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Effects of Electronic Cigarette Liquid Solvents Propylene Glycol and Vegetable Glycerin on User Nicotine Delivery, Heart Rate, Subjective Effects, and Puff Topography

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EFFECTS OF ELECTRONIC CIGARETTE LIQUID SOLVENTS PROPYLENE GLYCOL AND VEGETABLE GLYCERIN ON USER NICOTINE DELIVERY, HEART RATE, SUBJECTIVE EFFECTS, AND PUFF TOPOGRAPHY

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List of Abbreviations

ANOVA	analysis of variance
AUC	area under the curve
BP	blood pressure
CO	carbon monoxide
CReSS	Clinical Research Support System
ECIG	electronic cigarette
FTC	Federal Trade Commission
gLMS	general labeled magnitude scale
HSD	honestly significant difference
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
NRT	nicotine replacement therapy
PG	propylene glycol
ppm	concentration in parts per million
TSNAs	tobacco specific nitrosamines
VG	vegetable glycerin

Abstract

EFFECTS OF ELECTRONIC CIGARETTE LIQUID SOLVENTS PROPYLENE GLYCOL AND VEGETABLE GLYCERIN ON USER NICOTINE DELIVERY, HEART RATE, SUBJECTIVE EFFECTS, AND PUFF TOPOGRAPHY.

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Electronic cigarettes (ECIGs) are a class of tobacco products that use a heating element to aerosolize a liquid, typically containing nicotine, allowing for user inhalation. Despite their rapid growth in popularity, little is known about ECIGs including how certain device and liquid factors influence nicotine delivery, user physiological and subjective responses, and puffing behavior (puff topography). Limited pre-clinical research has demonstrated that the ratio of two solvents commonly found in ECIG liquids, propylene glycol (PG) and vegetable glycerin (VG), may have an influence on the nicotine content of ECIG aerosols. However, the extent to which PG:VG ratio in ECIG liquids influences acute effects experienced by ECIG users is unknown. The primary purpose of this clinical laboratory study was to examine the influence of PG:VG ratio on plasma nicotine concentration, heart rate (HR), subjective effects, and puff topography in experienced ECIG users.

Thirty ECIG-experienced individuals participated in four independent laboratory conditions that differed only by the PG:VG ratio in the ECIG liquid (100:0, 55:45, 20:80, and 2:98). In each condition, participants used a 3.3 volt "eGo" ECIG battery attached to a 1.5 Ohm dual coil "cartomizer" loaded with 1 ml of ECIG liquid (nicotine concentration: 18 mg/ml). Participants completed two ECIG use bouts (10 puffs with 30 sec inter-puff-interval) in each study condition. ECIG PG:VG ratio had a direct influence on nicotine delivery, subjective effects, and puff topography. Nicotine delivery and overall nicotine intake were highest following the use of the liquids containing mostly PG, despite participants taking significantly shorter and smaller puffs in these conditions, suggesting PG may be a more efficient nicotine-delivery vehicle than VG. Abstinence symptoms were suppressed similarly across all PG:VG ratios, and HR also increased in a similar fashion in all conditions following ECIG use. Participants reported significantly lower scores on items assessing sensory ECIG effects following use of the 100PG:0VG liquid, indicating a lower overall satisfaction with this liquid. Further evaluating the influence of PG and VG and other ECIG device and liquid characteristics on ECIG acute effects using clinical laboratory methodologies could inform regulations of these products.

Effects of Electronic Cigarette Liquid Solvents Propylene Glycol and Vegetable Glycerin on User Nicotine Delivery, Heart Rate, Subjective Effects, and Puff Topography

Overview

Despite the numerous well-documented deleterious health effects associated with combustible tobacco use such as cancer and cardiovascular disease (Mathers & Loncar, 2006), 15.1% of U.S. adults (Jamal, King, Neff, Whitmill, Babb, & Graffunder, 2016), 8.0% of high school students, and 2.2% of middle school students are current cigarette smokers (Jamal et al., 2017). As a result of the continued use of cigarettes, smoking remains the leading preventable cause of death in the United States (approximately 480,000 individuals annually; USDHHS, 2014) and worldwide (estimated 6 million individuals annually; Agaku, King, & Dube, 2014). Future generations may see the disease and death toll from tobacco consumption increase as a result of the plethora of alternative, unregulated tobacco products that have emerged recently in U.S. and global markets. One such class of products that has become increasingly popular among adolescents and adults in recent years is electronic cigarettes (ECIGs). Of particular concern, an increasing number of previously nicotine-naïve adolescents have tried ECIGs (Bunnell et al., 2014) and these individuals may be more likely to use tobacco cigarettes in the future (Soneji et al., 2017; Spindle, Hiler, Cooke, Eissenberg, Kendler, & Dick, 2017; Wills, Knight, Sargent, Gibbons, Pagano, & Williams, 2016).

All ECIGs share common components including a power source such as a battery and a heating element often referred to as an "atomizer" that aerosolizes a solution for user inhalation. The solutions used in ECIGs may or may not contain nicotine and various flavorants but almost always contain at least one of two solvents: propylene glycol (PG) and/or vegetable glycerin (VG). Despite the increasing prevalence of ECIGs, few clinical laboratory studies have examined how the various device and liquid features of these products influence their acute effects

including nicotine delivery, subjective effects, and user inhalation behavior (i.e., user "puff topography"). As demonstrated previously with numerous other nicotine/tobacco products (e.g., waterpipe/hookah: Blank et al., 2011; little cigars/cigarillos: Koszowski, Rosenberry, Kanu, Viray, Potts, & Pickworth, 2015; tobacco cigarettes: Benowitz, Porchet, Sheiner, & Jacob, 1988), using clinical laboratory methods to understand the nicotine delivery profiles, subjective effects such as abstinence symptom suppression, and puff topography associated with ECIG use can provide a better understanding of the abuse liability and the toxicant exposure that may be associated with using these products. The primary goal of this dissertation was to determine the extent to which two common ingredients found in ECIG liquids (PG and VG) influence the acute effects associated with ECIG use.

How Does Nicotine Influence Tobacco Consumption?

Nicotine, a psychomotor stimulant found in tobacco, is the primary constituent that is believed to be responsible for producing the reinforcing effects that promote continued use of tobacco products. Specifically, nicotine exerts effects on tobacco users by interacting with nicotinic acetylcholine receptors (nAChR), ligated-gated ion channel receptors located throughout the peripheral and central nervous systems (CNS; Brunzell, Stafford, & Dixon, 2015). When delivered to the brain, (i.e., the CNS) nicotine acts as an agonist, binding to nAChR receptors and ultimately resulting in the release of various neurotransmitters (e.g., dopamine; Benowitz, 2010). While nAChR receptors vary considerably in their structure, containing various combinations of α and/or β protein subunits, the $\alpha 4\beta 2$ nAChR receptors are believed to be the primary mechanism behind the development of nicotine dependence in tobacco users (Brunzell et al., 2015; Benowitz, 2010). When nicotine binds to the $\alpha 4\beta 2$ nAChR receptors in the mesolimbic area of the brain, connecting the ventral tegmental area (VTA), nucleus accumbens,

and prefrontal cortex, the neurotransmitter dopamine is released resulting in a pleasurable experience for the user (Benowitz, 2010). Importantly, nicotine-induced release of dopamine in the brain can promote positive reinforcement, one mechanism that fosters the continued use of tobacco products (Benowitz, 2008; Henningfield & Keenan, 1993, USDHHS, 2014).

Positive reinforcement occurs when a stimulus (e.g., nicotine) is elicited by a behavior (e.g., smoking) and the behaviorally-elicited stimulus increases the likelihood that the behavior will occur again (Stewart, De Wit, & Eikelboom, 1984; Glautier, 2004). Positive reinforcement is believed to occur in tobacco users as a result of nicotine producing a variety of effects upon administration, including mild euphoria, heightened arousal, and improved cognitive functioning (USDHHS, 2014; Watkins, Koob, & Markou, 2000). These effects that can occur after self-administration of nicotine are thought to be one factor that encourages continued tobacco consumption (Glautier, 2004). Indeed, non-human animals such as rats and primates will work to self-administer nicotine, providing further evidence that nicotine can serve as a positive reinforcer (Corrigall & Coen, 1989; Donny, Caggiula, Brown, & Knopf, 1995; Le Foll, Wertheim, & Goldberg, 2007).

The speed that nicotine is delivered to the user may affect the degree to which the effects associated with tobacco use maintain the use of these products. For example, nicotine when administered through inhalation to a user by way of the smoke from a combustible tobacco cigarette is absorbed by the lung's alveoli (Le Houezec, 2003; Stead et al., 2012) and delivered rapidly to the user's bloodstream and brain (Benowitz, 2008). Indeed, smoking a single cigarette can result in nearly complete saturation or occupancy of the aforementioned $\alpha 4\beta 2$ nAChR receptors that release the neurotransmitter dopamine in key areas associated with reward in the brain (e.g., VTA; Brody et al., 2006). Conversely, the nicotine delivery profile of other nicotine-

containing products such as the nicotine patch is much slower as they do not involve pulmonary absorption. For example, the nicotine patch delivers nicotine via the much slower transdermal route (Benowitz et al., 1988; Le Houezec, 2003). This difference in the rapidity of nicotine delivery between cigarettes and other nicotine-containing products (e.g., oral, non-combustibles) may result in differences in the onset and magnitude of the positive rewarding aspects associated with nicotine self-administration (e.g., Cobb, Weaver, & Eissenberg, 2010) and may partially explain why cigarettes are used at much higher rates than other products that deliver nicotine less rapidly and efficiently (Agaku et al., 2014).

Negative reinforcement may be another mechanism that facilitates the continued use of tobacco products. Negative reinforcement occurs when the performance of a particular behavior elicits the removal of an aversive stimulus and that behaviorally-elicited stimulus removal increases the probability of that particular behavior's re-occurrence (Jaffe, 1992; Eissenberg, 2004). For example, negative reinforcement is thought to occur when a tobacco product is self-administered and that product administration subsequently removes or suppresses a negative state (e.g., irritability, anxiousness; Hughes & Hatsukami, 1986) that often appears during periods of tobacco abstinence in long-term users of nicotine/tobacco (Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005; Vansickel, Cobb, Weaver, & Eissenberg, 2010).Importantly, the extent to which a product suppresses aversive tobacco abstinence symptoms, thereby increasing the likelihood of subsequent tobacco product administration via negative reinforcement, is a powerful determinant of whether someone will continue to use the product. For example, smokers often report that they continue to smoke because smoking can suppress the aversive symptoms they experience during abstinence (Gilbert, Sharpe, Ramanaiah, Detwiler, & Anderson, 2000). Indeed, abstinence symptom severity predicts smoking relapse (Baker,

Brandon, & Chassin, 2004; Droungas, Ehrman, Childress, & O'Brien, 1995; Gilbert et al., 2000). Analyses of two clinical trials examining the efficacy of the nicotine patch revealed that as abstinence symptom severity increased, the likelihood of participants' relapsing to tobacco cigarettes also increased (Piasecki, Fiore, & Baker, 1998).

The extent to which negative reinforcement maintains tobacco use may also be influenced by the speed with which nicotine is eliminated from a user's body. Specifically, the half-life of nicotine is approximately 1-2 hours for adults (Benowitz, 2008), meaning nicotine begins to be eliminated from a user's blood soon after it has been absorbed. This rapid elimination of nicotine also means that using a cigarette or other nicotine-containing product may suppress a user's aversive abstinence symptoms for a short period of time, necessitating subsequent and repeated nicotine self-administration (USDHHS, 2014). Taken together, the rapid delivery of nicotine to a user's blood when nicotine is inhaled (such as through tobacco smoke), accompanied by the relatively fast elimination of nicotine from a user's system after use, help explain why nicotine-containing products such as tobacco cigarettes are used continually by numerous individuals over long periods of time. As described in further detail below, the continued use of tobacco products is done in part to suppress the negative state associated with nicotine abstinence.

How Do Subjective Effects Influence Tobacco/Nicotine Consumption in Long-term Users?

During periods of abstinence from nicotine, tobacco product users often experience an aversive syndrome that affects their physiological (e.g., decreases in heart rate (HR) and increases in body weight), subjective (e.g., increased irritability and depressed mood) and cognitive state (e.g., increased difficulty concentrating; USDHHS, 2014). These abstinence symptoms have been examined experimentally in the clinical laboratory by researchers using

physiological recording equipment, various subjective questionnaires such as the Hughes-Hatsukami withdrawal scale and the Tiffany-Drobes Questionnaire of Smoking Urges (QSU), and tests of cognitive functioning such as the Digit Symbol Substitution Task (Eissenberg, Adams, Riggins, & Likness, 1999; Hughes & Hatsukami, 1986; McLeod, Griffiths, Bigelow, & Yingling, 1982; Tiffany & Drobes, 1991). For example, in one double-blind, Latin squared-ordered, repeated measures study, 32 tobacco cigarette smokers were instructed to use either nicotine-containing cigarettes, de-nicotinized cigarettes, or abstain from cigarettes over separate 5-day periods (Buchhalter et al., 2005). Outcomes associated with nicotine/tobacco abstinence were assessed on each of the five days of the study conditions. A mean increase in the visual analog scale (VAS) subjective item "Urge to Smoke" from the Hughes Hatsukami withdrawal scale (Eissenberg et al., 1999; Hughes & Hatsukami, 1986) was observed from day 1 (Mean = 57.4, SD = 20.9) to day 2 (Mean = 73.9, SD = 20.0) over the five days that participants abstained from cigarettes. Conversely, in the condition in which participants used nicotine-containing cigarettes, participants' "Urge to Smoke" did not differ across the entire five days. Interestingly, several subjective items that increased significantly over the course of the five days in the no cigarette condition (e.g., "QSU factor 1," "Urge to Smoke," "Hunger," "Desire for Sweets") did not change throughout the entire week in which nicotine-containing or de-nicotinized cigarettes were used. Similar abstinence symptom suppression between the nicotine-containing and de-nicotinized cigarette conditions suggest that non-nicotine behavioral stimuli associated with smoking, such as hand-to-mouth movements, the sight of smoke, and/or sensations at the back of the throat may also facilitate the suppression of certain abstinence symptoms (Buchhalter et al., 2005). As will be discussed later, these non-nicotine behavioral stimuli may account partially for the effectiveness of ECIGs at suppressing tobacco/nicotine abstinence symptoms.

As has been demonstrated in numerous clinical laboratory studies, tobacco/nicotine abstinence symptoms can be suppressed by administration of various nicotine-containing products, including tobacco cigarettes (Buchhalter et al., 2005; Vansickel et al., 2010), oral non-combustible products (Blank & Eissenberg, 2010; Cobb et al., 2010), and nicotine replacement therapies (NRTs) such as the nicotine gum and patch (Fagerström, Schneider, & Lunell, 1993; Kleykamp, Jennings, Sams, Weaver, & Eissenberg, 2008). Importantly, these studies have demonstrated that a product's nicotine delivery profile often is related directly to its effectiveness at suppressing abstinence symptoms. For example, as previously mentioned, tobacco cigarettes deliver nicotine in a fast and highly efficient manner (Benowitz, 2008). Numerous clinical laboratory studies have demonstrated that when a smoker self-administers nicotine using their own brand of cigarettes, abstinence symptoms are suppressed reliably and often to a greater magnitude compared to other nicotine-containing products that tend to deliver less nicotine, as indexed by participants' plasma nicotine concentration (Blank & Eissenberg, 2010; Cobb et al., 2010; Breland, Buchhalter, Evans & Eissenberg, 2002; Buchhalter & Eissenberg, 2000, Vansickel et al., 2010). For example, in one Latin-square ordered, repeated measures, clinical laboratory examination of 28 overnight abstinent cigarette smokers, participants completed seven conditions in which they used either their own brand of cigarettes, one of four oral non-combustible products ("Camel Snus," "Marlboro Snus," the dissolvable nicotine product "Ariva," or the "Commit" nicotine lozenge), low-nicotine cigarettes ("Quest"), or a sham (unlit) cigarette (Cobb et al., 2010). For each condition, the product was administered twice, separated by 60 minutes, and plasma nicotine was assessed at various times after each product administration. Significantly greater nicotine delivery was observed after the use of participants' own brand of cigarettes relative to the conditions in which the oral-noncombustible products or

the low-nicotine cigarettes were used. In addition to delivering more nicotine, participants' own brand of cigarettes suppressed numerous abstinence symptoms (e.g., "craving a cigarette/nicotine" and "QSU factor 1") to a greater extent relative to the oral-noncombustible products (Cobb et al., 2010).

Taken together, results from several human laboratory studies have revealed that nicotine-containing products often vary in the degree that they can suppress the aversive symptoms experienced by nicotine-dependent individuals during periods of nicotine abstinence and that this variability may be dependent on the effectiveness of the product at delivering nicotine. Importantly, non-nicotine behavioral stimuli (e.g., hand-to-mouth movements, the sight of smoke/aerosol, throat sensations) that are associated with smoking may also be relevant (Buchhalter et al., 2005; Vansickel et al., 2010). Another important factor to consider when examining nicotine-containing products is puff topography (i.e., detailed examinations of a user's puffing behaviors), as the manner in which an individual uses a particular product may have a direct influence on their exposure to nicotine as well as other toxicants (Blank, 2008).

How Does Puff Topography Influence Toxicant Exposure?

Puff topography measurement can consist of measuring puff number, puff duration, puff volume, inter-puff-interval (IPI), and flow rate during a tobacco product-use episode. For tobacco cigarettes, an individual's puffing behavior can be a principle determinant of the amount of nicotine and other carcinogens to which they are exposed (Herning, Jones, Benowitz, & Mines, 1983; Sutton, Russell, Iyer, Feyerabend, & Saloojee, 1982; Zacny, Stitzer, Brown, Yingling, & Griffiths, 1987). In a study that examined the influence of puff topography on toxicant exposure, participants (N = 9) were trained to control their puff volume at four different levels (15, 30, 45, and 60 ml) while other aspects of their puff topography (e.g., puff number,

IPI, breath hold duration) were all held constant (Zacny et al., 1987). This experimental design allowed a determination of how this individual puff topography variable influenced toxicant exposure. Results demonstrated that as participants' puff volume increased, the amount of nicotine and carbon monoxide (CO) delivered (as assessed via nicotine concentration in participants' blood plasma and CO concentration in exhaled breath) also increased. These findings highlight the importance of assessing puff topography associated with using tobacco cigarettes because they demonstrate unequivocally that the puff volume taken by a cigarette smoker has a direct influence on exposure to nicotine and CO (Zacny et al., 1987). In another repeated measures study, puff topography and nicotine delivery were examined in cigarette smokers (N = 11) in three conditions differing by the nicotine content of the cigarettes used (i.e., 0.4, 1.0, and 2.5 mg; Herning et al., 1983). The nicotine content of the cigarettes used in this study accounted for 27% of the variance associated with plasma nicotine concentrations that were observed. However, several puff topography variables that were measured including puff number, duration, volume, and IPI collectively accounted for an additional 20% of the variance associated with participants' plasma nicotine concentrations (Herning et al., 1983).

Puff topography can also assist in understanding the toxicant exposure associated with using various alternative tobacco products. For example, puff topography analyses were instrumental in understanding the toxicant exposure of so-called "low-yield" cigarettes. "Low-yield" cigarettes were marketed with claims that they could reduce smokers' exposure to harmful tobacco-related carcinogens resulting in the widespread public perception that these products were healthier than traditional cigarettes (Davis, 1987). In fact, when "low-yield" cigarettes are compared directly to "full flavor" cigarettes using a smoking machine and standardized puffing parameters such as the Federal Trade Commission (FTC) puffing protocol (i.e., 2 sec puff

duration, 58 sec IPI, and 35 ml puff volume puffs are performed until the cigarette reaches a length of 23 mm), the toxicant content of the smoke produced is lower for "low-yield" cigarettes (Hoffmann, Djordjevic, & Hoffman, 1997; FTC, 2000). However, numerous clinical laboratory studies that have analyzed puff topography of cigarette smokers have revealed that participants do not typically puff in a uniform manner and often increase the intensity with which they puff when switching from "full-flavor" to "low-yield" cigarettes (Benowitz, et al., 2005; Gust & Pickens, 1982; Zacny & Stitzer, 1988). As a result of this change in behavior, "low-yield" cigarettes can expose users to toxicant levels comparable to that of traditional "full flavor" cigarettes (Baldinger, Hasenfratz, & Battig, 1995; Gust & Pickens, 1982; Herning, Jones, Bachman, & Mines, 1981; Zacny & Stitzer, 1988). Furthermore, several studies have revealed that puff topography data obtained from tobacco cigarette smokers differ from the puffing parameters used in the FTC method (Djordjevic, Hoffman, Hoffman, 1997; Eissenberg et al., 1999). For example, in one study topography data was recorded in individuals (N = 12) using "low-yield" and "medium-yield" cigarettes in an *ad libitum* manner. Results demonstrated that puff volume ranged from 43 to 63 ml while IPI ranged from 18 to 53 seconds, deviating from the FTC parameters of 35 ml and 58 seconds (Djordjevic et al., 1997). Another study similarly demonstrated that puff duration values observed in men (Mean = 1.53 sec; SD = 0.55) and women (Mean = 1.19; SD = 0.49) differed from the FTC method's standardized 2 second puff duration (Eissenberg et al., 1999).

The observed increases in puff topography when switching from "full-flavor" to "low-yield" cigarettes, such as longer puff durations and/or greater puff volumes, often result in comparable toxicant exposure for users of these two products (Gust & Pickens, 1982; Zacny & Stitzer, 1988). For example, in one Latin-square-ordered study "full-flavor" cigarette smokers (N

= 10) completed five conditions, lasting 5 days each, that differed by the product used: usual own brand "full flavor" cigarettes (FTC nicotine yield: 1 mg), "full-flavored" cigarettes of a different brand (FTC nicotine yield: 1 mg), and three variations of "low-yield" cigarettes (FTC nicotine yields: 0.1, 0.4, and 0.7 mg; Zacny & Stitzer, 1988). In general, smokers exhibited greater puff volumes and smaller IPIs when using the "low-yield" cigarettes relative to the "full-flavored" cigarettes. As a result of participants' altering their puff topography when using "low-yield" cigarettes compared to "full-flavor," CO exposure did not differ between groups (with the exception of the lowest FTC nicotine-yield cigarettes, 0.1 mg). Furthermore, exposure to nicotine's primary metabolite cotinine, while significantly lower in the two lowest nicotine-yield cigarettes (0.1 and 0.4 mg), was not proportionate to what would be expected based on the FTC nicotine yields. That is, toxicant exposure theoretically should be reduced by 30, 60, and 90% as a result of switching to cigarettes of 0.7, 0.4, and 0.1 mg (given that "full flavored cigarettes" in this study had an FTC nicotine-yield of 1 mg). However, when using the three "low-yield" cigarettes, participants' cotinine was only reduced by 12% in the 0.7 mg condition, 25% in the 0.4 mg condition, and 40% in the 0.1 mg condition (Zacny & Stitzer, 1988).

These examinations of the puff topography associated with "low-yield" cigarettes have led to the understanding that these products provide few, if any, health benefits to cigarette smokers (Thun & Burns, 2001). Indeed, no convincing evidence exists to demonstrate that changes in cigarette design between the 1950s and 1980s when "low-yield" cigarettes were prominent on the market resulted in any significant decreases in disease and death rates for cigarette smokers or the population as a whole (Thun & Burns, 2001). Presently, tobacco companies are prohibited from making reduced harm claims for their products such as "low" or "light" without providing sufficient evidence that their product reduces a user's harm relative to

commercially available products and also benefits the general health of the population as a whole (USDHHS, 2014). Examinations of "low-yield" cigarettes serve as excellent examples of how science, particularly clinical laboratory research, can assist in understanding alternative tobacco products and ultimately inform regulatory action. Additional research is needed to understand and inform appropriate regulatory actions for ECIGs, another class of alternative tobacco products that are growing in popularity rapidly among adolescents and adults, despite remaining largely unregulated in the U.S.

Who is Using ECIGs and Why?

The modern ECIG was patented by Hon Lik in China in 2003 (Lik, 2003) but was not introduced into the U.S. market until 2007 (Regan, Promoff, Dube, & Arrazola, 2013). In recent years, substantial increases in the marketing of ECIGs have been observed using many advertising approaches that have been banned for tobacco cigarettes. Some of these marketing approaches include television advertising, endorsements by various celebrities, and sponsoring of music and sporting events (England, Bunnell, Pechacek, Tong, & Mcafee, 2015; Grana, Glantz, & Ling, 2011). In addition, ECIGs have become more widely available and accessible with ECIG shops opening in many major U.S. cities and smaller metropolitan areas (Lee & Kim, 2014; Wagoner et al., 2014). In traditional U.S. retail channels alone (i.e., excluding online and specialty ECIG or "Vape" shops), ECIG sales more than doubled, increasing from $273.6 million in 2012 to $636.2 million in 2013 (Giovenco, Hammond, Corey, Ambrose, & Delnevo, 2015). The increased marketing and availability of ECIGs in the U.S. has corresponded with increases in prevalence in use among adolescents and adults.

Adolescent ECIG Use. In recent years, ECIGs have surpassed tobacco cigarettes as the most commonly used tobacco product among adolescents. Data from the National Youth

Tobacco Survey (NYTS), have revealed that past 30-day use of ECIGs (i.e., at least one use in 30 days prior to participating in the study) increased from 0.6% in middle school and 1.5% in high school students in 2011 to 4.3% of middle school and 11.3% of high school students in 2016 (Jamal et al., 2017; see Figure 1). Similar increases in adolescent ECIG use have been reported in other nations including Poland (Goniewicz, Gawron, Nadolska, Balwicki, &Sobczak, 2014), New Zealand (White, Li, Newcombe, & Walton, 2015), Korea (Lee, Grana, &, Glantz, 2014), Germany (Hanewinkel & Isensee, 2015), Finland (Kinnunen, Ollila, El-Amin, Pere, & Lindfors, & Rimpelä, 2015), Canada (Czoli, Hammond, & White, 2014), Ireland (Babineau, Taylor, & Clancy, 2015), Italy (Gallus et al., 2014), and Switzerland (Douptcheva, Gmel, Studer, Deline, & Etter, 2013). Focus groups conducted with adolescent ECIG users in the U.S. have revealed that the primary reasons that these individuals initiate ECIG use is due to curiosity, appealing flavors, and peer influences (Kong, Morean, Cavallo, Camenga, & Krishnan-Sarin, 2015). Notably, smoking cessation typically is not cited as a reason for initiating ECIG use among adolescents (Kong et al., 2015).

Adult ECIG Use. ECIG use has also increased dramatically among U.S. adults in the last several years. In one study, four cross-sectional, nationally representative samples were obtained from 2010-2013 (King, Patel, Nguyen & Dube, 2015). Trends in tobacco cigarette and ECIG use were examined across the four samples. Ever ECIG use rates increased from 1.8% in 2010 to 13.0% in 2013. In 2013, current use of ECIGs was highest among young adults aged 18-24 (14.2%), second highest among adults aged 25-44 (8.6%), third highest among adults aged 45-64 (5.5%), and lowest among those 65 and older (1.2%). Additionally, in the 2013 sample, those who reported being cigarette smokers had the highest likelihood of being current ECIG users (King et al., 2015).

