HHS Public Access

Author manuscript

Tob Regul Sci. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

Tob Regul Sci. 2016 October ; 2(4): 426–439. doi:10.18001/TRS.2.4.12.

Clinical Laboratory Evaluation of Electronic Cigarettes/ Electronic Nicotine Delivery Systems: Methodological Challenges

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Abstract

Objective—Evaluating electronic cigarettes (ECIGs) in the clinical laboratory is critical to understanding their effects. However, laboratory evaluation of ECIGs can be challenging, as they are a novel, varied, and evolving class of products. The objective of this paper is to describe some methodological challenges to the clinical laboratory evaluation of ECIGs.

Methods—The authors gathered information about challenges involved in the laboratory evaluation of ECIGs. Challenges were categorized and solutions provided when possible.

Results—Methods used to study combustible cigarettes may need to be adapted to account for ECIG novelty and differences within the class. Challenges to ECIG evaluation can include issues related to 1) identification of ECIG devices and liquids, 2) determination of short -term ECIG abstinence, 3) measurement of use behavior, and 4) assessment of dependence. These challenges

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Human Subjects Statement
All studies included in this analysis were approved by the Institutional Review Board of Virginia Commonwealth University.

Conflict of InterestStatement
All authors of this article declare they have no conflicts of interest.

are discussed, and some suggestions to inform ECIG evaluation using clinical laboratory methods are provided.

Conclusions—Awareness of challenges and developing, validating, and reporting methods used to address them aids interpretation of results and replication efforts, thus enhancing the rigor of science used to protect public health through appropriate, empirically-based, ECIG regulation.

Keywords

electronic cigarette; electronic nicotine delivery system; clinical laboratory; methods; evaluation

INTRODUCTION

In recent years the use of electronic cigarettes (ECIGs, also electronic nicotine delivery systems, or ENDS) has been increasing rapidly in adolescents and adults.[1–3] The effects of ECIG use are beginning to be evaluated, and a complete understanding of them will require a variety of methods, including *in vitro* and *in vivo* non -human animal studies, clinical trials, qualitative work, and quantitative surveys. Used together, these methods can help reveal the influence of product design and user behavior on the long-term impact of ECIG use on individual and public health (for reviews of such work, see [4–7]).

The clinical laboratory is one setting in which many questions about ECIGs can be answered, as has been done for medication development (eg, [8–10]), other drugs of abuse[11–13] as well as many other tobacco products (eg, [14–16]). For example, studies conducted in the clinical laboratory showed that cigarette smokers change their puffing behavior (puff topography) when they switch from “full-flavor” to “light” cigarettes, and thus are exposed to similar levels of toxicants (eg, [17,18]). Indeed, epidemiological studies have confirmed that the health benefits of switching to “light” cigarettes are minimal at best (eg, [19–22]). Similarly, clinical laboratory-based studies have shown that a single episode of tobacco smoking using a waterpipe delivers significantly higher levels of CO than the smoking of a single cigarette[23,24], as well as that smokers’ use of some smokeless tobacco products reduces exposure to toxicants such as CO and the tobacco specific nitrosamine NNK[25] but fails to suppress withdrawal adequately. [26] Such findings from the clinical laboratory have direct implications for increasing understanding of the individual and public health burden of tobacco product use.

Of course, cigarettes, waterpipe, and smokeless tobacco differ on a variety of characteristics – ingredient types and amounts, design, packaging, method of use – and thus require different methods to evaluate their effects. Relative to other tobacco products, for example, mass market cigarettes are more homogenous and generally are more standardized with respect to packaging, size, shape, and nicotine content. Consequently, identification of product, quantification of cigarette use patterns, and other issues relevant to clinical laboratory research are relatively straightforward, increasing the validity of cross-study comparisons and aiding study replication. Also convenient for evaluating the effects of cigarettes, as well as other combusted tobacco products (eg, waterpipe, cigars), is that measurement of recent product use is possible via analysis of exhaled air carbon monoxide (CO). In contrast, non-combusted products that do not produce CO require the measurement

of other biological fluids for verification of abstinence (eg, [27,28]). Each tobacco product presents methodological challenges for the evaluation of its effects in the clinical laboratory.

