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Measurement of cardiovascular and pulmonary function endpoints and other physiological effects following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers

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ABSTRACT

Acute changes in select physiological parameters associated with cardiovascular physiology (systolic and diastolic blood pressure (BP) and heart rate (HR)), pulmonary function (FVC, FEV1, and exhaled CO and NO) and adverse events were measured in 105 clinically confined subjects who were randomized into groups that either completely or partially switched from conventional cigarettes to e-cigarettes or completely discontinued using tobacco and nicotine products altogether. Use of the e-cigarettes for five days under the various study conditions did not lead to higher BP or HR values, negative respiratory health outcomes or serious adverse health events. Reductions in BP and HR vital signs were observed in most of the participants that either ceased tobacco and nicotine products use altogether or switched completely to using e-cigarettes. Pulmonary function tests showed small but non-statistically significant improvements in FVC and FEV1 measurements in most use groups. Statistically significant ($p < 0.05$) benefits associated with smoking reduction were also noted in exhaled CO and NO levels. All study products were well tolerated. The study findings suggest that there are potential cardiovascular and pulmonary function benefits when smokers switch to using e-cigarette products. This further reinforces the potential that e-cigarettes offer smokers seeking an alternative to conventional tobacco products.

1. Introduction

Electronic cigarettes (e-cigarettes) are becoming an increasingly popular alternative to conventional tobacco cigarettes among smokers worldwide. E-cigarettes are battery-powered devices that deliver vaporized nicotine, propylene glycol and/or glycerol and flavorings to users from an "e-liquid". E-cigarettes do not contain tobacco, require combustion or generate side-stream emissions but simulate the visual, sensory, and behavioral aspects of smoking which conventional nicotine replacement therapy products do not (Nelson et al., 2015; Nides et al., 2014; Hajek et al., 2014a,b). E-cigarettes have also been found to deliver sufficient levels of nicotine to satisfy users (Vansickel and Eissenberg, 2013; Polosa et al., 2014; McNeill et al., 2015; Goniewicz et al., 2016) and there is also evidence that e-cigarettes can encourage quitting or cigarette consumption reduction even among those not intending to quit or rejecting other support (Caponnetto et al., 2013; McRobbie et al., 2014; McNeill et al., 2015).

In recent years, a credible and accumulating body of scientific evidence has shown that e-cigarettes are less harmful than smoking conventional tobacco cigarettes and may substantially reduce harm (e.g. Royal College of Physicians, 2016; Nutt et al., 2014). Public Health England, after reviewing all currently available evidence on the subject, concluded that it was reasonable to estimate that e-cigarettes are approximately 95% less harmful than smoking cigarettes (McNeill et al., 2015). While the precise percentage is difficult to quantify, such estimates are supported by previous studies which have reported reduced or undetectable levels of select harmful or potentially harmful constituents (HPHCs) in e-cigarette aerosols when assessed following machine-based aerosol

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generation (Goniewicz et al., 2014; Tayyarah and Long, 2014). Furthermore, studies of the major biomarkers of HPHCs or other chemicals in e-cigarette aerosols, have indicated substantially (9–450 times) lower levels compared to the smoke from cigarettes, cigars, hookah and other conventional tobacco cigarettes (Goniewicz et al., 2014; Hecht et al., 2015).

Two recent human studies measuring urine, blood and exhaled breath biomarkers of exposure to cigarette smoke toxicants and carcinogens in smokers who switched from tobacco cigarettes to e-cigarettes further support and extend the harm reduction potential of e-cigarettes by reporting that substituting tobacco cigarettes with e-cigarettes may significantly reduce exposure to HPHCs and numerous toxicants and carcinogens otherwise present in tobacco cigarettes (Goniewicz et al., 2016; O'Connell et al., 2016). More specifically, Goniewicz et al., 2016 showed that smokers who switched from tobacco cigarettes to e-cigarettes, were able to obtain similar levels of nicotine, but experienced statistically significant reductions in 12 out 17 measured urinary biomarkers of exposure (BoE) of tobacco smoke, with mean nitrosamine levels declining in all subjects by 64% by the end of the second week of product use. Reductions in levels of exhaled toxic gases such as carbon monoxide were also noted.

Similarly, O'Connell et al., 2016, reported that smokers who completely substitute conventional tobacco cigarettes with e-cigarettes are able obtain similar levels of nicotine but experience substantial reductions (29–95%) to numerous harmful toxicants reported to be significant contributors to smoking-associated disease risks. Together, both studies observed significant reductions in exposure to a total of 25 out of 30 tobacco-related human toxicants classified by FDA as HPHCs (USFDA, 2012) or by the International Agency for Research on Cancer (IARC) as Group 1 human carcinogens (e.g., tobacco-specific nitrosamines such as Nicotine-derived nitrosamine ketone (NNK); 1-3-butadiene; benzene; and ethylene oxide) (IARC, 2016) in smokers who either completely or partially replaced their tobacco cigarettes with e-cigarettes. The results of these studies provide biological evidence which shows that switching from tobacco cigarettes to e-cigarettes, in the short-term, provides smokers with comparable levels of nicotine, while also reducing their exposure to a variety of toxicants, otherwise present in tobacco cigarettes, which are believed to contribute to smoking related disease. This is encouraging as public health authorities such as the US Surgeon General suggest that reducing exposure to HPHCs found in tobacco smoke and discontinuing tobacco cigarette smoking can reduce the risks associated with diseases such as lung cancer, heart disease and emphysema (USDHHS, 2014).

To date, the scientific literature associated with the potential effects of e-cigarettes on cardiovascular and respiratory or lung function is growing and suggests that e-cigarettes may be less harmful than tobacco smoking. For example, a previous study comparing the immediate effects of tobacco cigarette and e-cigarette use on left ventricular (LV) myocardial function found that smoking one tobacco cigarette led to significant acute myocardial dysfunction, while the e-cigarette, which contained 1.1% nicotine, had no acute adverse effects on cardiac function (Farsalinos et al., 2014a). It was reported that smoking the tobacco cigarette led to important hemodynamic consequences, such as significant elevations in heart rate (HR), systolic and diastolic blood pressure (BP), but use of the e-cigarette only resulted in a slight increase in diastolic blood BP. Another clinical study (Yan and D'Ruiz, 2015) investigating the acute effects of e-cigarettes on BP and HR in comparison to tobacco cigarette smoking reported similar results. The study reported increases in systolic, diastolic BP and HR following acute use of both tobacco cigarettes and e-cigarettes, however, the increases associated with e-cigarette use were minimal and not clinically significant as compared to those of the cigarette smokers.

Furthermore, Farsalinos et al., 2016 investigated changes in BP and HR in smokers who reduced or quit smoking by using e-cigarettes for a 12-month period in a randomized control trial. The study reported that smokers (with elevated BP at baseline) who reduced smoking or quit smoking by switching to e-cigarettes experienced statistically significant reductions in systolic BP after 1 year. Similar changes in BP from baseline were observed in quitters who stopped using e-cigarettes compared to quitters who still used e-cigarettes. In addition, Benowitz and Burbank, 2016 investigated the cardiovascular safety of nicotine within the context of short-term e-cigarette use and concluded that the cardiovascular risks of nicotine from e-cigarettes are low in healthy users. It was also reported that while it is possible that people with established cardiovascular disease (CVD) might incur some increased risk from e-cigarette use, the risk is much less than that of smoking. Interestingly, the investigators also noted that in contrast to cigarette smoking which results in an arterial spike of nicotine, e-cigarette use is more intermittent and results in lower and more stable nicotine levels without arterial spikes. Moreover, this effect may possibly reduce the intensity of the pharmacologic effects associated with nicotine and subsequently result in less cardiovascular stress or impact for e-cigarette users as compared to conventional tobacco smokers.

Very few investigations exist which have focused on the effects of e-cigarettes on lung function. Most of the studies and surveys conducted to-date indicate that the use of e-cigarettes leads to a near normalization in toxic-levels of exhaled carbon monoxide (Farsalinos and Polosa, 2014; Polosa, 2015) and do not appear to support negative respiratory health outcomes under acute use conditions (Flouris et al., 2013; Polosa, 2015). It has also been recently suggested by Polosa, 2015 that smokers with preexisting asthma and COPD may benefit from regular e-cigarette use. Evidence for this is based on emerging medical case reports, which showed significant improvements in quality of life and reductions in the number of pulmonary disease exacerbations in patients who quit tobacco smoking on their own by switching to e-cigarettes (Caponnetto et al., 2011) and on the findings from a large internet survey of regular e-cigarette users diagnosed with asthma or COPD which largely corroborate the medical case report findings (Farsalinos et al., 2014b). In general, the internet survey showed that improvements in the symptoms of asthma and COPD were reported by 65.4% and 75.7% of the survey respondents diagnosed with pulmonary disease, respectively. Furthermore, it was also reported that after switching, the use of pulmonary disease medications was reported to have stopped in 18.4% of the respondents with asthma and COPD. Worsening conditions after switching were only reported by 1.1% of the asthmatics and 0.8% of the COPD respondents.

Moreover, findings from the first long-term (1 year) investigation of changes in spirometric indices and respiratory symptoms in smokers who reduced or quit smoking by switching to e-cigarettes also indicate e-cigarette use may have beneficial effects in relation to respiratory outcomes (Cibella et al., 2016). The study reported that smokers who quit smoking and substantially reduced their exposure to harmful cigarette smoke toxicants by switching to e-cigarettes, experienced a steady and progressive normalization of peripheral airways function, as measured by forced expiratory flow from 25% to 75% of vital capacity, ($FEF_{25-75\%}$) improvements from baseline. Improvements in respiratory symptoms were also noted.

Currently, further information is needed to augment our understanding of the impacts of acute e-cigarette use on key physiological parameters associated with cardiovascular and respiratory function. This information, together with the emerging evidence that has been presented above, will provide further insight as to

whether reducing exposure to the HPHCs found in tobacco smoke by discontinuing tobacco cigarette smoking and switching to e-cigarettes results in improved cardiovascular and pulmonary health under short and long-term use conditions. The cardiovascular vital signs, pulmonary function endpoints and exhaled breath biomarkers measured in this study are believed to be pertinent measures of human tobacco-smoke toxicant exposure and smoking-associated disease risks by public health authorities (USDHHS, 2014). As such, the purpose of this study was to measure changes in select physiological endpoints such as cardiovascular (systolic and diastolic BP and HR), pulmonary function (FVC, FEV1, and exhaled CO and NO) and safety and tolerability following short-term (5-day) *ad libitum* use of e-cigarettes by established adult smokers under exclusive use, dual use and discontinuance of all tobacco and nicotine product conditions.

Another goal of this study was to collect blood and urine samples from subjects in the various use groups for further research. Bio fluids collected from the various use groups in this study, and from other ongoing long-term studies, will be used in future studies assessing the acute and long-term impacts of e-cigarette use on important biological marker of effect endpoints such as inflammation and oxidative stress.