Increases in the use rates of ECIGs among adults also have been documented in the United Kingdom (Dockrell, Morison, Bauld, & McNeill, 2013), Italy (Gallus et al., 2014), Korea (Lee, Grana, & Glantz, 2014) and numerous other countries. Adult ECIG users report a variety of reasons for using these products including the perceived health benefits to themselves and those around them, social benefits, increased convenience such as the ability to use their product indoors, and various pleasurable effects such the alleviation of nicotine abstinence symptoms and better taste relative to tobacco cigarettes (Soule, Rosas, & Nasim, 2016). A primary reason adults report using ECIGs is to reduce their consumption of conventional cigarettes or as a smoking cessation aid (Berg, Haardoerfer, Escoffery, Zheng, & Kegler, 2015; Richardson, Pearson, Xiao, Stalgaitis, & Vallone, 2014; Soule et al., 2016).

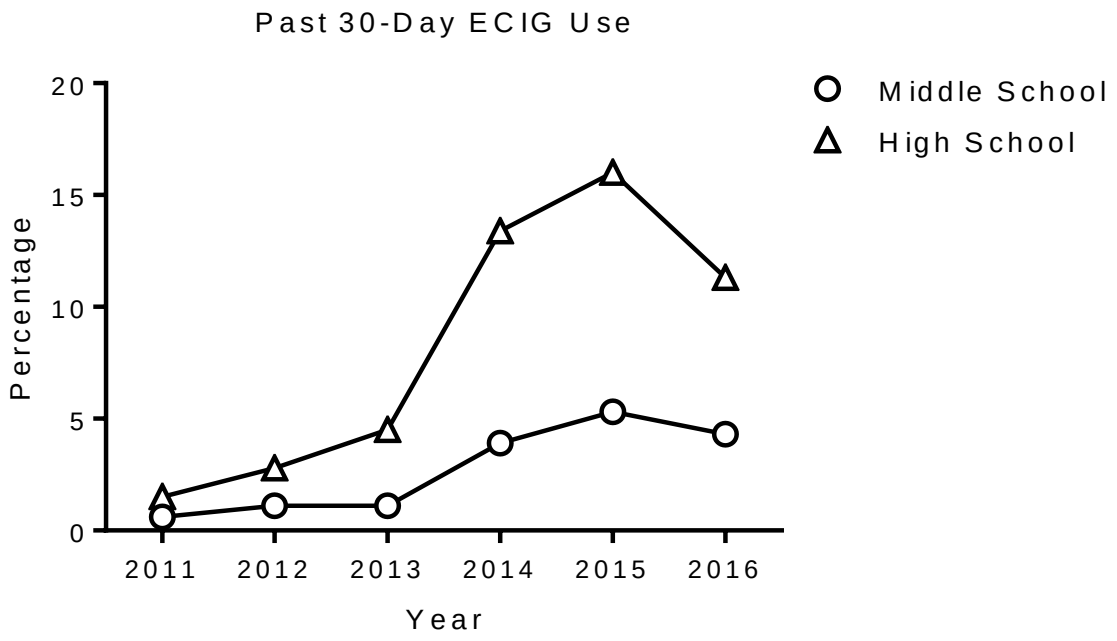

Figure 1. Trends of past 30-day ECIG use among U.S. middle and high school students from 2011-2016 (Jamal et al., 2017).

One of the most concerning aspects associated with the increase in prevalence of ECIG use is the rise in use of ECIGs among individuals who previously were nicotine-naïve, many of whom are adolescents or young adults. For example, NYTS data have indicated that the number of participants from this nationally representative sample who reported ever having used an ECIG despite never smoking a tobacco cigarette more than tripled from 79,000 in 2011 to 263,000 in 2013 (Bunnell et al., 2014). Furthermore, recent data from the National Health Interview Survey (NHIS) revealed that 9.7% of 18-24 year olds reported trying ECIGs despite never smoking tobacco cigarettes (Schoenborn & Gindi, 2015). In addition, recent longitudinal surveys have indicated that adolescents and young adults who use ECIGs despite never smoking cigarettes are more likely to begin smoking cigarettes in the future (Leventhal et al., 2015; Spindle et al., 2017; Wills et al., 2016). In one longitudinal study, surveys assessing use of various tobacco products were administered to high school students in Hawaii in 2013 and again in 2014 (N = 2,338). Results indicated that at timepoint 1, individuals who reported ever using an ECIG but never smoking a tobacco cigarette were more likely to have smoked a cigarette at timepoint 2 relative to those who had not tried ECIGs (Wills et al., 2016). Although the long-term health effects associated with ECIG use are currently unknown, the fact that ECIGs appear to be facilitating the use of tobacco cigarettes in adolescents could result in major public health consequences.

One limitation shared by the majority of survey-based studies examining ECIG use is that these studies often assess ECIG use as if ECIGs are one product. However as will be described below, ECIGs are actually a broad product category with great variability among them and their associated liquid solutions.

Components of the ECIG

ECIGs are typically battery-powered devices that use a heating element (often called an "atomizer") to heat a liquid solution and create an aerosol that the user can inhale. The liquid solution sometimes, but not always, contains nicotine and various flavorants, and some type of solvent (typically PG and/or VG). However, since their introduction, ECIGs have evolved into a class of products that vary by their appearance, device characteristics, and the contents of their associated liquids (Breland, Spindle, Weaver, & Eissenberg, 2014).

ECIG Devices. ECIGs differ greatly in their appearance and design features (Figure 2). Indeed, over 466 unique brands of ECIGs are available on the internet currently and this number continues to increase (Zhu et al., 2014). One of the most commonly advertised and popular models of ECIGs are so-called "cigalikes." These models are designed to bear a resemblance to tobacco cigarettes, often being of a similar size and shape of a cigarette and including a light-emitting diode at the non-mouth end that glows when the user inhales and activates the inner heating element (Figure 2). The liquid solution used by "cigalike" devices is stored in a cartridge. These cartridges, when containing the internal heating element, are referred to as "cartomizers." After a cartridge is depleted of the liquid, some "cigalike" devices allow the user to replace or re-fill the cartridge while others must be disposed of entirely by the user (Breland et al., 2014).

Other ECIG models often contain rechargeable batteries, store ECIG liquid in reservoirs (referred to commonly as "tanks") or pre-filled cartridges, and do not resemble tobacco cigarettes in their appearance (Figure 2). Many models of this nature require the user to press a button in order to activate the device's internal heating element and produce the inhalable aerosol. Importantly, in some of these models, users can alter characteristics of the device such as the

voltage supplied by the battery or the resistance of the heating element, as measured in Ohms (Ω). Altering voltage and/or resistance affects the power flowing through the heating element (as measured in Watts, W) and the content of the subsequent aerosol produced, as described in further detail later in this document (Breland et al., 2014; Breland, Soule, Lopez, Ramôa, El-Hellani, & Eissenberg 2017; Talih et al., 2015). ECIGs that are not "cigalikes" and thus not constrained by size limitations typically have larger, more powerful batteries accompanied with "tanks" that can store 1 ml of liquid or more. To date, limited data exists regarding use rates of specific types of ECIGs. However, in one nationally representative survey adult ECIG users were categorized as either using "closed systems" such as "cigalikes" that do not allow for users to fill the device with ECIG liquid or "open systems" such as tank-based models that enable users to fill the device with a liquid of their choosing. Overall, 51.4% of the sample currently used only "closed systems," 41.1% currently used only "open systems," while the remaining 7.4% currently used both types. Interestingly, individuals who were current "closed system" users were significantly more likely to be current cigarette smokers relative to "open system" users; individuals who had never smoked cigarettes were excluded from the sample (Chen, Zhuang, & Zhu, 2016). In another examination that pooled data from eight survey studies of adolescent and young adult ECIG users, 7.5% of the sample reported primarily using "closed system" disposable "cigalike" devices while 77% reported primarily using "later generation" or "open system" devices; remaining participants did not know or did not answer questions related to their device type (Barrington-Trimis et al., 2017). Differences across these studies in device preference may suggest adolescents/young adults are less likely to be users of "cigalike" devices relative to adult ECIG users, although this question has yet to be explored empirically. Adding to

the complexity of this product category, ECIG liquids available on the market are perhaps even more varied than the devices themselves.

ECIG Liquids. The liquid solutions intended for ECIGs vary markedly, often differing by flavoring, nicotine concentration, and the liquid solvent in which the nicotine is dissolved. One study estimated that over 7,000 unique flavors of ECIG liquids are available for purchase, and that this number increases regularly (Zhu et al., 2014). Data from studies recruiting ECIG users via the internet have demonstrated that individuals who use more advanced, tank-based ECIGs cite the ability to choose from a variety of flavors as one of the most important and alluring aspects of ECIG use (Yingst, Veldheer, Hrabovsky, Nichols, Wilson, & Foulds, 2015). Nicotine concentrations of ECIG liquids generally range from 0 to 36 mg/ml, but may also be available at much higher concentrations (e.g., 50 + mg/ml; Breland et al., 2017; Varlet, Farsalinos, Augsburger, Thomas, & Etter, 2015). Because nicotine used in ECIG liquids typically is extracted from the tobacco plant, known tobacco-related carcinogens such as tobacco-specific nitrosamines (TSNAs) have been detected across a wide variety of liquids at varying levels (Cheng, 2014; Kim & Shin, 2013; Han, Chen, Zhang, Liu, & Fu, 2015). Lastly, the ratio of PG to VG in ECIG liquids can be anywhere from 0:100 or 100:0, but these solvents are found in some combination in virtually all ECIG solutions (Etter, 2012). Indeed, PG and VG can comprise 80% to 97% of the total mass percentage of ECIG liquids (Han et al., 2015).

Importantly, advertised nicotine concentrations in ECIG liquids often differ from actual nicotine concentrations (Trehy et al., 2011). For example, numerous studies have detected nicotine in ECIG liquids advertised as containing 0 mg/ml (Hadwiger et al., 2010; Trehy et al., 2011; Kubica et al., 2013) and other reports have demonstrated that ECIG liquids may contain nicotine at much lower concentrations than advertised (Goniewicz et al., 2013; Goniewicz,

Kuma, Gawron, Knysak, & Kosmider, 2012; Spindle, Breland, Karaoghlanian, Shihadeh, & Eissenberg, 2015). Variation in nicotine concentrations even exists across cartridges of the exact same brand and manufacturer (Goniewicz, Hajek, & McRobbie, 2013; Cheah et al., 2014).

Actual concentrations of PG and VG in ECIG liquids also can vary from advertised concentrations considerably. For example, in one examination of 27 distinct ECIG liquids, actual concentrations of PG and VG differed from labeled concentrations consistently. Further, there were several instances in which actual PG and/or VG concentrations and labeled concentrations of these solvents differed by greater than 10% (Peace, Baird, Smith, Wolf, Poklis, & Poklis, 2016).

Regulation of ECIGs: The Tobacco Control Act

The considerable variability in the components of ECIG devices and contents of the associated liquids are a consequence of these products being unregulated for several years in many countries, including the U.S. (Breland et al., 2017). After the passing of the Family Smoking Prevention and Tobacco Control Act (FSPTCA) of 2009, the Food and Drug Administration (FDA) immediately began regulating the distribution, marketing, and manufacturing of certain tobacco products such as cigarettes, roll-your-own tobacco, and smokeless tobacco. For example, this regulatory power enables the FDA to prohibit the sale of tobacco products to adolescents, implement standards regarding product ingredients and labeling to prevent misbranding and/or product adulteration, restrict advertising that may be misleading or target specific vulnerable populations, and to regulate the amount of toxicants found in tobacco products including nicotine (Department of Health and Human Services, 2014).

Figure 2. Several different types of ECIG models.

Importantly, the FSPTCA also gave FDA the ability to regulate other tobacco products not initially included in the statute which are defined as: "any product made or derived from tobacco that is intended for human consumption, including any component, part or accessory of the tobacco product."

In fact, with a "deeming" announcement in May of 2016, FDA announced intentions to broaden its regulatory power to include ECIGs and numerous other alternative tobacco products (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). By exerting their authority to regulate the distribution, marketing, and manufacturing of ECIGs, FDA could reduce the harm associated with using these products in several ways such as by ensuring that: adolescents are unable to purchase them legally, the ingredients found in these products are labeled and controlled appropriately, these products are not marketed specifically to attract certain groups such as adolescents, and users cannot obtain nicotine in levels that may be toxic or foster physiological dependence. However, in order for the FDA to provide appropriate regulations for these products, understanding the respective influences the numerous components of ECIG devices and liquids have on the resulting aerosol produced by an ECIG and the acute effects that ECIG aerosol may have on a user is vital. This understanding may inform policy decisions regarding what contents should be allowable in an ECIG, the extent to which ECIGs should be accessible to the general public, and what labeling information should be required by those who manufacture ECIGs. Described below is a review of the pre-clinical studies that have explored the content of ECIG aerosols and the clinical studies that have examined the acute effects associated with ECIG use (i.e., nicotine delivery and subjective effects such as abstinence

symptom suppression) as well as a review of device/liquid factors and user characteristics (i.e., puff topography) that have been demonstrated to influence these outcome measures.

Pre-Clinical Analytical Chemistry Examinations of ECIGs

While examinations of the contents of ECIG liquids can be informative, analyses of the content of aerosols produced by ECIGs provide insight into airborne constituents, such as nicotine, that a user is exposed to when using an ECIG. Early pre-clinical laboratory studies sought to determine whether toxicants typically found in tobacco smoke (e.g., nicotine, CO, TSNAs) were present in aerosols produced from numerous commercially available brands of ECIGs and ECIG liquids (Goniewicz et al., 2014; McAuley, Hopke, Zhao, & Babaian, 2012; Schripp, Markewitz, Uhde, Salthammer, 2013). Generally, results of these studies demonstrated that many potentially hazardous compounds are present in aerosols generated from ECIGs, but at far lesser amounts relative to tobacco cigarettes. In one study, aerosols were generated from 11 different types of "cigalike" ECIGs, with cartridges containing varying nicotine concentrations (4 - 18 mg/ml), using a smoking machine and standardized puffing parameters (i.e., puff number: 150, puff volume: 70 ml, puff duration: 1.8 sec; IPI: 10 sec). Numerous toxicants were detected in the aerosols generated including volatile organic compounds (VOCs), TSNAs, and heavy metals, but at levels ranging from 9 to 450 times lower than those detected previously in aerosols generated from tobacco cigarettes (Goniewicz et al., 2014).

Importantly, because these early pre-clinical studies did not control individual characteristics of the ECIG devices and liquids tested, the results obtained provide little generalizability to the vast array of ECIG devices and liquids available on the market. For example, by not keeping the ECIG liquid consistent, these early pre-clinical studies were unable to determine if variability in toxicant yields across devices were in fact due to differences in a

particular device characteristic (e.g., battery voltage) or the result of the nicotine concentration and/or flavorings differing across the liquids used to generate the aerosols. Furthermore, the puffing parameters used by these early pre-clinical studies to generate ECIG aerosols were consistent with that of a tobacco cigarette smoker, but other studies have demonstrated that the puff topography of an experienced ECIG user may differ markedly from that of a tobacco cigarette smoker (Hiler et al., 2017; Spindle et al., 2015). Thus, by not using puffing parameters representative of an experienced ECIG user, the toxicant content of the aerosols generated in these early pre-clinical studies may not be indicative of an ECIG user's actual toxicant exposure.

Additional pre-clinical studies have explored ECIG aerosols in a systematic and controlled manner to determine how particular device and liquid features and user puffing behaviors affect the toxicant content of ECIG aerosols (El-Hellani et al., 2016; Kosmider et al., 2014; Kosmider, Sobczak, Knysak, Goniewicz, 2014; Ogunwale et al., 2017; Sleiman et al., 2016; Soussy, El-Hellani, Baalbaki, Salman, Shihadeh, & Saliba, 2016; Talih et al., 2015). For example, several pre-clinical studies have demonstrated that altering the battery voltage of an ECIG can influence the nicotine yield of the resulting aerosol produced (Kosmider, Sobczak, Knysak, Goniewicz, 2014; Talih et al., 2015). In one study, aerosols were generated from an ECIG set to one of three voltages (3.2, 4.0, and 4.8 V) and analyzed for nicotine content. Importantly, this study held constant puffing parameters (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate) and ECIG liquid nicotine concentration (18 mg/ml). Results demonstrated that as battery voltage was increased, the yield of nicotine found in the aerosol produced also increased (Kosmider, Sobczak, Knysak, Goniewicz, 2014). Results from another study that manipulated battery voltage in an identical manner and also used the same puffing parameters to produce ECIG aerosols demonstrated that increases in battery

voltage caused an increase in the yield of various carbonyl compounds with known toxic properties (e.g., formaldehyde, acetaldehyde; Kosmider et al., 2014). Overall, increasing ECIG power output either by increasing battery voltage or decreasing the resistance of an ECIG's heating element has been demonstrated to increase toxicant yields of nicotine and various carbonyl compounds across a variety of ECIG brands and device types (El-Hellani et al., 2016; Ogunwale et al., 2017; Talih, Salman, et al., 2017).

Other studies have demonstrated that manipulating characteristics of ECIG liquids and the puffing parameters used to generate ECIG aerosols also have an effect on toxicant yields (El-Hellani et al., 2016; Talih et al., 2015; Soussy et al., 2016; Stepanov & Fujioka, 2015; Talih et al., 2017). In one pre-clinical study, results demonstrated that ECIG device/liquid characteristics and puff topography variables each affected nicotine yield when systematically manipulated while other factors were held constant (Talih et al., 2015). For example, in one condition of the study, ECIG liquid nicotine concentration was varied from 8.53 to 15.73 mg/ml while several other factors such as device voltage and puffing parameters (i.e., puff number, duration, volume, IPI, and flow rate) were held constant. As a result of increasing the nicotine concentration of the ECIG liquid, nicotine yield approximately doubled. In addition, when experimenters altered puff topography profiles while holding constant device and liquid characteristics, nicotine yield was again altered. Several distinct puffing profiles were used to generate ECIG aerosols, each intended to simulate profiles of different types of users based on the findings from previous clinical examinations of ECIG puff topography (e.g., Spindle et al., 2015). Specifically, puffing profiles meant to represent a tobacco cigarette smoker (2 sec puff duration, 66 ml puff volume, 33 ml/sec flow rate), an average experienced ECIG user taking slow (4 sec puff duration, 68 ml puff volume, 17 ml/sec flow rate) or fast puffs (4 sec puff duration, 132 ml puff volume, 33

ml/sec flow rate), and an extreme experienced ECIG user taking slow (8 sec puff duration, 136 ml puff volume, 17 ml/sec flow rate) or fast puffs (8 sec puff duration, 264 ml puff volume, 33 ml/sec flow rate). Results revealed that after 15 puffs were taken using these distinct puffing profiles and holding device voltage and liquid nicotine concentration constant, mean (SD) nicotine yield was lowest when puffing parameters were mimicking a tobacco cigarette smoker 0.11 mg (0.02) and highest in the two conditions in which puffing profiles of an extreme experienced ECIG user were used: 0.68 mg (0.07) - 0.72 mg (0.10). These findings suggest that increasing puff duration results in an increase in nicotine yield. However, increasing flow rate has a minimal effect on nicotine yield, as evidenced by the lack of difference between the fast and slow extreme experienced ECIG user profiles (Talih et al., 2015).

While pre-clinical lab studies have allowed for further understanding of the content of ECIG aerosols, the amount of nicotine found in the aerosol produced from an ECIG may not necessarily reveal the amount of nicotine delivered to user (i.e., plasma nicotine concentration), as a variety of additional factors (e.g., aerosol pH, inhalation depth, metabolism of nicotine) can also influence nicotine delivery. Furthermore, these pre-clinical studies are unable to assess the subjective effects, user puff topography, or physiological changes (e.g., changes in HR) associated with ECIG use, as they do not employ human participants. Therefore, clinical laboratory studies in which nicotine delivery (as indexed by plasma nicotine concentration), HR, subjective effects, and puff topography are assessed in tandem among a relevant population of individuals (e.g., ECIG users) are critical to understanding the acute effects associated with using these products.

Clinical Laboratory Examinations of ECIGs

Importantly, the ECIG-related clinical lab studies conducted to date have used various methodologies developed originally to examine the acute effects associated with the use of tobacco cigarettes and other alternative tobacco products. Specifically, several of these studies have used standardized ECIG use bouts in which participants are instructed to take a fixed number of puffs, separated by fixed IPIs (e.g., Hiler et al., 2017; Fearon et al., 2017; Vansickel et al., 2010). These "directed bouts" (typically consisting of 10 puffs with 30 second IPI) have been used in examinations of a variety of tobacco products (e.g., little cigars/cigarillos: Blank, Nasim, Hart, & Eissenberg, 2013; ECIGs: Hiler et al., 2017; and tobacco cigarettes: Griffiths, Henningfield, & Bigelow, 1982), increasing the ability to make comparisons regarding toxicant exposure across different products. Furthermore, standardizing participants' puffing in this manner is useful because it reflects actual puffing parameters associated with using tobacco cigarettes *ad libitum*. That is, when smoking a single cigarette, smokers take an average of 10 puffs with roughly 30 second IPIs (Breland, 2005). Some of the studies that have examined ECIG acute effects have also included *ad libitum* ECIG use bouts in which participants are instructed to use their device at their own pace, as much as they prefer, for a fixed period (Dawkins, Kimber, Doig, Feyerabend, & Corcoran, 2016; Farsalinos, Spyrou, Tsimopoulou, Stefopoulos, Romagna, & Voudris, 2014; Spindle, Hiler, Breland, Karaoghlanian, Shihadeh, & Eissenberg, 2017; Vansickel & Eissenberg, 2013; Wagener et al., 2017). The section below summarizes ECIG clinical laboratory studies conducted to date that have used these directed and *ad libitum* puffing methodologies. Some of these studies have revealed that particular device/liquid characteristics that alter ECIG toxicant yields also influence ECIG acute effects.

Nicotine Delivery. Several clinical laboratory studies have examined the nicotine delivered from an ECIG by measuring nicotine concentration in users' blood plasma following ECIG use (Dawkins et al., 2016; Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Farsalinos et al., 2015; Fearon et al., 2017; Hajek, Przulj, Phillips, Anderson, & McRobbie, 2017; Hiler et al., 2017; Nides, Leischow, Bhatter, & Simmons, 2014; St. Helen, Havel, Dempsey, Jacob, & Benowitz, 2015; St. Helen, Ross, Dempsey, Havel, Jacob, & Benowitz, 2016; Vansickel et al., 2010; Vansickel & Eissenberg, 2013; Wagener et al., 2017; Yan & D'Ruiz, 2015). Collectively, these studies have revealed that the type of ECIG, nicotine concentrations of the ECIG liquid, and experience of the user all influence nicotine delivery.

Different types of ECIGs deliver nicotine with different effectiveness. For example, in one examination of ECIG-associated nicotine delivery, 23 experienced ECIG users completed two conditions, differing only by the type of ECIG: (1) "V2" "cigalike" device with a "cartomizer" (device wattage not reported) or (2) "EVIC" tank-based ECIG with "EVOD" atomizer set at 9 watts (Farsalinos et al., 2014). Identical ECIG liquids ("Flavourart Maxblend," 18 mg/ml nicotine concentration, PG:VG ratio: 34:66) and ECIG use bouts (one 10-puff, 30 sec IPI directed bout followed by one 60-min *ad libitum* bout) were used in the two conditions. Blood samples were taken and plasma nicotine concentration assessed immediately after the directed ECIG use bout and at 15-minute intervals in the *ad libitum* bout. Results from the 10-puff directed bouts revealed that the mean (SD) plasma nicotine concentration of 6.6 ng/ml (2.9) observed in the tank-based condition was significantly greater than the observed mean of 4.9 ng/ml (2.4) in the "cigalike" condition. Similarly, the mean (SD) plasma nicotine concentration at the end of the *ad libitum* bout in the tank-based condition of 23.5 ng/ml (9.1) was significantly greater relative to the mean plasma concentration of 15.8 ng/ml (5.8) in the "cigalike" condition

(Farsalinos et al., 2014). In another study, 20 experienced ECIG users completed a single laboratory session consisting of one directed bout (10 puffs, 30 sec IPI) followed by one 120 min *ad libitum* bout with their preferred product and ECIG liquid (Wagener et al., 2017). Nine of the experienced ECIG users in the study regularly used a tank-based device (referred to by the authors as "second generation") while the remaining 11 participants used more advanced ECIG devices (referred to by the authors as "third generation") that allow for more user customization such as the ability to manually alter the wattage of the device. The mean (SD) device wattage of 8.6 (1.9) observed in the "second generation" devices was significantly lower compared to mean wattage of 71.6 (50.0) seen in the "third generation" devices that were used. The mean (SD) liquid nicotine concentration used by the "second generation" users was significantly higher, 22.3 mg/ml (7.5), relative to the mean liquid nicotine concentration used by "third generation" users: 4.1 mg/ml (2.9). Results demonstrated that after the 10-puff directed bout, the mean (SD) plasma nicotine concentration of "third generation" users was significantly greater, 17.5 ng/ml (12.9), relative to the "second generation" users, 7.3 ng/ml (2.8). Given that puff number and IPI were held constant during the directed puffing bout and that, on average, the individuals using the "second generation" devices used a lower liquid nicotine concentration, the observed differences in nicotine delivery across the different device types is likely the result of the differences in device power (Wagener et al., 2017).

Nicotine delivery associated with ECIG use also is related to the nicotine concentration present in ECIG liquids (Dawkins et al., 2016; Hiler et al., 2017). In one four-condition, within-subject study, nicotine delivery was examined in experienced ECIG users (N = 33) who used an "eGo" ECIG (3.3 volts, 1000 mAh battery) and a 510 dual-coil "cartomizer" (1.5 Ω; device power 7.3 W) loaded with 1 ml of ECIG liquid (PG:VG ratio: 70:30) for two separate 10-puff,

30 sec IPI, directed ECIG use bouts. The four conditions differed only by the nicotine concentration of the ECIG liquid (mg/ml): 0, 8, 18, or 36. Results demonstrated that nicotine delivery in these ECIG users increased in a dose-dependent manner. Specifically, mean (SD) plasma nicotine boost (difference between baseline and post ECIG use plasma nicotine concentration) after the first directed bout increased as the nicotine concentration of the ECIG liquid was increased: 0 mg/ml: 0.01 ng/ml (1.5); 8 mg/ml: 8.2 ng/ml (7.8); 18 mg/ml: 13.0 ng/ml (6.2); 36 mg/ml: 17.9 ng/ml (17.2; Hiler et al., 2017). Another study also illustrated that ECIG liquid nicotine concentration can influence nicotine delivery: ECIG-experienced individuals (N=11) completed two sessions consisting of 60 minutes of *ad libitum* ECIG use (Dawkins et al., 2016). These two sessions differed only by liquid nicotine concentration used: either low (6 mg/ml) or high (24 mg/ml). Mean (SD) plasma nicotine concentration boost following 60 minutes of *ad libitum* ECIG use was 22.0 ng/ml (16.2) in the low liquid nicotine concentration condition and 43.6 ng/ml (34.8) in the high liquid nicotine concentration condition. Thus, participants' mean nicotine boost when using the high liquid nicotine concentration was nearly twice as large relative to the mean nicotine boost resulting from using the low liquid nicotine concentration.

