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Chemical Composition and Evaluation of Nicotine, Tobacco Alkaloids, pH and Selected Flavors in e-Cigarette Cartridges and Refill Solutions

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Abstract

Introduction—Electronic cigarette (e-cigarette) use is increasing dramatically in developed countries, but little is known about these rapidly evolving products. This study analyzed and evaluated the chemical composition including nicotine, tobacco alkaloids, pH and flavors in 36 e-liquids brands from four manufacturers.

Methods—We determined the concentrations of nicotine, alkaloids, and select flavors and measured pH in solutions used in e-cigarettes. E-cigarette products were chosen based upon favorable consumer approval ratings from online review websites. Quantitative analyses were performed using strict quality assurance/quality control (QC) validated methods previously established by our lab for the measurement of nicotine, alkaloids, pH and flavors.

Results—Three-quarters of the products contained lower measured nicotine levels than the stated label values (6% - 42% by concentration). The pH for e-liquids ranged from 5.1 – 9.1. Minor tobacco alkaloids were found in all samples containing nicotine, and their relative concentrations varied widely among manufacturers. A number of common flavor compounds were analyzed in all e-liquids.

Conclusions—Free nicotine levels calculated from the measurement of pH correlated with total nicotine content. The direct correlation between the total nicotine concentration and pH suggests that the alkalinity of nicotine drives the pH of e-cigarette solutions. A higher percentage of nicotine exists in the more absorbable free form as total nicotine concentration increases. A number of products contained tobacco alkaloids at concentrations that exceed U.S. Pharmacopeia limits for impurities in nicotine used in pharmaceutical and food products.

Keywords

Electronic Cigarette; Nicotine; Cigarette; Tobacco; Tobacco Alkaloids; Flavors

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DECLARATION OF INTERESTS

The authors do not declare any conflicting interests.

INTRODUCTION

Electronic cigarettes (e-cigarettes) or electronic nicotine delivery systems (ENDS) are rapidly gaining acceptance among consumers and becoming a lucrative product in the tobacco market.[1,2] Recently, the CDC reported that e-cigarette use doubled from January 2011 to January 2012 among teens.[3] E-cigarettes are battery powered aerosol generating devices that use a resistive heating coil to vaporize a solution containing propylene glycol, glycerin, flavors, frequently nicotine and sometimes ethanol and water. The solution, also known as e-liquid or e-juice, is contained in a disposable or refillable cartridge depending on the design of the e-cigarette. Solutions for e-cigarettes are available in many flavors that most often fall in five main categories: tobacco flavors (which are similar to cigarettes), fruit flavors (blueberry, peach, etc.), menthol flavors, sweet flavors (candy, chocolate, etc.) and other flavors (coffee, black tea, wine, etc.). E-liquids are available in varying nicotine concentrations that typically range from 0 mg/mL to 24 mg/mL nicotine.[4-7]

The typical e-cigarette often resembles a traditional cigarette and consists of three main parts: a battery, a cartridge and an atomizer containing a heating coil, though more recent versions of e-cigarettes have combined the cartridge and atomizer. When a user draws on an e-cigarette, a pressure switch/sensor activates the heating element to vaporize the e-liquid, the vapor then rapidly condenses to form an aerosol. A growing number of e-cigarette designs are currently on the market and these appear to be rapidly evolving to help facilitate the delivery of nicotine to the consumer in a pleasing manner. In addition to the original e-cigarette design, numerous new, larger versions, often referred to as tank systems are increasing in popularity. The tank e-cigarette devices are customizable and often bear no resemblance to a cigarette. They also usually have a manually activated switch that turns on the heating coil. Because the e-cigarette's nicotine delivery is directly related to the power delivery (wattage) of the device, tank e-cigarettes may incorporate a voltage tunable battery.[8] Users can then adjust or "tune" the voltage to deliver their differing amounts of nicotine. E-cigarettes have become highly customizable to meet the specific needs of users. Customizable features include replaceable heating coils with two or more wicks for better vaporization and multiple chamber atomizers that claim to produce a more "robust" vapor.[9]

Some manufacturers of e-cigarettes market the products as a safer alternative to combustible tobacco and in some cases imply that the products are free of harmful substances. While manufacturers do not promote e-cigarettes as cessation devices, they have been investigated for this purpose with mixed results. Some literature has shown that e-cigarettes have shown some promise as a potential cessation tool for smoking. [10] For example, a recent study from the United Kingdom found that when e-cigarettes were used as an aid for cessation, users were 60% more likely to sustain cessation when compared to conventional nicotine reduction therapies.[11] Despite those results, a number of other studies have shown that e-cigarette use was not associated with smoking cessation.[1,12,13] In addition, a recent study among Korean adolescents showed that "adolescents who tried to quit smoking were more likely to use e-cigarettes but less likely to no longer smoke, which suggests that e-cigarettes inhibit rather than promote cessation."[14] Similar results were observed for US adolescents.[15] Consumers may perceive that e-cigarettes are a safe alternative to cigarettes, which could increase experimentation.[16] However, the public health impact of using e-cigarettes cannot be adequately assessed given the relatively limited and inconsistent data on e-cigarettes currently available.

In order to assess claims about the safety of e-cigarettes, more research needs to be done to further examine the chemical contents of e-liquids. There are limited analytical data on chemicals in e-cigarette cartridges and refill solutions. Nicotine is the most widely studied constituent. Deviation between labeled and measured concentrations of nicotine in refill solutions has been reported.[17-26] The nicotine used in these devices is extracted from tobacco, and with it, other tobacco constituents are co-extracted. Other analytes of interest that have been tested in refill cartridges include tobacco-specific nitrosamines (TSNAs),[27] aldehydes,[28] tobacco alkaloids[18, 23, 25, 26] and flavors.[21] The aim of this study was to provide further analysis of potentially harmful substances contained in e-cigarettes. In order to help address the existing information gap, we measured pH as well as the concentration of nicotine, tobacco alkaloids, and selected flavors found in the cartridges and refill solutions of 36 varieties of e-cigarettes using robust, quantitative, and validated methods.

METHODS

Samples

E-cigarette materials were purchased online directly from four manufacturers (eSmoke, www.eSmoke.net; Premium, www.premiumecigarette.com; V2, www.v2cigs.com; South Beach Smoke, www.southbeachsmoke.com). A total of 36 varieties (South Beach Smoke, 7 samples; V2, 8 samples; Premium, 10 samples; eSmoke, 11 samples) were analyzed in this study. Brands were chosen based upon consumer approval ratings from online review websites (www.ecig-reviews.net, www.ecigcity.net) at the time of purchase. Upon receipt, samples were logged into a custom database, assigned barcodes with a unique barcoded ID, and stored in their original containers until analyzed. Samples in cartridge form were uncapped and the solution soaked contents were removed. The saturated reservoir material was compressed inside a 3-mL disposable syringe and the liquid was collected in a vial. Liquid refill samples were used as provided by the manufacturers. For each product, only one manufacturer lot was analyzed; thus lot-to-lot variability was not assessed.

Reagents and materials

Nicotine standards were purchased from AccuStandard (New Haven, Connecticut). Quinoline used as an internal standard for nicotine was purchased from Sigma-Aldrich (St. Louis, Missouri). *Nicotiana glauca* was purchased through Lab Depot (Dawsonville, Georgia). The pH calibration solutions were purchased from Control Company (Friendswood, Texas).