Clinical laboratory researchers now face similar challenges for evaluating the effects of ECIGs, a relatively new and fast-evolving product. Currently available clinical laboratory methods must be adapted, and new methods must be developed and validated, to provide the rigorous science that will inform regulation designed to protect individual and public health. The purpose of this paper is to describe some methodological challenges to the clinical laboratory evaluation of ECIGs, including: 1) identification of ECIG devices and liquids, 2) determination of short-term ECIG abstinence, 3) measurement of ECIG use behavior, and 4) assessment of ECIG dependence. Where possible, solutions to these problems are also discussed.

ECIG Devices and Liquids

ECIGs are a class of products that use an electrically-powered heating element to aerosolize a liquid that contains solvents such as propylene glycol (PG) and vegetable glycerin (VG), flavorants, and, usually, nicotine. There are at least 466 distinct ECIG brands and over 7,700 distinct liquid flavors that contain nicotine in concentrations ranging from 0 to 36 mg/ml or higher.[4,29] The vast number of combinations and permutations of these brands and liquids presents a challenge for systematic ECIG evaluation.

ECIG devices—ECIG systems can be “closed”, in which case the user cannot add any liquid (eg, cartridges are pre-filled by the manufacturer), or “open”, in which case the user adds liquid to the device as desired (eg, cartridges or reservoirs called “tanks” are filled by the user and are often reusable). Thus, the liquid ingredients and the amount of liquid available for use per ECIG episode is determined at least partially by which system is chosen. ECIGs may also vary in terms of device power (measured in Watts), which is a function of electromagnetic force (E, measured in Volts) provided by the battery, and resistance (R, measured, in Ohms) of the heating element (power is equal to E^2/R). Thus device power increases as voltage increases and resistance decreases, and increased power results in increased yield of nicotine and other toxicants.[30,31] In clinical laboratory investigations of ECIG effects, decisions regarding which device configurations to use likely will influence study outcome and may depend on study goals. Choices may differ if the goal is, for example, to generalize results to users of a commonly purchased ECIG type/brand, to study specific ECIG use behaviors (eg, dripping of liquid directly onto heater coil; [32–34]), or to ensure delivery of nicotine reliably (eg, [35]). Also important for consideration is whether the device chosen mirrors that used by the study participants, when those participants have previous experience with an ECIG. Whatever device configuration is used in clinical laboratory work, accurate measurement and reporting of voltage and resistance as well as any other relevant characteristics (eg, number of heating elements) is essential.

ECIG liquid—ECIG liquid can be purchased in pre-filled cartridges or in bottles of various volumes and nicotine concentrations via specialty shops and internet vendors. Unfortunately, however, product labeling may not reflect actual liquid content. For example, objective testing confirms variability in nicotine content across samples of products with the same

labeled concentration.[36–40] Samples of one brand of liquid labeled as 0 mg nicotine were found to contain from 0.07 to 21.8 mg nicotine per cartridge, while those labeled as 24 mg nicotine actually contained 0.09 to 20.6 mg nicotine per cartridge.[40] Thus, while some product labels may be accurate[41], others are not (eg, approximately 20% in difference in labeled versus actual nicotine concentration[38]). Such discrepancies between product labeling and liquid content necessitate that researchers confirm the nicotine concentration of liquid products purchased for use in clinical laboratory studies. In these situations, researchers are faced not only with the additional costs and time lost for independent testing, but also the determination of an acceptable margin of error for a given study. Another approach is to for researchers to make liquids themselves, thus affording complete control over all ingredients. This control may be important, as nicotine yield may be affected by other liquid ingredients. For instance, increased levels of PG, relative to VG, may result in increased nicotine yield.[31] Therefore, whether liquids are purchased from independent vendors or made in-house, verifying and reporting concentrations of each constituent is important.

