bondurant, S. (Eds.) (2001). Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction, National Academy Press: Washington, DC.

Talih, S., Balhas, Z., Eissenberg, T., Salman, R., Karaoghlanian, N., El Hellani, A., ... & Shihadeh, A. (2015). Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: measurements and model predictions. *Nicotine & Tobacco Research*, *17*, 150-157.

Trehy, M. L., Ye, W., Hadwiger, M. E., Moore, T. W., Allgire, J. F., Woodruff, J. T., ... & Westenberger, B. J. (2011). Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. *Journal of Liquid Chromatography & Related Technologies*, *34*, 1442-1458.

U.S. Department of Health and Human Services. 2014. The Health consequences of smoking—50 years of progress: A report of the surgeon general. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA.

Vansickel, A. R., Cobb, C. O., Weaver, M. F., & Eissenberg, T. E. (2010). A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and

cardiovascular and subjective effects. *Cancer Epidemiology Biomarkers & Prevention*, *19*, 1945-1953.

Vansickel, A. R., Weaver, M. F., & Eissenberg, T. (2012). Clinical laboratory assessment of the abuse liability of an electronic cigarette. *Addiction*, *107* (8), 1493-1500.

Vansickel, A. R., & Eissenberg, T. (2013). Electronic cigarettes: effective nicotine delivery after acute administration. *Nicotine & Tobacco Research*, *15*, 267-270.

Yan, X. S., & D'Ruiz, C. (2015). Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regulatory Toxicology and Pharmacology, 71*(1), 24-34.

Zhu, S. H., Sun, J. Y., Bonnevie, E., Cummins, S. E., Gamst, A., Yin, L., & Lee, M. (2014). Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. *Tobacco Control*, *23*, 3-9.

APPENDIX A

Telephone Screening Form

Introduction: This is a research study about e-cigarettes.

Purpose: To compare the effects of different nicotine doses on behavior and how you feel.

Study Details: If you are eligible for this study, you will be asked to visit our lab on the MCV campus for four sessions. These sessions will begin at approximately the same time each day, will take approximately 2.5 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, 10 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 using an e-cigarette. There is some risk of bruising at the catheter site, and there is a minimal risk of infection associated with any blood draw.

For each session, we will provide you with an e-cigarette that may contain nicotine or no nicotine. During the session we will ask you to use this e-cigarette at two separate times.

When you use the 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 use the e-cigarette (the size and number of the 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 $75 after completing the first session, $75 after completing the second session, $150 after completing the third session, and $200 after completing the fourth session. Thus, the total amount you could earn for the entire study is $500.

"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

Date _____

Interviewer _____

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?" _____ (feet and inches)

7. "What is your weight?" _____ (pounds)

8. "Which identifier best describes you?" Male or Female

9. "Did you graduate high school?" *Circle Yes or No*

If Yes: Skip the next question.

10. "Did you obtain your GED?": *Circle Yes or No*

General health status:

11. "Do you have any chronic health concerns or problems?" *Circle Yes or No*

If Yes: "Please describe the concern or problem":

12. "Are you under a doctor's care for a medical condition?" *Circle Yes or No*

If Yes: "Please describe the condition":

13. "Are you taking any prescription or over-the-counter medications?" *Circle Yes or No*

If Yes: "Please identify the medication":

14. Do you have any psychiatric conditions like depression or anxiety?
Circle Yes or No

If Yes: "Please describe the condition":

15. "Have you ever been diagnosed with high or low blood pressure?" *Circle Yes or No*

If Yes: "Please indicate whether it is high or low":

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?" _____ *(months or yrs)*

19. "Have you ever used an electronic cigarette?" *Circle Yes or No*

If Yes: ask the following questions

"Do you use an electronic cigarette regularly?" *Circle Yes or No*
If no, ask "how many times have you ever used an ECIG?" _____

"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 e-liquid do you vape per day? (Please indicate liquid or cartridge)"

_____

"For how long have you been using 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*

29. "What was the first day of your last period?" _____

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. Effects of electronic cigarette dose and user experience

VCU IRB Number: HM 20000629

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 (e-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 four, approximately 2.5-hour sessions here at the Clinical Behavioral Pharmacology Laboratory located on VCU's medical campus. The four sessions will begin at approximately the same time each day, will be separated by at least 48 hours, and will occur no more than twice per week. Before each session, we will ask you to abstain from **all** e-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 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, 10 samples each session). 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. Inserting a catheter can be challenging for some individuals with smaller veins or veins that are harder to see. In this laboratory we will attempt to insert a catheter no more than three times in one day and, if all three attempts are unsuccessful, we will discontinue the session and pay you for the time that you spent complying with study conditions before the session began ($15) and also for the time you spent in the laboratory ($15/hour).