Alkaloid standards nornicotine, myosmine, anabasine, anatabine, and isonicoteine were purchased from Toronto Research Chemicals (Toronto, Ontario; Canada). Standards were purchased as racemic mixtures, if applicable. Isotopically labeled internal standard, (+/−) nornicotine-2,4,5,6-d_4 (pyridine-d_4), was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada); DL-Nicotine (methyl-d_3) was obtained from Cambridge Isotope Labs (Andover, Massachusetts). These were added to samples and used for quantification.

Flavor standards (eucalyptol, camphor, menthol, methyl salicylate, pulegone, ethyl salicylate, cinnamaldehyde, eugenol, diphenyl ether and coumarin) were purchased from Sigma-Aldrich (St. Louis, Missouri). 3',4'-(methylenedioxy)-acetophenone (MDA) was also purchased from Sigma-Aldrich and was used as an internal standard for quantifying flavor analytes. Research cigarettes, 3R4F, were obtained from the University of Kentucky and were used as matrix blank for spiking calibration standards (Lexington, Kentucky). All other chemicals were of analytical grade and were purchased through Fisher Scientific unless otherwise indicated (Pittsburgh, Pennsylvania).

Sample Preparation and Analysis Procedures

Nicotine analysis was based on modifications to a previously reported method.[29] Modifications include the use of GC-MS/MS (rather than GC-MS) and a faster GC run time (2.3 minutes versus 3.7 minutes). Also, the sample size was adjusted from 1000 mg to 400 mg, and the corresponding standard and extraction solvent volumes were scaled appropriately.

The sample preparation for the nicotine method used a 400 mg (±2 mg) sample size. Samples of e-juice were weighed into a 15 mL amber vial, spiked with 50 μL of quinoline internal standard (10.5 mg/mL), and 100 μL of alkaloids internal standard consisting of D_3-nicotine (0.38 mg/mL) and D_4-nornicotine (0.41 mg/mL). A 1 mL aliquot of 2N NaOH was added, and the sample was allowed to stand at room temperature for 15 minutes. Afterwards, 10 mL of methyl tert-butyl ether was added and the vials were capped and placed on a Rugged Rotator (Glass-Col, Terre Haute, Indiana) to tumble at 70 revolutions/minute for 1 hour. After agitation, sample extracts were expressed through a 0.45 μM filter directly into individual GC vials. Samples were run in triplicate (N=3) and analyzed. The GC-MS/MS hardware setup is the same for both the nicotine and alkaloids and because the internal standard for alkaloids was also added, the same samples could be analyzed for minor tobacco alkaloids. The analysis of nicotine and minor alkaloids was performed using a separate injection and a separate method on the same instrument. Analysis was performed in triplicate (N=3). Nicotine concentrations were reported in mg/g rather than mg/mL because the exact ratio of propylene glycol/glycerin in each e-liquids was not known.

Triplicate (N=3) samples were prepared and analyzed for minor alkaloid concentrations using the methods previously outlined.[30] Flavors analysis was also performed on triplicate samples (N=3) using methods previously outlined by Lisko *et. al*.[31] The pH analyses were done using the method described in a Federal Register Notice,[32] and samples were analyzed in duplicate (N=2).

Instrumentation and Apparatus

Flavors GC/MS analysis was performed using an Agilent 7890 GC coupled with a 5975 MSD (Agilent Technologies; Newark, DE, USA). The chromatographic separation was accomplished using an Ultra-2 capillary column (25m × 0.32mm × 0.52μM) (Agilent Technologies; Andover, MA, USA) with research grade helium (>99.9999% purity) used as the carrier gas. Specific details of the previously validated method can be found in Lisko *et. al*.[31]

Alkaloids GC-MS/MS analyses were performed using an Agilent 7890 GC coupled with a 7000 Triple-Quad detector (Newark, DE). The chromatographic separation was accomplished using a DB-1701 capillary column (30m × 0.250 μM, 0.25 μM) (J&W Scientific) with research grade (>99.9999% purity) helium used as the carrier gas. Specific details of the previously validated method can be found in Lisko *et. al*.[30]

Nicotine GC-MS/MS analysis was performed using an Agilent 7890 GC coupled with a 7000 Triple-Quad detector (Newark, DE) equipped with a CTC autosampler (Agilent Technologies; Andover, MA), which injects 1 μL of the extract per vial for analysis. The split/splitless injector was maintained at 230 °C with a helium flow rate of 1.7 ml/min for 3 min. Injections were made with a split ratio of 300:1 with a solvent delay of 1.2 min. The chromatographic separation (Supplemental Figure 1) was accomplished using a DB-1701 capillary column (30m × 0.250 μM, 0.25 μM) (J&W Scientific) with research grade (>99.9999% purity) helium as the carrier gas. The GC ramp conditions were as follows: 175 °C for 0.1 min; ramp at 10 °C/min to 180 °C; and lastly ramp 75 °C/min to 240 °C. The total GC run time was 2.3 minutes and the transfer line temperature was set to 285 °C. Compounds were ionized using electron impact ionization (70eV) in positive mode and the ion source maintained at 280 °C. Mass measurements were made in Multiple Reaction Mode (MRM). The retention times and m/z transition values chosen for detection are provided in Supplemental Table 1.

Standard curves were constructed by the analysis of *Nicotiana glauca* matrix spiked with known amounts of nicotine. *N. glauca* is an anabasine-rich tobacco species that contains no nicotine, which makes it an ideal matrix for calibration. The calibration range for the nicotine method was 0.05 - 42 mg/g and the limit of detection (LOD) was found to be 0.05 mg/g. The calculation of LOD was estimated as $3s_0$, where s_0 is the estimate of the standard deviation at zero analyte concentration. The value of s_0 was taken as the y-intercept of a linear regression of standard deviation versus concentration as specified by Taylor *et al*.[40] The method was validated by measuring the precision and accuracy of nicotine at three concentration levels. Precision/accuracy data were obtained by spiking five blank matrix samples at low, medium and high concentration levels of nicotine. A blank control was prepared by spiking five *N. glauca* matrix samples with internal standard only. The precision and accuracy of the method were found to be 3.1-3.4% RSD and 93.9 – 97.9% recovery, respectively. A matrix comparison between *N. glauca* and propylene glycol was also performed to ensure there were no matrix effects that should be considered when evaluating samples. Standard curves were injected in triplicate and the slopes and intercepts were compared. Slope differences less than 5% indicate an absence of matrix effects. A summary of the matrix comparison as well as the validation parameters can be found in Supplemental Table 2.