Researchers must also make choices concerning the flavor of liquid used. Nearly 20% of adult ECIG users report use of flavored ECIG liquid.[42] Liquid flavor preference may differ as a function of cigarette smoking status, such that former smokers (ie, exclusive ECIG users) may be more likely to prefer fruit/sweet flavors while current smokers (ie, dual ECIG-cigarette users) may prefer tobacco flavors ([43] but see [44]) Users may also mix multiple flavors to create a unique taste profile[45] or switch between flavors often. [43] Flavor switching may occur be cause users perceive a blunting of the flavor with long term use,[43] or what has been referred to as “vaper’s tongue”.[46] For these reasons, the flavor(s) of liquid used in a clinical laboratory study is likely to affect outcomes such as subjective experience, choice behavior, and/or patterns of use.

Summary—Researchers should be mindful of the combination of device and liquid features chosen for study, recognizing their influence on study outcomes and the potential limitations of generalizability of study findings. Equally important is the accurate reporting of such features (eg, actual nicotine concentration of the liquid) and the ECIG-related characteristics of the sample (eg, previous experience with specific types of ECIG devices/liquids).

Determination of Short -Term ECIG Abstinence

The evaluation of tobacco product effects (eg, abstinence symptom suppression, toxicant exposure) often requires that study participants abstain from nicotine/tobacco either in the short- or long-term. Short-term (eg, <12 hours) nicotine/tobacco abstinence is often used to assess product-related nicotine delivery and/or abstinence symptom suppression (eg, [47–50]). Longer-term abstinence (eg, days or weeks) may be required as a negative control condition in studies designed to examine user toxicant exposure (eg, [14,26]), the effects of nicotine after more than 12 hours abstinence (eg,[51,52]), or product cessation outcomes (eg, [53,54]). Common measures of product abstinence include self-report and biochemical markers of exposure such as expired air carbon monoxide (CO) or nicotine or cotinine in body fluids.

Self-report—Self-report measures include items such as "*Have you used any nicotine-containing products in the past [12 hours/24 hours/7 days]?*" or similar (eg, [55–57]), depending on the abstinence interval of interest. However, the validity of self-reported smoking status/smoking behavior is sometimes questionable (eg, [58,59]), particularly when participation is a clinical laboratory study is contingent upon short-term abstinence. In such cases, some motivated participants may misrepresent their recent nicotine/tobacco use history. Moreover, given the ease of taking even only a few puffs from an ECIG, participants may be more likely to misremember recent ECIG use than cigarette use. Thus, to ensure that the required abstinence period is met, self-report alone may not be the ideal measure.

Expired air CO—CO is produced when tobacco or other carbon-containing compounds are burned incompletely, so usually it is inhaled by users of combustible tobacco products. In humans, CO has a relatively short half-life of 2–3 hours[60] and is therefore often used to verify short-term combustible tobacco abstinence.[23,61–63] In contrast, ECIGs do not burn anything when operated as intended, so these products do not produce CO and do not deliver it to users (eg, [64]). Thus, CO also is not a good index of recent ECIG use in individuals who use these products. For example, data from a study of ECIG-naïve cigarette smokers (N= 33) and experienced ECIG users(N = 25) who were instructed to abstain from all nicotine/tobacco demonstrates that, for cigarette smokers, low expired air CO concentration (Mean= 6.7 ppm; SD =2.3) was accompanied by low plasma nicotine concentration (Mean= 3.3 ng/ml; SD = 2.8). However, for the ECIG users, low CO concentrations (Mean = 3.0 ppm; SD= 2.1) were associated with plasma nicotine concentration that was significantly higher than the smokers (Mean =5.8 ng/ml; SD= 11.6), indicating that at least some ECIG users likely did not abstain for as long as the cigarette smokers did (See Figure 1; adapted from [65]). In particular, of the 25 experienced ECIG users participating in this multi -session study, 8 had at least one baseline plasma nicotine concentration greater than 10 ng/ml, despite mean CO levels of 3.5 ppm (SD= 2.5) at baseline. Plasma nicotine concentrations of greater than 10 ng/ml are consistent with recent tobacco/ECIG use.[64] Clearly, CO is not as informative a method for verifying pre-study abstinence in ECIG users as it is for users of combustible tobacco products. Indeed, combustible tobacco users may turn to ECIGs to help them comply with abstinence instructions, adding a level of complexity to studies of combustible products as well.