2. Material and methods

Details pertaining to the participants' characteristics, study design and methods have previously been described (O'Connell et al., 2016; D'Ruiz et al., 2016).

2.1. Ethics and consent to participate

All pertinent study documents received ethical clearance for research involving human participants by the institutional review board: Chesapeake Research Review, Inc. (CRRI), Columbia, MD. Study participants gave written informed consent prior to initiation of any study-specific procedures. The clinical trial was registered at: http://ClinicalTrials.gov: identifier: NCT02385227.

2.2. Study population

One hundred and five (105) subjects meeting the study eligibility criteria were enrolled into the study and randomized into one of six study groups. The main criteria for inclusion in the study were as follows: healthy adult male and female smokers, 21–65 years of age inclusive; a smoker for at least 12 months and currently smoked an average of 10 or more conventional manufactured tobacco cigarettes per day (any brand, flavor or style); consistent use of their current usual brand style for 14 days prior to check-in; positive urine cotinine at screening (≥500 ng/mL); and exhaled carbon monoxide CO > 12 ppm at screening. Exclusion criteria included: history or presence of clinically significant mental or physical health conditions; females who were pregnant or breast-feeding; high blood pressure; body mass index <18 kg/m^2 or >40 kg/m^2; acute respiratory illnesses requiring treatment within 2 weeks prior to check-in; use of prescription smoking cessation treatments, anti-diabetic or insulin drugs or medications; and positive urine screen for alcohol or drugs of abuse. Self-reported mouth-hold smokers (i.e., smokers who draw smoke from the conventional tobacco cigarette into the mouth and throat but do not inhale) were also excluded. Prior use of an e-cigarette was not an exclusion criterion, provided all other criteria were met; however, none of the subjects reported previous use of e-cigarettes.

2.3. Products tested

Test articles included both e-cigarettes and conventional tobacco cigarettes. Three commercially available closed system blu™ e-cigarette products purchased in 2014 (manufacturer, Fontem Ventures B.V., The Netherlands) were evaluated during this study: rechargeable tobacco flavor, rechargeable cherry flavor and disposable cherry flavor. All e-cigarette formulations were reviewed and characterized using conventional product stewardship and toxicological review practices. Given formulation similarities, estimates of the aerosols generated from the products were expected to be in line with those of our previous studies which reported reduced or undetectable levels of select harmful or potentially harmful constituents (HPHCs) in e-cigarette aerosols when assessed following machine-based aerosol generation (Tayyarah and Long, 2014). All e-cigarette products contained 24 mg/mL (2.4%) USP grade nicotine, USP grade vegetable glycerol (~50% in cherry flavor and ~80% in tobacco flavor), USP grade propylene glycol (~45% in cherry flavor and ~10% in tobacco flavor), distilled water, and flavorings. Each e-cigarette contained approximately 1 mL of e-liquid by volume. Subjects were provided unopened packs of their reported usual brand of conventional tobacco cigarettes for use during the study.

2.4. Study design

This was a randomized, open-label, forced-switch parallel arm study conducted at a single independent research center (Celerion, Lincoln, NE). Following successful screening and study qualification, subjects checked into the clinic on Day −2 and continued to smoke their usual conventional tobacco cigarette brand *ad libitum* through the evening of Day −1 (baseline). Subjects were confined in the research clinic for the entire duration of the study.

During enrollment, and as part of the study, participants completed several different questionnaires that measured nicotine dependence and a variety of subjective smoking-related effects over the course of the five-day study. These included a baseline smoking history survey; the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991; Fagerström, 2012) and the Brief Wisconsin Inventory of Smoking Dependence Motives (Brief WISDM) (Smith et al., 2010), which were all administered on Day −1 (baseline). A smoking urge questionnaire was administered to all subjects on Days −1 through 5 in the morning prior to the start of product use and in the evening using a simple and subjective 100 mm paper visual analog scale. The results associated with smoking desire were previously reported (D'Ruiz et al., 2016). All questionnaire responses and Brief WISDM subscale scores were listed by subject and summarized by product use group using descriptive statistics appropriate for the data point.

Baseline assessments occurred from the morning of Day −1 through the morning of Day 1 prior to the start of randomized product use and post-baseline assessments on the morning of Day 1 through the morning of Day 6. Bland, non-fried or grilled meals and snacks were served at standard times throughout the day. On the morning of Day 1, subjects were randomized into one of six groups (N = 15 each):

Exclusive E-Cigarette Use Groups
- Group A1 – Tobacco flavor rechargeable blu™ e-cigarette
- Group A2 – Cherry flavor rechargeable blu™ e-cigarette
- Group A3 – Cherry flavor disposable blu™ e-cigarette

Dual Use Groups
- Group B1 – Tobacco flavor rechargeable blu™ e-cigarette + usual brand combustible tobacco cigarette

- Group B2 – Cherry flavor rechargeable blu™ e-cigarette + usual brand combustible tobacco cigarette
- Group B3 – Cherry flavor disposable blu™ e-cigarette + usual brand combustible tobacco cigarette

Cessation Group

- Group C – Complete tobacco and nicotine product cessation

2.5. Product use

Use of the tobacco- or nicotine-containing e-cigarette products was only permitted as per the protocol and randomization during the entire duration of the study from check-in through discharge. Use of the assigned products was documented daily by clinic staff and subjects were monitored during clinical confinement to ensure that no illicit nicotine or tobacco products were used. Subjects randomized to the cessation group were housed in an area of the clinic separate from the other groups to minimize the chance for illicit product use and cross-contamination. With limited exceptions, all product use was *ad libitum* from 07:30 to 23:00 on Days −2 to 5. These exceptions included meals and questionnaire administration, 15 min prior to blood sampling and vital sign measurements, and 30 min prior to and during spirometry and exhaled CO and nitric oxide (NO) measurements.

Subjects randomized to receive the e-cigarette products were trained on how to use the e-cigarettes upon check-in and then again on Day −1. In general, this included instructions on what to do if the e-cigarette did not function and demonstrations on how to puff an e-cigarette (i.e., puff and inhale as one would a conventional cigarette). They were allowed to carry the e-cigarettes throughout the day in designated sections of the clinic. New e-cigarettes were supplied to the subjects each morning and throughout the day if the e-liquid solution was fully consumed. Puffing behavior and use topography were not recorded in this study. All e-cigarettes were weighed before and after use.

Two levels of conventional cigarette consumption reduction (100% and 50% from subject self-reports at Screening) were chosen to observe product use effects. Cigarette consumption was self-reported at screening and subjects in the dual use group were required to reduce their daily cigarette consumption on Days 1–5 by ~50% of that reported at baseline. Subjects randomized to the dual use group were required to request a cigarette product from the clinic staff and smoke only in specified sections of the clinic away from non-smoking subjects.

To assess how much nicotine was being delivered to the subjects, a rough estimate of the maximum amount of nicotine possibly delivered from each e-cigarette was calculated by utilizing the following simple mass-balance calculation:

Estimated Nicotine Delivery (mg) = Pre-weight - Post-weight difference (mg) X nicotine strength (%)

Each tobacco cigarette was assumed to deliver ~1 mg of nicotine for the purpose of estimating the amount of nicotine administered (FTC, 2012). The total estimated amount of nicotine delivered per day for a subject (in mg) was the sum total of the estimated nicotine delivery for all e-cigarette units and the number of cigarettes smoked on each day. As several factors may contribute to nicotine uptake from e-cigarettes as well as combustible cigarettes (e.g., particle size, depth of inhalation, breath holding following inhalation), it is unlikely that the full amount of nicotine in the volume of the e-cigarette solution indicated by the change in product weight before and after use was absorbed by the subjects. However, in the absence of a more precise method of estimating the actual dose of nicotine administered, the method used in this study was used to compare across study groups and should not be used to make a firm conclusion regarding nicotine uptake.

Product use data was listed by subject and day and was summarized by subject, product use group, and day using descriptive statistics (arithmetic mean, standard deviation, coefficient of variation, sample size, minimum, maximum, and median). A paired *t*-test was used to make within-group cohort comparisons of the daily estimated amount of nicotine delivered by the e-cigarettes and the number of cigarettes smoked per day.

2.6. Physiological assessments

2.6.1. Blood pressure and heart rate

Cardiovascular vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate) were measured by the study physician or appropriate clinical staff following at least 5 min of rest, prior to the start of product administration at ~6:45 in the morning and at ~17:50 in the evening at on Days −1 through 5. All measurements were preceded by a 30-min (minimum) abstention from study product use. A paired *t*-test was used to make within-group comparisons and a linear mixed model was used to compare between-group differences in the measured values. Descriptive statistics, including measured morning and evening value summaries and a mean change-from-baseline table for the data collected was provided.

2.6.2. Spirometry

Spirometry measures of the volume of air exhaled during a forced breath in one second (Forced Expiratory Volume - FEV1) and total volume of air exhaled (Forced Vital Capacity – FVC) were measured by the study physician or appropriate clinical staff in subjects to assess any impacts of product use on lung function. Reductions in such measures have previously been reported in tobacco cigarette smokers and patients with COPD **[32].** Baseline (Day −1) versus post-Baseline (Day 5) changes in FVC and FEV1 spirometry endpoints were performed in the afternoon on Days −1 and 5 using a KoKo® Spirometer and methods consistent with American Thoracic Society guidelines. FVC and FEV1 values were documented and descriptive statistics, including a measured value summary and measured value percentage change from baseline was provided for all data. A paired *t*-test was used to make within-group comparisons and a linear mixed model was used to compare between-group differences in FVC and FEV1.

2.6.3. Exhaled breath CO and NO

The concentration of CO and NO was measured in all subjects to assess the smoking status of subjects in the different product use groups. Exhaled CO and NO were measured during the study in the afternoon on Days −1 (Baseline), 1, 3 and 5 (prior to spirometry measurements on Days −1 and 5) using a Bedfont Micro + Smokerlyzer and Niox Mino, respectively. All physiological measurements were preceded by a 30-min (minimum) abstention from study product use. A paired *t*-test was used to make within-group comparisons and a linear mixed model was used to compare between-group differences and changes between baseline and Day 5 concentrations.

2.7. Safety and tolerability assessments

Safety and tolerability evaluations included assessments of adverse events (AEs). AEs spontaneously reported by the subjects or observed by the Principal Investigator (PI) or other study personnel were monitored from the time of check-in until the end-of-study (or early termination). Any concomitant medications taken from 30 days prior to check-in through the end-of-study (or

early termination) was also recorded. AEs were defined as any unwarranted medical occurrence (including an abnormal laboratory finding) experienced by a subject administered with a study product, whether or not considered study product-related by the investigator. AEs captured in the database were listed in by-subject data listings including verbatim term, coded term, cohort, severity, relationship to study product, and action; however, only product use-emergent AEs were summarized. AE seriousness, severity and relationship to study product were assessed by the PI. A study product use-emergent AE was defined as an AE that started or intensified at the time of or after study product usage. An AE that occurred during the washout period between study products was considered study product use-emergent for the last study product given. All AEs that occurred during this clinical trial were coded using the Medical Dictionary for Regulatory Activities (MedDRA®), Version 17.1.