The pH analysis was performed on a Sirius Vinotrate (Sirius Analytical, East Sussex, England) according to the method outlined in the Federal Register.[32] We dissolved 500 mg samples in 5 mL of distilled deionized water and determined an average pH measurement over a 1 hour period. Synthetic e-juice samples were prepared by dissolving a corresponding amount of commercially available nicotine (Sigma-Aldrich) in a 1:1 mixture of glycerin/ propylene glycol to reflect concentrations of nicotine similar to those found in commercially available e-juice. Samples for pH analysis were run in duplicate (n=2). The percentage of nicotine in the freebase form was calculated using the Henderson-Hasselbach equation according to previously established methods.[32]

RESULTS

Nicotine and Minor Tobacco Alkaloids

In agreement with previous literature reports, we found the measured nicotine concentration was often significantly lower than the labeled nicotine concentrations in the refill solutions and e-liquid cartridges. Using the student t-test, we observed that the measured nicotine levels were statistically lower than the stated label values for all varieties from three of the four manufacturers ($p < 0.03$). Measured nicotine concentrations were 5.8% - 41.7% lower than the labeled nicotine values for South Beach Smoke, V2 and Premium manufacturers. Premium 6 mg/mL e-liquid products were the least accurately labeled product tested, with 41.7% less nicotine in the liquid than specified on the product's labeling. Only one manufacturer, eSmoke, had nicotine levels on their labeling that was not statistically different than measured nicotine levels. Labeled nicotine concentrations for eSmoke products were within 3.4% of the measured nicotine concentration (Figure 1).

Inconsistencies among the measured nicotine concentrations among different flavors with the same labeled nicotine concentration were most evident in V2 and Premium varieties. The V2 12mg Sahara and Peppermint flavors had measured nicotine concentrations of 11 mg and 9.6 mg respectively. Similarly, Premium 24mg Tobacco and Peach flavors had measured concentrations that were quite different, 20.5 mg and 16.5 mg respectively. While other researchers[23] found measureable levels of nicotine in e-liquids labeled as containing no nicotine, we did not find measureable levels of nicotine in 0 mg refills and cartridges for the varieties tested from these four e-cigarette providers (LOD = 0.048 mg/g).

Minor tobacco alkaloids, nornicotine, myosmine, anabasine, anatabine and isonicoteine were found in all e-liquids tested that also contained nicotine (Table 1). In traditional tobacco, there are direct correlations between nicotine and minor alkaloid concentrations.[33] However, when examining the correlation of measured nicotine and minor alkaloids in e-liquids, the relationship was not as consistent. Because of the structural similarity of the minor tobacco alkaloids and nicotine, extracts from tobacco to obtain nicotine used in e-liquids likely contain differing concentrations of the minor alkaloids depending on purification or other manufacturing processes. This likely affects the relative concentrations of minor alkaloids with respect to nicotine.

Poor quality control is another explanation for the poor correlation between nicotine and minor alkaloids. Among the samples tested, a number of samples with similar measured nicotine concentrations had widely varying minor alkaloid concentrations. For example, V2 18 mg Menthol flavor and V2 18 mg Red flavor had anatabine levels of 23 and 193 μg/g, respectively. Also, eSmoke 11 mg Minty Menthol flavor and 11 mg Morning Coffee flavor had myosmine levels of 62.7 and 15.1 μg/g, respectively. Potentially, these flavors may have been made with different lots of nicotine solution but without knowing the manufacturing process, it is impossible to determine the cause of the variation.

The American e-Liquid Manufacturing Standards Association (AEMSA), an industry group with no regulatory authority, calls for the use of US Pharmacopeia Grade (USP) grade nicotine in their e-liquid products.[41] USP specifications of nicotine purity allow for a maximum of 0.5% (5 mg/g) of a single impurity and 1.0% (10 mg/g) total impurities.[42] For example, a product containing 15.0 mg/g of nicotine can have up to 75 μg/g of a single impurity and a maximum of 150 μg/g total impurities. For the products tested, the majority of products tested had impurities that did not exceed USP limits, however, total alkaloid concentrations found in eSmoke brand exceeded USP limits in all products (Table 1). The V2 Red 18 mg solution as well as Premium Pineapple 11 mg and Premium Peach 24 mg solutions each had a single impurity (anatabine) that exceeded USP limits. Total alkaloids for the V2 Red 18mg solution and the Premium Pineapple 11 mg solutions also exceed the proposed USP limits.

It is important to note, however, that when nicotine is exposed to air, oxidation can occur which results in the generation of minor alkaloids.[43, 44] Because the rate of oxidation in e-liquids has not been reported and the time between e-liquid production and testing is not known, it is difficult to assess the concentrations of alkaloids due to nicotine oxidation. Regardless of the source of alkaloids, whether the nicotine was exposed to air during manufacturing or an impure nicotine source was used, a number of samples were found to have alkaloid impurities that exceed USP specifications. While the health implications of select impurities are not known, we draw attention here to illustrate differences in the manufacturers approach to product design.

The minor tobacco alkaloid concentrations in e-liquids are generally much lower when compared to traditional cigarettes. Traditional cigarettes have minor tobacco alkaloid concentrations in the range of 659 – 986 μg/g for nornicotine, 8.6 – 17.3 μg/g for myosmine, 127 – 185 μg/g for anabasine, 927 – 1390 μg/g for anatabine and 23.4 – 45.5 μg/g for isonicoteine.[30] eSmoke e-liquids had the highest concentrations of the minor tobacco alkaloids (6.3 – 48.2 μg/g nornicotine, 8.7 – 62.7 μg/g myosmine, 21.2 – 152 μg/g anabasine, 63.1 – 485 μg/g anatabine and 2.4 – 20.7 μg/g isonicoteine). South Beach Smoke, V2 and Premium products contained considerably less alkaloid content, suggesting a either a more pure nicotine extract was used or nicotine oxidation was minimized for those refill cartridges.

Flavors

We tested the 36 e-cigarette products for ten flavor compounds commonly used as additives in tobacco products. These compounds included eucalyptol, camphor, menthol, methyl salicylate, pulegone, ethyl salicylate, cinnamaldehyde, eugenol, diphenyl ether and coumarin (Table 2). Measureable levels of eucalyptol (<LOD – 87 μg/g) and pulegone (<LOD - 119 μg/g) were found in the menthol flavored varieties for all manufacturers. Menthol concentrations ranged from 3700 – 12,000 μg/g in flavored e-liquids, which is similar to levels found in commercial cigarette filler.[34, 35] Menthol and pulegone are typical flavors found in mint products as well. Interestingly, menthol was also found at low concentrations in 40% of the tobacco-flavored non-menthol products tested in this study. Tobacco Gold flavor (South Beach Smoke) as well as Sahara and Red flavors (V2) contained low

concentrations of menthol (6.2 -14.7 µg/g). Added menthol may reduce harshness or more closely simulate the sensory experience of smoking traditional cigarettes.

pH

The pH values for each e-liquid correlated with the measured total nicotine concentration (Table 3). In general, higher total nicotine concentrations yielded higher pH values due to inherent alkalinity of nicotine. To test this hypothesis, synthetic e-liquids were prepared using a 1:1 mixture of propylene glycol and glycerin to create e-liquids with nicotine concentrations of 6 mg/mL, 11 mg/mL, 18 mg/mL and 24 mg/mL. A series of pH measurements were made on the laboratory prepared e-liquids and a direct relation between total nicotine concentration and pH was observed. When testing the commercial brands of e-liquid, a similar correlation between nicotine and pH exists. However, the commercial products contain a number of other flavor additives that could influence the resulting e-liquid pH, thus creating a weaker nicotine/pH relationship in commercial products. Nicotine free e-liquids were slightly acidic (pH 5.1 – 6.4), possibly due to the absence of nicotine and the presence of weakly acidic substances.