Nicotine—Nicotine can be measured in body fluids and has a half -life of 1–2 hours (eg [66–69]). However, nicotine measurement in body fluids involves analytical chemistry methods and therefore requires specific equipment and expertise that may not be available immediately to clinical laboratory researchers, introducing delays of days or weeks between sample collection and result reporting. Thus, while nicotine concentration is a useful tool for verifying short -term ECIG abstinence in theory, non-compliance may not be detected in practice until after a participant has completed a protocol. Depending on the study, data from a non-compliant participant may be unusable, thus decreasing power or requiring the expense and time associated with recruiting additional compliant participants to replace those found to be non-compliant.

Urine cotinine—Cotinine is a metabolite of nicotine, and has a half -life of approximately 16–19 hours.[70] Thus, it offers a better index of long -term product abstinence than nicotine or CO. For example, urine cotinine concentration has been used to confirm nicotine abstinence over days (eg, [14,26]) and weeks (eg, [71,72]), and is recommended for use in the biochemical verification of tobacco abstinence in cessation studies.[54] Importantly, cotinine can be measured in urine semi -quantitatively or quantitatively. Semi-quantitative measurement involves the use of a test strip that can be dipped into urine or saliva, providing an immediate estimate of cotinine concentration. However, over a 5-day period, this method can lead to mis-identification of abstinent tobacco users as non-abstinent if the test strips cross-react with the cotinine metabolite trans-3′-hydroxycotinine (eg, [73]). Quantitative measurement of cotinine using analytical chemistry methods is more specific, but, like nicotine analysis, requires the associated equipment, expertise, and time.

Summary—Overall, determining short -term abstinence from ECIGs in a time-and cost -efficient manner remains a challenge. Because of this ongoing challenge, studies in which short-term ECIG abstinence is critical may require a variety of procedures to enhance compliance, including the use of boguspipeline methods (eg, [74–77], observing participants for several hours before testing to ensure abstinence), and, ultimately, plasma nicotine analysis to eliminate data from non-compliant participants. Long-term ECIG abstinence can be determined by measuring saliva/urine cotinine concentration quantitatively. For studies involving participants who use a combination of combustible and non-combustible tobacco products, a combination of measures will likely be required. The field would be aided immensely by a valid, reliable, specific, rapid, and cost-effective method for the quantitative assessment of nicotine and cotinine in body fluids.

Measurement of ECIG Use Behavior

Clinical laboratory research with tobacco users requires an accurate understanding of an individual's tobacco product use history, broadly defined, to determine if potential study participants meet inclusion/exclusion criteria and characterize the study sample. Detailed use patterns (eg, puff topography measures) also are sometimes required to understand factors influencing self-administration of nicotine (and other toxicants). For tobacco cigarettes, instruments for measuring use behavior (eg, lifetime and current use; cigarettes/day; [3,78–80]) and topography[81–84] are well -established and similar instruments are becoming more standardized for other tobacco products, such as waterpipe.[85,86] For ECIGs, characterizing broad use patterns is challenging, though topography measurement is evolving.