ECIG-associated nicotine delivery also may be related to user experience. That is, individuals who have previous experience using ECIGs are often more effective at extracting nicotine from these products compared ECIG-naïve cigarette smokers (Farsalinos et al., 2015; Hiler et al., 2017). In one study, nicotine delivery was measured after the use of a tank-based ECIG (the "EVIC" with "EVOD" atomizer set at 9 watts) filled with an 18 mg/ml solution ("Flavourart Maxblend," PG:VG ratio: 34:66) in ECIG-experienced individuals (N = 24) and ECIG-naïve cigarette smokers (N = 23; Farsalinos et al., 2015). All participants completed one,

10-puff directed ECIG use bout followed immediately by a 60-minute *ad libitum* ECIG use bout. Results demonstrated that ECIG-experienced individuals obtained significantly more nicotine from the ECIG relative to ECIG-naïve cigarette smokers at each timepoint at which blood was sampled and plasma nicotine concentration measured (Farsalinos et al., 2015). In another study, nicotine delivery and puff topography was examined in experienced ECIG users (N = 33) and ECIG naïve cigarette smokers (N = 31) across four sessions each consisting of two 10-puff directed ECIG use bouts and differing only by liquid nicotine concentration (mg/ml): 0, 8, 18, or 36 (Hiler et al., 2017). Plasma nicotine concentration was dependent on user experience such that mean plasma nicotine concentration detected in experienced ECIG users was significantly greater relative to ECIG-naïve cigarette smokers in each active nicotine condition. For example, in the 36 mg/ml condition, mean (SD) plasma nicotine boost after the first 10-puff bout was 17.9 ng/ml (SD = 17.2) for experienced ECIG users and 6.9 ng/ml (SD = 7.1) for ECIG-naïve cigarette smokers (Hiler et al., 2017). As described in further detail later, the observed differences in nicotine delivery between ECIG-experienced and ECIG-naïve cigarette smokers in these studies are likely the result of the observed differences in puff topography between these two groups (Farsalinos et al., 2015; Hiler et al., 2017).

Of particular importance, in many clinical laboratory examinations of ECIGs, the nicotine delivery profiles observed were similar to those associated with the use of tobacco cigarettes (Hiler et al., 2017; Spindle et al., 2017; Wagener et al., 2017). Indeed, comparable or greater increases in plasma nicotine can be achieved after directed (i.e., 10 puffs, 30 sec IPI) or *ad libitum* ECIG use by experienced ECIG users relative to increases observed previously by tobacco cigarette smokers using their preferred brand of cigarettes under the same conditions. For example, in one repeated measures study, experienced ECIG users (N = 29) completed two

conditions differing by the presence or absence of a mouthpiece-based topography measurement device. In both conditions of the study, participants completed a 10-puff directed ECIG use bout and a 90-minute *ad libitum* ECIG use bout using their preferred battery and liquid (≥ 12 mg/ml nicotine concentration). Participants' blood was sampled and plasma nicotine concentrations assessed immediately after the directed bout and at 30, 60, and 90 minutes after the onset of the *ad libitum* bout. After 10 directed puffs from their preferred battery, ECIG users mean (SD) plasma nicotine concentration was 16 ng/ml (11.7) collapsed across condition and at the end of the *ad libitum* ECIG use bout, their mean (SD) plasma concentration was 35 ng/ml (23.4) collapsed across condition (Spindle et al., 2017). As demonstrated in previous clinical laboratory examinations including tobacco cigarette smokers, the increase in plasma nicotine concentration observed after smoking a single tobacco cigarette under similar conditions is also approximately 14-18 ng/ml (Breland, Buchhalter, Evans, & Eissenberg, 2002; Cobb et al., 2010; Kleykamp et al., 2008; Vansickel et al., 2010; Yan & D'Ruiz, 2015). Furthermore, mean plasma nicotine concentration observed in tobacco cigarette smokers smoking their preferred brand *ad libitum* over an extended period are also similar (e.g., 27.0 ng/ml, Foulds et al., 1992; 29.2 ng/ml, Yan & D'Ruiz, 2015) to the 35 ng/ml increase observed after 90 minutes of *ad libitum* ECIG use (Spindle et al., 2017). Given that ECIGs are capable of delivering nicotine at a similarly rapid rate to tobacco cigarettes, as evidenced by comparable nicotine delivery after 10 puffs from these products, ECIGs may produce positive nicotine-related rewarding effects in a user in a similar fashion to tobacco cigarettes. Similarly, this observed rapidity of nicotine delivery from ECIGs suggests that under certain conditions, ECIGs could suppress abstinence symptoms in a manner comparable to tobacco cigarettes.

Taken together, results of several clinical laboratory examinations of ECIGs have revealed that the type of device and liquid used, as well as the experience of the user all affect nicotine delivery. Furthermore, under certain conditions, ECIG users may be able to extract comparable levels of nicotine from their device as a tobacco cigarette smoker using their preferred brand of cigarettes (Hiler et al., 2017; Spindle et al., 2017; Vansickel et al., 2010). As described next, some of the same factors that influence ECIG-associated nicotine delivery also influence ECIG-related subjective effects, including abstinence symptom suppression.

Subjective Effects. Numerous clinical laboratory studies in which ECIGs have been examined have used subjective effect measures to assess the extent to which nicotine abstinence symptoms are suppressed in tobacco/nicotine-abstinent cigarette smokers and ECIG users following ECIG use (Dawkins & Corcoran, 2014; Dawkins et al., 2016; Farsalinos et al., 2014; Hiler et al., 2017; Nides et al., 2014; Spindle et al., 2015; Vansickel et al., 2010; Vansickel & Eissenberg, 2013; Wagener et al., 2017). In one examination of 25 cigarette smokers, subjective effects including nicotine abstinence symptoms were examined following a 10-puff directed ECIG use bout using the "NJOY" "cigalike" ECIG with menthol-flavored ECIG liquid (26 mg/ml nicotine concentration). After the directed ECIG use bout, several abstinence symptoms including "Anxious" and "Craving a Cigarette" were reduced significantly from baseline (Nides et al., 2014). In another study (Vansickel & Eissenberg, 2013), abstinence symptom suppression was examined in eight experienced ECIG users using the 11-item Hughes-Hatsukami withdrawal scale and the Tiffany-Drobes QSU-brief (Hughes & Hatsukami, 1986; Tiffany & Drobes, 1991). All participants were required to abstain from nicotine/tobacco for at least 12 hours prior to the study (verified retrospectively via baseline plasma nicotine concentrations at or below 2 ng/ml). Results demonstrated that following use of their preferred device, several items from the

Hughes-Hatsukami withdrawal scale were reduced significantly from baseline including "Anxious" and "Restlessness." In addition, the QSU Factor 1 assessing participants' intention to use their product was elevated at baseline, but reduced significantly at the end of a 60-minute *ad libitum* ECIG use bout (Vansickel & Eissenberg, 2013).

Interestingly, in addition to influencing ECIG-associated nicotine delivery, the type of ECIG also appears to influence abstinence symptom suppression following ECIG use (Lechner et al., 2015). In one repeated measures study, ECIG-naïve cigarette smokers (N = 22) completed two conditions on separate days in which they used an ECIG for one 5-minute *ad libitum* ECIG use bout. The two conditions differed only by the type of device (either the "cigalike" model "Blu" or a tank-based model, the "JoyeTech" "eGo C" with 3.2 V, 900 mAh battery). The nicotine concentration of the liquids used was held constant at 16 mg/ml. Findings revealed that abstinence symptoms were suppressed in both conditions, but at a greater magnitude in the condition in which participants used the tank-based model (Lechner et al., 2015). Another study also found that under controlled conditions (i.e., 10-puff, 30 sec IPI directed bouts) the use of a tank-based ECIG resulted in more positive subjective effects such as user satisfaction relative to when a "cigalike" device was used (Dawkins, Kimber, Puwanesarasa, & Soar, 2015). However, given that plasma nicotine concentration was not an outcome measure in these studies, the extent to which differential nicotine delivery across products led to the observed differences in subjective effects is unclear.

Similar to ECIG-related nicotine delivery, the contents of ECIG liquids may also affect ECIG-associated subjective effects such as the extent to which abstinence symptoms are suppressed following ECIG use. Specifically, the use of ECIGs with a nicotine-containing liquid results in greater abstinence suppression relative to non-nicotine containing liquids when other

relevant factors such as the type of device are held constant (Dawkins, Turner, & Crowe, 2013; Dawkins, Turner, Hasna, & Soar, 2012; Hiler et al., 2017). In one study, ECIG-naïve cigarette smokers (N = 20) completed two conditions in which they were instructed to use a cartridge-based ECIG (the "Tornado") *ad libitum* for 10 minutes. In both conditions, the cartridges were pre-loaded with tobacco flavored "Totally Wicked" ECIG liquid. However, the liquid contained 18 mg/ml of nicotine in one condition and 0 mg/ml in the other. The desire to smoke and abstinence symptoms were decreased to a greater extent in the condition in which nicotine was present in the ECIG liquid relative to the no nicotine placebo condition (Dawkins et al., 2013). In addition to the nicotine content of the ECIG liquid, the presence of flavorings may also alter the subjective effects associated with ECIG use. For example, in one study, adult cigarette smokers (N =32) sampled a "cigalike" ECIG ("V2") of five different concentrations of nicotine (0, 6, 12, 18, and 24 mg/ml) and varying levels of menthol flavoring (0%, 0.5%, 3.5%; Rosbrook & Green, 2016). The ratio of PG to VG was kept consistent at 70:30. After each ECIG use, two subjective questionnaires were administered including the general Labeled Magnitude Scale (gLMS) and the Labeled Hedonic Scale (LHS) in order to assess overall sensation, coolness/coldness, harshness/irritancy, and overall liking/disliking. The gLMS is a seven point scale of perceived sensation intensity with semantic labels ranging from "no sensation" to "strongest sensation of any kind" while the LHS scale is a bipolar scale of liking/disliking with five semantic labels, each placed on the scale based on their semantic magnitudes: "like/dislike slightly," "like/dislike moderately," "like/dislike very much," "like/dislike extremely," "most liked/dislike imaginable." Results demonstrated that the presence of menthol resulted in greater reductions in perceived airway irritation and harshness when the highest ECIG liquid nicotine concentrations was used (24 mg/ml) relative to the lower concentrations (0-18 mg/ml) and also resulted in greater product

satisfaction independent of the nicotine concentration of the ECIG liquid (Rosbrook & Green, 2016). These results suggest that the presence of flavors in ECIG liquids can result in a more favorable subjective effect profile by making high concentrations of nicotine more palatable and increasing overall product satisfaction independently and thus may increase the abuse liability of these products.

In addition to device and liquid characteristics, user experience also appears to influence the extent to which abstinence symptoms are suppressed following ECIG use (Hiler et al., 2017). In the aforementioned examination of 33 experienced ECIG users and 31 ECIG-naïve cigarette smokers consisting of four conditions differing only by liquid nicotine concentration (0, 8, 18, or 36 mg/ml), user experience also influenced the magnitude of abstinence symptom suppression observed. Overall, after each 10-puff ECIG use bout, experienced ECIG users reported greater reductions in certain abstinence symptoms relative to ECIG-naïve cigarette smokers. For example, immediately following the first 10-puff bout in the 36 mg/ml condition, ECIG experienced individuals' mean (SD) score for the item "Impatient" from the Hughes-Hatsukami withdrawal scale was 7.6 (14.0) while ECIG-naïve individuals' mean score at the same timepoint was 20.8 (25.7). These results may suggest that exclusive cigarette smokers attempting to switch to ECIGs may need to undergo a learning curve before they can suppress their nicotine abstinence symptoms in a manner comparable to their preferred brand of cigarettes (Hiler et al., 2017).

Notably, at least partial abstinence symptom suppression after ECIG use has been observed in instances when no nicotine was delivered to the user (Dawkins et al., 2015; Vansickel et al., 2010). In an examination of ECIG acute effects, ECIG-naïve cigarette smokers (N = 32) completed four conditions on separate days in which they used one of two different

types of “cigalike” ECIGs (the “NPRO” and “Hydro”), smoked their own brand of cigarette, or sham smoked using an unlit cigarette of their own brand for two, 10-puff (30 sec IPI) directed bouts. Nicotine delivery and subjective measures were assessed after each product administration. Despite the two “cigalike” products not delivering detectable levels of nicotine to participants, several items assessing abstinence symptoms were suppressed including “urge to smoke a cigarette” and “craving a cigarette” (Vansickel et al., 2010). The observed suppression of abstinence symptoms despite no nicotine being delivered to these participants suggest that ECIGs may suppress some tobacco abstinence symptoms partially by providing non-nicotine behavioral stimuli associated with smoking (e.g., hand-to-mouth movements, feeling at the back of the throat, and sight of smoke-like aerosol), as has been observed previously with de-nicotinized tobacco cigarettes (Rose, Behm, Westman, Bates, & Salley, 2003; Buchhalter et al., 2005). This apparent attenuation of nicotine/tobacco abstinence symptoms caused by non-nicotine behavioral stimuli, accompanied with the potential to deliver nicotine at levels comparable to tobacco cigarettes, may suggest that ECIGs can serve as positive reinforcers to nicotine-naïve individuals and positive and negative reinforcers to individuals who already are tobacco/nicotine dependent.

The results of the clinical laboratory examinations of ECIGs to date have revealed that nicotine delivery and the subjective effects associated with ECIG use (particularly abstinence symptom suppression) is highly variable and often dependent on several device and liquid characteristics. These observed discrepancies in ECIG-associated nicotine delivery and subjective effects, while partially explained by the aforementioned relevant device and liquid characteristics, also may be due to differences in puff topography among types of ECIG users (i.e., naïve vs experienced). Described below are the clinical laboratory examinations that have

evaluated ECIG puff topography, many of which have revealed that experienced ECIG users and ECIG naïve tobacco cigarette smokers exhibit differences in various puff topography parameters.

Puff Topography. Historically, puff topography has been examined in cigarette smokers using two measurement techniques: observational methods and mouthpiece-based recording devices. Described below are brief descriptions of observational methods and mouthpiece-based recording devices and an overview of the studies that have used these methods to examine ECIG puff topography.

Observational methods. Observational methods typically consist of video recording participants smoking in the laboratory, allowing for trained video scorers to subsequently view the videos and measure various topography variables (Blank, 2008; Frederiksen, Miller, & Peterson, 1997; Lichtenstein & Antonuccio, 1981). For example, video scorers can determine puff duration by subtracting the start of a puff (e.g., the exact point when the tip of a user's cigarette begins to glow red) from the end of a puff (e.g., the last point at which a user's lips are wrapped around their cigarette; Blank, Disharoon, & Eissenberg, 2009). Several studies have demonstrated the reliability and validity of observational methods for measuring cigarette smoker's puff topography (Blank et al., 2009; Lichtenstein & Antonuccio, 1981). Limitations to using observational methods to measure puff topography include the laborious nature of the video scoring process and the inability of these methods to capture puff volume and flow rate (Blank et al., 2009).

Despite these limitations, several studies have used observational methods to assess ECIG puff topography (Farsalinos, Romangna, Tsiapras, Kyrzopoulos, & Voudris, 2013; Hua, Yip, & Talbot, 2013; St. Helen et al., 2016). In one study, puff topography was examined observationally in 80 participants (35 ECIG naïve cigarette smokers, 45 experienced exclusive

ECIG users) using an "eGo-T" tank-based ECIG (liquid nicotine concentration: 9 mg/ ml). ECIG users completed one, 20-minute *ad libitum* bout while cigarette smokers completed two 10 minute *ad libitum* bouts, one with the "eGo-T" ECIG and the other with their preferred brand of cigarettes. Experienced ECIG users' mean puff duration was significantly longer (4.2 sec, SD = 0.7) relative to cigarette smokers when using their own brand (2.1 sec, SD = 0.4) and the "eGo-T" ECIG (2.4 sec; Farsalinos et al., 2013). Another observational study examined puff topography of ECIG users (N = 64) and cigarette smokers (N = 9) using YouTube videos and similarly found that ECIG users exhibited a longer mean puff duration (4.3 sec, SD = 1.5) relative to cigarette smokers (2.4 sec, SD = 0.8; Hua et al., 2013). In addition to these observational studies, other studies have attempted to measure ECIG puff topography using mouthpiece-based topography recording devices. Some of these studies have similarly demonstrated differences in puff topography between experienced ECIG users and ECIG-naïve cigarette smokers.

Mouthpiece-based Devices. Mouthpiece-based, computerized devices can measure puff topography when a product such as a cigarette is placed in a specialized mouthpiece capable of detecting flow-induced pressure changes that occur during inhalation. These pressure changes are converted to flow rate values (ml/sec) using previously calibrated software and used to calculate various topography variables (Blank, 2008). Again, these devices, such as the Clinical Research Support System for Laboratories (CReSS), have been demonstrated to be reliable and valid for measuring puff topography in tobacco cigarette smokers (Blank et al., 2009; Buchhalter & Eissenberg, 2000). Mouthpiece-based devices have several important features including increased precision and efficiency relative to observational methods and the ability to measure puff volume and flow rate (Blank, 2008).

Several studies conducted to date have used an extant mouthpiece-based device (the CReSSMicro), designed originally to measure cigarette smoker's puff topography, to examine ECIG puff topography (Behar, Hua, Talbot, 2015; Goniewicz, et al., 2013; Lee, Gawron, & Goniewicz, 2015; Norton, June, O'Connor, 2014). However, given that extant mouthpiece-based devices were not designed with parameters intended to provide adequate sensitivity for measuring ECIG users' puff topography, these devices may not be suitable for measuring ECIG puff topography (Spindle et al., 2015). More specifically, the flow-detecting threshold for the mouthpiece-based device CReSS is 15 ml/sec (Stewart, Vinci, Adams, Cohen, & Copeland, 2013), meaning that any portion of a puff below 15 ml/sec will not be recorded. Because experienced ECIG users puff at a lower mean flow rate relative to cigarette smokers (Behar et al., 2015; Eissenberg, 2014; Spindle et al., 2015) topography recording devices intended to measure puff topography of cigarette smokers' may not have a flow-detecting threshold sensitive enough to capture ECIG puff topography adequately. The inadequate sensitivity of extant mouthpiece-based devices for measuring ECIG puff topography may explain why experienced ECIG users displayed lower mean puff duration (2.7 sec) when using an ECIG with the CReSSMirco (Behar et al., 2015) relative to other reports (Farsalinos et al., 2013; Hua et al., 2013; Spindle et al., 2015).

To address the device sensitivity concerns associated with using existing topography recording devices to measure ECIG topography, researchers from the American University of Beirut (AUB) have developed a topography recording device designed to accommodate the low flow rate puffs exhibited typically by ECIG users. Specifically, the device is designed such that the pressure-sensing transducer and orifice dimensions of the mouthpiece are capable of detecting flow rates as low as 3 ml/sec (Spindle et al., 2017). This device has been used to

measure ECIG puff topography in several studies (Hiler et al., 2017; Spindle et al., 2015; Spindle et al., 2017). In one aforementioned examination of ECIG acute effects, this ECIG-specific computerized topography measurement device was used to measure puff topography in experienced ECIG users (N =29) who completed two conditions in which they used their preferred ECIG device and liquid. In both conditions, participants completed one 10-puff directed bout (30 sec IPI) and one 90-minute *ad libitum* ECIG use bout. The conditions differed only by the presence or absence of the mouthpiece-based recording device. Results demonstrated that ECIG acute effects (i.e., plasma nicotine and subjective effects) were not influenced by the presence of the topography recording device. Comparisons were also made between puff topography recorded in the directed bout to that of tobacco cigarette smokers from a previous study and significant differences puff duration, puff volume and flow rate were detected between groups (N = 123; Kleykamp et al., 2008). Specifically, ECIG users exhibited a mean (SD) puff duration of 4.5 sec (1.6), a mean puff volume of 124.6 ml (89.1), and a mean flow rate of 27.8 ml/sec (19.5) while cigarette smokers using their own brand of cigarettes exhibited a mean (SD) puff duration of 1.4 sec (0.4), a mean puff volume of 51.3 ml (19.2), and a mean flow rate of 38.0 ml/sec (9.7; Spindle et al., 2015; Spindle et al., 2017). Results from this study demonstrated the suitability of this ECIG-specific recording device for measuring ECIG users' puff topography while having minimal influence on the acute effects of ECIG use.

In the above-mentioned four-condition (0, 8, 18, or 36 mg/ml liquid nicotine concentration) within-subjects examination of 33 experienced ECIG users and 31 ECIG-naïve cigarette smokers, puff topography was also assessed across each study condition in all participants (Hiler et al., 2017). Collapsed across condition, experienced ECIG users exhibited a mean (SD) puff duration of 5.6 sec (3.0) which was significantly longer relative to ECIG-naïve

cigarette smokers who exhibited a mean puff duration of 2.9 sec (1.5) during the first directed ECIG use bout. These observed differences in puff topography likely provide an explanation for experienced ECIG users obtaining greater mean plasma nicotine concentrations relative to ECIG-naïve cigarette smokers at each non-zero liquid nicotine concentration examined in this study (Hiler et al., 2017).

Other Topography Measurement Methods. In addition to observational and mouthpiece-based methods, researchers have also attempted to measure ECIG puff topography by using ECIG devices (e.g., "eVIC" and "Smokio") programmed by the manufacturer to record variables such as puff number, duration, and time of puff occurrence (Dautzenberg & Bricard, 2015; Dawkins et al., 2016; Farsalinos et al., 2015). While the reliability and validity of these devices has not been examined empirically, mean puff duration values recorded from ECIG-experienced individuals with these devices are comparable (e.g., 3.84 – 5.2 sec; Dawkins et al., 2016) to those reported in other studies using observational methods (e.g., 4.2 sec; Farsalinos et al., 2013) or mouthpiece-based devices (e.g., 4.51 – 5.29 sec; Spindle et al., 2017).

Need for Systematic Evaluation of ECIG Device and Liquid Features

Of the aforementioned pre-clinical and clinical examinations, perhaps the most informative have been those that have examined the influence of one particular device or liquid characteristic in a systematic fashion while holding constant relevant device, liquid, and topography characteristics (e.g., Hiler et al., 2017; Talih et al., 2015). These studies have highlighted how particular device features (e.g., battery voltage), liquid components (e.g., liquid nicotine concentration), and puff topography variables (e.g., puff duration) alter the composition of the aerosols produced from ECIGs and/or the acute effects associated with ECIG use. Conversely, studies in which users have been permitted to use their preferred devices and liquids

in an *ad libitum* fashion (e.g., Spindle et al., 2017; Vansickel & Eissenberg, 2013; Wagener et al., 2017) have not allowed for investigators to elucidate the influence of individual device and liquid features on aerosol composition and/or ECIG acute effects. The present study seeks to examine the influence of two other common components of ECIG liquids (PG and VG) by varying the ratio of PG to VG in a systematic fashion while holding constant many device, liquid, and topography characteristics that may influence study outcomes.

Why Examine PG and VG?

Overview of PG and VG. PG and VG are the most common ingredients found in ECIG liquids, often accounting for upwards of 95% of the total contents of these solutions (Han et al., 2015). The primary function of these two ingredients in ECIG liquids is to facilitate the production of aerosol, thereby acting as a vehicle to carry nicotine and flavorants to the user's mouth, throat, and lungs. These two substances are used in a variety of other commercially available products such as cosmetics, foods, and beverages and are on the FDA's generally recognized as safe (GRAS) list for these purposes. PG and VG are also used commonly as humectants in other tobacco products such as cigarettes and waterpipe (hookah) tobacco in order to control and maintain moisture (Carmines & Gaworski, 2005; Schubert, Hahn, Dettbarn, Seidel, Luch, & Schulz, 2011). Several studies have demonstrated that exposure to PG (either orally, topically, or intravenously) in large quantities over a short period of time may result in lactic acidosis, acute kidney injury, hyperosmolarity, cardiac arrhythmia, hemolysis, and a sepsis-like syndrome (Lim, Poole, & Pageler, 2014; Miller, Forni, Yogaratnam, 2008; Zar, Graeber, & Perazellat, 2007). PG toxicity may be more likely in individuals with underlying renal insufficiencies or hepatic dysfunction (Zar et al., 2007). In addition, VG is also considered to be mildly toxic when administered either orally, subcutaneously, or intravenously in large

doses over a short period of time. For example, acute exposure to VG in animals such as mice, rats, guinea pigs, or rabbits can result in tremors, hyperemia in the lung, kidneys, and small intestines, vomiting, diarrhea, and ataxia (Deichmann, 1941; Hine, Anderson, Moon, Dunlap, & Morse, 1953).

Importantly, the extent to which PG and VG are safe for inhalation in an aerosolized form is unclear. Limited studies have examined passive exposure to PG via fog machines. In some of these reports, individuals participating in theatrical productions who were exposed to PG-containing fog reported more respiratory symptoms and mucous membrane irritation relative to those who participated in productions without the fog machine present (Burr, 1994; Moline, Golden, Highland, Wilmarth, & Kao, 2000). Another study demonstrated that individuals working near PG-based fog machines (within 10 feet) exhibited lower lung function, and increased incidences of headache, dizziness, shortness of breath, nasal symptoms, and tightness of the chest (Varughese, Teschke, Brauer, Chow, Netten, & Kennedy, 2005). Passive exposure to mist-containing PG can result in eye and respiratory irritation in addition to slight airway obstruction and upper respiratory irritation (Wieslander, Norback, & Lindgren, 2001). Also of concern, both PG and VG can undergo thermal decomposition when exposed to high temperatures, such as those achieved when ECIG liquids are aerosolized, which can result in the formation of potentially toxic and carcinogenic carbonyl compounds such as formaldehyde, acetaldehyde, and acrolein, each of which can also cause irritation and inflammation to the skin, eyes, respiratory tract, and mucous membranes during active or passive exposure (NCBI, 2018; Stein, Antal, & Jones, 1983). Overall, given the evidence demonstrating PG and VG's acute adverse effects and the potentially harmful toxicants formed as a result of heating these two

solvents at high temperatures, there is great uncertainty concerning the long-term health implications of repeated daily inhalations of aerosolized PG and VG.