2.8. Data analyses

Statistical analyses were performed using SAS procedures in SAS® Version 9.3. A paired *t*-test was used to make within-group comparisons between study days and a linear mixed model was used to assess between-group differences. Baseline values were included in the statistical models for the between-group comparisons as a covariate. Differences were considered statistically significant at an alpha level of 5% ($p < 0.05$).

3. Results

3.1. Study demographics and participant characteristics

A summary of the study demographics, participant baseline smoking history, and survey score results are presented in Table 1. The mean age of the study population was ~38 years, 65% males and 35% females. Baseline cigarettes smoked per day (CPD) ranged from ~15 to ~21 and years smoked ranged from ~15 to ~22. Menthol smokers made up 37% of the subject population. The FTND score provides an ordinal measure of nicotine dependence related to cigarette smoking. Mean baseline FTND scores were comparable across cohorts, ranging from 5.1 to 5.7, and, on average, indicated a moderate dependence. The Brief WISDM score provides a measure of tobacco smoking dependence. The Brief WISDM sub-score, scale, and total score means were broadly comparable across use groups, also indicating that smoking dependence was similar at baseline across groups.

3.2. Product use

Table 2 summarizes the number of cigarettes (CPD) smoked by the different user groups (including CPD reported at Screening) and the estimated quantity of nicotine delivered by the e-cigarette products. The mean reported cigarette consumption at Screening ranged from approximately 15 CPD to 21 CPD. Subjects randomized to exclusive use of e-cigarette products reported fewer CPD than those randomized to the dual use and cessation groups. To standardize cigarette consumption during the study, subjects in the dual use groups were required to reduce their daily cigarette consumption on Days 1–5 by ~50% of that reported at Screening. Overall, subjects smoked ~52% fewer cigarettes during the study compared to that reported at Screening.

At baseline, mean cigarette consumption ranged from approximately 14 to 18 CPD with the subjects to be randomized to the dual use group tending to smoke fewer cigarettes compared to the other use groups. From Day 1 to Day 5, cigarette consumption within the dual use group was consistent as subjects tended to smoke their entire daily allocated number of cigarettes each day. The estimated nicotine intake from the e-cigarette products increased over time, peaking in each of the use groups on Day 4 or Day 5. Large SD values indicate that there was variability in frequency of use within all of the groups.

On Day 5, the mean estimated quantity of nicotine consumed by the exclusive use groups was very consistent, varying only by 3.5 mg across the use groups. Results indicate that subjects using the tobacco flavored product received ~15% less nicotine than those using any of the cherry flavored e-cigarette products, but the differences were not statistically significant. In contrast, the difference in product use among dual users was larger, varying by approximately 5.7 mg across use groups. Subjects using the tobacco flavored product received approximately 81% and 40% more nicotine than from the rechargeable and disposable cherry flavored products, respectively. However, these apparently large differences were not statistically significant (Table 3).

Predictably, subjects in the exclusive use groups used the e-cigarettes more on average than the subjects in the dual use groups, who were able to continue smoking tobacco cigarettes. There were no statistically significant differences among the exclusive use groups or among dual users on Day 5.

By assuming that dual users received ~1 mg of nicotine per tobacco cigarette, over the course of the entire week, it was calculated that subjects in the dual use groups who used the tobacco rechargeable, cherry rechargeable and cherry disposable products theoretically consumed ~107, ~80, and ~89 mg of nicotine, respectively. In comparison, the respective exclusive use groups theoretically received ~86, ~99, and ~99 mg of nicotine. This indicates that smokers who switched completely to e-cigarettes were able to obtain a similar, or lesser amounts of nicotine, as those continuing to smoke and vape under the dual use conditions imposed by the study.

3.3. Cardiovascular effects (blood pressure and heart rate)

3.3.1. Systolic Blood Pressure (SBP)

Table 4 summarizes the Baseline and Day 5 morning and evening SBP values and statistical comparisons by user group. Baseline morning mean SBP values ranged from ~116 mmHg to ~124 mmHg across all groups. On Day 5, morning SBP mean values were ~3–7% lower for all groups and significantly lower for the exclusive tobacco flavored e-cigarette use group ($p = 0.0079$), dual cherry rechargeable ($p = 0.0368$) and disposable ($p = 0.0037$) product use groups.

Baseline evening mean SBP values ranged from ~119 mmHg to ~130 mmHg across all groups. Day 5 evening SBP mean values were slightly lower (~1%–5%) for all groups compared to baseline except for the exclusive cherry disposable use group, which experienced a slight (~1%) increase, which was not significant. Significantly lower means were observed in the dual use cherry rechargeable ($p = 0.0225$) and dual use cherry disposable ($p = 0.0106$) product use groups.

Mean SBP increased comparably across all use groups by ~2–9 mmHg from the morning to the evening on Day −1 as the subjects smoked their usual brand combustible cigarettes *ad libitum*. Increases in mean SBP from the morning to the evening on Day 5 were noted for all use groups and ranged from ~6% to ~10%, with statistically significant increases noted for all use groups except the exclusive cherry disposable ($p = 0.0614$) and dual tobacco ($p = 0.0684$) product use groups. Notably, the nicotine cessation group had the highest percent increase (~10%). This finding appears to be consistent with previously reported observations indicating that, while cigarette smoking causes an acute rise in blood pressure, on average, blood pressure is typically lower in cigarette smokers

Table 1
Demographics, baseline smoking history, FTND, and WISDM summarization by user group and overall.

	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation N = 15	Overall N = 105
	Tobacco Rechargeable N = 15	Cherry Rechargeable N = 15	Cherry Disposable N = 15	Tobacco Rechargeable N = 15	Cherry Rechargeable N = 15	Cherry Disposable N = 15		
Gender								
Female	6 (40%)	3 (20%)	9 (60%)	6 (40%)	3 (20%)	7 (47%)	3 (20%)	37 (35%)
Male	9 (60%)	12 (80%)	6 (40%)	9 (60%)	12 (80%)	8 (53%)	12 (80%)	68 (65%)
Race								
American Indian/Alaska Native	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)	1 (1%)
Black or African American	2 (13%)	6 (40%)	1 (7%)	2 (13%)	4 (27%)	1 (7%)	1 (7%)	17 (16%)
Black or African American, American Indian/Alaska	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7%)	1 (1%)
Hispanic or Latino	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	1 (7%)
White	13 (87%)	9 (60%)	14 (93%)	13 (87%)	11 (73%)	13 (87%)	13 (87%)	86 (82%)
Age (years)								
Mean	37.1	40.1	33.9	36.6	36.8	39.3	41.1	37.8
SD	11.4	10.6	11.8	10.8	11.6	10.6	11.2	11.1
Weight (kg)								
Mean	82.7	78.0	83.5	86.5	83.4	82.0	85.6	83.1
SD	17.3	18.0	19.2	19.4	18.0	16.3	17.0	17.6
BMI (kg/m^2)								
Mean	28.2	26.2	28.7	28.9	27.2	27.8	27.8	27.8
SD	5.5	6.5	5.7	5.5	4.7	5.1	4.9	5.4
CPD								
Mean	18.4	17.3	15.4	18.7	20.5	21.1	20.4	18.8
SD	7.1	6.2	3.3	6.6	7.3	5.8	7.5	6.5
Years Smoked								
Mean	19.2	20.3	15.0	19.3	14.6	21.7	21.3	18.8
SD	12.9	10.5	10.9	10.1	11.6	8.7	10.6	10.8
Usual Brand Flavor								
Menthol	6 (40%)	7 (47%)	8 (53%)	3 (20%)	7 (47%)	5 (33%)	3 (20%)	39 (37%)
Non-Menthol	9 (60%)	8 (53%)	7 (47%)	12 (80%)	8 (53%)	10 (67%)	12 (80%)	66 (63%)
FTND Score								
Mean	5.3	5.1	5.3	5.5	5.7	5.2	5.6	5.4
SD	1.5	2.0	1.5	2.0	1.1	1.7	2.0	1.7
WISDM Scores (mean scores)								
Affiliative Attachment	2.6	2.1	3.6	3.2	2.4	2.9	3.2	2.9
Automaticity	4.4	3.9	4.6	4.1	3.8	4.6	4.4	4.2
Loss of Control	3.6	3.4	4.4	4.8	4.1	4.5	3.9	4.1
Cognitive Enhancement	3.6	3.2	4.8	4.0	3.7	4.4	3.8	3.9
Craving	4.7	4.5	5.0	5.2	4.9	5.2	4.6	4.9
Cue Exposure/Associative Processes	4.4	4.1	4.9	4.6	4.5	4.8	4.5	4.6
Social/Environmental Goads	4.0	4.5	4.8	4.8	5.0	4.8	4.7	4.6
Taste	4.5	4.5	4.6	4.8	4.7	4.9	4.1	4.6
Tolerance	5.4	4.5	4.8	5.6	5.1	5.1	5.3	5.1
Weight	2.5	2.2	3.0	2.3	2.6	2.9	2.3	2.5
Affective Enhancement	3.9	3.4	4.5	3.9	4.2	4.4	3.9	4.0
Primary Dependence Motives Scale	4.5	4.1	4.7	4.9	4.5	4.8	4.5	4.6
Secondary Dependence Motives Scale	3.7	3.4	4.3	3.9	3.9	4.1	3.8	3.9
Total Score	43.6	40.4	49.0	47.3	44.9	48.4	44.6	45.5

than non-smokers (Mikkelsen et al., 1997; Green et al., 1986).

Table 5 summarizes the Day 5 and Day 5 change from baseline morning and evening systolic blood pressure and the statistical comparisons between product use groups. No statistically significant differences were observed in the Day 5 morning or evening SBP or the Day 5 change from baseline morning and evening SBP comparisons.

In terms of overall mean percentage change observations (Table 4), by Day 5, reductions in systolic blood pressure were observed, with morning decreases from baseline ranging from ~3% to ~7% for all product use groups and evening changes ranging from an ~1% increase to an ~5% decrease. By Day 5, the nicotine cessation group experienced the greatest increase in SBP from the morning to the evening on Day 5 (9.8% change) followed by the exclusive use (9.3%) and dual use (6.4%) groups, respectively. However, the differences between groups were not statistically significant. Fig. 1 provides an illustration of the change in morning and evening SBP values from baseline to Day 5 by product use group.