Measuring broad use patterns—The measurement of broad ECIG use patterns has presented several challenges to extant monitoring systems (see Table 1 for a listing of common frequency/consumption measures). First, the terminology used to describe and identify ECIGs is not yet established and appears to be shifting with marketing/industry influence.[29] Evolving terms to describe these products include "vape pens", "e-hookahs", and "vaporizers".[87] The variability in device characteristics (eg, voltage, resistance), which even some users have difficulty distinguishing between[88], and their influence on nicotine delivery adds additional complexity. Second, there is no standardized ECIG measurement unit that is analogous to a single cigarette. For example, some ECIG users may use their

devices constantly throughout the day[89], and/or may use multiple device types, making less meaningful the concept of a "single ECIG use episode" and complicating items assessing daily or weekly use. It is possible that broad categories of ECIG use behavior may exist. In-depth assessment of a large group of users may be necessary to quantify and describe these patterns. Third, to measure the intensity of ECIG consumption accurately, an important concern is whether a potential participant uses an open or closed system: with an open system the unit of measure may include ml used/day, and for a closed system the unit of measure may include number of disposable ECIGs or prefilled cartridges used (see Table 1).[90–92] Liquid nicotine concentration is also relevant to intensity of use, at least inasmuch as nicotine dependence is a criterion for inclusion into a study. Obtaining this information may be difficult given the variability in manufacturer labeling, accuracy of that labeling, lack of knowledge on the part of the ECIG user, and the ability of ECIG users to prepare and mix their own liquid.[89] The ease of purchase and preparation of ECIG liquid base constituents (propylene glycol, vegetable glycerin, nicotine base) can make this determination more difficult for some participants and perhaps those who mix their own liquids may need to be excluded from some studies.

Measuring ECIG puff topography—Another methodological obstacle faced by researchers interested in studying ECIGs concerns the measurement of puff topography. Puff topography is the detailed examination of puffing behaviors, including puff number, duration, volume, inter-puff-interval (IPI), and flow rate.[93] Puff topography measurement is critical to understanding the effects of tobacco products because nicotine intake, and the intake of other harmful smoke or aerosol constituents, is determined in part by how a product is used.[94,95] Historically, topography measurement has been assessed in laboratory settings using observational methods and mouthpiece-based computerized devices, both of which are reliable and valid approaches for cigarette smokers.[93,96,97] Both may present challenges to researchers interested in measuring topography in ECIG users.

Observational measurement of puff topography typically consists of trained scorers measuring variables from smoking bouts previously video-recorded in the laboratory. Unfortunately, this method of topography measurement presents challenges for the study of ECIG use that are similar to those encountered in studies of cigarette smoking: it is labor intensive and does not allow measurement of some critical variables (eg, puff volume; see [93]). Moreover, operational definitions developed for measurement of combustible cigarette topography may not be adapted easily for measurement of ECIG topography. For cigarettes, distinct cues can be used to determine reliably the start and stop of a puff, such as the red glow at the burning cigarette end that becomes more pronounced upon inhalation and/or the contact of a user's lips with the mouth-end of their cigarette.[93,98] For ECIGs, however, characterizing the start and stop of ECIG puffs is made difficult by the wide variability in design features. For instance, some closed system ECIGs include a light-emitting diode (LED) on the non-mouth end to simulate the glow of a cigarette tip, while others have no LED or one that glows whenever a button is pressed to activate the battery.[99] Indeed, LED activation in some instances does not represent puff onset.[100,101] For example, users may activate their device (therefore turning on the LED) before or after placing it in their mouth, or may leave their device in their mouth while not puffing actively.[100] Some

users also clearly inhale from their ECIG before LED activation, presumably in “cigalike” devices that require user inhalation to activate.[101] Thus, neither the glow of a LED nor users’ lip contact with their ECIG appear to be as well-correlated with puff onset/offset as these cues are with the onset/offset of a puff from a tobacco cigarette.