PG and VG's influence on ECIG use. Interestingly, anecdotal evidence from ECIG users suggests that different ratios of PG to VG may influence some aspects of ECIG use. In one examination of communications among ECIG users on the popular forum website Reddit, the purported influence of different liquid PG:VG ratios and flavors on various aspects of ECIG use were assessed (Li, Zhan, Wang, Leischow, & Zeng, 2016). Overall, 3,605 Reddit posts containing references to ECIG liquid PG:VG ratio, nicotine concentration, and flavors were identified and included in the final analyses (published by 2,394 unique users). Results revealed that ECIG users reported that liquids containing higher proportions of PG and higher nicotine concentrations provided a better "throat hit." Interestingly, ECIG liquids containing mostly PG tended to be tobacco, menthol or beverage flavor while those containing mostly VG tended to be fruit, cream, or nut flavor. Out of all liquids referenced in the various posts, "high VG" liquids were mentioned most frequently (52%), "balanced but high VG" were the second highest (25%), "balanced" was the third highest (14%), "balanced but high PG" was the fourth highest (5%), and "high PG" was the least referenced PG:VG ratio (3.5%; Li et al., 2016).

Examinations of individual posts on websites such as Reddit also provide insight regarding the effect of PG:VG ratio on ECIG use. For example, ECIG users describing the influence of PG and VG report: "higher VG = more vapour production with a muted flavour compared to PG. Higher PG = less vapour production but improved flavour. Higher PG might also give you a better throat hit. VG is thicker than PG, so the vapour it produces tends to be thicker, and smoother" (whateverdaheva, 3/26/15, Reddit.com). In addition, ECIG vendors often include information in marketing materials describing the effects of PG and VG to potential

consumers: “According to some vapers, PG gives the user more of a throat hit, although VG produces more vapor. Vapers may also notice a marginally sweeter taste from the VG-based liquid” (goldengatevapor.com).” Thus, some ECIG users report that liquids with higher levels of PG typically provide a greater throat hit, more pronounced nicotine/flavor delivery, and produce less exhaled aerosol while predominantly VG liquids tend to provide a “smoother” throat sensation while also producing more visible exhaled aerosol.

In addition to the anecdotal evidence, some empirical evidence suggests that the content of PG and VG in ECIG liquids can influence the particle size, toxicant content, and visibility of ECIG aerosols. For example, when aerosols are produced in a preclinical setting and assessed for particle size using specialized equipment (e.g., an electrical mobility particle sizer spectrometer), ECIG liquids with higher concentrations of VG produce larger particles than those containing PG (Baassiri et al., 2017; Meng, Son, Kipen, Schwander, & Delnevo, 2017; Zhang, Sumner, & Chen, 2013). Particle size is a main predictor of the extent to which a particular aerosol will deposit in the lungs of a user. In general, aerosols with smaller particles are deposited to a greater extent in a user’s lungs, meaning the contents of the aerosol will be absorbed more readily into the bloodstream (Heyder, 2004; Zhang et al., 2013). Given that high PG liquids generate aerosols of a smaller particle size relative to those produced from liquids containing mostly VG, PG liquids may also result in more nicotine and/or flavorants being delivered to a user relative to liquids containing VG predominantly.

PG and VG can also influence the toxicant content of ECIG aerosols in several important ways (Bitzer et al., 2017; Geiss, Bianchi, & Barrero-Moreno, 2016; Jensen, Strongin, & Peyton, 2017; Kosmider et al., 2014; Kosmider, Sobczak, Knysak, Goniewicz, 2014). In one pre-clinical examination, the influence of differing ratios of PG and VG on the yield of carbonyl compounds

(known tobacco-related toxicants) was explored across ten commercially available ECIG liquids with liquid nicotine concentrations ranging from 18-24 mg/ml. Puffing parameters (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate) and the ECIG used to generate the aerosols ("eGo-3" with "crystal 2 clearomizer," 3.2 V battery) were kept consistent across all liquids examined. Study findings revealed that the yield of numerous carbonyl compounds was greatest in solutions containing high amounts of PG (Kosmider et al., 2014). In addition, liquids containing more PG produce more free radicals (Bitzer et al., 2017), toxicants found in tobacco smoke that can induce oxidative stress and contribute to development of smoking-related diseases (USDHHS, 2014). Interestingly, other studies have revealed that while PG and VG can both contribute to the formation of certain carbonyl compounds such as formaldehyde, the presence of other carbonyl compounds in some ECIG aerosols may be attributed primarily to PG or VG (Geiss et al., 2016; Wang et al., 2017). For example, in another pre-clinical study, researchers systematically varied ECIG liquid PG:VG ratio and device wattage and determined that acetaldehyde was formed primarily by PG while acrolein was formed primarily by VG and both solvents contributed to the formation of formaldehyde (Geiss et al., 2016). Indeed, in tobacco cigarettes, increasing VG can also increase selectively the levels of acrolein present in the resulting smoke (Carmines & Gaworski, 2005). Interestingly, acetaldehyde has been demonstrated to enhance the reinforcing effects of nicotine; rodents will self-administer approximately five times more nicotine if it is mixed with acetaldehyde at concentrations that approximate those found in tobacco cigarettes (Talhout, Opperhuizen, van Amsterdam, 2007). Thus, nicotine delivered from liquids containing more PG may be more reinforcing to users given that aerosols formed from liquids high in PG will also likely contain more acetaldehyde, although no studies have examined this possibility to date.

PG:VG ratio can also influence the amount of nicotine present in ECIG aerosols (Baassiri et al., 2017; Kosmider, Sobczak, Knysak, Goniewicz, 2014; Talih et al., 2017). For example, in one pre-clinical study, aerosols were generated using ECIG liquids of three different PG:VG ratios (0:100, 50:50, 100:0) and nicotine yield was examined. Several factors known to influence toxicant yield were held constant including: puff topography (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate), battery voltage (3.2 V), and ECIG liquid nicotine concentration (18 mg/ml). Results indicated that as PG:VG ratio was increased, the nicotine yield of the aerosols produced also increased (Figure 3). That is, the ECIG liquid with PG as the only solvent produced the highest yield of nicotine in the subsequent aerosol, while the liquid with VG as the principal solvent produced the lowest amount of nicotine (Kosmider, Sobczak, Knysak, Goniewicz, 2014). In another pre-clinical examination experimenters systematically examined the extent to which a variety of device/liquid factors and puffing conditions influenced nicotine flux (the amount of nicotine emitted from an ECIG per unit time; Talih et al., 2017). Aerosols were generated, captured, and examined from over 100 sessions of varying device, liquid, and puffing conditions. Results demonstrated that across two different device wattages (4 W and 11 W), increasing the PG content of the ECIG liquid used resulted in an increase in nicotine flux, albeit to a lesser extent with the 11 W condition, while holding other relevant liquid (e.g., liquid nicotine concentration) and puffing parameters (e.g., puff duration) constant (Talih et al., 2017).

Finally, as referenced in anecdotal reports, ECIG liquid PG:VG ratio can influence the visibility of the resulting aerosol produced. In one pre-clinical study, particle size, total particulate matter, and visibility of ECIG aerosols was examined across three different PG:VG ratios (100PG:0VG, 70PG:30VG, or 0PG:100VG) while numerous device factors (e.g., device

wattage) and puff topography variables (e.g., puff duration, volume) were held constant. Results demonstrated that liquids with greater VG had a greater light-scattering coefficient, due to a larger particle size, thus increasing the visibility of these aerosols relative to mostly PG liquids. Despite producing particles of a greater size and visibility, aerosols produced from predominantly VG liquids had a smaller mass (and thus lower total particulate matter concentrations) relative to liquids high in PG. In addition, the smallest light-scattering coefficient was observed in the 100PG:0VG condition, demonstrating the liquids containing mostly PG would likely produce aerosols with very minimal visibility (Baassiri et al., 2017). Also contributing to differences in visibility, particles produced from predominantly VG liquids tended to evaporate more slowly relative to those produced from high PG liquids, meaning VG-containing aerosols remained visible for longer (Baassiri et al., 2017).

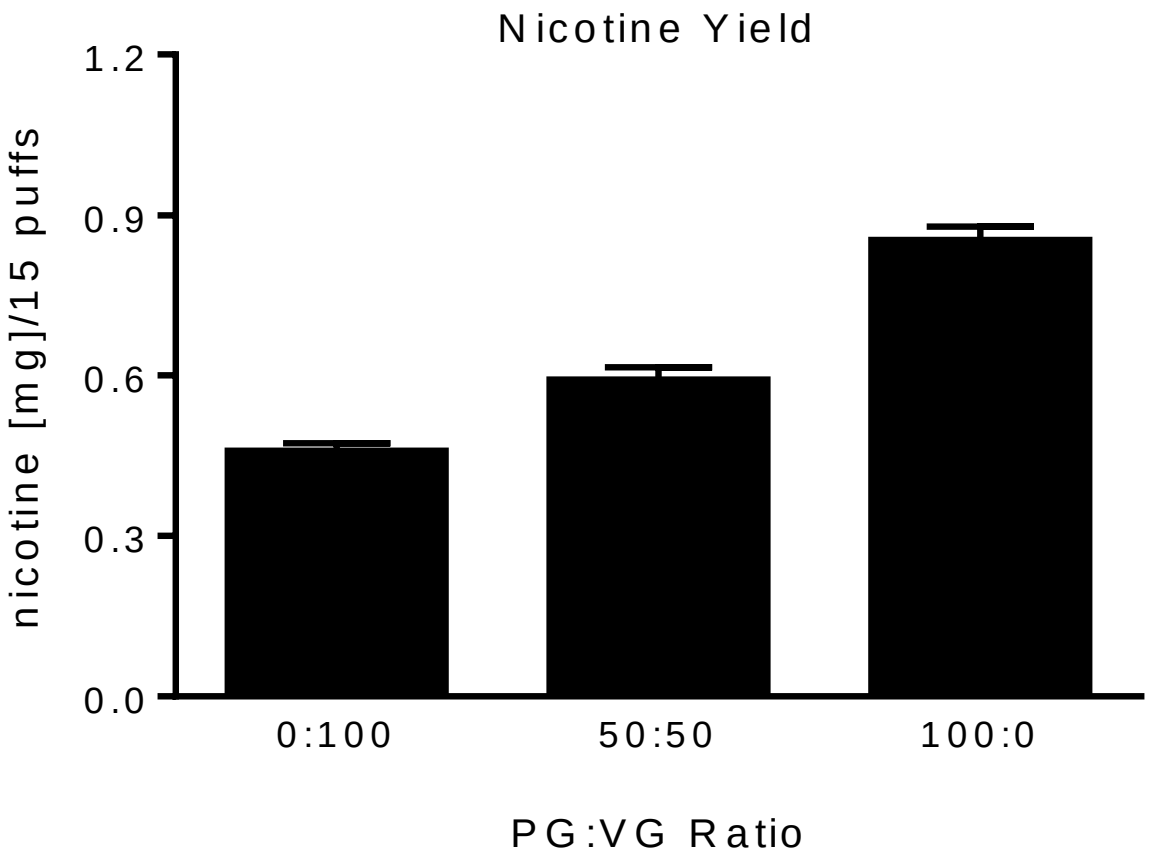

Figure 3. Mean nicotine yield (mg; + SEM) from three sessions in which aerosols were produced from an ECIG differing only by the ratio of PG to VG. The PG:VG ratios used were: 0PG:100VG, 50PG:50VG, and 100PG:0VG. Numerous factors including the device (3.2V "eGo-3"), liquid nicotine concentration (18 mg/ml), and puffing parameters (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate) were held constant. Figure adapted from Kosmider, Sobczak, Knysak, Goniewicz (2014).

Another study explored whether the differences in aerosol visibility produced by different combinations of PG and VG affected smoking urges in tobacco cigarette smokers (King, Howe, Newell, McNamara, & Cao, 2017). In this study, participants (N = 53) were randomized to one of two experimental conditions in which they interacted with a confederate using an ECIG ("Eleaf iStick Pico," 40 W, 0.27Ω) *ad libitum* for 20 minutes with either a 100PG:0VG liquid, thus producing little to no visible aerosol, or a very high VG liquid (23PG:73VG) capable of producing a visible aerosol. In each study condition, participants also viewed the same confederate drinking a bottle of water prior to watching them use the ECIG. Participants were asked to rate their desire to smoke a cigarette at three timepoints: after watching the confederate drink water, and at 5 and 20 minutes after the onset of the confederate's ECIG use bout. Results demonstrated that participants desire to smoke was significantly higher in the high VG condition in which a visible aerosol was present relative to the 100PG:0VG condition in which no visible aerosol was produced (King et al., 2017). These findings suggest that the sight of smoke-like aerosol produced by ECIGs may evoke smoking urges in tobacco cigarette smokers but the extent to which the sight of aerosol facilitates ECIG users' suppression of abstinence symptoms remains unclear. Nonetheless, differing degrees of visibility across ECIG aerosols may influence the extent to which ECIGs can suppress abstinence symptoms. As mentioned previously, at least partial abstinence symptom suppression can still be achieved when ECIGs (e.g., Vansickel et al, 2010) or cigarettes (e.g., Buchhalter et al., 2005) do not deliver nicotine suggesting that the sight of aerosol and other non-nicotine behavioral stimuli may also contribute to abstinence symptom suppression from ECIGs. Therefore, ECIGs may be most effective at suppressing abstinence symptoms when delivering nicotine and producing visible aerosol, although this research question has yet to be explored.

Taken together, results from anecdotal evidence, as well as the few empirical examinations conducted, indicate that the ratio of PG to VG in ECIG liquids alters particle size, the content of ECIG aerosols and other potentially important aspects of ECIG use such as aerosol visibility. Given these findings and the ubiquity of PG and VG in ECIG liquids, systematic clinical laboratory studies are needed in order to understand more precisely the influence of PG and VG on the acute effects associated with ECIG use. Further understanding of how ECIG liquid PG:VG ratio influences nicotine delivery, subjective effects (such as nicotine abstinence symptom suppression), and puff topography associated with ECIG use may be necessary for determining appropriate regulations for these products. Specifically, if results from this study demonstrate that liquid PG:VG ratio has a profound influence on nicotine delivery and the other outcomes of interest, FDA may need to consider regulating liquids such that only one PG:VG ratio is allowable in order to avoid liquid PG:VG ratio serving as a moderator to the amount of nicotine an ECIG delivers. That is, if FDA ultimately regulates other aspects of ECIGs such as liquid nicotine concentration or device wattage in an attempt to limit the amount of nicotine a user may obtain from their device, but PG:VG ratio remains unregulated, ECIG users and manufacturers may be able to circumvent these attempts to limit nicotine delivery by altering liquid PG:VG ratio themselves. Furthermore, subjective effects results from this study may demonstrate whether certain PG:VG ratios are more desirable to users than others, providing the FDA with information that may be useful should regulators decide to select a single, standard PG:VG ratio for all ECIG liquids.

Statement of the Problem

ECIGs have become increasingly popular in adolescent and adult populations but little systematic, empirical research has been conducted on them to date. The limited available pre-

clinical and clinical research has revealed that several factors including device characteristics (e.g., device wattage), ECIG solution contents (e.g., liquid nicotine concentration), and user experience can affect the toxicant content of the aerosols produced from ECIGs in addition to the acute effects associated with ECIG use. For example, newer generation devices operating at higher wattages produce more nicotine in the resulting aerosol, deliver more nicotine to users, and suppress abstinence symptoms more effectively relative to low wattage "cigalike" devices. However, more systematic and controlled clinical laboratory studies are necessary to understand fully the individual influences of ECIG device and liquid characteristics on important outcomes such as nicotine delivery, subjective effects, and puff topography, as the influence of many device and liquid components are not well understood. For example, limited pre-clinical research and anecdotal evidence suggests that two ubiquitous solvents used in ECIG liquids (PG and VG) may influence some aspects of ECIG use including nicotine yield, "throat hit," and aerosol visibility, but the effects of these two commonly used ingredients have not been explored systematically in actual ECIG users. Thus, the extent to which PG and VG alter the acute effects of ECIG use remains unknown.

The Present Study

In this clinical laboratory study, experienced ECIG users completed four conditions in which they used an ECIG that differed by the ratio of PG to VG: 100:0, 55:45, 20:80, and 2:98. In each study condition, several factors were held constant including the ECIG ("eGo" with 3.3 V, 1000 mAh battery), "cartomizer," (510-style, dual-coil, 1.5 Ω), liquid nicotine concentration (18 mg/ml), ECIG liquid flavor ("Virginia Pure" tobacco flavor; liquids were purchased from the same vendor), puff number (10), and IPI (30 sec). Primary outcome measures were nicotine delivery, HR, subjective effects, and puff topography. Secondary outcome measures included the

particulate matter recorded during each study session, an index of the amount of aerosol exhaled from participants across each liquid PG:VG ratio, and the overall amount of liquid consumed by participants over the course of each session.

Statement of the Hypothesis

The main hypotheses of the present study were as follows: (1) nicotine delivery would increase as the concentration of PG increases with the greatest nicotine delivery coming from the 100PG:0VG condition, and (2) the most favorable subjective effect profiles, including the most pronounced abstinence symptom suppression and most positive sensory ECIG effects, would be observed in the 55PG:45VG condition. This hypothesis was based on the assumption that the 100PG:0VG liquid would likely not produce a visible aerosol, a non-nicotine stimulus that could contribute to reducing nicotine/tobacco abstinence symptoms or other subjective effects associated with ECIG use.

Method

Selection of Participants

Thirty ECIG-experienced community volunteers completed this within-subjects design. For the outcome measure plasma nicotine, a power analysis conducted prior to the study revealed that this number of participants would be sufficient to obtain power of at least 0.80 (i.e. provide an 80% chance of detecting an effect, if an effect exists). The power analysis conducted for plasma nicotine was performed using the means and standard deviations from three pre-clinical studies that examined the extent to which altering ECIG liquid PG:VG ratio influenced nicotine yield, as no prior studies have examined the influence of PG:VG ratio on nicotine delivery in human participants (Table 1; Baassiri et al., 2017; Kosmider, Sobczak, Knysak, Goniewicz, 2014; Talih, personal communication). In each of these pre-clinical studies, aerosols were generated using ECIG liquids of three different PG:VG ratios (100PG:0VG, 50PG:50VG, 0PG:100VG) analogous to those used in the present study (100PG:0VG, 55PG:45VG, 2PG:98VG). One of the studies included a fourth condition (20PG:80VG; Baassiri et al., 2017) also used in the present study. Each of these studies held constant: liquid nicotine concentration at 18 mg/ml (as in the present study), device wattage (4.27 - 4.5 W), and several puff topography variables including puff number, duration, volume, IPI, and flow rate (see Table 1 for details). Where possible, effect sizes derived using the means and standard deviations from these three studies were averaged to create a single effect size for each possible within group comparison. For example, given that each of these three studies compared nicotine yields produced from a 100PG:0VG liquid to those produced from a 0PG:100VG liquid, the effect size for this comparison from each respective study was calculated and these three effect sizes were then averaged to produce a single effect size. Results of this power analysis revealed that a sample

size of 8 participants would be sufficient to achieve power > 80% for any possible within group comparison assuming an alpha error probability (i.e., Type 1 error rate) of less than 0.05 and a small ($r \geq 0.30$) to moderate ($r \geq 0.50$) correlation among repeated measures.

Another power analysis was performed for the additional outcome measures in the present study (i.e., subjective effects, puff topography, particulate matter, and amount of liquid consumed) and revealed that 27 participants were required to detect moderate effect sizes ($f > 0.35$) and obtain power of at least 0.80, assuming a moderate correlation among repeated measures (i.e., $r \geq 0.50$), and an alpha error probability of less than 0.05 (Barcikowski & Robey, 1985). Given that no prior studies have examined the influence of ECIG liquid PG:VG ratio on these additional outcome measures, effect sizes from previous studies could not be drawn upon when conducting this power analysis. Thus, using the criteria described above, 30 participants were sufficient to detect within-group differences for all outcome measures in the present study.

Participants were recruited by Institutional Review Board (IRB)-approved advertisements and word-of-mouth. All experimental sessions occurred 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). To be eligible for the study, participants had to be healthy, weigh over 110 pounds, aged 18-55, use < 5 conventional tobacco cigarettes daily, use ≥ 1 ml of ECIG liquid daily, use regularly ECIG solution with a nicotine concentration ≥ 6 mg/ml, and have been using their ECIG for ≥ 3 months (all according to self-report). Participants were also eligible if they used 10 ml per day of any liquid nicotine concentration other than 0 mg/ml. Finally, participants had to agree to abstain from all nicotine/tobacco products for at least 12 hours prior to each study session.

Table 1.

Mean (SD) Nicotine Yield Data from Three Pre-Clinical Studies Used for Plasma Nicotine Power Calculation.

PG:VG Ratio	Study 1[a]	Study 2[b]	Study 3[c]
0:100	0.46 (0.09)	0.52 (0.19)	0.13 (0.02)
20:80	N/A	N/A	0.17 (0.01)
50:50	0.59 (0.14)	1.16 (0.07)	0.33 (0.01)
100:0	0.85 (0.16)	1.73 (0.20)	0.58 (0.02)

Note: N/A = condition not included in that study

[a]Kosmider, Sobczak, Knysak, Goniewicz, 2014. Factors held constant: liquid nicotine concentration: 18 mg/ml; device wattage: 4.27; puffing parameters: 15 puffs of 2 sec duration, 17 sec IPI, 50 ml volume, and 25 ml/sec flow rate.

[b]Talih, personal communication (unpublished preliminary data). Factors held constant: liquid nicotine concentration: 18 mg/ml; device wattage: 4.54; puffing parameters: 15 puffs of 4 sec duration, 10 sec IPI, 66.67 ml volume, and 16.67 ml/sec flow rate.

[c]Baassiri et al., 2017. Factors held constant: liquid nicotine concentration: 18 mg/ml; device wattage: 4.3; puffing parameters: 15 puffs of 4 sec duration, 10 sec IPI, 66.67 ml volume, and 16.67 ml/sec flow rate.

Exclusion criteria included: history of chronic disease or psychiatric condition, regular use of a prescription medication, marijuana use >10 and alcohol use >25 days in the past 30, and any use of other illicit drugs (e.g., cocaine, opioids, benzodiazepines, and methamphetamine) in the past 30 days (all according to self-report). Lastly, a positive test for pregnancy (via urinalysis at screening) was exclusionary for women.

Screening and Informed Consent Procedures

Prospective participants took part in a screening process consisting of two parts. The first involved a phone interview or online screen in which potential participants were asked about their health status and ECIG/tobacco use. Individuals meeting the study requirements were asked to come to the laboratory for a second in-person screening, where they provided additional information about their health, ECIG/tobacco use, use of other licit and illicit drugs, and demographic information. During the in-person screening, participants also gave their informed consent to participate in the study and women provided a urine sample for a pregnancy test.

A total of 41 individuals consented to participate in the study. Of these 41 individuals, 11 did not complete the study and thus were not included in the final analyses. Of these 11 individuals who consented but did not complete the study, four were determined to be ineligible during in-person screening process (i.e., two had been using an ECIG < 3 months, one used < 1 ml of ECIG liquid per day, and one weighed < 110 pounds) while the remaining seven began the study but were discontinued prior to study completion (i.e., three failed to attend study sessions, venous access could not be attained in three participants, and one participant exhibited an elevated HR after using the ECIG). Of the 30 participants who completed the study, 29 were males and the mean (SD) age of these individuals was 26.9 years (7.1). The self-reported races of the 30 individuals who completed the study were as follows: 21 “White/Caucasian,” four

"Asian," two "Black/African American," two "Other," and one "more than one race." Regarding self-reported ethnicity, 29 participants reported being "Not Hispanic or Latino" while the remaining participant identified as "Hispanic or Latino." The individuals who completed the study had been using ECIGs for mean (SD) length of 16.6 months (12.3), regularly used a mean (SD) liquid nicotine concentration of 8.5 mg/ml (4.2), used a mean (SD) volume of liquid per day of 6.3 ml (5.7), and used a mean (SD) of 0.03 cigarettes per day (0.2). Notably, only one participant reported current cigarette use. Specifically, this participant reported using one cigarette per day. Twenty-two participants were former cigarette smokers, although two of these individuals reported less than five lifetime uses of cigarettes (see Table 2 for further details on screening data). Thus, eight participants reported never using cigarettes in their lifetime. Additional information gathered from these participants at screening concerning their preferred ECIG device settings and liquid characteristics are reported in Table 3.

Participant Safety and Rights

Methods and procedures of this study involved no more than minimal risk for individuals who already use ECIGs daily, and had been conducted many times at the CBPL without the occurrence of a serious adverse event. Participants were informed that they may experience some mild discomfort after abstaining from nicotine for 12 hours, but that this discomfort would not be medically dangerous. Procedures involving the drawing of participants' blood intravenously also posed minimal risk such as the potential for bruising and/or infection at the catheter site. However, the risks associated with drawing blood were minimized by the trained nursing staff, aseptic procedures, and use of sterile disposable equipment. Overall, risks and side effects associated with using ECIGs/nicotine were typical for the target population and no unanticipated adverse events occurred.

Table 2.

Overall Demographic and Screening Data for Study Completers (N=30).

	Mean or N	SD
Age (years)	26.9	7.1
Screen CO	2.9	2.2
Cigarettes/day	0.03	0.2
Former regular smokers	20	
Past number of cigarettes/day[a]	12.6	10.3
Length of time smoking (years)[a]	6.6	6.2
Months abstinent from cigarettes[a]	21.1	18.0
Volume ECIG liquid used/day (ml)	6.3	5.7
Liquid concentration (mg/ml)	8.5	4.2
Duration ECIG use (months)	16.6	12.3
Fagerström TND [b]	3.7	2.4
Penn State Dependence[c]	8.8	4.8

[a]Data from 20 participants who were former regular cigarette smokers but were not current smokers (Note: two additional participants reported < 5 lifetime cigarettes uses and were therefore not considered former regular smokers).

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

[c]Penn State Electronic Cigarette Dependence Index (Foulds et al., 2015).

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	Device Wattage	Liquid Flavor
1	Kangertech mini	6	80/20	29	Tobacco
2	IPV 3 (Pioneer)	6	30/ 70	50	Dessert
3	eGo-T	12	unknown	unknown	Peach
4	Cuboid mini	12	50/50	12	Black Currant Tart
5	eGo-T	12	unknown	unknown	Blueberry
6	Cool Fire IV	12	70/30	16	Mango
7	Kangertech EVOD	18	70/30	75	Strawberry Vanilla
8	eGo	18	50/50	unknown	Tobacco
9	eVic VTC Mini	9	20/80	35	Kiwi Custard
10	eVic VTC Mini	3	70/30	45	Coffee
11	Congestus Mod	3	20/80	unknown	Grape
12	eGo	12	50/50	6.8	Mixed Berry
13	Kangertech	6	unknown	15	Vanilla
14	Wismec RX200	3	30/70	60	Berry Citrus
15	Sigelei	3	20/80	50	Strawberry Crème Pie.
16	eGo (AIO)	6	unknown	22.8	Watermelon
17	Aspire (Cleito)	3	40/60	42	Apricot Horchata
18	Wismec RX 2/3	6	30/70	55.5	Peach/Blueberry
19	Aspire	12	0/100	30.5	Fruit
20	Wismec Rx200	6	30/70	85.6	Grape
21	Vape forward	6	40/60	75	Tangerine/Blueberry
22	SmokTech (Alien)	6	30/70	37	Cookies
23	SmokTech	6	20/80	65	Cherry Mountain Dew
24	SmokTech	12	20/80	55	Strawberry
25	SmokTech	9	20/80	40	Fruit
26	eVic (AIO)	6	70/30	60	Kiwi
27	SmokTech	12	20/80	unknown	Fruit
28	eleaf	12	70/30	50	Strawberry
29	Sigelei	12	20/80	150	Fruit
30	Wismec	6	45/65	204	Milk & Berries

Trained staff including a registered nurse and on-call medical monitor, ensured protection of participants' safety and rights throughout the study. HR and blood pressure (BP) were monitored continuously and sessions were ended if a participant's systolic BP dropped below 90 or above 140 or if their HR dropped below 50 or above 120 bpm (one participant was discontinued for a HR above 120 bpm). Data were treated with professional standards regarding confidentiality; all data were identified by an alphanumeric code only and stored in locked rooms and computers only available to CBPL staff.