The percentage of nicotine in the free (unprotonated) form can be calculated using the Henderson-Hasselbach equation based on measured pH and total nicotine.[32] The free or unprotonated form of nicotine is more readily absorbed by the user than protonated forms, increasing the rate of uptake of nicotine received by the user.[36] Generally, all e-liquids that contained nicotine had free-base nicotine concentrations in the range of 60-90%, and there was a trend toward increasing free-base nicotine concentrations as the measured total nicotine concentrations increased. Because it was determined that the pH is driven by the alkalinity of nicotine in laboratory prepared e-liquids, this observation was expected (Figure 2). The correlation between pH, measured nicotine and free-base nicotine is not as strong (R^2 = 0.827 for commercial products versus R^2 = 0.965 for laboratory prepared e-liquids), likely due to flavors and other additives found in the various e-liquids. For the nicotine-containing products tested, the free-base nicotine percentages plateaued at approximately 90%.

DISCUSSION

We evaluated pH, nicotine, flavors and minor tobacco alkaloids in e-liquid found in cartridges and refill solutions of four e-cigarette manufacturers: South Beach Smoke, Premium, V2 and eSmoke. The measured nicotine levels for South Beach Smoke, Premium and V2 were all significantly lower than the labeled concentrations. Because labels are inaccurate, an inherent consumer risk exists in that consumers do not know how much nicotine they may be exposed to when using e-cigarettes. Although results from this study found measured nicotine levels lower than labeled concentrations, other studies have found more nicotine than labeled concentrations.[17-26] Regardless of the inaccuracies on the label, most of the e-liquids tested had a high percentage (60 – 90%) of nicotine existing in free or unprotonated form. The amount of nicotine in e-liquids can result in adverse medical effects if ingested[37] and as a result, calls to poison control centers about exposures to e-cigarette products have increased dramatically.[38] Minor tobacco alkaloids were found in all nicotine containing e-liquid varieties, which suggests the nicotine in the e-liquids is extracted from tobacco. In some cases, minor alkaloid levels indicate that the nicotine used in certain e-liquids exceeded USP impurity specifications. The limitation of this observation is that the oxidation rate of nicotine is unknown, thus the source of impurities cannot be identified with certainty. Products from all four manufacturers tested contained measureable levels of flavors. Flavors have been shown to play an important role in helping enhance the experience for the e-cigarette user, as well as potentially aiding with smoking abstinence.[45,46] Although flavored e-cigarette products are popular with adult users, sweet and candy-like flavors may make e-cigarettes attractive to children.[39] The pH of e-liquids that were examined was largely driven by the concentration of nicotine due to its alkalinity. A direct correlation was found between pH, measured total nicotine concentration, and free nicotine (%) in e-liquids.

This research assessed single manufacturer lots of 36 different e-liquids from four manufacturers; much more research is needed to more fully characterize e-cigarettes and assess potential public health concerns resulting from increased use of e-cigarettes and other electronic nicotine delivery devices. Our evaluation of the e-liquids provides insight into constituents and additives in current brands, but given the number of brands and the dynamic market, and we believe routine analytical testing of products is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Adkison SE, O'Connor RJ, Bansal-Travers M, Hyland A, Borland R, Yong HH, et al. Electronic nicotine delivery systems: International tobacco control four-country survey. Am. J. Prev. Med. 2013; 44(3 SUPPL. 3):207–215. doi: 10.1016/j.amepre.2012.10.018. [PubMed: 23415116]
2. Koch W. E-cigarettes: no smoke, but fiery debate over safety. USA Today. Sep 17.2012
3. Centers for Disease Control and Prevention (CDC). Notes from the Field: Electronic Cigarette Use Among Middle and High School Students – United States, 2011-2012. MWWR. Sep 6; 2013 62(35):729–730. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6235a6.htm.
4. Cahn Z, Siegel M. Electronic cigarettes as a harm reduction strategy for tobacco control: A step forward or a repeat of past mistakes? J.Public. Health Pol. 2011; 32(1):16–31. doi: 10.1057/jphp.2010.41.
5. Etter JF, Bullen C. Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. Addiction. 2011; 106(11):2017–2028. doi: 10.1111/j.1360-0443.2011.03505.x. [PubMed: 21592253]
6. Henningfield JE, Zaatari GS. Electronic Nicotine Delivery Systems: Emerging Science Foundation for policy. Tob. Control. 2010; 19:89–90. doi: 10.1136/tc.2009.035279. [PubMed: 20378582]
7. Pauly J, Li Q, Barry MB. Tobacco-free electronic cigarettes and cigars deliver nicotine and generate concern. Tob. Control. 2007; 16(5):357. doi: 10.1136/tc.2006.019687. [PubMed: 17897997]

8. Farsalinos KE, Spyrou A, Tsimopoulou K, Stefopoulos C, Romagna G, Voudris V. Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. Sci. Reports. 2014; 4:4133. doi: 10.1038/srep04133.
9. Liu, Q. Electronic Cigarette and Electronic Cigarette Device. US20140060527 A1. Mar 6. 2014
10. Bullen C, Howe C, Laugesen M, McRobbie H, Parag V, Williman J, et al. Electronic cigarettes for smoking cessation: a randomised controlled trial. Lancet. 2013; 382(9905):1629–37. doi: 10.1016/S0140-6736(13)61842-5. [PubMed: 24029165]
11. Brown J, Beard E, Kotz D, Michie S, West R. Real-world effectiveness of e-cigarettes when used to aid smoking cessation: a cross-sectional population study. Addiction. 2014; 109:1531–1540. doi: 10.1111/add.12623. [PubMed: 24846453]
12. Regan AK, Promoff G, Dube SR, Arrazola R. Electronic nicotine delivery systems: adult use and awareness of the ‘e-cigarette’ in the USA. Tob. Control. 2013; 22(1):19–23. doi: 10.1136/tobaccocontrol-2011-050044. [PubMed: 22034071]
13. Pearson JL, Richardson A, Niaura RS, Vallone DM, Abrams DB. e-Cigarette Awareness, Use, and Harm Perceptions in US Adults. Am. J. Public Health. 2012; 102(9):1758–1766. doi: 10.2105/AJPH.2011.300526. [PubMed: 22813087]
14. Lee S, Grana RA, Glantz SA. Electronic Cigarette Use Among Korean Adolescents: A Cross-Sectional Study of Market Penetration, Dual Use, and Relationship to Quit Attempts and Former Smoking. J. Adolescent Health. 2013 doi: 10.1016/j.jadohealth.2013.11.003.
15. Dutra LM, Glantz SA. Electronic Cigarettes and Conventional Cigarette Use Among US Adolescents: A Cross-sectional Study. JAMA Pediatrics. 2014; 168(7):610–617. doi: 10.1001/jamapediatrics.2013.5488. [PubMed: 24604023]
16. Choi K, Forster JL. Beliefs and Experimentation with Electronic Cigarettes: A Prospective Analysis Among Young Adults. Am. J. Prev.Med. 2014; 46(2):175–178. doi: 10.1016/j.amepre.2013.10.007. [PubMed: 24439352]
17. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, et al. Levels of Selected Carcinogens and Toxicants in Vapour from Electronic Cigarettes. Tob. Control. 2013 Published online March 6, 2013. doi: 10.1136/tobaccocontrol-2012-050859.
18. Etter JF, Zather E, Svensson S. Analysis of refill liquids for electronic cigarettes. Addiction. 2013; 108(9):1671–9. doi: 10.1111/add.12235. [PubMed: 23701634]
19. Kirschner RI, Gerona R, Jacobitz KL. Nicotine content of liquid for electronic cigarettes [Abstract #240]. 2013 Annual Meeting of the North American Congress of Clinical Toxicology (NACCT). Clin. Tox. 2013; 51:684.
20. Cameron JM, Howell DN, White JR, Andrenyak DM, Layton ME, Roll JM. Variable and potentially fatal amounts of nicotine in e-cigarette nicotine solutions. Tob. Control. 2013 doi: 10.1136/tobacccocontrol-2012-050604.
21. Pellegrino RM, Tinghino B, Mangiaracina G, Marani A, Vitali M, Protano C, et al. Electronic cigarettes: an evaluation of exposure to chemicals and fine particulate matter (PM). Ann. Ig. 2012; 24(4):279–288. [PubMed: 22913171]
22. Cheah NP, Chong NW, Tan J, Morsed FA, Yee SK. Electronic nicotine delivery systems: regulatory and safety challenges: Singapore perspective. Tob.Control. Dec 1.2012 Published Online First. doi: 10.1136/tobaccocontrol-2012-050483.
23. Trehy ML, Ye W, Hadwiger ME, Moore TW, Allgire JF, Woodruff JT, et al. Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. J. Liq. Chrom Rel. Tech. 2011; 34(14):1442–58. doi: 10.1080/10826076.2011.572213.
24. Cobb NK, Byron MJ, Abrams DB, Shields PG. Novel nicotine delivery systems and public health: The rise of the “e-cigarette.”. Am. J. Public Health. 2010; 100:2340–42. doi: 10.2105/AJPH.2010.199281. [PubMed: 21068414]
25. Westenberger, BJ. Evaluation of e-cigarettes. U S Food and Drug Administration; 2009. http://www.fda.gov/downloads/drugs/scienceresearch/ucm173250.pdf [accessed 25 Oct 2013]
26. Westerberger, BJ. Evaluation of Johnson Creek Liquids for E-cigarette Fills. U S Food and Drug Administration; 2009.