Mouthpiece-based computerized devices measure tobacco cigarette user puff topography with the aid of specialized mouthpiece capable of detecting flow-induced pressure changes that occur during inhalation; the cigarette must be placed in the mouthpiece which is designed to hold it. Stationary and portable versions of these computerized topography devices are available to researchers[93] and the portable version has been used to examine ECIG topography. [37,102–104] This approach has presented several methodological challenges for characterizing ECIG puffing behavior. First, one commonly used device stops recording data after 43 puffs have been measured, and while this cutoff far exceeds the average number of puffs taken from a single tobacco cigarette, may not exceed the number of puffs taken during a single ECIG use episode, resulting in incomplete data capture for some participants.[102,104] Second, when long puff durations are taken with existing portable topography devices, ECIG liquid may be drawn into the mouthpiece and alter device sensitivity.[102] Third, measuring ECIG topography with a mouthpiece-based device requires that the ECIG fit firmly into the mouthpiece. Many ECIG models do not meet this requirement, limiting the types of ECIGs for which topography can be measured. Last, design parameters of existing mouthpiece-based devices may not provide adequate sensitivity for measuring ECIG topography.[105] Typically, existing topography devices detect flow rate values at or above 15 ml/sec[106], well below average values usually observed in cigarette smokers.[104,105] However, experienced ECIG users puff with lower flow rates than cigarette smokers, sometimes close to 15 ml/sec.[102,105,107] Thus, commercially available topography recording devices may not have design parameters sensitive enough to measure ECIG puff topography accurately.[105]

To address concerns associated with using existing computerized devices to measure ECIG topography, researchers have created mouthpiece-based topography devices designed specifically for ECIGs.[105,108] For example, devices have been designed without puff number recording limitations[108] and with sufficient sensitivity for recording puffs with low flow rates. [105] Yet these instruments share some limitations with those they are intended to replace. ECIG aerosols can condense inside the mouthpiece and affect topography measurement and mouthpiece shape limits the types of ECIGs that can be studied (as in [105]). Future research may benefit from topography measurement instrumentation that does not require a specialized mouthpiece, permitting participants to use their preferred device without modification and likely resulting in more naturalistic ECIG topography assessments.

Summary—Measuring ECIG use behavior accurately and reliably is critical for clinical laboratory researchers so that they can screen potential participants, report participant use history, and study factors that influence user toxicant exposure. Future work in this area offers opportunities for testing and development of measures for clinical laboratory use that assess broad patterns of ECIG consumption with validity and reliability. Qualitative data may be useful for addressing product terminology (as in [88]), as with other tobacco products (eg, cigars; [109]). Prospective methods are needed for characterizing those ECIG use patterns

that differ between device categories or types of users. Ecological momentary assessment[110] would be particularly informative, as this technique would allow individuals to record their ECIG use in real-time and in their natural environment. Of course, each of these measurement methods has limitations (eg, underreporting of ECIG use for some ecological momentary assessment methods[111]), though their combined use should elucidate users' actual patterns of consumption. There is an ongoing need for instrumentation that allows topography measurement across all ECIG devices in order to characterize user behavior and toxicant exposure more completely and, potentially, to relate these outcomes to dependence and other health-related outcomes.

Measurement of ECIG Dependence

ECIG nicotine delivery is a function of device design, liquid constituents, and user behavior[112] and, while there is considerable variability, at least some ECIGs deliver physiologically active doses of nicotine under some conditions.[105,113] Because nicotine is a dependence-producing, psychoactive drug, those ECIGs that deliver nicotine effectively may support nicotine dependence. As with product use history, clinical laboratory researchers may need to measure dependence to ensure that potential participants meet study inclusion/exclusion criteria, to describe groups of participants, and/or to study the factors that are influenced by or that influence dependence level. While there are numerous scales for measuring dependence in cigarette smokers and SLT users[114–117], development of a valid and reliable instrument that can be used to measure dependence in ECIG users has begun only recently.[118–120] This ongoing development effort is complicated by all of the issues related to devices, liquids, and ECIG use patterns discussed above. That is, nicotine delivery profile varies widely as a function of device power and liquid constituents, and ECIG use episodes cannot be characterized easily and likely differ considerably across individuals. In addition, self-report items commonly used to measure dependence in cigarette smokers may not be adapted easily for use in measuring ECIG dependence. For example, some items assess smokers' difficulty with abstaining from cigarettes in places where smoking is prohibited (eg, [116]). ECIG use is sometimes permitted in locations where cigarettes are not[121], and, more importantly, ECIG users have developed techniques to use these products covertly[122,123], thus rendering an item assessing difficulty abstaining from ECIG use in certain settings potentially meaningless (although self-reports of this covert use behavior may themselves be indicative of dependence). All of these issues may be related to the observation that ECIG users' scores on existing dependence instruments are lower than those for cigarette smokers[118–120]. Of course, some ECIGs, particularly those that deliver nicotine inefficiently (eg, [124]) simply may be less likely to produce/maintain dependence (see [118]). Exploring dependence in ECIG users empirically likely will require a valid and reliable instrument that is specific to this population.[125]