Materials

In each of the four study sessions, participants used an "eGo" ECIG (3.3 volt, 1000 mAh battery) with a 510-style, dual-coil "cartomizer" (1.5 Ω). The "cartomizer" was pre-loaded by research staff with 1 ml of ECIG solution ("Virginia Pure" tobacco flavor), containing 18 mg/ml of nicotine. This flavor was also chosen based on the results of another study (Hiler et al., 2017) in which 33 ECIG-experienced users were given the option of using tobacco or menthol flavor after sampling each of these flavors with the same ECIG and cartomizer used in the present study (these liquids were provided by the same vendor used in the present study). Twenty-one of the 33 completers in this study chose the tobacco flavor over the menthol flavor (Hiler et al., 2017). All ECIG liquids were made by and purchased from a local ECIG vendor, Avail (Richmond, VA). All "eGo" ECIG batteries were purchased from the same local vendor. The 510-style "cartomizers" were produced by SmokTech (Shenzhen, China) and purchased online. The ratio of PG to VG in the ECIG solution loaded into the "cartomizer" differed by session. The PG:VG ratios as labeled by the vendor were: 100:0, 70:30, 30:70, and 0:100. Upon subsequent independent verification (using procedures described in Peace et al., 2016), the ratios were determined to be 100:0, 55:45, 20:80, and 2:98. Liquid nicotine concentrations were also verified

prior to administration and determined to be ± 1 mg/ml of the intended nicotine concentration (18 mg/ml). The resistance, (Ω), of all "cartomizers" used were also verified and determined to be ± 0.1 Ω of the intended resistance (1.5 Ω) in all cases.

Procedures

Figure 4 illustrates the study procedure for each of the four experimental sessions. Once screening procedures and informed consent were completed, participants attended the CBPL on VCU's medical campus on four separate days (separated by a minimum of 48 hours) for four, single-blind, independent laboratory sessions, each lasting approximately 3.5 hours. Sessions were ordered by Latin square, and differed only by the PG:VG ratio of the liquid placed in the "cartomizer" (100:0, 55:45, 20:80, and 2:98). Participants were unaware of the liquid PG:VG ratio in each condition. "Cartomizers" were weighed before and after each experimental session in order to assess the amount of liquid consumed by each participant. In order to verify twelve hours of abstinence from combustible products, participants were asked to provide expired air CO concentration at the beginning of each session (≤ 10 ppm, as in Breland et al., 2002). Given that ECIGs do not typically produce CO, abstinence from ECIGs and other non-combustible nicotine products were verified retrospectively by examining participants' baseline plasma nicotine concentration. Due to noncompliance with abstinence requirements by ECIG users in other studies (e.g., Hiler et al., 2017), the present study also required all participants to undergo a one-hour observation period prior to the onset of each study session during which they were not permitted to use any nicotine/tobacco product. Because nicotine has a relatively short half-life of 1-2 hours (Benowitz, 2008), this additional hour likely increased the chances that a participant who was not abstinent for the full 12 hours would still be experiencing nicotine-abstinence symptoms when the session commenced and served to further decrease participants' baseline

plasma nicotine concentration (Blank, Breland, Cobb, Spindle, Ramôa, & Eissenberg, 2016). After the measurement of participants' expired air CO and the one-hour observation period, an intravenous catheter was inserted into a forearm vein, and monitoring of physiological responses (HR and BP) commenced. After the intravenous catheter was inserted successfully, an AM510 Sidepak personal aerosol monitor (Shoreview, Minnesota, USA) was placed in the room at eye level with the participant in order to obtain 30 minutes of baseline particulate matter readings. Thirty minutes after the insertion of the intravenous catheter, 7 ml of blood was sampled to establish baseline plasma nicotine concentration and participants responded to several computerized questionnaires intended to assess nicotine abstinence symptoms and other subjective effects. Thus, the baseline blood sample was taken approximately one hour and 30 minutes after the participant arrived for their session. After responding to the baseline subjective questionnaires, participants completed the first of two directed ECIG use bouts (10 puffs, 30 sec IPI). IPI was defined as the time between the onset of one puff and the onset of a subsequent puff (as in Farsalinos et al., 2014; Hiler et al., 2017; Vansickel et al., 2010). For both of the directed ECIG use bouts, trained research staff instructed participants when to puff and verified compliance. Immediately following the tenth and final puff, another 7 ml of blood was sampled and participants responded to subjective questionnaires again. Blood was sampled again and subjective questionnaires responded to at 15, 30, 45, and 55 minutes after the onset of the first directed bout. After this sixth blood sample, the second directed ECIG use bout began, at exactly 60 minutes after the first directed bout. A seventh blood sample was taken immediately after the second ECIG use bout, followed by administration of subjective questionnaires. Three final rounds of blood samples and subjective questionnaires occurred 15, 30, and 45 minutes after the onset of the second directed ECIG use bout. After the ninth blood draw and subjective

questionnaire administration (30 minutes after the final ECIG use bout), the Sidepak personal aerosol monitor was removed from the room and particulate matter measurements concluded. After the completion of the tenth set of subjective measures (45 minutes after the final ECIG use bout), 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; see Figure 4).

Outcome Measures

Physiological Measures. Blood samples were centrifuged, stored at -70°C, and sent to VCU's Bioanalytical Analysis Core Laboratories for analysis of nicotine concentration, limit of quantitation (LOQ) = 2 ng/ml (see Breland, Kleylamp, & Eissenberg, 2006). HR and BP were monitored continuously using Criticare Systems model 507, fitted with pulse oximeter, and expired air CO was measured via a BreathCO monitor (Vitalograph, Lenexa, KS).

Puff Topography. Puff topography variables including puff volume, duration, number, IPI, and flow rate were measured using a mouthpiece-based ECIG topography recording device developed and manufactured at AUB and used in several other clinical laboratory examinations of ECIGs (e.g., Hiler et al., 2017; Spindle et al., 2015). This instrument functions similar to commercially available cigarette topography measurement devices such as CReSS (see introduction and Blank, 2009). Importantly, this device does not interfere with nicotine delivery or abstinent symptom suppression in experienced ECIG users (Spindle et al., 2017) and provides sensitivity sufficient to ensure valid measurements at flow rates as low as 3 ml/sec, as commercially available cigarette topography measurement devices may not be sensitive enough to measure ECIG topography accurately (Spindle et al., 2017). Several mouthpieces were manufactured for the device, each of which was calibrated separately using a custom built automatic digital flow calibrator. The instrument was calibrated prior to each session.

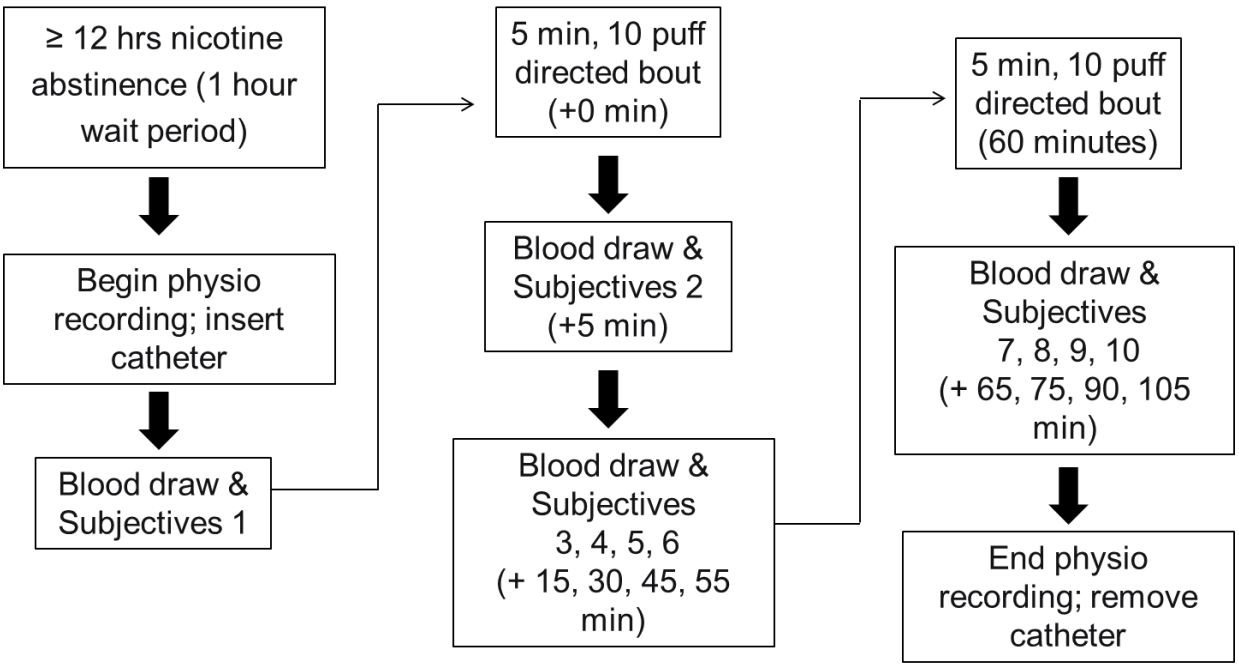

Figure 4. Session procedure involving participants visiting the laboratory for four, 3.5-hour sessions differing only by ECIG liquid PG:VG ratio.

Subjective Questionnaires. Four of the five subjective questionnaires were administered to participants using a computerized visual analog scale (VAS). These VAS scales consisted of a word or phrase being centered on a horizontal line with "not at all" on the left and "extremely" on the right. Participants recorded their responses by moving a mouse cursor and clicking at any point on the horizontal line, with scores being expressed as a percentage of total line length. Where necessary, questionnaires were modified from the original versions intended for tobacco cigarette smokers, such that the words "cigarette" or "smoking" were replaced by "e-cigarette" or "vaping." The remaining questionnaire (the gLMS) was administered with a paper copy and participants were instructed to mark their responses on the vertical line using a pen, with scores expressed as a percentage of total line length.

Hughes-Hatsukami Withdrawal Scale. Nicotine abstinence symptoms were assessed using the Hughes-Hasukami withdrawal scale. This scale consisted of 11 items including: "Anxious," "Craving and e-cigarette/nicotine," "Depression," " Difficultly concentrating," "Drowsy," "Hunger," "Impatient," "Irritable," "Restlessness," "Desire for sweets," and "Urge to use an ECIG" (Hughes & Hatsukami, 1986). Two items from the original questionnaire ("Increased eating" and "Insomnia/Disturbed sleep") were omitted for the present study.

Tiffany Drobes-QSU Brief. Abstinence symptom suppression also was assessed using the Tiffany Drobes-QSU Brief. This scale consisted of ten items, each presented on the screen as a phrase centered above seven boxes ranging from ("strongly disagree") to ("strongly agree"). The ten items were follows: "I have a desire for an ECIG right now," "Nothing would be better than smoking an ECIG right now," If it were possible, I would probably use an ECIG right now," I could control things better right now if I could use an ECIG," "All I want right now is an ECIG," "I have an urge for an ECIG," "An ECIG would taste good now," "I would do almost

anything for an ECIG now," "Smoking an ECIG would make me less depressed," and " I am going to use an ECIG as soon as possible" (Cox, Tiffany, & Christen, 2001). These items form two factors: (1) intention to use one's product (0-30) and (2) anticipation from relief from abstinence symptoms (0-24; Tiffany & Drobes, 1991).

Direct Effects of Nicotine. The direct effects of nicotine scale was used to further assess the effects of nicotine delivery from ECIGs and nicotine–related side effects on participants. This scale consists of 10 items: "Confused," "Dizzy," "Headache," "Heart Pound," "Lightheaded," "Nauseous," "Nervous," "Salivation," "Sweaty," and "Weak" (Evans, Blank, Sams, Weaver, & Eissenberg, 2006).

Direct Effects of ECIG Use. This 10-item scale was adapted from the "Direct Effects of Tobacco" scale, which was developed with items reported in studies assessing the subjective effects of smoking (e.g. Foulds et al., 1992; Pickworth, Bunker, & Henningfield, 1994). The 10 items of this scale included: "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?"

General Labeled Magnitude Scale. This category-ratio scale consisted of seven semantic labels of increasing sensitivity including: "no sensation," "barely detectable," "weak," "moderate," "strong," "very strong," and "strongest imaginable sensation of any kind" (Green, Shaffer, & Gilmore, 1993). As in Rosbrook and Green (2015) participants were asked to rate the overall sensation of the flavoring, the harshness/irritancy, and the "throat hit" provided by the ECIG after the two product administrations in each session.

Particulate Matter. Particulate matter was measured during each session using an AM510 SidePak personal aerosol monitor (Shoreview, Minnesota, USA). This device is capable of measuring concentrations of particles with a diameter between 0.1(or 100 nm) and 2.5 micrometers (μm) in ambient air. Hereafter, particulate matter recorded in the present study will be referred to as $PM_{2.5}$, a common abbreviation for particulate matter falling between 0.1 and 2.5μm. The SidePak operates by drawing ambient air into the device where the $PM_{2.5}$ present then scatters the light from an internal laser. Mass concentrations of the particles drawn into the device are then derived based on the extent of light scattering detected. The Sidepak was set to a one-minute log interval, meaning 60 consecutive one-second measurements of $PM_{2.5}$ were averaged to produce a single value for each minute the device was recording. Prior to each session, the SidePak was zero-calibrated using a particulate air (HEPA) filter and the device's impactor was lubricated per the manufacturer's guidelines (as in Cobb, Vansickel, Blank, Jentink, Travers, & Eissenberg, 2013; Hyland, Travers, Dresler, Higbee, & Cummings, 2008). This device and corresponding settings/procedures have also been used in other studies to assess indoor air quality (i.e., $PM_{2.5}$) associated with passive exposure to ECIGs (Soule, Maloney, Spindle, Rudy, Hiler, & Cobb, 2017), tobacco cigarettes (Hyland et al., 2008) and waterpipe or hookah (Cobb et al., 2013).

Amount of Liquid Consumed. In order to determine the total amount of liquid consumed, each "cartomizer" was weighed at the beginning and end of each session. The difference between the baseline and post-session "cartomizer" weights represented the total amount of liquid consumed (in grams) for each session (as in Wagener et al., 2017). In order to derive the amount of liquid consumed in milliliters, the total amount of liquid consumed in grams from each session was divided by the density of the liquid being used in that particular

condition; the different concentrations of PG and VG resulted in slightly different densities across liquids. The respective densities (g/ml) of the four different liquids used in this study were as follows: 100PG:0VG: 1.036, 55PG:45VG: 1.137, 20PG:80VG: 1.216, and 2PG:98VG: 1.257.

Data Analysis Plan

Data preparation. For plasma nicotine data, instances in which the measurement value was lower than the assay's LOQ was replaced with the LOQ (2 ng/ml; as in Vansickel et al., 2010), providing a more conservative approach than assuming that each value below the LOQ was zero. For plasma nicotine, area under the curve (AUC) was calculated for both 10-puff directed bouts (bout 1 AUC: timepoints 1-5; bout 2 AUC: timepoints 6-10) within each condition using the linear trapezoidal method (as in Benowitz et al., 1988; see Vaughan & Dennis, 1978). Prior to analysis, HR data were averaged to produce a single value for the five minutes prior to each ECIG use bout and prior to each blood draw (10 values in total). The software of the topography device integrated flow rate data to produce the values for the topography variables puff number, puff duration, puff volume, IPI, and mean flow rate (see Shihadeh, Azar, Antonios, & Haddad, 2004 for details). Prior to analysis, the software performed two data cleaning procedures to correct for transducer noise. These cleaning procedures consisted of combining into a single puff any two puffs that are separated by 300 ms or less and deleting any puffs with a duration 300 ms or less. Remaining data for each variable were averaged for all participants to produce single values for each 10-puff directed bout. Prior to analysis, $PM_{2.5}$ data were averaged to produce three values for each participant and experimental session: one for the 30 minutes prior to the first 10-puff directed bout, one for the 10 minutes during the two 10-puff bouts, and one for the 30 minutes after the last puff in the second 10-puff bout. As in other studies assessing indoor air quality associated with ECIGs (e.g., Soule et al., 2017), a calibration factor of 0.32

was applied to the raw $PM_{2.5}$ data (recorded in μg) collected by the SidePak in order to produce more precise estimates of $PM_{2.5}$ (see Hyland et al., 2008). Specifically, each individual value was multiplied by 0.32 prior to analysis.

Data Analyses. Repeated measures analysis of variance (ANOVAs) were used to examine data for plasma nicotine, HR, subjective measures, puff topography, $PM_{2.5}$, and total amount of liquid consumed. Four (condition) by ten (time) repeated measures ANOVAs were used to examine plasma nicotine and HR. Plasma nicotine AUC analysis contained two levels of time (one for each 10-puff directed ECIG use bout). For the Hughes-Hatsukami, Tiffany Drobes-QSU Brief, Direct Effects of Nicotine, and Direct Effects of ECIG use scales, each questionnaire item (or factor in the case of the QSU Brief) was examined individually with condition (four levels) and time (10 levels) as the two within-subjects factors; the Direct Effects of ECIG use scale only had nine levels of time, as participants could not provide a baseline score for these items prior to ECIG use. Items from the gLMS were also examined individually with the same two factors (condition and time) but the time factor only consisted of two levels. Across all plasma and subjective data, less than 0.002% of data were missing. For the few instances of missing data for these measures, values were imputed by averaging the value before and the value after the missing cell.

Puff topography variables including puff duration, puff volume, and flow rate were analyzed using condition and time as the two within-subjects factors. The time factor for topography analyses consisted of two levels, as these data were averaged to produce a single value for each variable within each directed ECIG use bout. Due to an equipment malfunction, one participants' topography data was not recorded in two separate sessions and thus this individual was excluded from all topography analyses. Puff number and IPI were held constant

in this study, and thus are not outcome measures. The repeated measures ANOVA conducted on $PM_{2.5}$ data also contained condition and time as the two factors, but these data contained three timepoints (the average of the 30 minutes prior to the first ECIG use bout, the average of the 10 minutes during the two bouts, and the average of the 30 minutes after the second bout). Median $PM_{2.5}$ values were also calculated (as in Cobb et al., 2013; Soule et al., 2017), in order to lessen the influence of potential outliers and to allow for comparisons to studies that did not report mean $PM_{2.5}$ values. Finally, the total amount of liquid consumed by participants (in ml) was analyzed using a one-way repeated measures ANOVA with condition (four levels) as the lone factor.

Violations of sphericity were adjusted using Huynh-Feldt corrections. In order to maintain statistical power and limit type 1 error rate for plasma nicotine, HR, and subjective effects, planned contrasts (paired samples t-tests) were also conducted across conditions at the two timepoints immediately following each ECIG-use bout (e.g., timepoints 2 and 7 for plasma nicotine). At these two post-ECIG use timepoints, the mean value for each outcome measure in the 100PG:0VG condition was compared to the corresponding mean values in the 2PG:98VG, 20PG:80VG, and 55PG:45VG conditions. Because these comparisons were non-orthogonal, a Bonferroni correction was applied (Keppel, 1991). Given that there were three comparisons made at each timepoint, the threshold for statistical significance for these planned comparisons was an alpha error probability level of less than .017. Tukey's Honestly Significant Difference (HSD) was used to explore all other significant main effects and interactions. Statistical analyses were performed using IBM SPSS (Version 24.0).

Prior to conducting analyses for all aforementioned outcome measures, baseline plasma nicotine data were examined retrospectively to ensure that participants complied with the study

requirement of ≥ 12 hours abstinence from all nicotine-containing products prior to each session. Participants with baseline plasma values of 5.0 ng/ml or higher were considered to have not complied with abstinent requirements (as in Hiler et al., 2017; Spindle et al., 2017). Ultimately, three of the 30 participants who completed the study were considered to have not abstained prior to at least one experimental session (two participants did not abstain prior to one of their four sessions while the remaining participant did not abstain prior to three of their four sessions). In order to determine whether non-compliance by these three participants influenced study outcomes, analyses on all outcome measures were conducted with and without these three individuals and the two sets of results were compared. Exclusion of these individuals largely did not influence study results. Indeed, of the 40+ repeated measures ANOVAs conducted with and without non-abstainers, results only differed for three subjective items when these individuals were excluded. Ultimately, the three non-abstinent participants were included in all final analyses because the vast majority of results were unaffected by their omission and the higher N resulted in greater statistical power.

Results

Results from all outcome measures including plasma nicotine, HR, subjective measures, puff topography, $PM_{2.5}$, and amount of liquid consumed are described below. Table 4 displays results from the statistical analyses (main effects and interactions) for all physiological and subjective effect measures.

Physiological Measures

Plasma Nicotine. Figure 5 depicts the mean plasma nicotine results for each condition and timepoint. As indicated in Table 4, there was no time by condition interaction observed for this outcome measure, although there was a trend towards significance [F (27, 783) = 1.41, p <.08]. However, significant main effects of time and condition were observed for plasma nicotine. For the main effect of time, post-hoc analyses (Tukey's HSD) revealed that, collapsed across condition, mean (SD) plasma nicotine concentration increased significantly from 2.60 ng/ml (1.85) at baseline to 10.40 ng/ml (8.11) immediately after the first bout and to 11.11 ng/ml (6.80) immediately after the second bout.

Planned contrasts were also conducted to examine more precisely the influence of PG:VG ratio on participants' plasma nicotine concentrations immediately after each directed ECIG use bout. No significant differences were detected across conditions immediately after bout 1 (timepoint 2). However, immediately after bout 2 (timepoint 7) mean (SD) plasma concentration of 13.40 ng/ml (8.99) in the 100PG:0VG condition was significantly higher relative to the mean (SD) plasma nicotine concentrations of 9.59 ng/ml (7.95) in the 20PG:80VG condition [t (29) = 2.56, p < .017] and 8.58 ng/ml (5.41) in the 2PG:98VG condition [t (29) = 2.72, p < .017]. No significant differences were detected between the two highest PG conditions after bout 2.

In order to assess total nicotine exposure within each ECIG use bout, AUC was also examined for plasma nicotine in each condition. Main effects of condition and time, but no time by condition interaction, were observed for AUC data (Table 4). Collapsed across condition, mean AUC was significantly higher in bout 2 relative to bout 1. AUC values for the two 10-puff directed bouts within each condition are displayed in Figure 6. For bout 1 AUC, post-hoc analyses (Tukey's HSD) revealed that mean (SD) AUC for the 100PG:0VG condition (276.75 ng•min/ml; 221.49) was significantly higher relative to the mean AUC for the 2PG:98VG condition (178.32 ng•min/ml; 183.76). Mean AUC values for bout 1 did not differ significantly across other conditions. For bout 2 AUC, post-hoc analyses (Tukey's HSD) revealed that mean (SD) AUC for the 100PG:0VG condition (373.24 ng•min/ml; 274.09) was significantly higher relative to the mean AUC for the 2PG:98VG (257.75 ng•min/ml; 217.26) and 20PG:80VG conditions (251.93 ng•min/ml; 224.49; Tukey's HSD, $p < .05$). Mean AUC values for bout 2 did not differ significantly across other conditions (Figure 6).

Table 4.

Statistical Analyses Results for Physiological and Subjective Measures.

Outcome measures	Condition (*C*) *F*	*p*	η^2_p	Time (*T*) *F*	*p*	η^2_p	*C* × *T* *F*	*p*	η^2_p
Plasma Nicotine[a]	5.11	<.01*	0.15	32.81	<.001*	0.53	1.41	*ns*	0.05
Area Under the Curve	5.14	<.01*	0.15	30.03	<.001*	0.51	0.79	*ns*	0.03
Heart Rate[a]	1.62	*ns*	0.05	48.27	<.001*	0.63	0.97	*ns*	0.03
Subjective Measures									
Hughes-Hatsukami[a]									
Anxious	0.28	*ns*	0.01	7.87	<.01*	0.21	1.18	*ns*	0.04
Craving	0.34	*ns*	0.01	16.15	<.001*	0.36	0.97	*ns*	0.03
Depression	0.69	*ns*	0.02	3.06	*ns*	0.10	0.96	*ns*	0.03
Difficulty Concentrating	0.32	*ns*	0.01	8.12	<.001*	0.22	0.89	*ns*	0.03
Drowsy	0.52	*ns*	0.02	9.90	<.001*	0.26	1.32	*ns*	0.04
Hunger	2.73	*ns*	0.09	6.83	<.01*	0.19	0.68	*ns*	0.02
Impatient	0.59	*ns*	0.02	5.43	<.01*	0.16	1.04	*ns*	0.04
Irritable	0.42	*ns*	0.01	3.73	<.05*	0.11	0.85	*ns*	0.03
Restless	0.73	*ns*	0.02	2.89	<.05*	0.91	1.00	*ns*	0.03
Sweets	0.58	*ns*	0.02	1.88	*ns*	0.06	2.04	*ns*	0.05
Urge to Vape	0.70	*ns*	0.02	15.97	<.001*	0.36	0.71	*ns*	0.02
Direct Effects of Nicotine[a]									
Confused	2.30	*ns*	0.07	0.65	*ns*	0.02	1.17	*ns*	0.04
Dizzy	0.29	*ns*	0.01	3.35	<.05*	0.10	0.57	*ns*	0.02
Headache	0.42	*ns*	0.01	1.25	*ns*	0.04	1.09	*ns*	0.04
Heart Pound	0.34	*ns*	0.01	1.97	*ns*	0.06	0.67	*ns*	0.02
Lightheaded	0.55	*ns*	0.02	6.84	<.05*	0.19	1.09	*ns*	0.04
Nauseous	0.59	*ns*	0.02	1.51	*ns*	0.05	1.28	*ns*	0.04
Nervous	0.21	*ns*	0.01	2.00	*ns*	0.07	0.47	*ns*	0.02
Salivation	0.40	*ns*	0.01	0.96	*ns*	0.03	0.62	*ns*	0.02
Sweaty	1.08	*ns*	0.04	1.29	*ns*	0.04	0.64	*ns*	0.02
Weak	2.75	*ns*	0.09	1.19	*ns*	0.04	0.50	*ns*	0.02
Direct Effects of Vaping[b]									
Awake	5.53	<.01*	0.16	3.77	<.01*	0.12	2.25	<.05*	0.07
Calm	3.26	<.05*	0.10	7.32	<.001*	0.20	1.09	*ns*	0.04
Concentrate	5.03	<.01*	0.15	1.49	*ns*	0.05	1.58	*ns*	0.05
Dizzy	2.90	*ns*	0.09	5.00	<.01*	0.15	1.00	*ns*	0.03
Pleasant	6.94	<.01*	0.19	2.80	<.05*	0.09	0.71	*ns*	0.02
Reduce hunger	2.09	*ns*	0.07	3.68	<.01*	0.11	0.66	*ns*	0.02
Right Now	0.11	*ns*	0.01	14.65	<.001*	0.34	0.41	*ns*	0.01
Satisfying	3.98	<.05*	0.12	4.70	<.01*	0.14	0.56	*ns*	0.02
Sick	0.49	*ns*	0.02	0.16	*ns*	0.01	0.81	*ns*	0.03
Taste Good	3.14	<.05*	0.10	0.93	*ns*	0.03	0.69	*ns*	0.02
Gen. Linear Magnitude[c]									
Flavor	1.86	*ns*	0.06	0.56	*ns*	0.02	0.97	*ns*	0.03
Harshness	4.74	<.01*	0.14	0.92	*ns*	0.03	0.82	*ns*	0.03
Throat Hit	11.47	<.001*	0.28	1.53	*ns*	0.05	1.44	*ns*	0.05
Tiffany-Drobes[a]									
Factor 1 (Intention)	0.74	*ns*	0.03	19.65	<.001*	0.40	1.15	*ns*	0.04
Factor 2 (Anticipation)	3.04	<.05*	0.10	9.71	<.001*	0.25	1.11	*ns*	0.04

Note: ns = not significant.