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 e-cigarette.

For each session, we will ask you to use an e-cigarette that we provide. The e-cigarette may contain nicotine or no nicotine. Neither you nor the study staff will know what each e-cigarette contains. During each session we will ask you to use the e-cigarette at two separate times. Each time, we will ask you to take only 10 puffs, and we will tell you when to take each of these puffs. At each of these two times we need you to remain seated in a comfortable chair while you are using the e-cigarette.

When you use the 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 are using the e-cigarette (the size and number of the puffs that you take). Your participation in this study will help us understand how people use e-cigarettes and what effects e-cigarettes produce. You will have an opportunity to experience all of the questionnaires and see all of the equipment before your first session.

Risks and Discomforts: You may experience some discomfort during abstinence from e-cigarettes and nicotine before the session or while using e-cigarettes during the session. Side effects from products that contain nicotine can include sweating, lightheadedness, dizziness, nausea, and nervousness. These effects are unlikely in individuals who use nicotine-containing products regularly. Side effects from tobacco/nicotine abstinence can include irritability, anxiety and restlessness, excessive hunger, difficulty concentrating, and sleep disturbance. Though these potential side effects have not been characterized in e-cigarettes users, they are common abstinence symptoms in cigarette smokers. Though uncomfortable, these feelings are not medically dangerous. You may also feel some discomfort when the nurse inserts or withdraws the needle, or when blood samples are taken. There is some risk of bruising at the catheter site, and there is a minimal risk of infection associated with any blood draw. We try very hard to minimize your discomfort at these times, and the use of a trained nurse and sterile, disposable equipment enhances comfort while reducing the risk of bruising and infection. If you find any effects or data collection procedures unacceptable, you may stop your participation at any time. You should not donate blood 4 weeks before or 4 weeks after this study.

Benefits. You will derive no personal benefit from this study. However, your participation will help us in the future as we try to improve our measuring equipment.

Costs of Participation. There is no cost to you for participation except for your time. Participating in this study will take about 14 hours in the laboratory.

Payment for Participation. You will be paid for the time that you are not using tobacco prior to each session and for your time in the laboratory: you will receive $75 after completing the first session, $75 after completing the second session, $150 after completing the third session, and $200 after completing the fourth session. Thus, the total amount you could earn for the entire study is $500. If you choose to leave the study early, you will keep what you have earned up to that point. For example, if you complete one session, you will earn $75.

In the event a session is begun but not completed (for reasons beyond your control), you will not receive full payment for a completed session. Instead, you will receive partial payment for the

time spent complying with study conditions before the session began ($15) and also for the time spent in the laboratory ($15/hour).

Alternatives. This is not a therapeutic study. You have the alternative not to participate.

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.

Confidentiality of your records will be maintained by keeping all data in a locked file and in a coded database. Release of this information will be withheld, consistent with the law, unless you give permission to release this information. The information obtained in this study may be published, but your identity will not be revealed.

Compensation for Injury. Virginia Commonwealth University and the VCU Health System (formerly known as the Medical College of Virginia Hospitals) have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study. If you are injured or if you become ill as a result of your participation in this study, contact your study nurse immediately. Your study nurse will arrange for short term emergency care or referral if it is needed. Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

Pregnancy. Every effort will be made to have women enter this study on an equal basis with men. Tobacco use may be harmful to a fetus, and pregnant women may not participate in this study. If you suspect that you are pregnant, or if you are currently breast-feeding a baby, please inform the investigator now and do not participate. We will conduct a urine pregnancy test during the screening evaluation visit to ensure that pregnant women do not participate.

Voluntary Participation and Withdrawal. You do not have to participate in this study. If you choose to participate you may stop at any time without any penalty. You may also choose not to answer particular questions that are asked in this study. The investigators will answer any questions that you may have. If you choose not to participate or to discontinue your participation, this choice will in no way affect any medical care you receive now or in the future at this institution. If during the course of the study you experience adverse effects, or if you do not comply with the study restrictions, your participation may be stopped by Dr. Eissenberg without your consent. Any significant new findings that develop during the course of the research study that may affect your willingness to continue to participate will be provided to you.

Questions. You can call Dr. Eissenberg at 827-3562 for information about the research or about research-related injury.