DISCUSSION

Some ways of addressing the issues raised here are outlined below, as well as summarized in Table 2. With regard to device characteristics and liquid constituents, the ever-changing ECIG landscape presents an ongoing issue that requires independent measurement and thorough reporting of all relevant variables, to the extent possible. At the least, published

work should specify that battery voltage, heater resistance, heating element number/design, and liquid nicotine concentration were verified. Studies that require participants to meet short-term ECIG abstinence criterion may need to include an observation period of several hours' duration to ensure that criterion is met. Otherwise, biochemical verification after the fact (ie, plasma nicotine concentration below some pre-defined criterion) may be the only option, though it is costly and often not timely. Measurement of use patterns and assessment of dependence likely requires development of ECIG-specific instruments, and this development effort is one in which clinical laboratory researchers have much to contribute. For example, laboratory studies of *ad libitum* use behavior over an extended period can inform definitions of ECIG use episodes and help understand use patterns generally. Clinical laboratory studies can reveal the extent to which several hours of observed and verified ECIG abstinence reveals hallmarks of dependence such as compulsion to use an ECIG, aversive symptoms suppressed by subsequent ECIG use, evidence of tolerance, and preoccupation with use (eg, [126]). Such work will be important for understanding ECIG effects, and likely will also aid design of clinical trials and epidemiological studies.

Clinical laboratory evaluation of ECIGs and their effects is necessary but presents many challenges only some of which are detailed here: space constraints do not allow an elaboration of the difficulties in recruiting exclusive ECIG users (many ECIG users also report concurrent cigarette use[127]), challenges in providing study participants with their own brand/flavor of ECIG liquid when many users consume several flavors within a single day, and the possibility that drugs other than nicotine may be found in ECIG liquids.[128,129] The rapid evolution of products and use behaviors and the lack of regulatory control over ECIGs in many countries suggests that these and other issues will continue to remain an important feature of clinical laboratory studies of ECIG effects.

IMPLICATIONS FOR TOBACCO REGULATION

The effects of electronic cigarette (ECIG) use are beginning to be evaluated, and a complete understanding of them will require a variety of methods, including *in vitro* and *in vivo* non-human animal studies, clinical trials, qualitative work, and quantitative surveys. Used together, these methods can help reveal the influence of product design and user behavior on the long-term impact of ECIG use on individual and public health. The clinical laboratory is one setting in which many of the questions about ECIGs can be answered, and clinical laboratory researchers face challenges evaluating the effects of ECIGs, as these are a relatively new and fast-evolving product group. Currently available clinical laboratory methods must be adapted, and new methods must be developed and validated, to provide the rigorous science that will inform regulation designed to protect individual and public health. These future regulations of ECIGs may involve restrictions on devices, liquids, and flavors, and thus, researchers must use methods that clearly elucidate the effects of each ECIG component. Indeed, the May, 2016 announcement by the U.S. Food and Drug Administration (FDA) that ECIGs and their components are to be regulated by the FDA, and that some products may be required to undergo premarket review, highlights the need for addressing the challenges discussed here.[130] In general, awareness of challenges and developing, validating, and reporting methods used to address them aids interpretation of

results and replication efforts, thus enhancing the rigor of science used to protect public health through appropriate, empirically-based, ECIG regulation.