[a]df C = (3,87); df T = (9,261); df C x T (27,783).

[b]df C = (3,87); df T = (8,232); df C x T (24,696).

[c]df C = (3,87); df T = (1,29); df C x T (3,87).

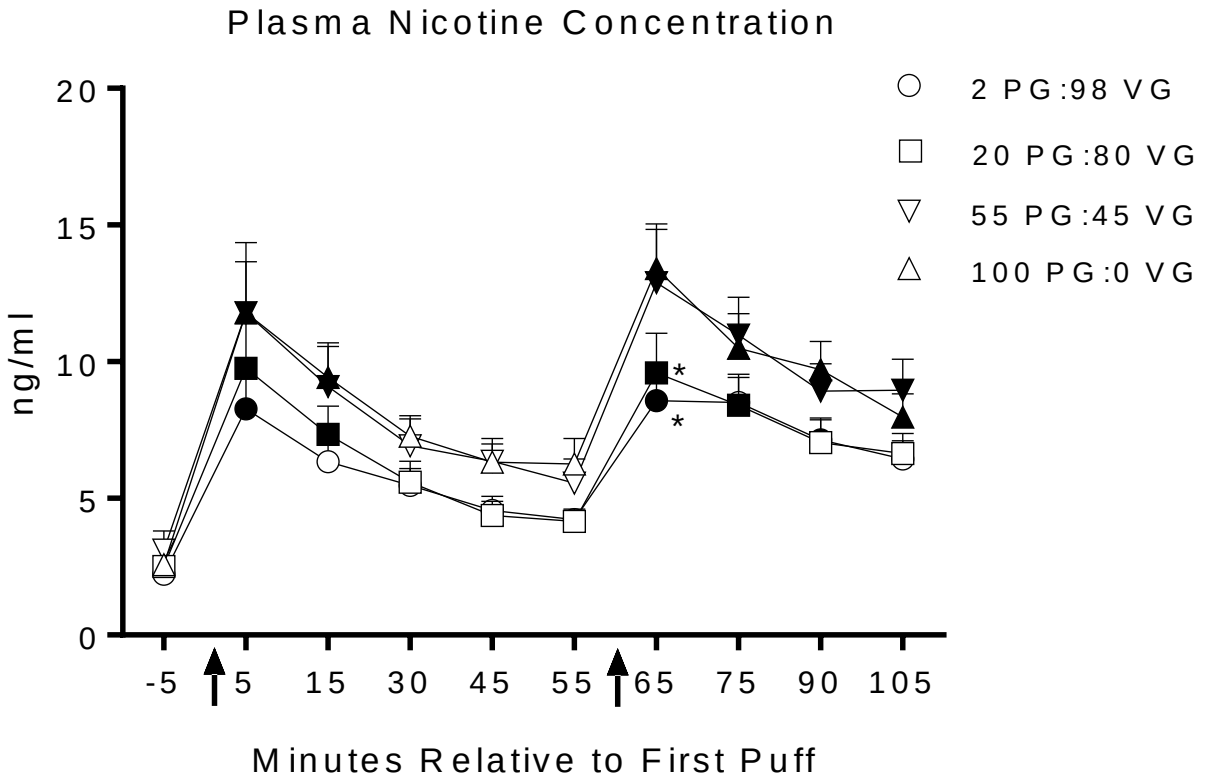

Figure 5. Mean plasma nicotine concentration (+ SEM) from 30 ECIG-experienced participants during four independent sessions that differed only by PG:VG ratio. Arrows beneath the x-axis indicate the onset of each 10-puff ECIG use bout. Filled symbols indicate a significant difference from baseline (-5 timepoint). Asterisks (*) indicate significant differences from the 100PG:0VG condition at that timepoint (planned contrasts with Bonferroni correction: *p*s < .017).

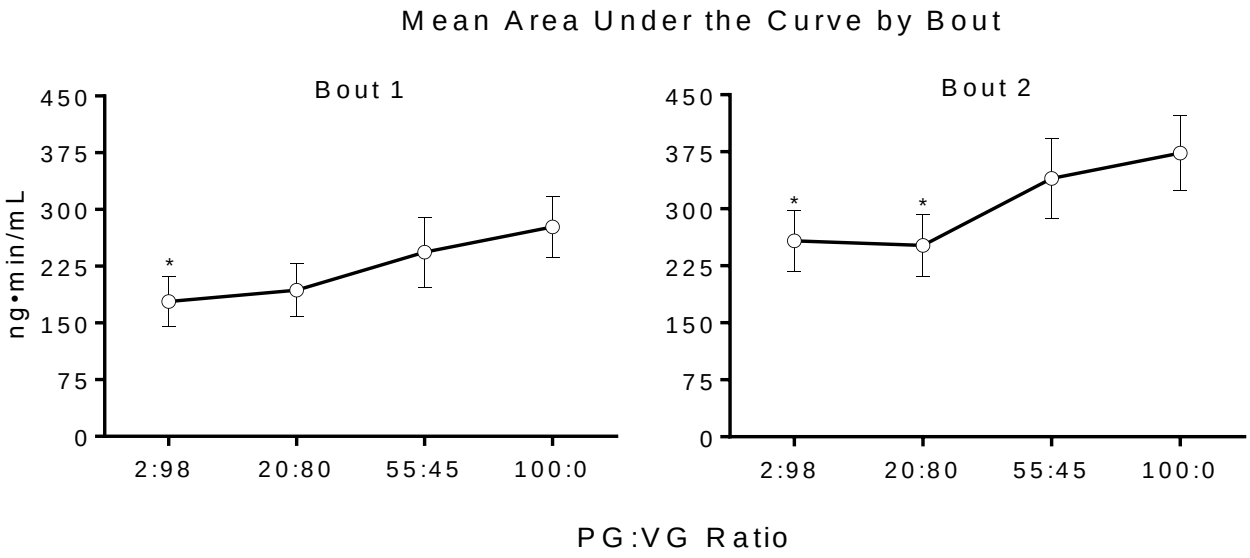

Figure 6. Mean area under the curve (+/- SEM) for plasma nicotine data from 30 ECIG-experienced participants during four independent sessions that differed only by PG:VG ratio. Asterisks (*) indicate significant differences from the 100PG:0VG condition within that bout (Tukey's HSD, *p*s < .05).

Heart Rate. As indicated in Table 4, no significant condition by time interaction or main effect of condition was observed for HR, though there was a significant main effect of time. Collapsed across condition, mean (SD) HR increased significantly from 63.71 beats per minute (bpm; 5.37) at baseline to 70.98 bpm (5.87) immediately after bout 1 and 70.92 bpm (6.86) immediately after bout 2 (Tukey's HSD, $p < .05$; Figure 7). Planned contrasts conducted at the two timepoints immediately after each bout (timepoints 2 and 7) did not reveal any significant differences across conditions.

Subjective Measures

Hughes-Hatsukami Withdrawal Scale. As indicated in Table 4, significant main effects of time (but no significant main effects of condition or condition by time interactions) were observed for the items "Anxious," "Craving an e-cigarette/nicotine," "Difficulty concentrating," "Drowsy," "Hunger," "Impatient," "Irritable," "Restlessness," and "Urge to use an ECIG." Figure 8 shows the results for "Craving" and "Difficulty Concentrating" (two of the items with the largest *F* values). Post-hoc analyses revealed that VAS scores for the items "Anxious," "Craving an e-cigarette/nicotine," "Difficultly Concentrating," "Drowsy," and "Urge to use an ECIG" were reduced significantly following both ECIG use bouts relative to baseline (Tukey's HSD, *p*s < .05). For example, collapsed across condition, mean (SD) VAS score for the item "Urge to use an ECIG" decreased significantly from 53.83 (32.71) at baseline to 28.49 (25.76) after bout 1 and 29.29 (26.97) after bout 2. Subsequent post-hoc tests (Tukey's HSD) for the items "Hunger," "Impatient," "Irritable," and "Restlessness" did not reveal any significant reductions in VAS scores from baseline. Planned contrasts conducted at the two timepoints immediately after bouts 1 and 2 did not detect any significant differences across conditions.

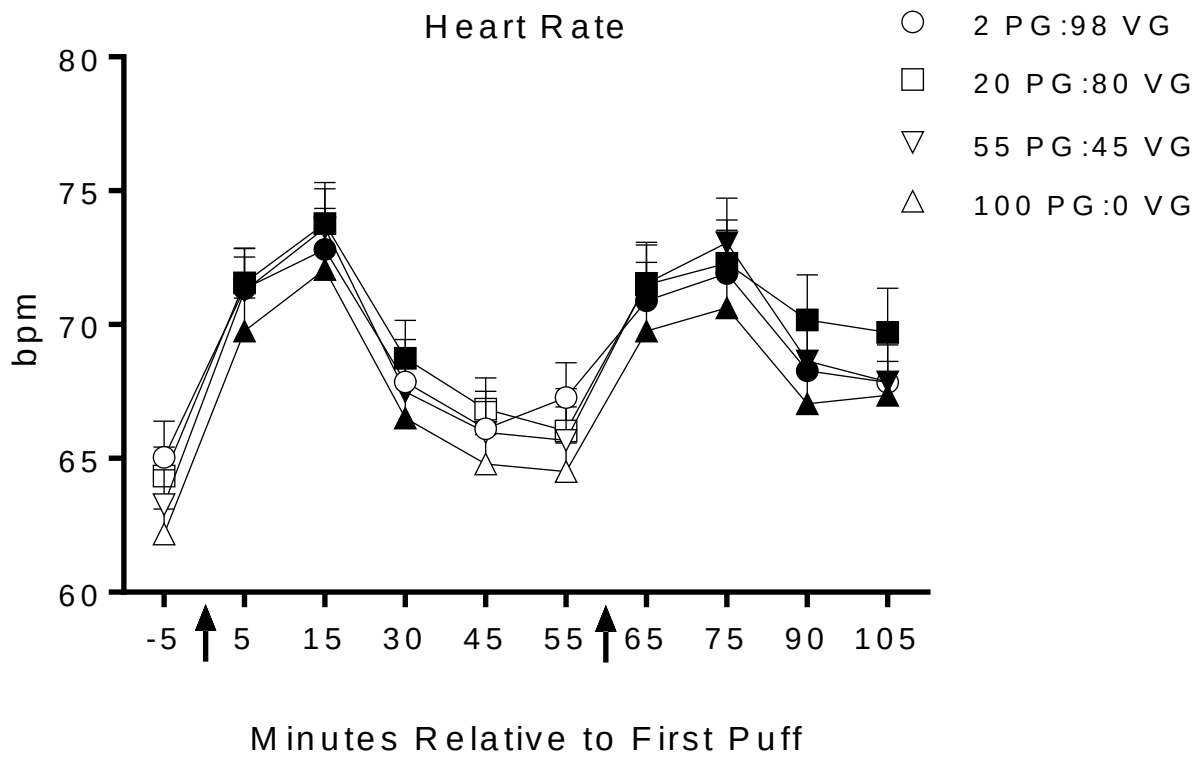

Figure 7. Mean (+ SEM) HR values (bpm) from 30 ECIG-experienced participants during four independent sessions that differed only by PG:VG ratio. In all other respects the figure is identical to Figure 5.

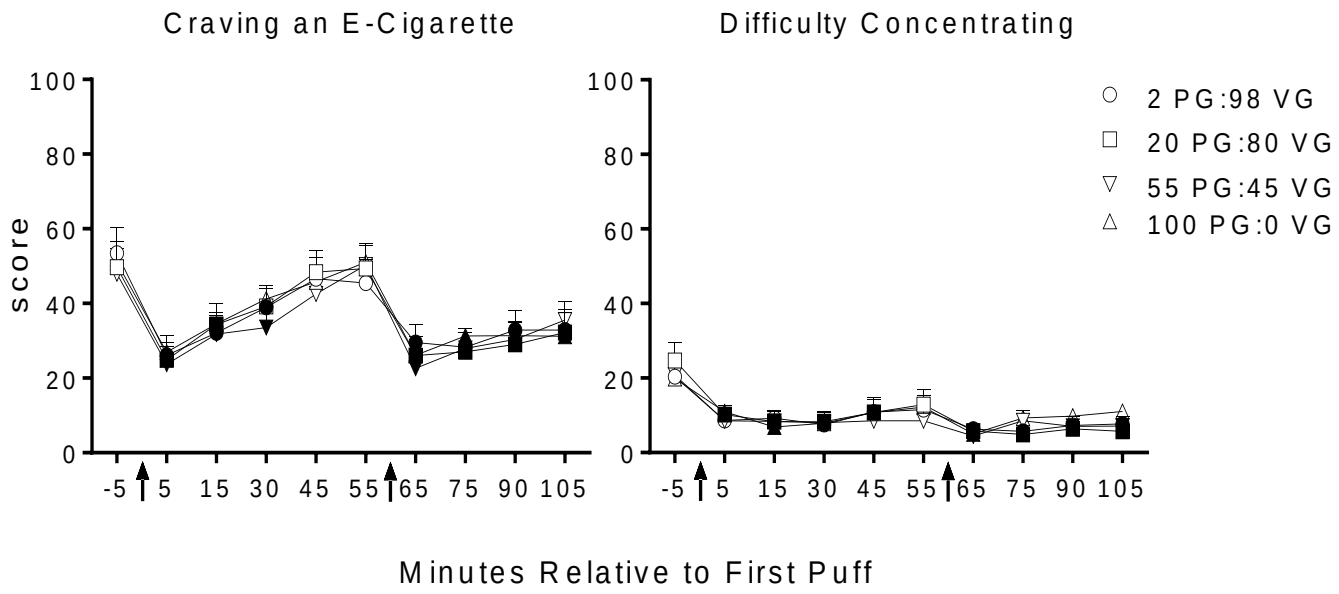

Figure 8. Mean ratings (+ SEM) for two visual analog scale items, "Craving an e-cigarette" (left) and "Difficulty Concentrating" (right), from the Hughes-Hatsukami withdrawal scale. In all other respects the figure is identical to Figure 5.

Tiffany Drobes QSU Brief. No significant interactions of time and condition were observed for the two QSU factors (Table 4). However, significant main effects of time were observed for both QSU factors and a main effect of condition was also observed for factor 2. Planned contrasts conducted at timepoints 2 and 7 also did not reveal any significant differences across conditions for either factor. Subsequent post-hoc tests revealed that, for both factors, scores were reduced significantly after each directed ECIG use bout relative to baseline (Tukey's HSD, *p*s < .05). For example, collapsed across condition, the mean (SD) score for factor 1 was reduced significantly from 21.34 (8.28) at baseline to 13.96 (7.71) after bout 1 and 12.62 (7.73) after bout 2.

Direct Effects of Nicotine. No significant interactions of time and condition or main effects of condition were detected for any item from the Direct Effects of Nicotine scale. As shown in Table 4, significant main effects of time were observed for the items "Dizzy" and "Lightheaded." Planned contrasts did not reveal any differences across conditions for any item. Post-hoc testing for the items "Dizzy" and Lightheaded" did not detect differences from baseline for any timepoint (Tukey's HSD, *p*s < .05).

Direct Effects of ECIG Use. A significant time by condition interaction was observed for the item "Awake." This interaction of condition and time is explained by significant differences in mean VAS scores across conditions at the two timepoints immediately following each ECIG use bout (see Figure 9). At the timepoint immediately following bout 1, the mean (SD) score on this measure was 41.3 (29.29) in the 2PG:98VG condition which was significantly higher than the mean score of 28.2 (24.98) reported in the 100PG:0VG condition (Tukey's HSD, $p < .05$). For the timepoint immediately following bout 2, mean (SD) score for the item "Awake" was 37.3 (34.60) in the 55PG:45VG condition which was significantly higher relative to the

mean score of 24.63 (26.85) reported in the 100PG:0VG condition (Tukey's HSD, $p < .05$). Planned contrasts conducted at the timepoints immediately following bouts 1 and 2 did not reveal any additional significant differences across conditions after the Bonferroni correction was applied.

Figure 9 displays four items ("Awake," "Calm," "Pleasant," and "Satisfying") for which significant main effects of condition and time were observed. Significant main effects of condition were detected for the items "Awake," "Calm," "Concentrate," "Pleasant," "Satisfying," and "Taste Good." Significant main effects of time were also observed for the items "Awake," "Calm," "Dizzy," "Pleasant," "Reduce Hunger," "Right Now," and "Satisfying." Because there was no true baseline timepoint for this subjective questionnaire (i.e., a timepoint prior to any product administration) no comparisons to baseline were possible. Planned contrasts conducted at the two timepoints immediately following bouts 1 and 2 revealed significant differences across conditions for the items "Awake," "Calm," "Concentrate," "Pleasant," and "Satisfying." For the item "Calm" mean (SD) VAS score in the 100PG:0VG condition was 33.13 (25.94) after bout 1 and this score was significantly lower relative to the mean score of 47.67 (31.27) observed in the 20PG:80VG condition after bout 1 [t (29) = -2.75, $p < .017$]. In addition, the mean (SD) VAS score in the 100PG:0VG condition of 26.93 (25.25) after bout 2 was also significantly lower relative to the mean score in the 2PG:98VG condition of 42.93 (30.69) at the same timepoint [t (29) = -3.23, $p < .017$]. For the item "Concentrate" the mean (SD) VAS score reported in the 100PG:0VG condition after bout 2 was 20.23 (22.10) which was significantly lower relative to the mean score of 32.70 (32.66) observed in the 55PG:45VG condition after bout 2 [t (29) = -2.74, $p < .017$]. For the item "Pleasant" the mean (SD) VAS score in the 100PG:0VG condition was 43.33 (28.81) immediately after bout 1 and

43.67 (26.83) immediately after bout 2. These mean post-bout scores observed in the 100PG:0VG condition were significantly lower relative to all other conditions at the corresponding post-bout timepoints with the exception of the 20PG:80VG condition after bout 1 [*ts* (29) < -2.61, *ps* < .017]. Finally, for the item "Satisfy" the mean (SD) VAS scores detected in the 100PG:0VG condition of 52.60 (30.98) after bout 1 and 53.27 (29.87) after bout 2 were significantly lower relative to the mean scores detected in the 2PG:98VG condition of 67.70 (25.83) after bout 1 and 67.57 (29.26) after bout 2 [*ts* (29) < -2.97, *ps* < .017]. In addition, the mean (SD) score for the 100PG:0VG condition after bout 2 of 53.27 (29.87) was also significantly lower relative to the mean score of 66.97 (28.83) in the 55PG:45VG condition after bout 2 [*t* (29) = -2.91, *p* < .017].

General Labeled Magnitude Scale. As indicated by Table 4, significant main effects of condition were observed for the items "Harshness/Irritancy" and "Throat Hit." For the item "Harshness" planned contrasts revealed that immediately after bout 2 the mean (SD) score observed in the 100PG:0VG condition of 47.75 (25.60) was significantly higher relative to the mean (SD) score observed in the 2PG:98VG condition of 32.63 (18.62) and the mean score of 33.33 (23.18) observed in the 20PG:80VG condition [*ts* (29) > 3.07, *ps* < .017]. Figure 10 displays results for the item "Throat Hit." The mean (SD) VAS scores observed for "Throat Hit" in the 100PG:0VG condition of 51.17 (28.67) after bout 1 and 52.0 (24.67) after bout 2 were significantly greater relative to the mean scores observed in the 2PG:98VG condition of 37.93 (26.54) after bout 1 and 32.43(21.46) after bout 2 [*ts* (29) > 3.32, *ps* < .017] and also the mean scores observed in the 20PG:80VG condition of 34.87 (23.23) after bout 1 and 32.47 (21.23) after bout 2 [*ts* (29) > 3.63, *ps* < .017].

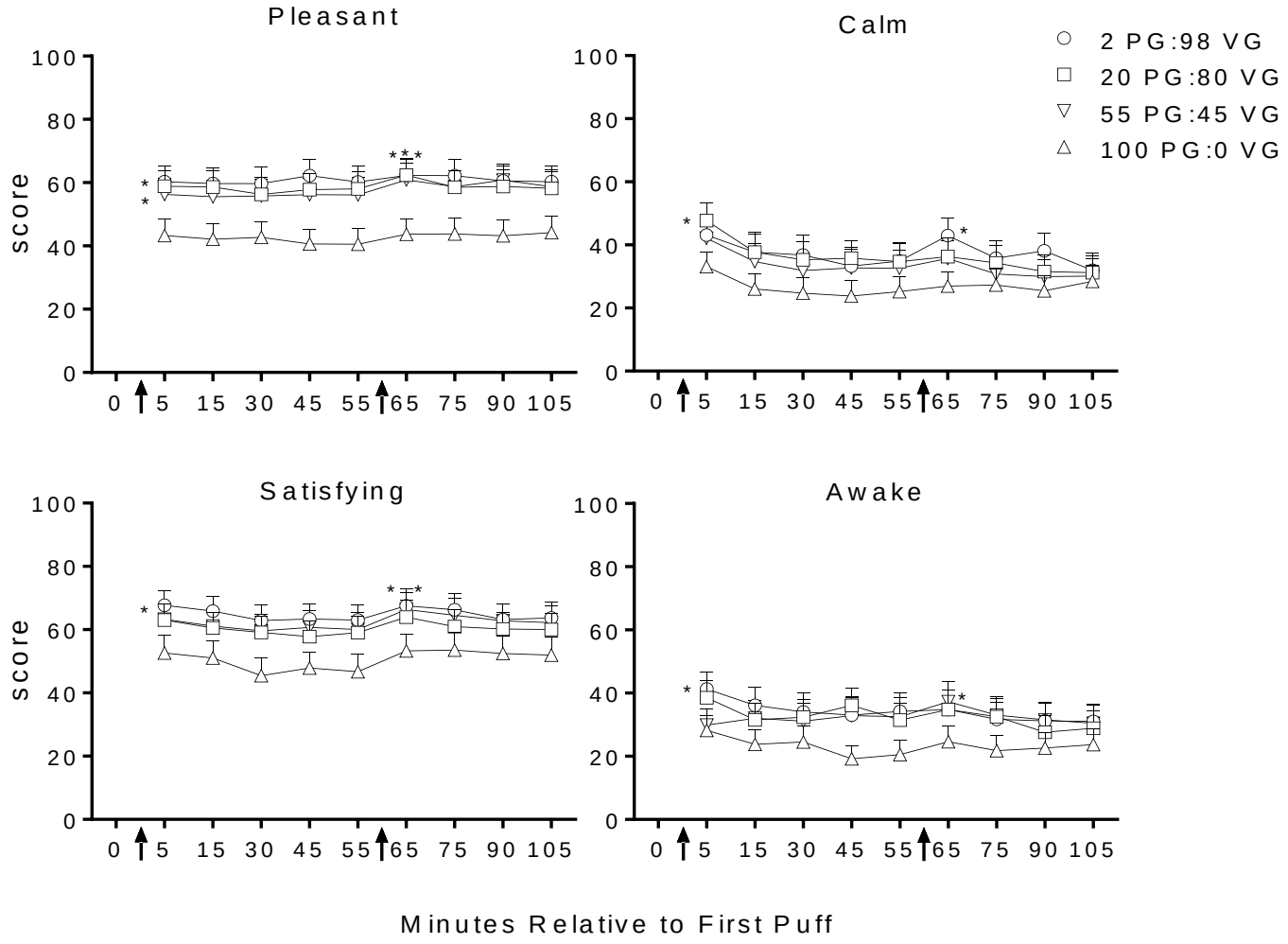

Figure 9. Mean ratings (+ SEM) for four visual analog scale items from the Direct Effects of ECIG use scale: "Was the ECIG pleasant?" (top left), "Did the ECIG make you feel more calm?" (top right), "Was the ECIG Satisfying?" (bottom left), and "Did the ECIG make you feel more awake?" (bottom right). In all other respects the figure is identical to Figure 5.

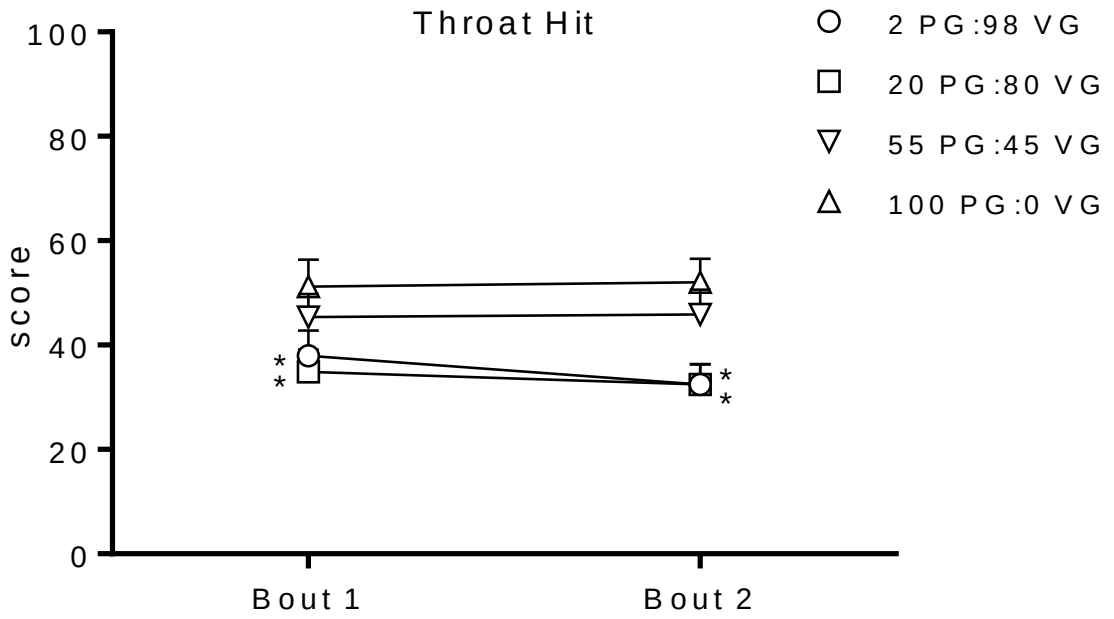

Figure 10. Mean ratings (+ SEM) for the item "Throat Hit" from the general Labeled Magnitude scale (gLMS). Note that this item was only administered on two occasions: immediately after the first and second ECIG use bouts. In all other respects the figure is identical to Figure 5.