3.3.2. Diastolic Blood Pressure (DBP)

Table 6 summarizes the baseline and Day 5 morning and evening SBP values and statistical comparisons by user group. Baseline morning mean DBP values ranged from ~74 mmHg to ~79 mmHg across all groups. On Day 5, the morning mean values were lower for all groups (range ~0.1%–7%) compared to baseline. Mean values were significantly lower for the exclusive tobacco rechargeable

Table 2
Summary of the number of cigarettes smoked and estimated amount of nicotine delivered.

		Exclusive E-Cigarette Use			Dual Use			Nicotine Cessation
		Classic Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Classic Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	–
Screening	Cigarettes	18.4 ± 7.1	17.3 ± 6.2	15.4 ± 3.3	18.7 ± 6.6	20.5 ± 7.3	21.1 ± 5.8	20.4 ± 7.5
Day −1	Cigarettes	16.9 ± 5.1	15.8 ± 5.4	14.9 ± 1.5	14.5 ± 4.5	14.2 ± 2.0	14.8 ± 2.4	17.5 ± 4.9
Day 1	Cigarettes	NA	NA	NA	8.9 ± 3.1	9.6 ± 2.7	9.8 ± 2.0	NA
	E-cigarette nicotine	14.9 ± 15.2	17.7 ± 16.9	13.6 ± 11.0	8.9 ± 6.9	4.6 ± 3.3	5.6 ± 3.4	NA
Day 2	Cigarettes	NA	NA	NA	8.9 ± 2.9	9.5 ± 2.8	9.9 ± 1.9	NA
	E-cigarette nicotine	17.2 ± 15.1	17.6 ± 14.3	19.6 ± 16.8	11.1 ± 7.8	6.5 ± 5.5	7.4 ± 7.2	NA
Day 3	Cigarettes	NA	NA	NA	8.9 ± 3.0	9.5 ± 2.5	10.1 ± 2.0	NA
	E-cigarette nicotine	16.3 ± 13.4	19.8 ± 16.9	19.4 ± 20.0	12.4 ± 9.5	6.2 ± 6.5	9.4 ± 9.8	NA
Day 4	Cigarettes	NA	NA	NA	9.0 ± 3.2	9.7 ± 2.9	10.0 ± 2.0	NA
	E-cigarette nicotine	18.4 ± 14.6	20.8 ± 14.2	23.4 ± 21.0	13.3 ± 10.7	7.5 ± 8.3	9.9 ± 9.1	NA
Day 5	Cigarettes	NA	NA	NA	9.0 ± 3.3	9.6 ± 2.8	10.4 ± 2.2	NA
	E-cigarette nicotine	19.4 ± 16.6	22.9 ± 16.6	22.9 ± 20.5	12.7 ± 11.6	7.0 ± 9.3	9.1 ± 8.6	NA
Day 1 through Day 5 Mean	Cigarettes	NA	NA	NA	44.7 ± 15.4	47.9 ± 13.6	50.5 ± 9.5	NA
	E-cigarette nicotine	86.1 ± 70.5	98.9 ± 75.1	99.0 ± 86.8	61.8 ± 45.9	31.9 ± 30.1	38.9 ± 36.4	NA

Values are presented as mean ± SD mg nicotine or cigarettes smoked. NA = Not available.
Cessation group subjects reported smoking 20.4 ± 7.5 CPD during screening and smoked 17.5 ± 4.9 cigarettes on Day −1.

Table 3
Statistical Comparisons of the Day 5 Estimated Nicotine Delivered by e-Cigarettes Between Use Groups.

Comparison	First LSM (mg)	Second LSM (mg)	Difference (mg)	p-Value
A1 vs B1	19.40	12.74	6.66	0.2140
A2 vs B2	22.86	7.04	15.83	**0.0038**
A3 vs B3	22.91	9.08	13.83	**0.0125**
A1 vs A2	19.40	22.86	−3.46	0.5170
A1 vs A3	19.40	22.91	−3.51	0.5117
A2 vs A3	22.86	22.91	−0.04	0.9934
B1 vs B2	12.74	7.04	5.70	0.2873
B1 vs B3	12.74	9.08	3.66	0.5011
B2 vs B3	7.04	9.08	−2.04	0.7074

LSM = Least-square means.
Use Groups:
A1: Exclusive Tobacco flavor rechargeable e-cigarette.
A2: Exclusive Cherry flavor rechargeable e-cigarette.
A3: Exclusive Cherry flavor disposable e-cigarette.
B1: Dual Tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
B2: Dual Cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
B3: Dual Cherry flavor disposable e-cigarette and usual brand combustible cigarette.
Bold indicate statistical significance, $p < 0.05$.

($p = 0.0080$), exclusive cherry disposable ($p = 0.0417$), dual cherry rechargeable ($p = 0.0439$), and dual cherry disposable product use groups. Changes in evening DBP (Day 5 vs D-1) were ~1–5% lower for all groups except for the dual use tobacco group which had a slight (~1.7%) increase. Values were statistically lower only for the dual use cherry rechargeable user group ($p = 0.0393$).

Table 7 provides the Day 5 and Day 5 change from baseline morning and evening DBP and statistical comparisons between product use groups. In general, no statistically significant differences or trends were observed in the morning or evening DBP at Day 5 or Day 5 versus baseline changes.

Overall, the change in mean DBP values from the morning to the evening on Day −1 (Table 6) were comparable across user groups. Values ranged from no change to an ~2 mmHg increase during the period when all subjects smoked their usual brand combustible cigarettes *ad libitum*. In terms of overall mean percent change observations, by Day 5, reductions in DBP were observed. Morning values from baseline ranged from no change to ~7% across all product use groups. Changes in DBP in the evening all decreased by ~1%–~5%, except for one dual tobacco rechargeable use group, which experienced an increase of 1.7%. By Day 5, the morning to evening changes ranged from slight decreases to a 5% increase. However, none of the changes were statistically significant (Table 7). Fig. 2 provides an illustration of the change in morning and evening DBP values from baseline to Day 5 by product use group.

3.3.3. Heart rate

Heart rate (HR) was measured in all subjects in the morning and evening of all study days. Table 8 summarizes the Day −1 through Day 5 morning, evening and change from baseline HR values and statistical comparisons for all product use groups.

The baseline morning mean HR values were comparable across use groups, though the exclusive and dual tobacco groups had slightly higher HR than the other groups. By Day 5, all groups except for the dual use cherry disposable group experienced lower morning HRs compared to baseline. The nicotine cessation group experienced the largest reduction in HR (~9%), followed by the exclusive e-cigarette use group (~2%–~7%) and the dual use groups (~3% reduction to ~2% increase). A reduction in morning HRs on Day 5 were statistically significant for the cessation ($p = 0.0483$) and exclusive tobacco ($p = 0.0207$) use groups.

The baseline evening mean HR rate values were comparable across use groups, with the exclusive and dual tobacco use groups experiencing slightly higher HRs than the other groups. By Day 5, the nicotine cessation group and each of the exclusive use groups had evening mean HRs that were lower than baseline. With the nicotine cessation group exhibiting ~10% reductions and the exclusive group ~5%–7% reductions. Statistically significant reductions in HR were observed in the nicotine cessation group ($p = 0.0054$), the exclusive tobacco ($p = 0.0115$) and cherry rechargeable ($p = 0.0203$) product use groups. In contrast, the dual use group experienced increases in the evening mean HRs ranging from ~1% to 5%, though none were statistically significant.

Table 9 summarizes the Day 5 morning, evening HRs and change from baseline HR statistical comparisons between product use groups. On the morning of Day 5, the nicotine cessation group had a mean HR that was statistically significantly lower than the dual classic tobacco product use group ($p = 0.0007$). No other consistent trends were observed between the use groups. Among the Day 5 change from baseline morning HR comparisons, only the difference between the nicotine cessation group and the dual cherry disposable product use group were found to be statistically significant

Table 4
Systolic Blood Pressure Summary and Day 5 vs Day −1 Statistical Comparisons.

Day	Time Point	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
		Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
−1	Morning	118.8 ± 15.4	123.9 ± 9.3	116.3 ± 13.8	123.3 ± 13.0	119.1 ± 12.7	121.4 ± 14.7	119.5 ± 13.8
	Evening	122.9 ± 11.3	130.1 ± 11.4	118.9 ± 14.5	126.6 ± 12.5	127.0 ± 16.9	125.7 ± 11.1	128.9 ± 15.7
5	Morning	110.9 ± 9.2	115.9 ± 10.8	112.0 ± 10.9	117.9 ± 15.0	113.1 ± 10.3	111.3 ± 10.0	113.3 ± 10.1
	Evening	120.6 ± 12.6	126.5 ± 14.9	119.9 ± 15.1	124.6 ± 12.5	119.5 ± 11.2	118.1 ± 10.0	124.3 ± 12.5
Morning Day 5 Change from Day -1 Systolic Blood Pressure								
N		15	15	15	15	15	14	14
Absolute change		−7.9 ± 9.9	−8.0 ± 14.7	−4.3 ± 12.8	−5.3 ± 16.4	−6.0 ± 10.1	−8.5 ± 9.0	−5.4 ± 14.1
p-value		**0.0079**[a]	0.0531	0.2110	0.2278	**0.0368** [a]	**0.0037** [a]	0.1797
% change		−6.0 ± 7.4	−5.9 ± 12.2	−3.1 ± 9.8	−3.7 ± 13.2	−4.5 ± 8.9	−6.7 ± 7.0	−3.6 ± 11.6
Evening Day 5 Change from Day -1 Systolic Blood Pressure								
N		15	15	15	15	15	14	14
Absolute change		−2.3 ± 8.8	−3.6 ± 10.3	1.0 ± 11.7	−2.0 ± 9.0	−7.5 ± 11.4	−5.9 ± 7.4	−3.1 ± 10.0
p-value		0.3221	0.1962	0.7450	0.4011	**0.0225**	**0.0106**	0.2607
% change		−1.8 ± 7.2	−2.7 ± 7.9	1.1 ± 9.6	−1.3 ± 7.0	−5.2 ± 8.2	−4.7 ± 6.0	−2.0 ± 8.5
Day 5 Evening Change from Morning Systolic Blood Pressure								
N		15	15	15	15	15	14	14
Absolute change		9.7 ± 9.4	10.6 ± 11.6	7.9 ± 15.0	6.7 ± 13.1	6.4 ± 10.2	6.9 ± 6.7	11.0 ± 8.5
p-value		**0.0012** [a]	**0.0033** [a]	0.0614	0.0684	**0.0290** [a]	**0.0020** [a]	**0.0003** [a]
% change		8.9 ± 8.4	9.3 ± 9.9	7.5 ± 13.0	6.5 ± 11.4	6.0 ± 9.4	6.4 ± 6.2	9.8 ± 8.0

Day −1 and 5 and absolute change values are presented as arithmetic mean ± SD in mmHg.
% change presented as arithmetic mean ± SD.
Bold indicate statistical significance, $p < 0.05$.
[a] Statistically significant.