Participants' Rights Information. If you have questions about your rights as a research participant, you may contact:

Office for Research Subjects Protection
Virginia Commonwealth University
Virginia Biotechnology Research Park, BioTech One
800 East Leigh Street, Suite 115, P.O. Box 980219

Richmond, VA 23298-0219
Telephone: 804-828-0868

If you agree to join this study, please print and sign your name below. You will receive a copy of this consent form.

Consent. I have read this consent form. I understand the information about this study. All my questions about the study and my participation in it have been answered. I freely consent to participate in this research study.

By signing this consent form I have not waived any of the legal rights which I otherwise would have as a participant in a research study.

_____
Participant's Printed Name

_____ _____
Signature of Participant Date

_____ _____
Signature of Person Performing Consent Date

_____
Witness's Printed Name

_____ _____
Signature of Witness Date

_____ _____
Signature of Investigator Date

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

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

APPENDIX D

Direct Effects of ECIG Use Scale

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 by drawing a vertical mark anywhere along the horizontal line.

	Not at All	Extremely
1. Was the e-cig satisfying?		
2. Was the e-cig pleasant?		
3. Did the e-cig taste good?		
4. Did the e-cig make you dizzy?		
5. Did the e cig calm you down?		
6. Did the e-cig help you concentrate?		
7. Did the e-cig make you feel more awake?		

8. Did the e-cig reduce your hunger for food?	
9. Did the e-cig make you sick?	
10. Would you like to use another e-cig RIGHT NOW?	

APPENDIX E

Acceptability Questionnaire

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 by drawing a vertical mark anywhere along the horizontal line.

Did the ECIG/equipment that you used today:

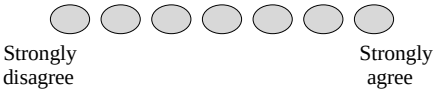
	Not at All	Extremely
1. Alter your vaping behavior?		
2. Make you less likely to want to vape today?		
3. Reduce your vaping enjoyment today?		
4. Affect the taste of the ECIG that you smoked today?		
5. Make vaping more difficult?		
6. Increase your awareness of how much you vaped today?		

APPENDIX F

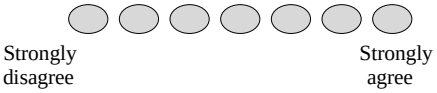
Questionnaire of Smoking Urges- Brief

For each item, please indicate how you feel RIGHT NOW

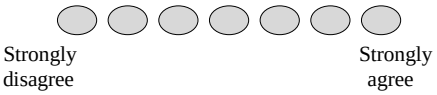
I have a desire for a cigarette right now.



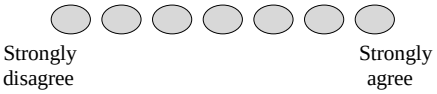
Nothing would be better than smoking a cigarette right now.



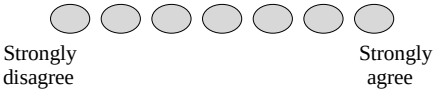
If it were possible, I probably would smoke now.



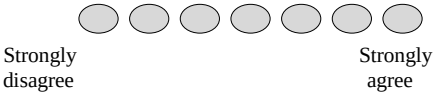
I could control things better right now if I could smoke.



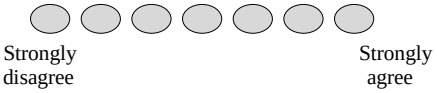
All I want right now is a cigarette



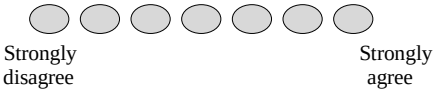
I have an urge for a cigarette.



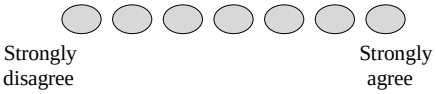
A cigarette would taste good now.



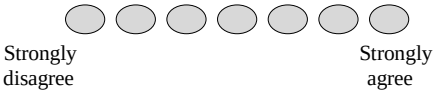
I would do almost anything for a cigarette now.



Smoking would make me less depressed.



I am going to smoke as soon as possible.



Vita

Marzena M. Hiler was born in Krakow, Poland on December 8, 1989. She is a graduate of Canyon high school in Anaheim, CA and has a B.A. in psychology from the University of California Irvine (UCI) in Irvine, CA, which she received in 2012. She began the Health psychology doctoral program at Virginia Commonwealth University in August, 2014.