27. Kim HJ, Shin HS. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography-tandem mass spectrometry. J. Chrom. A. 2013; 1291:48–55. doi: 10.1016/j.chroma.2013.03.035.

28. Lim HH, Shin HS. Measurement of aldehydes in replacement liquids of electronic cigarettes by headspace gas chromatography-mass spectrometry. Bull. Kor.Chem. Soc. 2013; 34(9):2691–6. doi: 10.5012/bkcs.2013.34.9.2691.

29. Stanfill SB, Jia LT, Ashley DL, Watson CH. Rapid and chemically selective nicotine quantification in smokeless tobacco products using GC-MS. J. Chrom.Sci. 2009; 47(10):902–909. doi: 10.1093/chromsci/47.10.902.

30. Lisko JG, Stanfill SB, Duncan BW, Watson CH. Application of GC-MS/MS for the Analysis of Tobacco Alkaloids in Cigarette Filler and Various Tobacco Species. Anal.Chem. 2013; 85:3380–3384. doi: 10.1021/ac400077e. [PubMed: 23394466]

31. Lisko JG, Stanfill SB, Watson CH. A GC/MS Method for the Quantitation of Ten Flavor Compounds in Smokeless Tobacco Products and Cigar Filler. Anal. Meth. 2014; 6:4698. doi: 10.1039/c4ay00271g.

32. Department of Health and Human Services. Federal Register. 1999; 64(55):14086.

33. Sisson VA, Severson RF. Alkaloid composition of *Nicotiana* species. Beitr. Tabakforsch. Int. 1990; 14:327–39.

34. Celebucki CC, Wayne GE, Connolly GN, Pankow JF, Chang EI. Characterization of measured menthol in 48 US cigarette sub brands. Nicotine Tob. Res. 2005; 7(4):523–531. 2005. doi: 10.1080/14622200500186270. [PubMed: 16085523]

35. Benowitz NL, Herrera B, Jacob P. Mentholated cigarette smoking inhibits nicotine metabolism. J. Pharmacol. Exp.Ther. 2004; 310(3):1208–1215. doi: 10.1124/jpet.104.066902. [PubMed: 15084646]

36. Tomar SL, Henningfield JE. Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. Tob. Control. 1997; 6:219–225. doi: 10.1136/tc.6.3.219. [PubMed: 9396107]

37. U.S. Department of Health and Human Services. The Health Consequences of Smoking – 50 Years of Progress. A Report of the Surgeon General. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; Atlanta, GA: 2014. p. 111-112.Printed with corrections, January 2014http://www.surgeongeneral.gov/library/reports/50-years-of-progress/index.html [accessed August 20, 2014]

38. Centers for Disease Control and Prevention (CDC). Notes from the Field: Calls to Poison Control Centers for Exposures to Electronic Cigarettes – United States, September 2010-February 2014. MWWR. Apr 4; 2014 63(13):292–293. http://www.cdc.gov/mmwr/PDF/wk/mm6313.pdf.

39. Friedman D. E-cigarette makers are targeting minors, hooking them on nicotine, say congressional Democrats. New York Daily News. Apr 14.2014

40. Taylor, JK. Quality Assurance of Chemical Measurements. Lewis Publishers; Chelsea, MI: 1987.

41. American E-Liquid Manufacturing Standards Association. [Accessed: September 27, 2014] E-Liquid Manufacturing Standards Version 2.0/2.14.2014. 2014. p. 6Article IIhttp://www.aemsa.org/wp-content/uploads/2014/09/AEMSA-Standards_Version-2-01.pdf

42. USP Monographs: Nicotine. U. S. Pharmacopeia; www.pharmacopeia.cn/v29240/usp29nf24s0_m56620.html [Accessed: September 27, 2014]

43. Kisaki T, Maeda S, Koiwai A, Mikami Y, Sasaki T, Matsushita H. Transformation of Tobacco Alkaloids. Beitr. Tabakforsch. Int. 1978; 9:308–316.

44. Linnell RH. The Oxidation of Nicotine. I. Kinetics of the Liquid Phase Reaction Near Room Temperature. Tobacco Science. 1960; 4:89–91.

45. Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Spyrou A, Voudris V. Impact of Flavour Variability on Electronic Cigarette Use Experience: An Internet Survey. Int. J. Environ. Res. Public Health. 2013; 10:7272–7282. doi: 10.3390/ijerph10127272. [PubMed: 24351746]

46. Dawkins L, Turner J, Roberts A, Soar K. ‘Vaping’ profiles and preferences: an online survey of electronic cigarette users. Addiction. 2013; 108:1115–1125. doi: 10.1111/add.12150. [PubMed: 23551515]

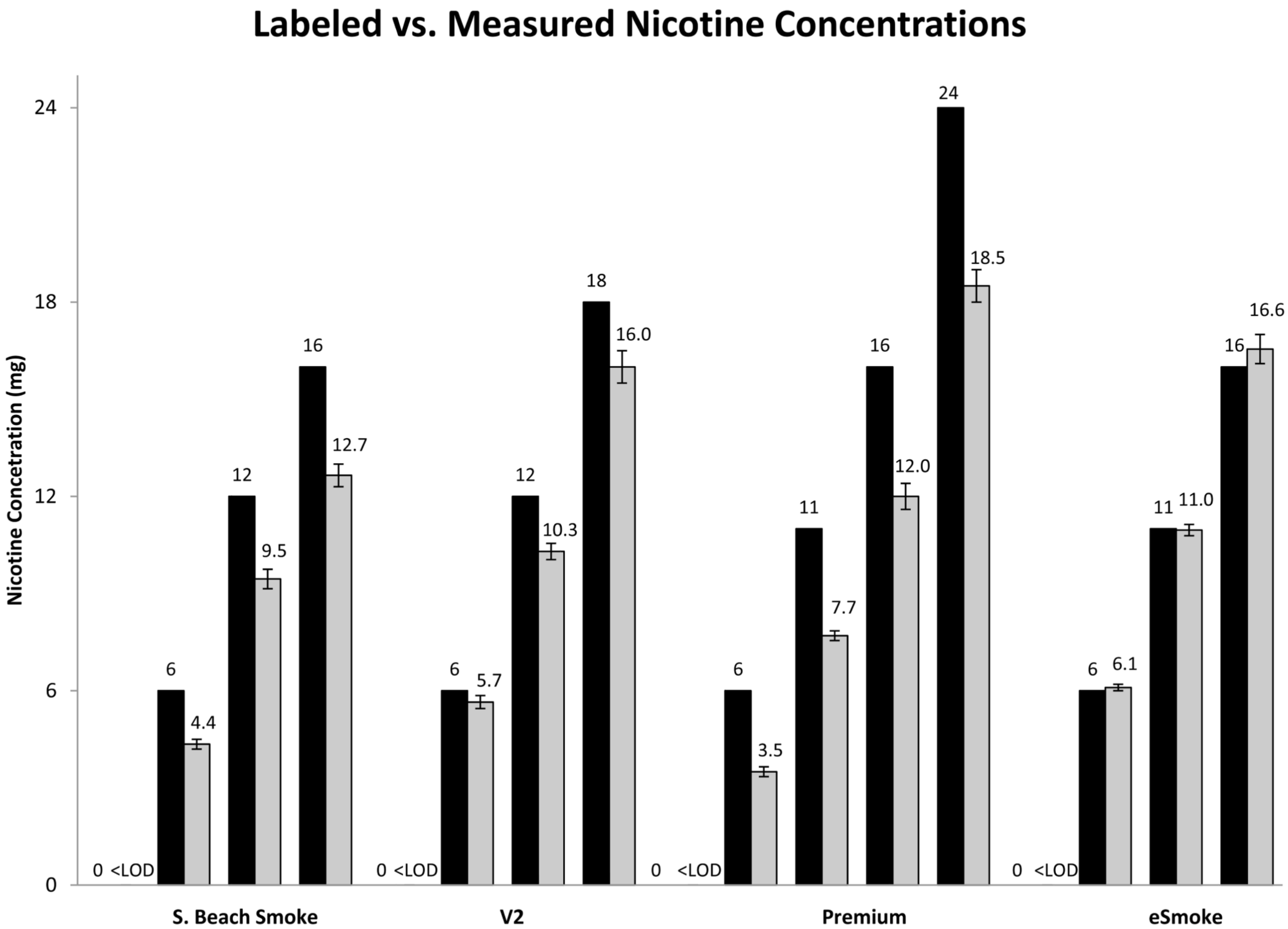

Figure 1.
Measured nicotine concentrations were consistently lower than labeled amounts for all brands tested except eSmoke.

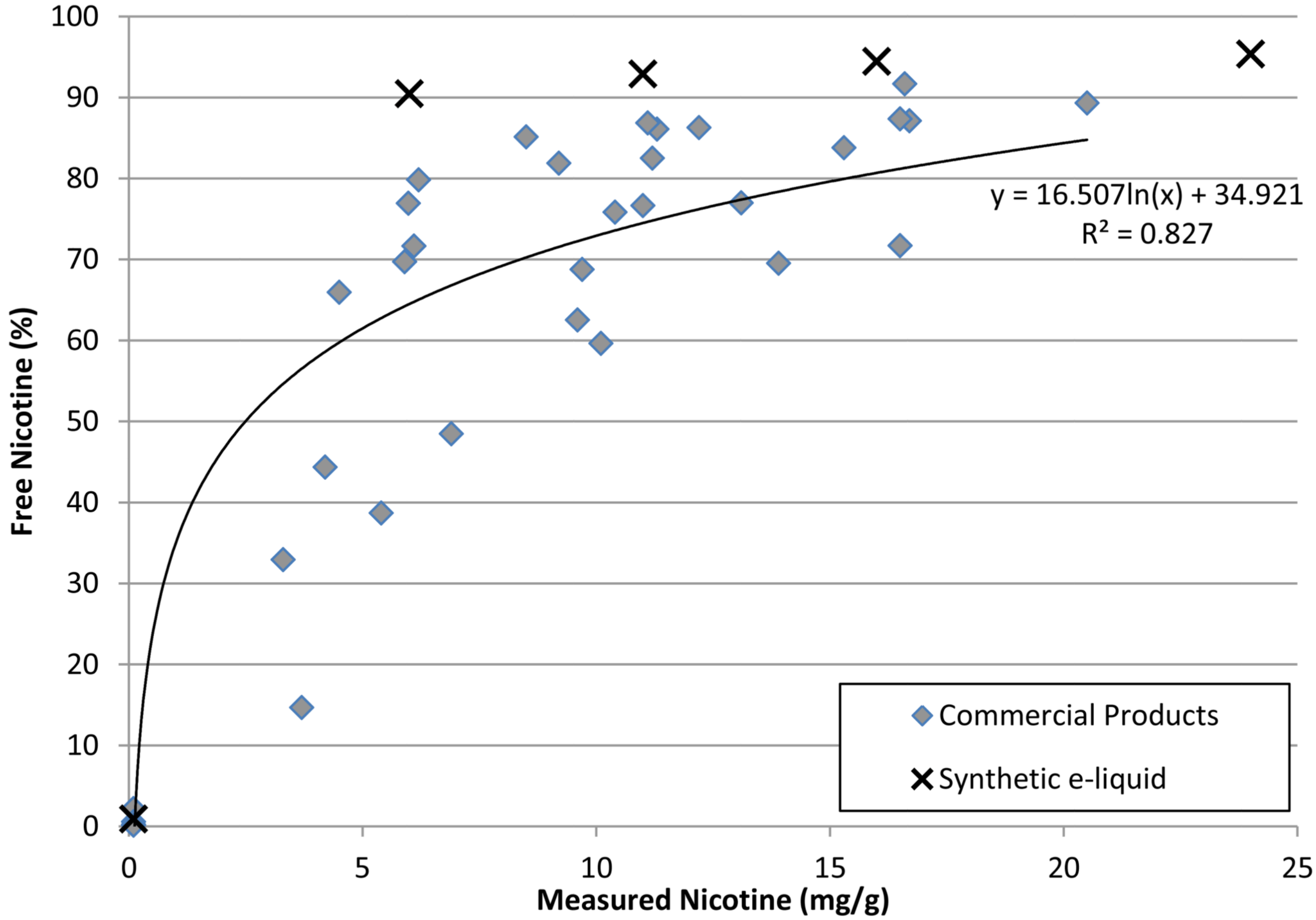

Figure 2.