Table 5
Day 5 and day 5 change from baseline morning and evening systolic blood pressure statistical comparisons.

Group Comparison	Day 5 Comparisons				Day 5 Change from Day −1 Comparisons			
	First LSM [a] (mmHg)	Second LSM (mmHg)	Difference (mmHg)	p-Value	First LSM (mmHg)	Second LSM (mmHg)	Difference (mmHg)	p-Value
Morning Systolic Blood Pressure								
A1 vs C	111.32	113.80	−2.48	0.4990	−7.93	−5.36	−2.58	0.5868
A2 vs C	114.36	113.80	0.56	0.8799	−8.00	−5.36	−2.64	0.5772
A3 vs C	113.41	113.80	−0.39	0.9151	−4.33	−5.36	1.02	0.8289
B1 vs C	116.66	113.80	2.86	0.4385	−5.33	−5.36	0.02	0.9960
B2 vs C	113.42	113.80	−0.38	0.9167	−6.00	−5.36	−0.64	0.8920
B3 vs C	111.36	113.80	−2.44	0.5127	−8.50	−5.36	−3.14	0.5146
Evening Systolic Blood Pressure								
A1 vs C	122.21	122.80	−0.59	0.8606	−2.33	−3.14	0.81	0.8264
A2 vs C	123.17	122.80	0.37	0.9135	−3.60	−3.14	−0.46	0.9014
A3 vs C	124.27	122.80	1.47	0.6679	1.00	−3.14	4.14	0.2633
B1 vs C	123.69	122.80	0.88	0.7931	−2.00	−3.14	1.14	0.7569
B2 vs C	118.28	122.80	−4.52	0.1815	−7.53	−3.14	−4.39	0.2360
B3 vs C	118.97	122.80	−3.83	0.2660	−5.93	−3.14	−2.79	0.4587
Day 5 Evening vs Morning Systolic Blood Pressure								
A1 vs C	–	–	–	–	9.82	10.74	−0.93	0.8232
A2 vs C	–	–	–	–	10.53	10.74	−0.21	0.9592
A3 vs C	–	–	–	–	8.07	10.74	−2.68	0.5219
B1 vs C	–	–	–	–	6.81	10.74	−3.93	0.3453
B2 vs C	–	–	–	–	6.21	10.74	−4.54	0.2715
B3 vs C	–	–	–	–	6.93	10.74	−3.81	0.3665

Group A1: Exclusive tobacco flavor rechargeable e-cigarette.
Group A2: Exclusive cherry flavor rechargeable e-cigarette.
Group A3: Exclusive cherry flavor disposable e-cigarette.
Group B1: Dual tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B2: Dual cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B3: Dual cherry flavor disposable e-cigarette and usual brand combustible cigarette.
Group C: Nicotine cessation.
[a] LSM = least square means.

($p = 0.0350$).

Amongst the use groups, the Day 5 evening HR was statistically lower for the nicotine cessation group compared to all the dual use groups (dual tobacco rechargeable: $p = 0.0003$; dual cherry rechargeable: $p = 0.0015$; dual cherry disposable: $p = 0.0001$) but not compared to the exclusive use groups. Similar statistically significant differences were also noted in the Day 5 change from baseline measurements for evening HRs. The evening HRs for the nicotine cessation group were statistically lower compared to all the dual use groups (Table 9).

Statistical comparison of the Day 5 evening versus morning HR showed that the nicotine cessation group experienced the smallest

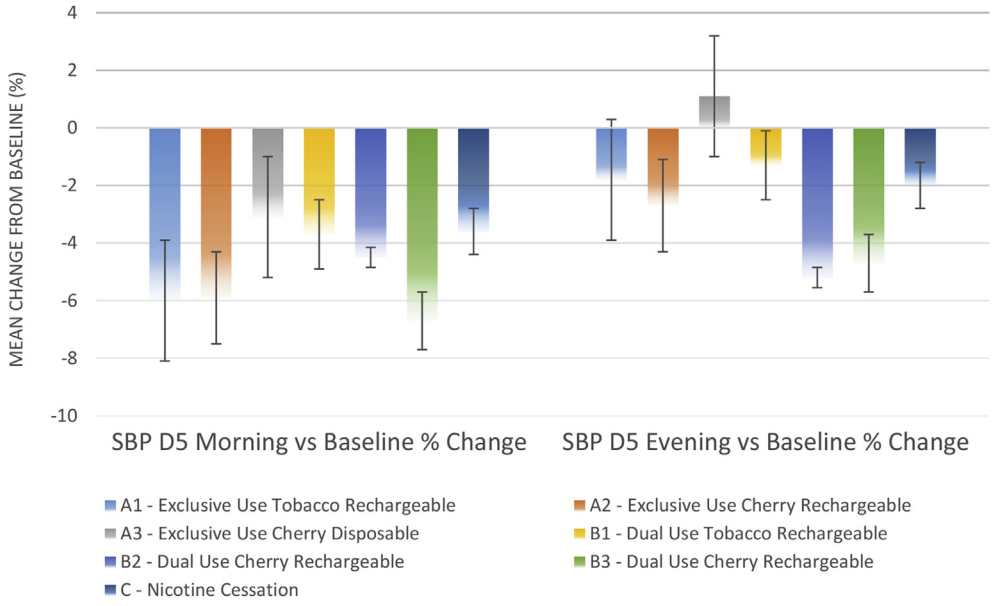

Fig. 1. Summary of morning and evening systolic blood pressure (mmHg) Changes from baseline by use group from baseline to day 5.

Table 6
Diastolic Blood Pressure Summary and Day 5 vs Day −1 Statistical Comparisons.

Day	Time Point	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
		Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
−1	Morning	74.9 ± 6.8	79.0 ± 8.3	73.8 ± 11.1	78.4 ± 8.8	75.1 ± 10.7	77.4 ± 8.5	75.9 ± 8.1
	Evening	73.2 ± 6.9	77.5 ± 9.1	72.5 ± 11.0	76.3 ± 7.5	75.3 ± 10.1	75.3 ± 7.9	76.2 ± 8.1
5	Morning	70.5 ± 6.9	72.8 ± 11.0	69.0 ± 9.2	74.5 ± 12.7	71.3 ± 10.3	71.9 ± 6.8	75.1 ± 8.1
	Evening	70.2 ± 7.7	74.2 ± 7.9	71.6 ± 11.2	77.5 ± 10.8	70.7 ± 7.2	71.5 ± 7.1	74.8 ± 6.8
Morning Day 5 Change from Day -1 (baseline) Diastolic Blood Pressure								
N	15	15	15	15	15	14	14	
Absolute change	−4.4 ± 5.5	−6.2 ± 12.0	−4.8 ± 8.3	−3.9 ± 11.9	−3.8 ± 6.7	−4.9 ± 7.9	−0.3 ± 6.4	
p-value	**0.0080**[a]	0.0644	**0.0417** [a]	0.2287	**0.0439** [a]	**0.0355** [a]	0.8707	
% change	−5.7 ± 6.7	−7.3 ± 14.3	−5.8 ± 10.3	−4.5 ± 13.5	−4.6 ± 9.2	−5.8 ± 9.9	−0.1 ± 8.5	
Evening Day 5 Change from Day -1 (baseline) Diastolic Blood Pressure								
N	15	15	15	15	15	14	14	
Absolute change	−3.0 ± 7.6	−3.3 ± 7.5	−0.9 ± 8.3	1.1 ± 9.3	−4.6 ± 7.8	−3.4 ± 10.4	−1.1 ± 6.3	
p-value	0.1484	0.1141	0.6901	0.6424	**0.0393** [a]	0.2385	0.5328	
% change	−3.7 ± 10.4	−3.6 ± 10.1	−0.6 ± 11.3	1.7 ± 12.4	−5.3 ± 10.0	−3.7 ± 13.6	−1.0 ± 8.0	
Day 5 Evening Change from Morning Diastolic Blood Pressure								
N	15	15	15	15	15	14	14	
Absolute change	−0.3 ± 6.9	1.4 ± 11.4	2.6 ± 9.0	2.9 ± 9.1	−0.5 ± 7.2	−0.4 ± 5.9	−0.3 ± 4.8	
p-value	0.8546	0.6413	0.2799	0.2338	0.7793	0.8252	0.8287	
% change	−0.1 ± 9.7	3.5 ± 15.1	4.3 ± 12.4	5.0 ± 12.7	0.0 ± 9.2	−0.3 ± 8.7	−0.0 ± 6.4	

Day −1 and 5 and absolute change values are presented as arithmetic mean ± SD in mmHg.
% change presented as arithmetic mean ± SD.
[a] Statistically significant.

increase in mean HR from the morning to the evening of Day 5. The exclusive use and dual use groups followed respectively. Statistically significant differences were observed between the cessation group and dual cherry rechargeable and disposable product use groups ($p = 0.0307$ and 0.0418, respectively).

Overall, mean HRs increased comparably by ~9–12 bpm from the morning to the evening on Day −1 across all use groups as the subjects smoked their usual brand combustible cigarettes *ad libitum*. Statistically significant increases in mean HR from the morning to the evening on Day 5 were noted in all use groups, with the increases ranging from ~12% to ~23% (Table 8). Fig. 3 provides an illustration of the change in HR values from baseline to Day 5 by product use group.

3.4. Pulmonary effects (spirometry (FEV1 and FVC) and exhaled CO and NO)

3.4.1. Forced Vital Capacity (FVC)

Observed changes in measured FVC from baseline to Day 5 were small, ranging from ~-0.5% to 3.1%. Statistically significant increases were noted for the exclusive tobacco ($p = 0.0207$) and cherry rechargeable ($p = 0.0113$) product use groups (Table 11). No

Table 7
Day 5 and day 5 change from baseline morning and evening diastolic blood pressure statistical.