Puff Topography

Mean (SD) puff duration, puff volume, and flow rate data are displayed in Table 5. For puff duration, a significant condition by time interaction was observed [F (3, 84) = 3.45, p <.05] in addition to significant main effects of time [F (1, 28) = 28.33, p <.001] and condition [F (3, 84) = 12.34, p < .001]. Post-hoc tests (Tukey's HSD) revealed that during bout 1, participants took significantly longer puffs in the 2PG:98VG condition (Mean = 5.26 sec; SD = 1.95) relative to the 55PG:45VG (Mean = 4.47 sec; SD = 1.52) and 100PG:0VG conditions (Mean = 4.32 sec; SD = 1.35). In addition, during bout 2, participants took significantly longer puffs in the 2PG:98VG condition (Mean = 5.90 sec; SD = 2.26) relative to all other conditions: 20PG:80VG: (Mean = 5.32 sec; SD = 2.23), 55PG:45VG (Mean = 4.91 sec; SD = 1.58), 100PG:0VG (Mean = 4.48 sec; SD = 1.44). Additional post-hoc tests (Tukey's HSD) revealed that within all conditions except the 100PG:0VG condition, participants took longer puffs, on average, in the second bout relative to the first. For example, in the 2PG:98VG condition, participants mean (SD) puff duration increased from 5.26 secs (1.95) in bout 1 to 5.90 secs (2.26) in bout 2 (Table 5).

For puff volume, no significant time by condition interaction was observed. However, significant main effects of time [F (1, 28) = 33.78, p <.001] and condition [F (3, 84) = 3.97, p < .05] were observed. Post-hoc tests (Tukey's HSD) revealed that during bout 1, participants took significantly larger puffs, on average, in the 2PG:98VG condition (Mean = 115.45 ml; SD = 58.28) relative to the 55PG:45VG (Mean = 96.81 ml; SD = 51.61) and 100PG:0VG conditions (Mean = 100.25 ml; SD = 47.11). In addition, during bout 2, participants took significantly larger puffs, on average, in the 2PG:98VG condition (Mean = 133.92 ml; SD = 67.22) relative to all other conditions: 20PG:80VG: (Mean = 121.69 ml; SD = 68.63), 55PG:45VG (Mean = 110.89

ml; SD = 55.14), 100PG:0VG (Mean = 103.09 ml; SD = 50.86). Additional post-hoc tests (Tukey's HSD) revealed that again within all conditions except the 100PG:0VG condition, participants exhibited longer mean puff volume in the second bout relative to the first. For example, in the 2PG:98VG condition, participants mean (SD) puff volume increased from 115.45 ml (58.28) in bout 1 to 133.92 ml (67.22) in bout 2 (Table 5). Lastly, no significant main effects or interactions were detected for the puff topography variable flow rate (ml/sec).

Particulate Matter ($PM_{2.5}$)

A significant time by condition interaction was observed for the outcome exhaled $PM_{2.5}$ [F (1, 28) = 33.78, p <.001]. Main effects of time [F (2, 58) = 12.23, p <.01] and condition [F (3, 87) = 4.10, p <.05] were also observed. As Figure 11 shows, the interaction is explained by differences in mean $PM_{2.5}$ concentrations measured during the two directed ECIG use bouts across conditions. Specifically, the mean (SD) $PM_{2.5}$ concentration detected during the two ECIG use bouts in the 100PG:0VG condition of 1.45 $\mu g/m^3$ (0.79) was significantly lower relative to the mean concentration of 57.63 $\mu g/m^3$ (68.02) detected in the 20PG:80VG condition and the mean concentration of 62.03 $\mu g/m^3$ (143.68) detected in the 2PG:98VG condition (Tukey's HSD, ps < .05). Additional post-hoc tests (Tukey's HSD) revealed that within the 20PG:80VG and 2PG:98VG conditions, mean $PM_{2.5}$ concentrations increased significantly during the two ECIG use bouts relative to baseline. For example, the mean (SD) $PM_{2.5}$ concentrations in the 2PG:98VG condition increased significantly from 1.58 $\mu g/m^3$ (1.11) at baseline to 62.03 $\mu g/m^3$ (143.68) during the two ECIG use bouts (see Figure 11). All mean (SD) and median $PM_{2.5}$ values detected before, during, and after the two ECIG use bouts for the four experimental conditions are presented in Table 6.

Amount of Liquid Consumed

The mean (SD) amount of liquid consumed was 0.12 ml (0.07) in the 100PG:0VG condition, 0.13 ml (0.12) in the 55PG:45VG condition, 0.09 ml (0.05) in the 20PG:80VG condition and 0.11 ml (0.09) in the 2PG:98VG condition, with no significant differences observed across conditions [F (3, 87) = 2.37, p = ns].

	ECIG Liquid PG:VG Ratio							
	Bout 1				Bout 2			
	2:98	**20:80**	**55:45**	**100:0**	**2:98**	**20:80**	**55:45**	**100:0**
Puff Duration (sec)	5.26* (1.95)	4.99* (1.99)	4.47 (1.52)	4.32 (1.35)	5.90*+ (2.26)	5.32*+ (2.23)	4.91*+ (1.58)	4.48 (1.44)
Puff Volume (ml)	115.45* (58.28)	108.85* (51.84)	96.81 (51.61)	100.25 (47.12)	133.92*+ (67.22)	121.69*+ (68.63)	110.89*+ (55.14)	103.09 (50.86)
Flow Rate (ml/s)	21.88 (8.12)	21.84 (6.56)	21.52 (7.85)	22.97 (6.89)	22.66 (7.21)	22.57 (8.97)	22.43 (8.59)	22.86 (7.41)
IPI (sec)	24.48 (2.05)	24.97 (1.99)	25.36 (1.75)	25.48 (2.11)	24.17 (2.45)	24.56 (2.22)	24.90 (2.37)	25.55 (1.68)
Puff Number	9.93 (0.37)	10.07 (0.37)	10.03 (0.19)	10.03 (0.89)	10.07 (0.25)	10.13 (0.57)	10.17 (0.91)	10.03 (0.18)

Table 5. Mean (SD) puff parameters for ECIG-experienced (N = 29) individuals for two 10-puff directed ECIG use bouts (30 sec IPI). A malfunction of the topography recording device resulted in incomplete data for one participant out of the 30 completers who were included in all other analyses. Note, IPI (30 s) and puff number (10) were controlled experimentally (see method). Asterisks (*) indicate significant differences from the 100PG:0VG condition within that bout and plus signs (+) indicate differences from bout 1 within that condition (Tukeys HSD; *p*s < .05).

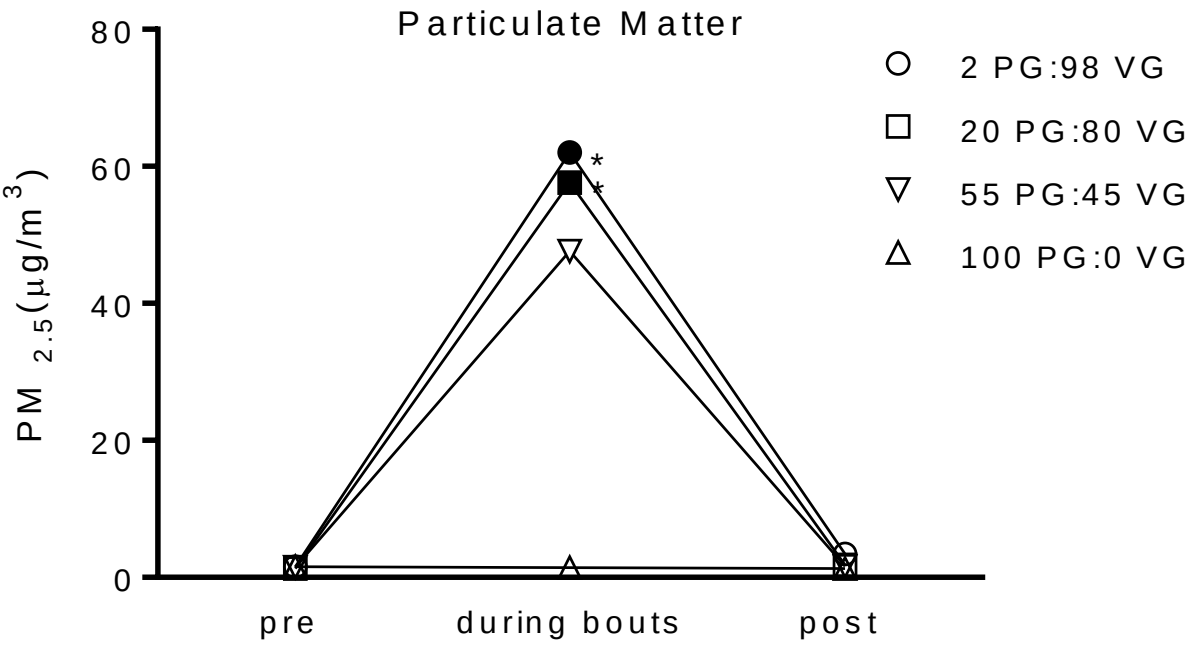

Figure 11. Exhaled mean $PM_{2.5}$ concentrations detected in each condition for 30 minutes prior to the first ECIG use bout (pre), for the 10 minutes during the two 10-puff directed ECIG use bouts, and for 30 minutes after the last puff of the second bout. In all other respects the figure is identical to Figure 5.

Table 6.

Mean (SD) and Median Particulate Matter ($PM_{2.5}\mu g/m^3$) for Each Study Condition.

	Pre-ECIG Use[a]		During ECIG Use[b]		After ECIG Use[c]	
PG:VG Ratio	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
2:98	1.58 (1.11)	1.34	62.03 (143.77)	17.88	3.45 (6.59)	1.69
20:80	1.32 (0.63)	1.25	57.63 (68.02)	26.87	1.82 (1.37)	1.46
55:45	1.48 (0.85)	1.28	47.66 (90.44)	7.84	1.63 (0.85)	1.42
100:0	1.60 (1.26)	1.28	1.45 (0.79)	1.30	1.28 (0.64)	1.17

[a]30 minutes prior to the first 10-puff directed bout.

[b]10 minutes during the two 10-puff bouts.

[c]30 minutes after the second 10-puff bout.

Discussion

ECIGs are a class of tobacco products that have increased exponentially in popularity since their introduction into the U.S. marketplace. ECIGs vary substantially in their device features and liquid contents and this variability can influence the acute effects of ECIG use. For example, previous clinical laboratory studies revealed that varying certain device (e.g., power; Wagener et al., 2017) and liquid factors (e.g., liquid nicotine concentration; Hiler et al., 2017) can influence nicotine delivery, subjective effects, and puff topography in ECIG users. Additionally, non-nicotine liquid ingredients such as the solvents PG and VG may also influence ECIG acute effects: some evidence from pre-clinical studies suggests that different combinations of PG and VG can alter the amount of nicotine emitted from ECIGs (i.e., nicotine yield; Kosmider, Sobczak, Knysak, Goniewicz, 2014). However, no prior studies have explored systemically the extent to which ECIG liquid PG:VG ratio influences acute effects experienced by ECIG users.

In order to investigate the influence of PG and VG on ECIG acute effects, 30 participants completed this within-subject study that had four conditions, each consisting of two 10-puff ECIG use bouts (30 sec IPI) and differing only by ECIG liquid PG:VG ratio: 100:0, 55:45, 20:80, and 2:98. In addition to varying PG:VG ratio systematically, the present study held numerous other ECIG device and liquid factors constant across study conditions (e.g., battery voltage, heater resistance, liquid nicotine concentration). The discussion below focuses on the primary outcome measures of the present study including nicotine delivery, HR, subjective effects, and puff topography and also the secondary outcome measure, $PM_{2.5}$. Implications of the effects observed are discussed, as well as potential study limitations.

ECIG Effects

Physiological Measures. Physiological outcome measures in the present study included plasma nicotine concentration and HR and thus provide evidence regarding the extent to which acute nicotine exposure and cardiovascular effects associated with ECIG use are influenced by liquid PG:VG ratio. Overall, plasma nicotine concentration and HR increased after the two ECIG use bouts during each experimental session. In addition, participants obtained significantly more nicotine when using the liquids comprised mostly of PG but HR was not influenced by ECIG liquid PG:VG ratio.

The present study was the first to provide data supporting a direct relationship between ECIG liquid PG:VG ratio and plasma nicotine concentration when other relevant device and liquid factors are held constant. Immediately after the first 10-puff bout, the mean (SD) plasma nicotine concentration (ng/ml) was 11.79 (10.19) in the 100PG:0VG condition, 11.79 (14.07) in the 55PG:45VG condition, 9.77 (9.01) in the 20PG:80VG condition, and 8.27 (6.97) in the 2PG:98VG condition. Immediately after the second 10-puff bout, the mean (SD) plasma nicotine concentration (ng/ml) was 13.40 (8.99) in the 100PG:0VG condition, 12.89 (10.72) in the 55PG:45VG condition, 9.59 (7.95) in the 20PG:80VG condition, and 8.58 (5.41) in the 2PG:98VG condition. Planned contrasts conducted between the 100PG condition and all other conditions at the two timepoints immediately following each ECIG use bout revealed that immediately after bout 2, the mean plasma nicotine concentration in the 100PG:0VG condition was significantly greater than mean values observed in the 20PG:80VG and 2PG:98VG conditions. Further, total nicotine exposure, as indexed by AUC for plasma nicotine, was significantly higher in the 100PG:0VG condition for bouts 1 (timepoints 1-5) and 2 (timepoints 6-10) relative to the corresponding AUC values in the 2PG:98VG condition; for bout 2 AUC

also significantly differed between the 100PG:0VG and 20PG:80VG conditions. Thus in general, participants obtained more nicotine when using the ECIG liquids comprised mostly of PG than when using the liquids consisting mostly of VG. These findings are consistent with pre-clinical data demonstrating that increasing PG in ECIG liquids results in a corresponding increase in the amount of nicotine found in the subsequent aerosol produced when relevant device, liquid, and topography variables are held constant (Kosmider, Sobczak, Knysak, Goniewicz, 2014). Therefore, PG:VG ratio should be added to the mounting list of device (e.g., battery voltage/heater resistance; Wagener et al. 2017) and liquid factors (liquid nicotine concentration; Hiler et al., 2017) that have been shown to influence ECIG user nicotine delivery.

In all study conditions, the nicotine delivered during the two 10-puff ECIG use bouts was physiologically active, as indexed by corresponding increases in HR. However, despite the observed influence of ECIG liquid PG:VG ratio on nicotine delivery in the present study, HR did not increase differentially across conditions. For example, in the 2PG:98VG condition the mean (SD) HR (bpm) observed at baseline of 62.18 (5.15) increased significantly to 69.76 (6.77) after bout 1 and 69.77 (6.87) after bout 2 and in the 100PG:0VG condition the mean HR at baseline of 63.27 (7.18) increased significantly to 71.26 (6.90) after bout 1 and 71.54 (7.81) after bout 2. Similar HR increases also were observed in the other two conditions following the two ECIG use bouts. Thus, nearly identical increases in HR of ~7-8 bpm were observed within all conditions suggesting that observed differences in nicotine delivery across certain conditions (e.g., 100PG:0VG and 2PG:98VG conditions) were not pronounced enough to alter the magnitude of change in HR following ECIG use. Given the wide range of PG:VG ratios used in the present study, these data suggest that using any combination of PG and VG in ECIG liquids can result in

the delivery of physiologically active doses of nicotine to the user, at least at the liquid nicotine concentration (18 mg/ml) and device power (7.3 W) used in this study.

Interestingly, the observed differences in nicotine delivery across the four ECIG liquid PG:VG ratios were not as pronounced as would be expected based on the results of previous systematic pre-clinical studies. For example, in one pre-clinical study, aerosols were generated using ECIG liquids of four different PG:VG ratios (100PG:0VG, 50PG:50VG, 20PG:80VG, 0PG:100VG) analogous to those used in the present study (100PG:0VG, 55PG:45VG, 20PG:80VG, 2PG:98VG) and nicotine yield was examined (Baassiri et al., 2017). Across each of these conditions the same liquid nicotine concentration (18 mg/ml) was used as in the present study. Device power (4.3 W) and several puff topography variables were also held constant (15 puffs of 4 sec puff duration, 10 sec IPI, 66.67 ml puff volume, and 16.67 ml/sec flow rate) across conditions. The mean (SD) nicotine yield (mg) detected was 0.58 (0.02) in the 100PG:0VG condition, 0.33 (0.01) in the 50PG:50VG condition, 0.17 (0.01) in the 20PG:80VG condition, and 0.13 (0.02) in the 0PG:100VG condition (Baassiri et al., 2017). Thus, holding all other relevant factors constant, the amount of nicotine present in the aerosols generated when using the 100PG:0VG liquid was over four times the amount of nicotine detected when the 0PG:100VG liquid was used. However, as depicted in Table 7, the observed changes in the nicotine delivered to participants as a result of manipulating PG:VG ratio in the present study were not proportional to the observed changes in nicotine yield in this pre-clinical examination. For example, immediately after bout 1, the mean plasma nicotine concentrations observed in the 100PG:0VG (11.79; SD = 10.19) and 2PG:98VG (8.27; SD = 6.97) conditions did not differ significantly and the mean plasma nicotine concentrations were identical in the 100PG:0VG and 55PG:45VG conditions.

One likely explanation for PG:VG ratio having less of an influence on nicotine delivery in the present study relative to nicotine yield in previous pre-clinical studies is that the present study did not control certain puffing parameters (e.g., puff duration, volume) that were controlled in other pre-clinical examinations (e.g., Baassiri et al., 2017). Indeed, as detailed below, participants in the present study took, on average, puffs that were significantly shorter and smaller when using the 100PG:0VG liquid relative to the other liquids containing various amounts of VG. Other ECIG clinical laboratory studies have demonstrated that shorter puff durations and/or smaller puff volumes can result in decreases in nicotine delivery when all other relevant factors are held constant (Hiler et al., 2017). For example, in one clinical lab study, experienced ECIG users and ECIG naïve cigarette smokers completed four conditions consisting of two 10-puff directed (30 sec IPI) ECIG use bouts differing only by liquid nicotine concentration (0, 8, 18, or 36 mg/ml) while relevant device and liquid factors were held constant. Results from this study revealed that the ECIG-naïve individuals took significantly shorter and smaller puffs relative to the experienced ECIG users and these shorter and smaller puffs resulted in less pronounced nicotine delivery among ECIG-naïve individuals at each active nicotine concentration (Hiler et al., 2017). Therefore, the shorter puff durations and smaller puff volumes observed by participants when using the 100PG:0VG liquid in the present study likely offset, to some extent, the effects of greater PG concentration on ECIG-associated nicotine delivery. These results highlight the importance of controlling for all relevant factors (e.g., device power, liquid nicotine concentration, puff topography) in systematic examinations of how a single factor (e.g., PG:VG ratio) influence ECIG-associated nicotine delivery (see limitations section below) and suggest that using real-world puff topography data to generate ECIG aerosols may result in more precise estimates of user toxicant exposure.

In addition to the observed differences in puff duration and volume across conditions, the use of a device with a higher wattage (7.3 W) in the present study relative to devices used in previous examinations of nicotine yield (e.g., 4.3 W; Baassiri et al., 2017) also may have decreased the magnitude with which PG:VG ratio influenced nicotine delivery. Generally, PG has a lower temperature threshold for evaporation and is more volatile relative to VG, meaning PG is vaporized preferentially over VG from ECIG liquids containing both solvents. Further, liquids high in PG will vaporize more rapidly and produce higher nicotine yields relative to liquids high in VG (Talih et al., 2017). However, increasing the internal temperature of an ECIG, which can be achieved by increasing the overall device wattage, causes the PG and VG in a given ECIG liquid to be vaporized more uniformly (Talih et al., 2017), decreasing the influence of liquid PG:VG ratio on ECIG aerosol nicotine yields (Kosmider, Sobczak, Knysak, Goniewicz, 2014). Thus, since the device used in the present study had a higher wattage relative to previous pre-clinical studies (and consequently a higher internal temperature), the vaporization of PG and VG was likely more similar in this study resulting in comparable nicotine delivery across certain PG:VG ratios (e.g., the 100PG:0VG and 55PG:45VG conditions). Further examinations are necessary to determine the extent to which ECIG liquid PG:VG ratio influences nicotine yield/delivery from ECIGs operating at higher power settings (e.g., > 10 W), particularly given the increased popularity of devices with far greater wattages than those used in the present study (Rudy, Leventhal, Goldenson, & Eissenberg, 2017; Wagener et al., 2017).

Table 7.

Comparison of Mean (SD) Nicotine Yield Results from Baassiri et al., 2017 and Mean (SD) Plasma Nicotine Concentration After Bout 1 from the Present Study.

Baassiri et al., 2017[a]				The Present Study (Bout 1)[b]			
PG:VG Ratio	Nicotine Yield (mg/15 puffs)	Puff Duration (sec)	Nicotine Yield Percentage Increase Relative to 0:100	PG:VG Ratio	Plasma Nicotine (ng/ml)	Puff Duration (sec)	Plasma Nicotine Percentage Increase Relative to 2:98
0:100	0.13 (0.02)	4.0	N/A	2:98	8.27 (6.97)	5.26 (1.95)	N/A
20:80	0.17 (0.01)	4.0	+30.7%	20:80	9.77 (9.01)	4.99 (1.99)	+18.1%
50:50	0.33 (0.01)	4.0	+153.8%	55:45	11.79 (14.07)	4.47 (1.52)	+42.6%
100:0	0.58 (0.02)	4.0	+346.2%	100:0	11.79 (10.19)	4.32 (1.35)	+42.6%

[a]Factors held constant: liquid nicotine concentration: 18 mg/ml; device wattage: 4.3; puffing parameters: 15 puffs of 4 sec puff duration, 10 sec IPI, 66.67 ml puff volume, and 16.67 ml/sec flow rate.

[b]Factors held constant: liquid nicotine concentration: 18 mg/ml; device wattage: 7.3; puffing parameters: 10 puffs, 30 sec IPI.

Subjective Measures. Abstinence symptom suppression, nicotine-specific effects, and sensory ECIG effects were assessed using various questionnaires in this study. Overall, results demonstrated that abstinence symptoms were suppressed following each ECIG use bout, and the magnitude of suppression did not differ across the different PG:VG ratios. However, participants reported significantly lower scores on several subjective measures assessing sensory effects of ECIG use when using the 100PG:0VG liquid suggesting lower overall satisfaction with this liquid.

Each ECIG liquid PG:VG ratio used in the present study produced reliable abstinence symptom suppression, as indexed by reductions in VAS scores for several subjective items from the Hughes-Hatsukami withdrawal scale and Tiffany-Drobes QSU brief. For the Hughes-Hatsukami withdrawal scale, VAS scores at baseline for the items "Anxious," "Craving and e-cigarette/nicotine," "Difficultly concentrating," "Impatient," "Irritable," "Restlessness," and "Urge to use an ECIG" decreased approximately by a factor of two following each product administration. For example, collapsed across condition, mean (SD) rating for the item "Craving an e-cigarette" decreased significantly from 50.52 (35.27) at baseline to 25.43 (25.76) immediately after bout 1and also decreased from 49.03 (31.59) immediately prior to bout 2 to 26.65 (24.47) immediately after bout 2 (VAS scale: 0-100). Similarly pronounced post-ECIG use reductions were observed for scores from both factors from the Tiffany-Drobes QSU brief. For factor 2, collapsed across condition, mean (SD) score at baseline of 7.40 (6.69) decreased to 4.06 (4.39) immediately after bout 1 and also decreased from 6.72 (5.96) immediately prior to bout 2 to 3.53 (4.24) immediately after bout 2 (cumulative score: 0-24). A similar pattern was observed for factor 1 from this scale. Notably, there was no evidence that ECIG liquid PG:VG ratio

influenced the suppression of abstinence symptoms, as subjective ratings following product administration did not differ across conditions for any items from these two questionnaires.

Although PG:VG ratio did not appear to influence abstinence symptom suppression, some sensory effects differed across PG:VG ratio following ECIG use. For example, participants reported that the 100PG:0VG liquid provided less favorable sensory effects, as evidenced by lower post ECIG use VAS scores for items from the Direct Effects of ECIG use scale in this condition. For example, immediately after bout 1, participants rated the item "Was the ECIG pleasant?" higher in the 2PG:98VG, 20PG:80VG, and 55PG:45VG conditions relative to the 100PG:0VG condition. Participants also reported significantly lower mean ratings in the 100PG:0VG condition following ECIG use for the items "Awake," "Calm," "Concentrate," and "Satisfying" relative to one or more of the other conditions (see Results section; Figure 9). Additional sensory effects were influenced by PG:VG ratio, as evidenced by two items from the gLMS differing across conditions. Specifically, participants reported that the 100PG:0VG liquid resulted in significantly more "Harshness/Irritancy" and provided a significantly greater "Throat Hit" following ECIG use relative to the two mostly VG liquids. Higher ratings of "Harshness/Irritancy" are often associated negatively with product satisfaction. Indeed, one study demonstrated that across numerous different ECIG flavors (e.g., tobacco, menthol, cherry, vanilla, piña colada, and peach schnapps) of the same liquid nicotine concentration (12 mg/ml), higher ratings of "Harshness/Irritancy" on the gLMS were associated with greater disliking and less overall satisfaction with the product (Kim et al., 2016). The extent to which higher ratings of "Throat Hit" can be viewed as a positive or negative sensory effect in this study is unclear. Some evidence suggests that experienced ECIG users prefer devices that can provide a greater throat hit for a variety of reasons such as the perception that ECIGs that provide a greater throat hit are

more efficacious at alleviating abstinence symptoms (Etter, 2016). In addition, surveys of ECIG users have demonstrated consistently that greater throat hit is associated with positive sensory effects such as overall device/liquid satisfaction and better taste (Etter, 2016; McQueen, Tower, & Sumner, 2011; Pokhrel, Herzog, Muranaka, & Fagan, 2015). However, in the present study higher ratings of "Throat Hit" did not coincide with more positive sensory effects and other experimental studies have demonstrated that throat hit is inversely associated with product satisfaction among young adult ECIG users (Goldenson et al., 2016). Because the 100PG:0VG liquid produced less positive sensory effects overall, the higher ratings of "Throat Hit" observed in this condition is likely another example of a negative sensory effect resulting from the use of this liquid.