Comparison of measured nicotine and free base nicotine for commercial and synthetic e-juice indicate nicotine's alkalinity drives pH and the subsequent free-nicotine (%) levels. A logarithmic fit was chosen based on the characteristics of the Henderson-Hasselbach equation.

Table 1

Nicotine and minor tobacco alkaloid concentrations in 36 e-cigarette cartridges and refill solutions (mean ± SD) of triplicate measures of a single manufacturer lot. USP maximum limits (0.5% for a single impurity (USP single), 1.0% total impurities (USP total)) for impurities in nicotine have been calculated based on the measured nicotine concentrations.

Flavor	Nicotine Label Conc.	NIC	NNIC	MYOS	ANAB	ANAT	ISONIC	Total Minor Alkaloids	USP single	USP total
South Beach Smoke		mg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Vanilla	0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Tobacco	6	4.5 ± 0.1	5.6 ± 0.2	5.8 ± 0.2	3.8 ± 0.2	6.6 ± 0.2	1.31 ± 0.01	23.0	22.5	45.0
Tobacco Blue	6	4.2 ± 0.2	6.4 ± 0.1	8.8 ± 0.3	7.5 ± 0.2	11.2 ± 0.1	1.78 ± 0.03	35.7	21.0	42.0
Tobacco Gold	12	9.7 ± 0.4	5.6 ± 0.1	13.3 ± 0.6	5.5 ± 0.2	7.1 ± 0.3	1.19 ± 0.04	32.6	48.5	97.0
Peppermint	12	9.2 ± 0.2	4.8 ± 0.2	11.2 ± 0.4	8.5 ± 0.2	9.5 ± 0.2	0.54 ± 0.01	34.5	46.0	92.0
Menthol	16	13.1 ± 0.5	7.3 ± 0.2	11.7 ± 0.3	9.6 ± 0.4	15.0 ± 0.6	3.74 ± 0.1	47.4	65.5	131.0
Peach	16	12.2 ± 0.2	6.8 ± 0.2	25.5 ± 0.7	14.5 ± 0.4	22.5 ± 0.3	0.72 ± 0.02	70.0	61.0	122.0
V2										
Menthol	0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Peppermint	0	NT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Sahara	6	5.4 ± 0.2	1.85 ± 0.03	2.9 ± 0.1	9.5 ± 0.3	17.4 ± 0.4	0.84 ± 0.02	32.4	27.0	54.0
Red	6	5.9 ± 0.2	5.6 ± 0.1	4.9 ± 0.2	8.2 ± 0.2	19.1 ± 0.3	0.70 ± 0.02	38.4	29.5	59.0
Sahara	12	11.0 ± 0.2	3.2 ± 0.1	5.2 ± 0.2	21.6 ± 0.7	41.6 ± 0.7	1.39 ± 0.02	73.0	55.0	110.0
Peppermint	12	9.6 ± 0.3	2.8 ± 0.1	6.0 ± 0.3	20.0 ± 0.5	33.5 ± 0.6	1.17 ± 0.01	63.5	48.0	96.0
Menthol	18	15.3 ± 0.4	2.3 ± 0.1	10.4 ± 0.2	14.6 ± 0.5	23.0 ± 0.2	4.42 ± 0.1	54.7	76.5	153.0
Red	18	16.7 ± 0.6	18.7 ± 0.2	26.1 ± 0.8	62.8 ± 3.2	193.1 ± 4.6	7.97 ± 0.3	308.6	83.5	167.0
Premium										
Cherry	0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Coffee	0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Blueberry	6	3.7 ± 0.2	5.2 ± 0.4	5.8 ± 0.3	6.7 ± 0.2	12.8 ± 0.4	0.44 ± 0.02	31.0	18.5	37.0
Watermelon	6	3.3 ± 0.1	4.7 ± 0.1	5.0 ± 0.2	6.2 ± 0.1	11.2 ± 0.3	0.37 ± 0.01	27.4	16.5	33.0
Pineapple	11	6.9 ± 0.2	13.0 ± 0.1	15.2 ± 0.5	17.9 ± 0.5	62.1 ± 2.1	13.6 ± 0.3	121.8	34.5	69.0

Flavor	Nicotine Label Conc.	NIC	NNIC	MYOS	ANAB	ANAT	ISONIC	Total Minor Alkaloids	USP single	USP total
South Beach Smoke		**mg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**
Menthol	11	8.5 ± 0.1	4.16 ± 0.01	7.2 ± 0.2	16.2 ± 0.2	23.3 ± 0.4	1.24 ± 0.02	52.1	42.5	85.0
Pear	16	10.1 ± 0.4	12.8 ± 0.1	18.9 ± 0.1	21.9 ± 0.7	40.1 ± 0.9	1.49 ± 0.02	95.1	50.5	101.0
Vanilla	16	13.9 ± 0.4	3.8 ± 0.1	9.0 ± 0.3	19.6 ± 0.6	30.3 ± 0.4	1.42 ± 0.02	64.2	69.5	139.0
Tobacco	24	20.5 ± 0.9	8.6 ± 0.1	18.3 ± 0.1	42.9 ± 1.9	82.4 ± 0.4	2.8 ± 0.1	154.9	102.5	205.0
Peach	24	16.5 ± 0.1	12.9 ± 0.2	17.3 ± 0.3	44.2 ± 1.3	84.8 ± 1.2	2.33 ± 0.03	161.5	82.5	165.0
eSmoke										
Morning Coffee	0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	NA	NA
Red El Toro	6	6.0 ± 0.1	15.2 ± 1.0	17.3 ± 0.7	37.7 ± 1.5	231.3 ± 9.1	9.9 ± 0.8	311.4	30.0	60.0
Morning Coffee	6	6.1 ± 0.1	6.3 ± 0.2	9.7 ± 0.4	21.2 ± 0.5	63.1 ± 1.8	2.6 ± 0.1	102.9	30.5	61.0
Green Apple	6	6.2 ± 0.1	4.5 ± 0.2	8.7 ± 0.2	21.7 ± 0.6	68.8 ± 2.9	2.38 ± 0.03	106.0	31.0	62.0
Tobacco RY4	11	11.3 ± 0.1	14.4 ± 0.4	14.5 ± 0.7	40.6 ± 0.5	130.9 ± 4.8	5.6 ± 0.2	206.0	56.5	113.0
Minty Menthol	11	10.4 ± 0.1	11.5 ± 0.2	62.7 ± 2.1	70.6 ± 2.0	361.1 ± 13.6	20.7 ± 0.5	526.7	52.0	104.0
C. Coconut	11	11.1 ± 0.3	8.0 ± 0.2	25.5 ± 1.1	53.0 ± 2.7	171.6 ± 6.4	4.4 ± 0.2	262.5	55.5	111.0
Morning Coffee	11	11.2 ± 0.2	11.3 ± 0.2	15.1 ± 0.4	42.4 ± 0.6	131.7 ± 3.3	6.1 ± 0.1	206.7	56.0	112.0
Morning Coffee	16	16.5 ± 0.6	19.7 ± 0.2	28.7 ± 1.0	87.4 ± 0.3	274.9 ± 11.5	8.8 ± 0.1	419.4	82.5	165.0
MTN Mist	16	16.6 ± 0.3	32.3 ± 0.7	35.6 ± 0.9	92.2 ± 1.7	300.3 ± 8.3	7.1 ± 0.1	467.6	83.0	166.0
Red El Toro*	24	NT	48.2 ± 2.8	41.5 ± 0.7	152.2 ± 3.7	485.4 ± 5.7	13.2 ± 0.1	740.4	120.0	240.0

Key: NIC = Nicotine, NNIC = Nornicotine, MYOS = Myosmine, ANAB = Anabasine, ANAT = Anatabine, ISONIC = Isonicoteine, NT = Not tested, NA = Not Applicable

*USP calculated values are based on the labeled nicotine concentration since the sample was not available for nicotine testing.