Group Comparison	Day 5 Comparisons				Day 5 Change from Day −1 Comparisons			
	First LSM [a] (mmHg)	Second LSM (mmHg)	Difference (mmHg)	p-Value	First LSM (mmHg)	Second LSM (mmHg)	Difference (mmHg)	p-Value
Morning Diastolic Blood Pressure								
Exclusive Use Tobacco Rechargeable vs. Cessation	71.27	75.56	−4.29	0.1499	−4.40	−0.29	−4.11	0.2083
Exclusive Use Cherry Rechargeable vs. Cessation	71.15	75.56	−4.41	0.1411	−6.20	−0.29	−5.91	0.0717
Exclusive Use Cherry Disposable vs. Cessation	70.41	75.56	−5.16	0.0845	−4.80	−0.29	−4.51	0.1677
Dual Use Tobacco Rechargeable vs. Cessation	73.24	75.56	−2.33	0.4351	−3.87	−0.29	−3.58	0.2730
Dual Use Cherry Rechargeable vs. Cessation	71.93	75.56	−3.63	0.2218	−3.80	−0.29	−3.51	0.2819
Dual Use Cherry Disposable vs. Cessation	71.51	75.56	−4.05	0.1810	−4.93	−0.29	−4.64	0.1631
Evening Diastolic Blood Pressure								
Exclusive Use Tobacco Rechargeable vs. Cessation	71.20	74.37	−3.17	0.2399	−3.00	−1.07	−1.93	0.5302
Exclusive Use Cherry Rechargeable vs. Cessation	72.92	74.37	−1.45	0.5894	−3.27	−1.07	−2.20	0.4751
Exclusive Use Cherry Disposable vs. Cessation	73.00	74.37	−1.37	0.6099	−0.87	−1.07	0.20	0.9468
Dual Use Tobacco Rechargeable vs. Cessation	76.80	74.37	2.43	0.3654	1.13	−1.07	2.20	0.4732
Dual Use Cherry Rechargeable vs. Cessation	70.60	74.37	−3.77	0.1606	−4.60	−1.07	−3.53	0.2520
Dual Use Cherry Disposable vs. Cessation	71.58	74.37	−2.79	0.3067	−3.43	−1.07	−2.36	0.4509
Day 5 Evening vs Morning Diastolic Blood Pressure								
Exclusive Use Tobacco Rechargeable vs. Cessation	–	–	–	–	0.28	0.43	0.16	0.9585
Exclusive Use Cherry Rechargeable vs. Cessation	–	–	–	–	1.44	0.43	1.87	0.5363
Exclusive Use Cherry Disposable vs. Cessation	–	–	–	–	2.62	0.43	3.05	0.3136
Dual Use Tobacco Rechargeable vs. Cessation	–	–	–	–	3.02	0.43	3.45	0.2558
Dual Use Cherry Rechargeable vs. Cessation	–	–	–	–	0.66	0.43	0.23	0.9402
Dual Use Cherry Disposable vs. Cessation	–	–	–	–	0.29	0.43	0.14	0.9625

[a] LSM = least square means.

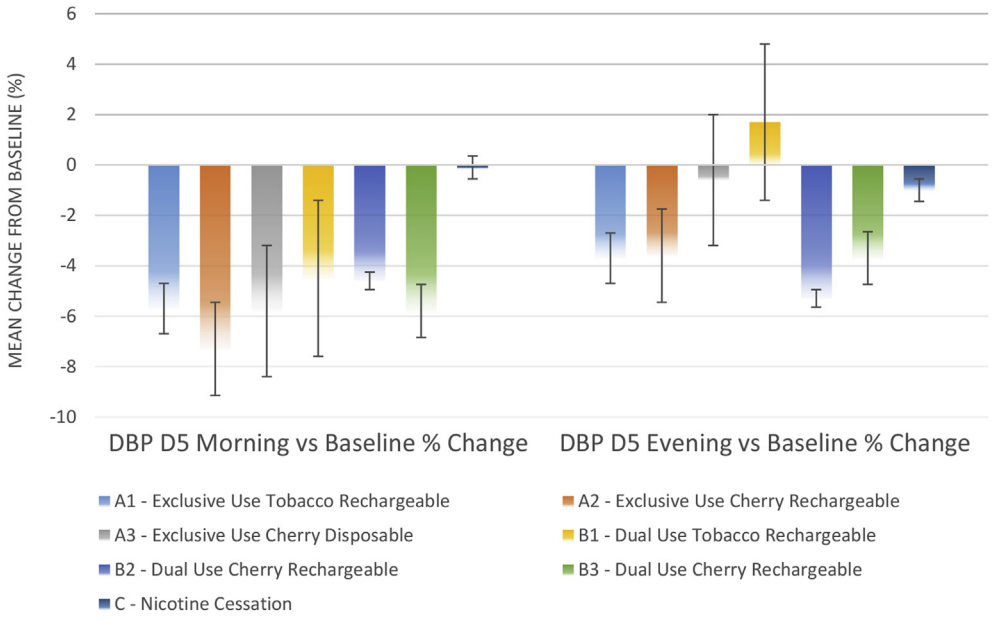

Fig. 2. Summary of morning and evening diasystolic blood pressure (mmHg) Changes from baseline by use group from baseline to day 5.

statistically significant differences were observed in measured FVC between any of the product use groups and the nicotine cessation group (Table 12).

3.4.2. Forced Expiratory Volume (FEV1)

Changes in measured FEV1 from baseline to Day 5 ranged from ~-1.5% to ~6%. Statistically significant increases were observed in the exclusive tobacco ($p = 0.0148$), exclusive cherry rechargeable ($p = 0.0276$), and dual cherry rechargeable product use groups ($p = 0.0191$) (Table 13). However, no statistically significant differences in measured FEV1 between any of the product use groups and the nicotine cessations group were noted (Table 14). Fig. 4 provides an illustration of the change in the FVC and FEV1 values from baseline to Day 5 by product use group. As seen in Fig. 4, the performance of the subjects who were exclusive users of the Cherry disposable device (A3) appeared to have different outcomes than those who were exclusive Cherry rechargeable (A2) and Tobacco rechargeable e-cigarettes. It is not known if these differences were due to device performance or differences in puffing profiles and is an area of further research.

3.4.3. Exhaled CO and NO

Physiological changes associated with smoking reduction were observed in the study exhaled CO and NO endpoints, with all groups experiencing statistically significant decreases in exhaled CO at Day 5 compared to baseline (Table 15). Decreases in the

Table 8
Heart Rate Summary and Day 5 vs Day −1 Statistical Comparisons Within Use Groups.

Day	Time Point	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
		Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
−1	Morning	76.4 ± 9.4	71.0 ± 11.8	70.7 ± 10.5	76.7 ± 10.4	66.5 ± 9.5	66.1 ± 9.3	70.0 ± 7.9
	Evening	85.9 ± 11.1	81.7 ± 9.9	82.5 ± 7.5	85.8 ± 10.2	76.3 ± 11.0	76.5 ± 8.0	79.2 ± 10.3
1	Morning	75.0 ± 8.7	70.8 ± 14.8	70.5 ± 8.1	77.4 ± 10.9	69.7 ± 12.2	67.1 ± 8.7	72.1 ± 9.1
	Evening	76.9 ± 11.4	77.3 ± 12.8	74.9 ± 11.0	82.7 ± 11.0	76.6 ± 9.2	73.8 ± 10.3	69.5 ± 8.1
2	Morning	75.8 ± 10.6	69.9 ± 9.8	69.3 ± 9.7	77.1 ± 12.1	66.4 ± 10.9	67.1 ± 7.39	66.1 ± 9.6
	Evening	82.5 ± 9.6	78.9 ± 9.9	77.9 ± 11.6	88.0 ± 10.8	78.8 ± 10.4	78.9 ± 10.0	70.1 ± 11.7
3	Morning	72.7 ± 9.4	69.1 ± 9.6	70.5 ± 10.0	75.5 ± 9.1	64.9 ± 11.1	67.4 ± 10.0	64.3 ± 6.5
	Evening	79.6 11.5	80.3 ± 10.6	79.9 ± 9.9	88.6 ± 8.1	76.9 ± 11.0	79.1 ± 8.5	71.2 ± 10.3
4	Morning	70.2 ± 8.6	70.5 ± 12.2	69.9 ± 9.1	77.4 ± 7.0	67.8 ± 12.8	67.9 ± 9.0	63.7 ± 6.8
	Evening	77.4 ± 9.5	77.7 ± 10.5	77.3 ± 8.9	88.5 ± 10.5	79.7 ± 13.4	77.4 ± 9.2	71.6 ± 10.0
5	Morning	70.4 ± 7.7	68.0 ± 10.3	68.3 ± 7.1	76.1 ± 8.6	64.3 ± 11.2	66.9 ± 7.2	62.8 ± 8.3
	Evening	79.7 ± 8.5	77.4 ± 11.8	78.7 ± 10.2	86.2 ± 9.4	77.8 ± 10.5	80.1 ± 12.5	69.9 ± 9.6
Morning Day 5 Change from Day -1 (baseline) Heart Rate								
N	15	15	15	15	15	14	14	
Absolute change	−6.0 ± 8.9	−3.0 ± 9.8	−2.3 ± 9.7	−0.5 ± 9.8	−2.1 ± 8.5	1.0 ± 7.0	−6.6 ± 11.3	
p-value	***0.0207*** [a]	0.2571	0.3655	0.8364	0.3457	0.6023	***0.0483*** [a]	
% change	−7.2 ± 10.8	−3.1 ± 14.4	−2.2 ± 11.6	0.3 ± 13.7	−3.0 ± 12.8	2.4 ± 10.6	−8.6 ± 14.6	
Evening Day 5 Change from Day -1 (baseline) Heart Rate								
N	15	15	15	15	15	14	14	
Absolute change	−6.3 ± 8.4	−4.3 ± 6.4	−3.8 ± 8.3	0.4 ± 8.9	1.5 ± 6.2	3.9 ± 9.8	−8.7 ± 9.8	
p-value	***0.0115*** [a]	***0.0203*** [a]	0.0964	0.8649	0.3559	0.1567	***0.0054*** [a]	
% change	−6.8 ± 7.7	−5.4 ± 8.2	−4.5 ± 9.9	1.1 ± 10.8	2.4 ± 7.9	5.4 ± 13.4	−10.4 ± 12.5	
Day 5 Evening Change from Morning Heart Rate								
N	15	15	15	15	15	14	14	
Absolute change	9.3 ± 8.6	9.4 ± 6.9	10.3 ± 9.7	10.1 ± 7.2	13.5 ± 6.5	13.3 ± 9.0	7.1 ± 5.8	
p-value	***0.0010*** [a]	***0.0001*** [a]	***0.0010*** [a]	***<0.0001*** [a]	***<0.0001*** [a]	***0.0001***	***0.0006*** [a]	
% change	13.8 ± 11.8	14.2 ± 11.0	15.6 ± 13.9	13.7 ± 10.3	22.5 ± 14.4	19.9 ± 13.3	11.5 ± 9.7	

Days −1 and 5 and absolute change values are presented as arithmetic mean ± SD in bpm.
% change presented as arithmetic mean ± SD.
[a] Statistically Significant.

cessation and exclusive use groups ranged from ~88% to ~89% and in the dual use group by ~26%–~32%. Furthermore, there were no differences between the cessation and exclusive use group's measurements on Day 5, whereas the dual use groups had significantly higher exhaled CO compared to cessation. Exhaled NO was observed to increase from baseline to Day 5 in the cessation and exclusive use groups (~46%–~63%), whereas the dual use groups experienced minimal changes. Fig. 5 provides an illustration of the change in CO and NO values from baseline to Day 5 by product use group.