Taken together, the subjective effects results from the present study have several important implications. First, consistent with previous reports (e.g., Etter & Eissenberg, 2015; Foulds et al., 2015), ECIG users in the present study exhibited signs of nicotine dependence. That is, participants reported nicotine-abstinence symptoms following 12 hours of ECIG abstinence and reductions in these symptoms were observed following ECIG use. Notably, the emergence of a physiological withdrawal state upon ceasing use of a drug and/or using a substance with the intention to alleviate this state are diagnostic criteria for dependence syndrome in the International Classification of Disease and Health Problems (ICD-10; WHO, 1992). Second, the reductions in abstinence symptoms following ECIG use suggest that any ECIG liquid PG:VG ratio can be effective at maintaining nicotine dependence when paired with the device (7.3 W) and liquid nicotine concentration (18 mg/ml nicotine concentration) reported here, although comparisons to participants' normal reduction in abstinence symptoms was not possible in this study. Future examinations should also include a condition in which participants

use their preferred ECIG device/liquid in order to compare the relative effectiveness of abstinence symptom suppression between the experimental ECIG and participants' preferred product. Third, the lower ratings for several subjective effects observed in the 100PG:0VG condition suggests that overall, participants had a lower preference for this ECIG liquid relative to the other liquids. Given that the 100PG:0VG liquid actually delivered the most nicotine on average and suppressed abstinence symptoms similarly to the other liquids, the lower preference for this liquid could be explained by one of two mechanisms: (1) the increased "Harshness/Irritancy" and "Throat Hit" provided by this liquid caused participants to report lower ratings for other subjective effects such as "Satisfaction" and "Pleasantness" or (2) as reported elsewhere (Baassiri et al., 2017; King et al., 2017) 100PG:0VG liquids produce little to no visible exhaled aerosol which may have prompted participants' to report less favorable subjective effects in this condition. Non-nicotine related behavioral stimuli such as the sight of exhaled aerosol/smoke can contribute to positive sensory effects and the suppression of abstinence symptoms for other tobacco products such as conventional cigarettes (Rose, Behm, Westman, & Johnson, 2000; Buchhalter et al., 2005). Additional research whereby "Harshness/Irritancy" and/or "Throat Hit" are kept constant (possibly by induction of throat analgesia) and participants are blinded as to whether their product is producing an aerosol (possibly with the use of a blind fold or a completely darkened room) may help to reveal the importance of aerosol visibility on ECIG user subjective effects.

Puff Topography. Puff topography variables including puff duration, puff volume, and flow rate were assessed during each 10-puff (30 sec IPI) ECIG use bout. Overall, results demonstrated that PG:VG ratio significantly influenced puff duration and volume such that participants took significantly shorter and smaller puffs when using the 100PG:0VG liquid (see

Table 5). These observed differences in puff topography could be explained by two mechanisms. First, as revealed by the subjective effects results, participants rated the 100PG:0VG liquid significantly higher in "Harshness/Irritancy" and "Throat Hit." Increased perceptions of "Harshness/Irritancy" and "Throat Hit" may have made the 100PG:0VG liquid more difficult to inhale relative to liquids containing mostly VG, resulting in shorter and smaller puffs in this condition. Conversely, participants may have altered their puff topography as a means of obtaining more nicotine from the ECIG, particularly when using the liquids containing mostly VG. That is, given that nicotine delivery in the present study was lower overall when liquids containing mostly VG were used, participants may have increased their puff durations and puff volumes in these conditions in an attempt to extract more nicotine from the ECIG. This idea is further supported by the observation that mean puff duration increased significantly from bout 1 to bout 2 when the mostly VG liquids were used, but stayed consistent across bouts when the 100PG:0VG liquid was used. Again, further examinations whereby "Harshness/Irritancy" and/or "Throat Hit" are kept consistent, and PG:VG ratio varied systematically may elucidate the mechanism behind the shorter and smaller puffs observed when 100PG:0VG liquid was used in the present study.

Interestingly, despite participants taking significantly shorter and smaller puffs when using the 100PG:0VG liquid and consuming a similar amount of liquid relative to the other liquids, mean plasma nicotine concentration following ECIG use was still higher in this condition. This finding has several important implications. First, given that decreasing puff duration and/or puff volume typically decreases nicotine yield (Talih et al., 2017) and nicotine delivery (Hiler et al., 2017; Zacny et al., 1987) from tobacco products, greater nicotine delivery from the 100PG:0VG liquid despite shorter and smaller puffs in this condition suggests that PG

may be a more efficient vehicle for ECIG nicotine delivery relative to VG. This greater efficiency of nicotine delivery from high PG liquids may be due to the fact that particles found in aerosols generated from liquids containing mostly PG tend to be much smaller than those found in the aerosols generated from liquids high in VG (Baassiri et al., 2017; Meng et al., 2017). Importantly, smaller particles can be deposited to a greater extent in a user's lungs and consequently absorbed more readily into the bloodstream (Heyder, 2004; Zhang et al., 2013). Second, longer puff durations and puff volumes associated with liquids high in VG could suggest a less favorable toxicant profile for users of high VG liquids. ECIG users also exhibit longer and larger puffs when using lower liquid nicotine concentrations (Dawkins et al., 2016; Hiler et al., 2017) and these compensatory puffing behaviors can result in greater production of harmful toxicants (Kosmider, Kimber, Kurek, Corcoran, & Dawkins, 2017). For example, in one clinical lab study ECIG users completed two conditions consisting of 60 minutes of *ad libitum* ECIG use with either a low (6 mg/ml) or high (24 mg/ml) liquid nicotine concentration. Results from this study revealed that, when using the lower liquid nicotine concentration, users exhibited a greater mean puff number and puff duration and also consumed more liquid overall (Dawkins et al., 2016). Using the mean puff topography data recorded in each condition, aerosols were produced, captured, and analyzed for toxicant content in a subsequent pre-clinical examination. Results demonstrated that the puffing profile associated with using the lower liquid nicotine concentration produced higher levels of carbonyl compounds such as formaldehyde, possibly because longer puff durations can increase the internal temperature of an ECIG and thus increase the thermal decomposition of PG and VG (Kosmider et al., 2017). Consequently, in the present study, the longer and larger puffs observed when using liquids containing mostly VG may suggest that over time, this more intensive puffing profile could also result in greater toxicant

production and user toxicant exposure. Ultimately, further pre-clinical research whereby aerosols are produced using the puff topography data from the present study and analyzed for toxicant content is necessary to estimate more precisely the respective toxicant profiles participants were exposed to under each study condition. Future clinical examinations assessing biomarkers of exposure to toxicants known to be formed from the thermal degradation of PG and/or VG (e.g., formaldehyde, acrolein, & acetaldehyde) in ECIG users of different PG:VG ratios may also help elucidate whether certain PG:VG ratios are more harmful than others.

Particulate Matter ($PM_{2.5}$). $PM_{2.5}$ was assessed in the present study in each condition before, during, and after ECIG use. The range of particles assessed in the present study (i.e., 0.1 – 2.5μm) are clinically important because they are sufficiently small to be inhaled deeply into the lungs (Zhang et al., 2013). Indeed, the majority of particles emitted from combustible tobacco cigarettes also fall into this range and have been demonstrated to cause a variety of adverse cardiovascular and respiratory effects to users and individuals exposed to secondhand smoke (USDHHS, 2014). Results from the present study revealed that during the 10-puff ECIG use bouts with the three liquids containing some VG, $PM_{2.5}$ was exhaled and detected in the ambient air. The mean (SD) $PM_{2.5}$ concentrations in these conditions increased from less than 2 $\mu g/m^3$ for the 30 minutes prior to the first ECIG use bout to 47.66 $\mu g/m^3$ (90.44) in the 55PG:45 VG condition, 57.63 $\mu g/m^3$ (68.02) in the 20PG:80VG condition, and 62.03 $\mu g/m^3$ (143.77) in the 2PG:98VG condition during ECIG use. The $PM_{2.5}$ concentrations detected in these three conditions during ECIG use were above the recommendations set by the U.S. Environmental Protection Agency (EPA) stating that individuals' daily average $PM_{2.5}$ exposure should not exceed 35$\mu g/m^3$ (EPA, 2006). However, the $PM_{2.5}$ concentrations observed in the present study are markedly lower than those detected in locations that permit the use of tobacco cigarettes

(Travers et al., 2004), waterpipe (Cobb et al., 2013), and ECIGs (Soule et al., 2017). For example, in separate studies examining $PM_{2.5}$ levels in waterpipe cafés (Cobb et al., 2013; N = 17), hospitality venues allowing cigarette smoking (Travers et al., 2004; N = 22), and an event held for ECIG users (Soule et al., 2017; assessed on six separate occasions over two days) mean $PM_{2.5}$ concentrations were 374 $\mu g/m^3$ among waterpipe cafés, 324 $\mu g/m^3$ among establishments allowing cigarette smoking, and 607.12 $\mu g/m^3$ at the ECIG user event. Because each these studies assessed $PM_{2.5}$ oftentimes while dozens of individuals used their respective products for upwards of 30 minutes, their results likely do not provide an adequate comparison to the present study that measured $PM_{2.5}$ from a single participant taking 10 puffs over 5 minutes from the ECIG provided.

Notably, negligible amounts of $PM_{2.5}$ were detected when participants used the 100PG:0VG liquid. Given that the SidePak personal aerosol monitor used in the present study could only detect particles between 0.1 (or 100nm) and 2.5μm, these results could suggest that particles emitted from ECIGs containing liquids high in PG are smaller than 100 nm (also referred to as ultrafine particles). This assertion is supported by previous examinations (e.g., Baassiri et al., 2017; Melstrom et al., 2017; Mikheev, Brinkman, Granville, Gordon, & Clark, 2016) in which ECIG particulate matter was assessed using equipment sensitive enough to detect ultrafine particulate matter. Generally, these studies have revealed that unlike tobacco cigarette smoke, ECIG aerosols are comprised of a relatively large amount of ultrafine particulate matter (Melstrom et al., 2017; Mikheev et al., 2016; Schripp et al., 2013). Furthermore, as more PG is added to an ECIG liquid, the subsequent aerosol generated will contain more particles in the ultrafine range (Baassiri et al., 2017). For example, in one pre-clinical examination PG:VG ratio was varied systematically and particulate matter was examined using equipment capable of

detecting ultrafine particles (Baassiri et al., 2017). Of the various PG:VG ratios tested, the 100PG:0VG liquid produced aerosols with the most total particulate matter that contained almost exclusively ultrafine particles while the 0PG:100VG liquid produced the least total particulate matter and contained approximately equal proportions of $PM_{2.5}$ and ultrafine particles. As a consequence of only containing ultrafine particles, aerosols produced from the 100PG:0VG liquid also scattered light less effectively and were therefore less visible relative to aerosols produced from mostly VG liquids that contained more $PM_{2.5}$ (Baassiri et al., 2017). Thus, in the present study, use of the 100PG:0VG liquid likely resulted in users exhaling predominantly ultrafine particles of minimal visibility that could not be detected by the Sidepak personal aerosol monitor. In addition, the smaller particles produced by the 100PG:0VG liquid were likely absorbed more readily in the lungs of users in the present study, contributing to the lower levels of exhaled particulate matter observed in this condition. Future examinations should be conducted to characterize the content of ECIG $PM_{2.5}$ and ultrafine particles and determine their respective effects on the user and bystanders, as total particulate matter concentrations such as those recorded in the present study provide no insight into the content of the particles detected or their toxicological profile.

Regulatory Implications

ECIGs are now subject to regulation by the FDA after the "deeming" declaration of the FSPTCA, meaning manufactures and retailers intending to market these products will be required to obtain authorization from FDA to do so. Specifically, this premarket authorization can be obtained by either: (1) providing evidence supporting the claim that an ECIG is substantially equivalent to a tobacco product that was commercially available in the U.S. prior to February 15, 2007 or (2) submitting a premarket tobacco product application containing

information such as the components, ingredients, and additives of the product, any information demonstrating the health risks of the product (including the extent to which the product presents less risk relative to other tobacco products), and a full description of the methods and facilities used to manufacture the 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). Because there were relatively few ECIGs on the market in 2007, there will likely be few predicate products that will be considered substantially equivalent to a modern ECIG. Thus, FDA will likely receive many premarket tobacco product applications for a variety of ECIG devices and liquids until the application deadline on August 8th, 2022.

In order to review adequately these product applications and make regulatory decisions that will have a positive impact on individual and public health, FDA will require a comprehensive understanding of how individual ECIG device characteristics, liquid components, and user behavior can influence the aerosols emitted from these products and the acute effects these products may have on the user. Importantly, with results from the present study, empirical evidence now exists demonstrating that liquid nicotine concentration (Dawkins et al., 2016; Hiler et al., 2017), device power (Farsalinos et al., 2014; Wagener et al., 2017), user puffing behavior (Farsalinos et al., 2015; Hiler et al., 2017), and ECIG liquid PG:VG ratio can all influence ECIG acute effects such as nicotine delivery. Therefore, regulations attempting to control ECIG acute effects will need to consider numerous factors in order to be effective. Conversely, regulations that attempt to control ECIG acute effects by considering only one factor could be circumvented easily by ECIG users and/or manufactures. For example, one regulation implemented by the European Union in 2014 attempted to limit ECIG nicotine delivery to levels comparable to a tobacco cigarette by prohibiting the sale of ECIG liquids with nicotine concentrations over 20

mg/ml. However, because other device, liquid, or user factors remain unregulated, users who are forced by this regulation to lower their liquid nicotine concentration could simply use a liquid with a higher PG content, increase the intensity of their puffs, and/or increase device power to increase their nicotine delivery to levels that may match or exceed the nicotine delivery of a tobacco cigarette. Indeed, several studies have demonstrated that users are capable of obtaining cigarette-like doses of nicotine using liquids much lower than 20 mg/ml (e.g., Dawkins et al., 2016; Spindle et al., 2017; Wagener et al., 2017). Given that manipulating device, liquid, and user puffing behaviors can also alter other acute ECIG effects (e.g., subjective effects; Hiler et al., 2017) and characteristics of the aerosols produced by these products (e.g., particulate matter and toxicant yields; Baassiri et al., 2017; Talih et al., 2015) the most effective regulatory actions pertaining to ECIGs will account for the influence of all of these factors.

Nicotine flux, or the amount of nicotine emitted from an ECIG per a given unit of time, is one model that has been proposed as a means to regulate ECIG nicotine delivery (Shihadeh & Eissenberg, 2014). The nicotine flux of a given ECIG can be derived with a high degree of precision by entering specific parameters such as liquid PG:VG ratio and nicotine concentration, battery voltage, heater resistance, and puff duration into a mathematical model (Talih et al., 2017). Thus, rather than attempting to limit nicotine delivery by regulating relevant factors individually, like the recent European Union regulation attempting to limit nicotine delivery by controlling liquid nicotine concentration, regulations that address nicotine flux focus on the rate of ECIG nicotine output, allowing device power, liquid nicotine concentration, and user behavior free to vary, so long as nicotine flux stays within the regulated range. Theoretically, the nicotine flux allowable in an ECIG could be restricted to a level that delivers enough nicotine to suppress nicotine abstinence symptoms adequately in current cigarette smokers, and that is also low

enough so as to encourage non-nicotine users to initiate nicotine use with an ECIG (Shihadeh & Eissenberg, 2014). Regulatory bodies such as the FDA would benefit from further clinical laboratory research in which nicotine flux levels are manipulated and subjective effects examined in order to determine the optimal flux level for achieving such a goal. Overall, regulating the acute effects of ECIGs and/or the content of their aerosols will require models such as the proposed nicotine flux model that are capable of considering simultaneously all of the device, liquid, and user factors that can influence nicotine delivery, non-nicotine toxicant delivery, and subjective effects.

Limitations

There were several limitations to the present study. First, while the 30 participants who completed the present study were all experienced ECIG users, 10 of these individuals were not former cigarette smokers while the remaining 20 were former cigarette smokers. These two groups differed in ways that may have influenced some results of the present study. For example, the individuals who had never smoked cigarettes reported being less dependent on their ECIG, as evidenced by significantly lower dependence scores on the Penn State Dependence questionnaire [t (28) = -2.73, $p < .05$]. For this questionnaire, former cigarette smokers had a mean (SD) dependence score of 10.35 (4.63) while individuals who were not former smokers had a mean dependence score of 5.8 (3.55; Table 8). Notably, these two groups did not differ in the amount of liquid they used per day, preferred liquid nicotine concentration or device power, or in the length of time they had been using ECIGs. One possible explanation for these observed differences in ECIG dependence is that the former cigarette smokers were highly dependent on their tobacco cigarettes, and this dependence level was maintained when they switched to ECIGs. Conversely, given that former cigarette smokers were on average significantly older

(Mean = 29.57; SD = 7.19) relative to never smokers (Mean = 21.53; SD = 2.51) [t (28) = -3.41, $p < .01$], these individuals may have reported greater dependence simply because they had been using nicotine-containing products for a greater length of time (Table 8).

Importantly, likely due to their higher levels of dependence, former cigarette smokers also reported experiencing more pronounced nicotine-abstinence symptoms at baseline relative to those who were not former smokers following 12 hours of abstinence from their ECIG. For example, collapsed across condition former smokers reported significantly higher mean baseline scores for the items "Craving an e-cigarette" (Mean = 63.99; SD = 35.46) and "Anxious" (Mean = 24.86; SD =29.26) from the Hughes Hatsukami withdrawal scale relative to individuals who were not former cigarette smokers ("Craving an e-cigarette": Mean = 23.58; SD = 26.65; "Anxious": Mean = 5.40; SD = 7.83; VAS scale: 0-100; Table 8). As a result of these baseline differences in abstinence symptoms, the overall magnitude of abstinence symptom suppression following ECIG use may have been underestimated, principally for former cigarette smokers. Future research may benefit from adding a minimum dependence score to the study inclusion criteria (e.g., dependence scores below a certain threshold will be exclusionary) or investigating never smokers and previous smokers separately in order to understand each group's respective ECIG-associated nicotine delivery and subjective effect profiles.

Table 8.

Results of Statistical Analyses for Demographic and Subjective Effects Data by Former Smoking Status.

	Former Cigarette Smokers N = 20	Never Smokers N = 10		
	Mean (SD)	Mean (SD)	*t*-statistic[a]	*p* value
Age (years)	29.6 (7.2)	21.5 (2.5)	3.4	<.05
Volume Liquid Used/day (ml)	6.7 (6.5)	5.4 (3.5)	0.6	n.s.
Nicotine Concentration (mg/ml)	9.3 (4.6)	6.9 (3.2)	1.5	n.s.
Duration ECIG use (months)	16.6 (13.5)	16.6 (10.1)	0.1	n.s.
Penn State Dependence[b]	10.4 (4.6)	5.8 (3.6)	2.7	<.05
Fagerström TND[c]	4.2 (2.5)	2.6 (1.8)	1.8	n.s.
Baseline "Craving"	64.0 (30.0)	23.6 (9.5)	12.1	<.001
Baseline "Urge to use an ECIG"	66.4 (27.7)	28.7 (8.8)	12.3	<.001
Baseline "Anxious"	24.9 (22.4)	5.4 (22.5)	5.0	<.01
Baseline "Irritable"	16.3 (17.4)	3.5 (17.4)	3.6	n.s.
Baseline "Depression"	4.1 (4.5)	2.6 (4.4)	0.8	n.s.
Baseline "Difficulty Concentrating"	11.7 (11.6)	5.6 (11.4)	1.8	n.s.
Baseline "Drowsy"	15.6 (17.0)	12.8 (17.1)	0.2	n.s.
Baseline "Hunger"	27.6 (21.0)	22.3 (21.2)	0.4	n.s.
Baseline "Impatient"	12.7 (11.6)	5.0 (11.4)	3.1	n.s.
Baseline "Restless"	14.9 (15.2)	7.8 (15.2)	1.5	n.s.
Baseline "Desire for Sweets"	8.8 (14.8)	11.8 (14.5)	0.3	n.s.
Baseline "QSU Factor 1"	13.6 (8.9)	18.2 (4.4)	3.4	n.s.
Baseline "QSU Factor 2"	5.7 (4.9)	3.7 (4.7)	1.2	n.s.

Note: n.s. = not significant; Baseline subjective scores were collapsed across condition.

[a]df = 28.

[b] Penn State Electronic Cigarette Dependence Index (Foulds et al., 2015).

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

Another limitation of the present study was that certain puff topography variables such as puff duration and puff volume were not controlled. As a result of allowing these puffing parameters to vary, participants exhibited significantly shorter and smaller puffs when using the 100PG:0VG liquid relative to the other liquids. These observed differences in puff duration and puff volume likely reduced the influence of PG:VG ratio on nicotine delivery, given that manipulating PG:VG ratio can influence nicotine yield to a greater extent when puff duration and puff volume are also held constant in pre-clinical examinations (e.g., Baassiri et al., 2017). Future examinations may want to consider also holding puff duration and puff volume constant, as in previous clinical laboratory examinations of tobacco cigarettes (e.g., Zacny & Stitzer, 1988). However, allowing puff duration and puff volume to vary across participants alternatively could be considered a strength of the study because this methodological approach likely increased its external validity. That is, results from the present study may suggest that prior pre-clinical examinations may have overestimated the influence of ECIG liquid PG:VG ratio on nicotine delivery, analogous to the example of "low-yield" cigarettes. In this example, despite pre-clinical examinations demonstrating that "low-yield" cigarettes would produce fewer toxicants relative to "full flavor" cigarettes, subsequent clinical laboratory studies revealed that these two products exposed users to comparable toxicant levels as a result of individuals increasing their puffing intensity when using the "low-yield" cigarettes (Hoffman et al., 1997; FTC, 2000). Collectively, findings from the present study and those from examinations of "low-yield" cigarettes highlight the importance of pre-clinical studies using puff topography data recorded from human participants when generating aerosols in order to assess toxicant yields more accurately. In addition, the present study's findings highlight the importance of controlling multiple puff topography variables (including puff duration and puff volume) in order to

elucidate more precisely the influence of a particular ECIG device or liquid characteristic on nicotine yield and/or delivery.

Additional limitations of the present study are noteworthy. First, participants were not permitted to use their preferred device and liquid, as these parameters were held constant in order to assess the influence of liquid PG:VG ratio on study outcomes. The extent to which outcomes such as participants' nicotine delivery, puff topography, and subjective effects may have differed with the use of their preferred device, liquid nicotine concentration, and flavor is unknown. However, given the extreme variability across devices and liquids and the fact that these factors can all influence ECIG acute effects, enabling participants to use their preferred device and liquid would have detracted severely from the internal validity of the study and made the interpretability of study findings more difficult. Second, the Sidepak personal aerosol monitor used in the present study was only capable of detecting particles between 0.1 (100 nm) and 2.5μm and thus could not detect so-called ultrafine particles that are smaller than 100 nm in diameter. ECIG aerosols tend to be comprised of a high proportion of ultrafine particulate matter, regardless of the PG:VG ratio used to generate them (Melstrom et al., 2017; Mikheev et al., 2016; Schripp et al., 2013). Further, aerosols generated from liquids high in PG are comprised principally of ultrafine particles (Baassiri et al., 2017). Thus, total particulate matter concentrations were likely underestimated in all study conditions, with the greatest underestimations occurring during the use of the 100PG:0VG liquid. However, further examinations are needed to understand better the clinical relevance of these ultrafine particles, as no investigations have elucidated the composition of these particles or whether they can have adverse health effects on ECIG users or bystanders via secondhand exposure. Third, the short-term nature of the study with controlled puffing parameters (i.e., 10-puff directed puffing bouts)

may have altered study outcomes. ECIG users may exhibit different puff topography under *ad libitum* puffing conditions relative to directed such that they may increase their mean puff number, duration, and volume (Spindle et al., 2017). Thus, PG:VG ratio may have further influenced ECIG acute effects under *ad libitum* puffing conditions. However, directed puffing parameters were necessary in this study to maintain internal validity and increase the interpretability of the results. Fourth, the majority of participants in the present study (29 out of 30) were males. While, several nationally-representative survey studies have demonstrated that males are more likely to be ECIG users than females (e.g., Jamal et al., 2017; Syamlal, Jamal, King, & Mazurek, 2016), this may have limited the generalizability of the present study's findings. Lastly, the laboratory setting could be considered a limitation. However, results from previous clinical laboratory studies have predicted subsequent real-world outcomes. For example, clinical laboratory examinations conducted on the heat-not-burn product the Accord demonstrated that this product was relatively ineffective at suppressing nicotine abstinence symptoms, suggesting that this product would likely be an inadequate substitution for conventional tobacco cigarettes (Breland et al., 2002). Subsequent examinations demonstrated that when cigarette smokers were given the Accord, this device did not substitute for tobacco cigarettes completely and increased participants' overall use of nicotine products (Hughes & Keely, 2004). Thus, despite the laboratory setting of the present study, the findings likely will translate to real-world outcomes.

Conclusions

This clinical laboratory study examined the influence of ECIG liquid PG:VG ratio on nicotine delivery, HR, subjective effects, puff topography, $PM_{2.5}$, and overall liquid consumption. Results demonstrated that when relevant device, liquid, and puffing parameters are

held constant and PG:VG ratio is manipulated systematically, nicotine delivery, ECIG sensory effects, puff topography, and $PM_{2.5}$ are influenced directly. Similar to previous pre-clinical examinations of the influence of PG:VG ratio on nicotine yield, post-ECIG use mean plasma nicotine concentration was highest in the 100PG:0VG condition. However, as a result of participants exhibiting significantly shorter and smaller puffs when using the 100PG:0VG liquid, differences in nicotine delivery across the different PG:VG ratios were less pronounced than nicotine yield differences observed in similar pre-clinical studies that held these puffing parameters constant. Abstinence symptoms were suppressed similarly across all ECIG PG:VG ratios but sensory effects of ECIG use were the least favorable in the 100PG:0VG condition. Lastly, concentrations of $PM_{2.5}$ increased relative to baseline during use of the three liquids containing VG, but did not increase during use of the 100PG:0VG liquid. Taken together, findings from the present study suggest that ECIG liquid PG:VG ratio can influence the acute effects of ECIG use and the aerosols emitted from these devices. Regulations intended to control acute effects of ECIG use such as nicotine delivery should consider ECIG liquid PG:VG ratio in addition to other device characteristics (e.g., device wattage), liquid components (e.g., liquid nicotine concentration), and user behaviors (e.g., puff duration) also known to influence these outcomes.

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