Table 2

Concentrations (μg/g, N=3) of selected flavor analytes* in e-Cigarette cartridges or refill solutions.

Flavor	Nicotine Label	EUC	CAM	MEN	PUL	CINN	ESAL
South Beach Smoke							
Vanilla	0 mg	-	-	-	-	-	-
Tobacco	6 mg	-	-	-	-	-	-
Tobacco Blue	6 mg	-	-	-	-	-	-
Tobacco Gold	12 mg	-	10.2 ± 2.1	6.2 ± 0.8	-	-	-
Peppermint	12 mg	-	-	3670 ± 161	25.7 ± 1.0	47.1 ± 0.9	-
Peach	16 mg	-	-	-	-	-	-
Menthol	16 mg	24.5 ±0.4	-	7780 ± 141	28.2 ± 0.4	-	-
V2							
Menthol	0 mg	21.6 ± 0.5	-	11200 ± 428	119 ± 3.8	-	-
Menthol	18 mg	39.4 ± 0.8	-	11100 ± 246	50.1 ± 0.9	-	-
Sahara	6 mg	-	-	14.7 ± 5.4	-	-	-
Sahara	12 mg	-	-	13.1 ± 1.8	-	-	-
Red	6 mg	-	-	-	-	-	-
Red	18 mg	-	-	13.6 ± 1.0	-	-	-
Peppermint	0 mg	-	5.9 ± 0.4	9770 ± 307	78.3 ± 1.7	37.6 ± 0.2	-
Peppermint	12 mg	-	5.8 ± 0.5	9530 ± 281	82.7 ± 1.9	10.4 ± 0.4	-
Premium							
Cherry	0 mg	-	1310 ± 75.3	-	-	-	13.0 ± 0.6
Coffee	0 mg	-	-	-	-	-	-
Watermelon	6 mg	-	-	-	-	-	-
Blueberry	6 mg	-	278 ± 8.9	-	-	-	-
Pineapple	11 mg	-	13.3 ± 2.0	-	-	-	-
Menthol	11 mg	86.8 ± 3.4	-	12400 ± 468	115 ± 2.8	98.6 ± 2.2	-
Pear	16 mg	-	-	-	-	-	-
Vanilla	16 mg	-	-	-	-	-	-
Tobacco	24 mg	-	-	-	-	-	-
Peach	24 mg	-	-	-	-	-	-
eSmoke							
Morning Coffee	0 mg	-	-	-	-	-	-
Morning Coffee	6 mg	-	-	-	-	-	-
Morning Coffee	11 mg	-	-	-	-	-	-
Morning Coffee	16 mg	-	-	-	-	-	-
Red El Toro	6 mg	-	-	-	-	-	-
Green Apple	6 mg	-	-	-	-	-	-
Tobacco	11 mg	-	-	-	-	-	-

Flavor	Nicotine Label	EUC	CAM	MEN	PUL	CINN	ESAL
Minty Menthol	11 mg	20.3 ± 0.7	-	4860 ± 150	10.5 ± 0.6	-	-
Caribbean Coconut	11 mg	-	-	-	-	-	-
MTN Mist	16 mg	-	9.9 ± 1.6	-	-	-	-
Red El Toro	24 mg	-	-	-	-	-	-

EUC: Eucalyptol, CAM: Camphor, MEN: Menthol, PUL: Pulegone, CINN: Cinnamaldehyde, ESAL: Ethyl Salicylate

- Denotes < LOD.

*All e-Cigarette samples were also tested for Diphenyl Ether, Coumarin, Methyl Salicylate and Eugenol but these flavor analytes were not detected.

Table 3

Nicotine (N=3), pH (N=2), and Free-Base nicotine of commercial and laboratory-prepared e-liquid.

Flavor	Nicotine Label Conc. (mg)	Nicotine (mg/g)	% Difference from Label	pH	Free Nicotine (%)
South Beach Smoke					
Vanilla	0	<LOD	NA	5.3	NA
Tobacco	6	4.5	−25.0	8.3	65.9
Tobacco Blue	6	4.2	−30.0	7.9	44.4
Tobacco Gold	12	9.7	−19.2	8.4	68.8
Peppermint	12	9.2	−23.3	8.7	81.9
Menthol	16	13.1	−18.1	8.5	77.0
Peach	16	12.2	−23.8	8.8	86.3
V2					
Menthol	0	<LOD	NA	6.4	NA
Sahara	6	5.4	−10.0	7.8	38.7
Red	6	5.9	−1.7	8.4	69.7
Sahara	12	11	−8.3	8.5	76.6
Peppermint	12	9.6	−20.0	8.2	62.5
Menthol	18	15.3	−15.0	8.7	83.8
Red	18	16.7	−7.2	8.9	87.1
Premium					
Cherry	0	<LOD	NA	5.3	NA
Coffee	0	<LOD	NA	5.8	NA
Blueberry	6	3.7	−38.3	7.3	14.7
Watermelon	6	3.3	−45.0	7.7	32.9
Pineapple	11	6.9	−37.3	8.0	48.5
Menthol	11	8.5	−22.7	8.8	85.1
Pear	16	10.1	−36.9	8.2	59.6
Vanilla	16	13.9	−13.1	8.4	69.5
Tobacco	24	20.5	−14.6	8.9	89.3
Peach	24	16.5	−31.3	8.4	71.7
eSmoke					
Morning Coffee	0	<LOD	NA	5.1	NA
Red El Toro	6	6.0	0.0	8.5	76.9
Morning Coffee	6	6.1	1.7	8.4	71.6
Green Apple	6	6.2	3.3	8.6	79.8
Tobacco RY4	11	11.3	2.7	8.8	86.1
Minty Menthol	11	10.4	−5.5	8.5	75.8
Caribbean Coconut	11	11.1	0.9	8.8	86.9

Flavor	Nicotine Label Conc. (mg)	Nicotine (mg/g)	% Difference from Label	pH	Free Nicotine (%)
Morning Coffee	11	11.2	1.8	8.7	82.5
Morning Coffee	16	16.5	3.1	8.9	87.4
MTN Mist	16	16.6	3.8	9.1	91.7
Laboratory-prepared e-juice					
1:1 PG*/Glycerin	0			6.0	NA
	6			9.0	90.5
	11			9.1	92.9
	16			9.3	94.5
	24			9.3	95.4

*PG: Propylene Glycol