3.5. Tolerability and adverse events

The number of subjects who experienced product use-emergent AEs and number of AEs are presented in Table 16. Overall, 72 mild product-use emergent AEs were experienced by 30% of subjects. The number of subjects reporting AEs ranged from 2 to 7 subjects each across groups receiving study products and only 1 subject in the cessation group. The most frequently reported AE was headache. Other common AEs included cough and dry throat. Moreover, there were no serious AEs and no subjects were withdrawn from the study due to adverse events related to the product used. A summary of the incidence of product use-emergent AEs classified according to MedDRA® Version 17.1 are provided in supplementary file S1.

4. Discussion

4.1. Impact of observed cardiovascular effect findings

Previous research has reported that increases in HR are associated with a higher risk of CVD (Bowman et al., 2007; Groppelli et al., 1992; Najem et al., 2006; Palatini and Julius, 2004; Singh, 2003). Elevated SBP has also been identified as a risk factor for cardiovascular disease (Pastor-Barriuso et al., 2003; Li et al., 2014; Kannel, 2000), with researchers reporting that increases in heart rate by 10 beats per minute and increases in systolic blood pressure by 10 mm Hg increases the risk of cardiac death by at least 20% (Perret-Guillaume et al., 2009).

In general, reductions in blood pressure and heart rate vital signs were observed mostly in the groups that either ceased using tobacco and nicotine products altogether or switched completely to using e-cigarettes. By Day 5, small changes in systolic blood pressure were observed, with morning decreases from Day −1 ranging from ~3% to ~7% and evening changes ranging from an ~1% increase to a ~5% decrease. A similar pattern was noted in the diastolic blood pressure measurements.

Moreover, morning and evening heart rates on Day −1 were comparable across use groups, as were the increases from morning to evening (range from ~11% to ~14%). By the evening of Day 5, subjects in the cessation and exclusive use group experienced small, but typically statistically significant, reductions in heart rates ranging from ~5% to ~10%. In contrast, the dual use groups tended to experience small increases (~1%–~5%) in the evening compared to Day −1. These evening values tended to be statistically significantly higher than in the cessation and exclusive use groups.

Although not all the results were statistically significant, our findings suggest that there were no immediate acute adverse effects associated with e-cigarette use over a 5-day period. In addition, potential cardiovascular benefits, notably reductions in HR, were observed in the study groups that either discontinued using nicotine products or switched completely to e-cigarette products. As previously noted, similar effects were also noted in recent e-cigarette study investigating the impacts of e-cigarettes on

Table 9
Day 5 morning, evening and change from baseline heart (pulse) rate statistical comparisons.

Comparison	Day 5 Comparisons				Day 5 Change from Day −1 (baseline) Comparisons			
	First LSM [a] (bpm)	Second LSM (bpm)	Difference (bpm)	p-Value	First LSM (bpm)	Second LSM (bpm)	Difference (bpm)	p-Value
Morning Heart Rate								
A1 vs C	67.98	63.51	4.47	0.1240	−6.00	−6.57	0.57	0.8699
A2 vs C	67.99	63.51	4.48	0.1169	−3.00	−6.57	3.57	0.3074
A3 vs C	68.47	63.51	4.96	0.0828	−2.33	−6.57	4.24	0.2263
B1 vs C	73.60	63.51	10.09	***0.0007***[b]	−0.53	−6.57	6.04	0.0860
B2 vs C	66.35	63.51	2.84	0.3202	−2.13	−6.57	4.44	0.2053
B3 vs C	69.14	63.51	5.63	0.0543	1.00	−6.57	7.57	***0.0350***[b]
Evening Heart Rate								
A1 vs C	76.25	71.61	4.64	0.1222	−6.27	−8.71	2.45	0.4309
A2 vs C	76.93	71.61	5.32	0.0719	−4.33	−8.71	4.38	0.1601
A3 vs C	77.68	71.61	6.07	0.0409	−3.80	−8.71	4.91	0.1155
B1 vs C	82.87	71.61	11.26	***0.0003***[b]	0.40	−8.71	9.11	***0.0040***[b]
B2 vs C	81.17	71.61	9.56	***0.0015***[b]	1.53	−8.71	10.25	***0.0013***[b]
B3 vs C	83.55	71.61	11.94	***0.0001***[b]	3.93	−8.71	12.64	***0.0001***[b]
Day 5 Evening vs Morning Heart Rate								
A1 vs C	–	–	–	–	9.35	7.21	2.14	0.4573
A2 vs C	–	–	–	–	9.30	7.21	2.09	0.4698
A3 vs C	–	–	–	–	10.06	7.21	2.85	0.3253
B1 vs C	–	–	–	–	10.22	7.21	3.01	0.2978
B2 vs C	–	–	–	–	13.51	7.21	6.30	***0.0307***[b]
B3 vs C	–	–	–	–	13.24	7.21	6.03	***0.0418***[b]

Group A1: Exclusive tobacco flavor rechargeable e-cigarette.
Group A2: Exclusive cherry flavor rechargeable e-cigarette.
Group A3: Exclusive cherry flavor disposable e-cigarette.
Group B1: Dual tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B2: Dual cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B3: Dual cherry flavor disposable e-cigarette and usual brand combustible cigarette.
Group C: Nicotine cessation.
[a] LSM = least square means.
[b] Statistically significant.

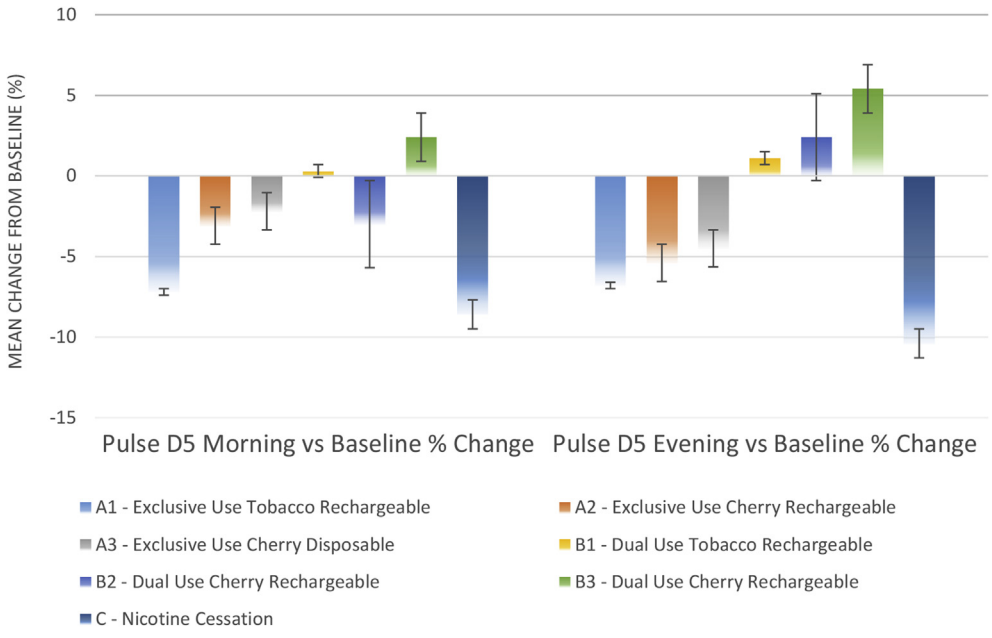

Fig. 3. Summary of pulse (heart) rate changes from baseline by use group from baseline to day 5.

cardiovascular health (Farsalinos et al., 2014a). Our results are also similar to those obtained in studies evaluating nicotine replacement therapies (NRTs), which showed no increases in blood pressure when comparing nicotine nasal sprays or transdermal nicotine with placebo conditions (Benowitz et al., 2002).

It is also interesting to note that the results obtained in this study are contrary to those obtained in a previous clinical study (D'Ruiz et al., 2015), conducted on similarly formulated e-cigarette products, which reported increases in SBP, DBP and heart rate following acute and exaggerated clinical use conditions (see Table 10). That study differed from the current study in that it was designed to characterize e-cigarette users' exposure to nicotine, and also measured the acute cardiovascular effects e-cigarettes in comparison with conventional tobacco cigarettes following an

Table 10
SBP, DBP and HR results from previous E-cigarette study (Yan and D'Ruiz, 2015).

Group	Systolic Blood Pressure (mmHG)				Diastolic Blood Pressure (mmHG)				Heart Rate (bpm)			
	Before use	After use	Change	*p* Value	Before use	After use	Change	p Value	Before use	After use	Change	*p* Value
A	119 ± 13.13	120 ± 11.83	1.13 ± 11.13	0.63	71 ± 9.51	78 ± 10.10	6.83 ± 6.69	6.77E-05	72 ± 8.55	75 ± 9.03	2.30 ± 9.03	0.057
B	120 ± 12.77	123 ± 11.92	2.83 ± 11.32	0.24	70 ± 10.25	77 ± 9.80	6.78 ± 6.46	4.83E-05	71 ± 8.95	75 ± 8.63	3.61 ± 5.97	0.008
C	119 ± 12.89	123 ± 13.45	3.96 ± 9.97	0.07	73 ± 8.61	76 ± 11.11	3.17 ± 7.28	0.048	70 ± 7.07	74 ± 7.14	4.09 ± 5.70	0.002
D	118 ± 10.27	124 ± 12.46	5.83 ± 10.03	0.02	70 ± 8.94	77 ± 8.45	6.78 ± 3.80	1.90E-08	72 ± 9.38	74 ± 8.68	1.87 ± 7.38	0.24
E	118 ± 11.29	122 ± 11.09	3.78 ± 10.70	0.1	72 ± 7.21	76 ± 9.33	4.39 ± 4.65	0.00017	71 ± 7.82	73 ± 7.53	2.22 ± 5.85	0.08
F	120 ± 12.56	126 ± 12.96	5.74 ± 12.37	0.04	71 ± 9.50	78 ± 9.53	6.78 ± 7.08	0.00014	70 ± 5.92	74 ± 8.64	4.26 ± 5.37	0.001

User Groups:
Product A: Tobacco flavored e-cigarette (2.4% nicotine, ~75% glycerin)/(N = 23).
Product B: Tobacco flavored e-cigarette (2.4% nicotine, ~50% glycerin ~20% propylene glycol)/(N = 23).
Product C: Menthol flavored e-cigarette (2.4% nicotine, ~75% glycerin)/(N = 23).
Product D: Tobacco flavored e-cigarette (1.6% nicotine, ~75% glycerin)/(N = 23).
Product E: Tobacco flavored e-cigarette (1.6% nicotine, ~50% glycerin/~20% propylene glycol)/(N = 23).
Product F: Tobacco Cigarette/(N = 24).
Source: X.S. Yan, C. D'Ruiz/Regulatory Toxicology and Pharmacology 71 (2015) 24–34.

Table 11
Measured FVC Summary and Day 5 vs Day −1 Statistical Comparisons.