Acknowledgments

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

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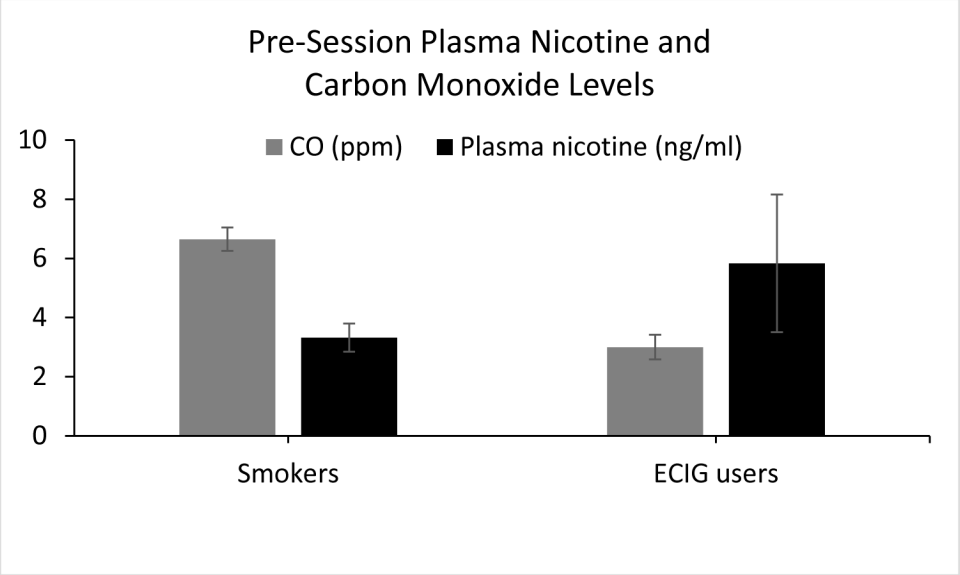

Figure 1.

Mean plasma nicotine and CO levels (± SEM) for 33 daily cigarette smokers and 25 daily ECIG users, after ~12 hours of self-reported tobacco/nicotine abstinence (includes cigarettes and ECIGs; methods fully described in [35 and 136] and presented in [65]. Asterisk indicates a significant group difference for plasma nicotine level, using an independent samples t-test ($p < .05$).

Table 1

Common ECIG Frequency/Consumption Measures

Measure	Representative empirical reports
Frequency of daily ECIG use	
Number of puffing episodes	119
Number of puffs	44,131–133
Number of hours for one refill/cartridge (hours)	131,133
Intensity (amount) of daily ECIG use	
Number of refills per day	131,133
mL of liquid per day	44,113,120,134
cartridges per day	134
ECIG device and liquid characteristics	
Preferred type/brand	92,105,119,133–135
Preferred battery brand	135
Preferred cartridge/liquid nicotine concentration/strength	92,119,133,134
Preferred cartridge/liquid brand	92
Preferred cartridge/liquid flavor	92,105,133

Table 2

Potential Solutions to the Methodological Challenges of Clinical Laboratory Electronic Cigarette Evaluation

Challenge	Potential solutions		
Device and liquid variability	Measure and report device characteristics (eg, battery voltage, heater resistance).	Report liquid characteristics (eg, flavor additives, PG: VG ratio).	Verify liquid nicotine concentration. Consider implications of flavor choice, if it is restricted.
Abstinence verification	Use a combination of bogus pipeline procedures and biological fluid assays.	Observe participant for several hours before beginning testing.	Develop reliable, valid, specific, sensitive, quick, and cost-effective methods of measuring recent nicotine exposure.
Use behavior measurement	Operationalize product terms and user behavior patterns.	Measure device and liquid characteristics used by respondents.	Report topography device specifications (eg, flow rate threshold to determine puff onset/offset; puff number cutoffs)
Dependence measurement	Consider behaviors specific to ECIG use.	Measure device and liquid characteristics used by respondents.	Develop reliable and valid ECIG dependence measurement instrument.