Day	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
−1	4.5 ± 1.1	4.4 ± 1.1	4.6 ± 0.9	4.5 ± 0.8	5.0 ± 1.0	4.4 ± 1.1	4.7 ± 0.8
5	4.6 ± 1.1	4.5 ± 1.1	4.4 ± 0.9	4.6 ± 0.9	5.1 ± 1.0	4.4 ± 1.1	4.8 ± 0.8
Day 5 Change from Day -1 (baseline)							
N	15	15	14	15	15	14	14
Absolute Change	0.1 ± 0.1	0.1 ± 0.2	−0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.3	−0.0 ± 0.1	0.0 ± 0.1
p-Value	***0.0207***[a]	***0.0113***[a]	0.4017	0.0615	0.1288	0.2440	0.4266
% Change	1.9 ± 2.3	3.1 ± 4.1	−0.9 ± 5.4	2.6 ± 4.7	3.0 ± 7.9	−0.8 ± 2.8	0.5 ± 2.3

Day −1 and 5 and absolute change values are presented as arithmetic mean ± SD in L.
% change presented as arithmetic mean ± SD.
[a] Statistically significant.

Table 12
Measured FVC statistical comparisons.

Comparison	Day 5 Comparisons				Day 5 Change from Day −1 Comparisons			
	First LSM [a] (L)	Second LSM (L)	Difference (L)	p-Value	First LSM (L)	Second LSM (L)	Difference (L)	p-Value
A1 vs C	4.68	4.62	0.06	0.4602	0.09	0.03	0.06	0.4362
A2 vs C	4.72	4.62	0.10	0.2074	0.13	0.03	0.10	0.1810
A3 vs C	4.52	4.62	−0.10	0.2206	−0.07	0.03	−0.10	0.2293
B1 vs C	4.70	4.62	0.08	0.2986	0.11	0.03	0.09	0.2745
B2 vs C	4.73	4.62	0.11	0.1800	0.13	0.03	0.10	0.1865
B3 vs C	4.56	4.62	−0.06	0.4315	−0.03	0.03	−0.06	0.4609

Group A1: Exclusive tobacco flavor rechargeable e-cigarette.
Group A2: Exclusive cherry flavor rechargeable e-cigarette.
Group A3: Exclusive cherry flavor disposable e-cigarette.
Group B1: Dual tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B2: Dual cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B3: Dual cherry flavor disposable e-cigarette and usual brand combustible cigarette.
Group C: Nicotine cessation.
[a] LSM = least square means.

intensive 30-min use period (one puff every 30 s), which was then followed by a 1-h natural use period. Although that study showed no statistically significant differences in HR increases amongst the products, the data trend implied a good correlation between the nicotine plasma level and increased HR ($p < 0.05$). In comparing the results obtained from both studies, it appears as though the longer-term use of e-cigarette products results in a more favorable cardiovascular profile than that of very short, acute, and exaggerated and somewhat less realistic use profile. This possibly underscores the importance of users becoming familiar with how to use the devices prior to the start of the study.

Furthermore, previous studies have reported that cigarette smoking causes an acute elevation in carboxyhemoglobin levels (COHb) and that COHb is an important risk factor for cardiovascular dysfunction (Yan and D'Ruiz, 2015; Flouris et al., 2013). All product use groups in this study experienced significant reductions in blood levels of COHb. The greatest reductions were observed in the exclusive e-cigarette use (~79%–~83%) and in the nicotine cessation (~75%) groups, with the dual use also experiencing a lesser decrease of ~9–23%. As expected, given that e-cigarettes lack the combustion by-products of convention tobacco cigarette products, our findings are consistent with those of earlier clinical studies which have reported reductions or no changes in blood COHb levels following short-term e-cigarette use (Van Staden et al., 2013; Farsalinos et al.,

Table 13
Measured FEV1 Summary and Day 5 vs Day −1 Statistical Comparisons.

Day	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
−1	3.4 ± 0.8	3.3 ± 1.0	3.4 ± 0.7	3.2 ± 0.5	3.8 ± 0.8	3.3 ± 0.9	3.5 ± 0.7
5	3.6 ± 0.9	3.4 ± 1.0	3.4 ± 0.8	3.3 ± 0.7	3.9 ± 0.8	3.2 ± 0.9	3.7 ± 0.7
Day 5 Change from Day -1							
N	15	15	14	15	15	14	14
Absolute Change	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.1	−0.0 ± 0.1	0.1 ± 0.2
p-Value	***0.0148***[a]	***0.0276***[a]	0.0986	0.1040	***0.0191***[a]	0.1735	0.2143
% Change	6.0 ± 8.6	2.8 ± 4.6	3.2 ± 6.8	4.6 ± 9.6	2.7 ± 4.2	−1.5 ± 3.5	1.6 ± 5.0

Day −1 and 5 and absolute change values are presented as arithmetic mean ± SD in L.
% change presented as arithmetic mean ± SD.
[a] Statistically significant.

Table 14
Measured FEV1 statistical comparisons.

Comparison	Day 5 Comparisons				Day 5 Change from Day −1 Comparisons			
	First LSM [a] (L)	Second LSM (L)	Difference (L)	p-Value	First LSM (L)	Second LSM (L)	Difference (L)	p-Value
A1 vs C	3.65	3.50	0.14	0.0789	0.20	0.06	0.14	0.0844
A2 vs C	3.52	3.50	0.02	0.7871	0.08	0.06	0.02	0.8255
A3 vs C	3.55	3.50	0.04	0.6012	0.10	0.06	0.04	0.6247
B1 vs C	3.59	3.50	0.09	0.2732	0.15	0.06	0.08	0.3026
B2 vs C	3.53	3.50	0.03	0.6896	0.10	0.06	0.04	0.6568
B3 vs C	3.40	3.50	−0.10	0.2098	−0.05	0.06	−0.11	0.1831

Group A1: Exclusive tobacco flavor rechargeable e-cigarette.
Group A2: Exclusive cherry flavor rechargeable e-cigarette.
Group A3: Exclusive cherry flavor disposable e-cigarette.
Group B1: Dual tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B2: Dual cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
Group B3: Dual cherry flavor disposable e-cigarette and usual brand combustible cigarette.
Group C: Nicotine cessation.
[a] LSM = least square means.

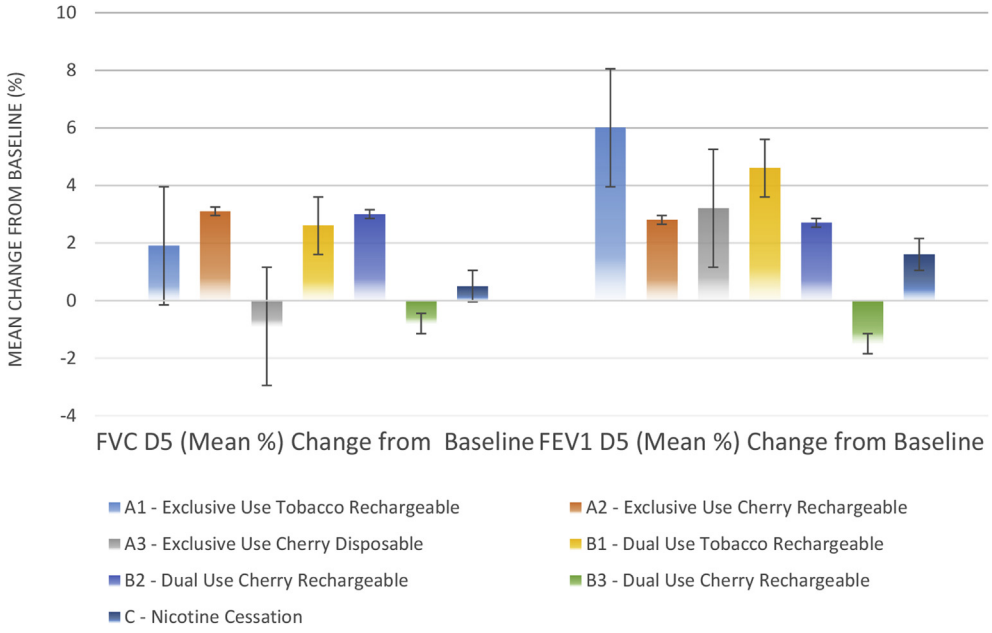

Fig. 4. Summary of FVC and FEV1 changes from baseline by use group from baseline to day 5.

2013). Altogether, these findings suggest the potential that e-cigarettes have in reducing exposure to HPHCs, which are reported to be significant contributors to smoking-associated cardiovascular disease risks.

4.2. Impacts of observed spirometry (FEV1 and FVC) findings on lung function

Smoking has been associated with diseases such as emphysema, which forms part of COPD. It is established that COPD is a

Table 15
Exhaled CO and NO Summary and Day 5 vs Day −1 Statistical Comparisons.

	Exclusive E-Cigarette Use Groups			Dual Use Groups			Nicotine Cessation
	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	Tobacco Rechargeable	Cherry Rechargeable	Cherry Disposable	
Day							
−1	27.2 ± 10.5	27.3 ± 6.9	26.9 ± 6.4	25.1 ± 7.3	25.4 ± 7.7	24.7 ± 5.5	29.3 ± 10.4
1	6.3 ± 2.0	7.9 ± 3.0	7.5 ± 1.8	18.1 ± 5.2	20.3 ± 6.7	18.5 ± 5.8	9.9 ± 3.1
3	2.7 ± 0.8	3.0 ± 0.9	2.7 ± 1.2	17.2 ± 4.9	17.3 ± 5.1	17.6 ± 5.3	3.1 ± 0.9
5	2.9 ± 0.8	2.9 ± 0.8	2.7 ± 0.9	17.3 ± 5.7	16.1 ± 3.3	18.2 ± 5.7	2.8 ± 0.7
Exhaled CO Day 5 Change from Day -1							
N	15	15	15	15	15	14	14
Absolute Change	−24.3 ± 10.4	−24.4 ± 6.9	−24.3 ± 6.4	−7.8 ± 8.0	−9.3 ± 6.7	−6.6 ± 4.7	−26.6 ± 10.8
p-Value	***<0.0001***[a]	***<0.0001***[a]	***<0.0001***[a]	***0.00 21***[a]	***<0.0001***[a]	**0.0002**[a]	***<0.0001***[a]
% Change	−88.2 ± 5.3	−88.9 ± 4.3	−89.4 ± 4.6	−26.5 ± 27.1	−31.5 ± 22.4	−26.4 ± 16.4	−89.4 ± 4.1
Exhaled NO Summary and Day 5 vs Day -1							
Day							
−1	14.8 ± 12.8	11.5 ± 4.8	10.0 ± 4.0	14.9 ± 11.1	10.6 ± 4.6	14.3 ± 13.5	11.3 ± 4.0
1	17.4 ± 15.4	10.1 ± 5.3	10.3 ± 4.0	14.1 ± 9.6	8.9 ± 2.9	13.2 ± 12.7	11.0 ± 5.5
3	24.9 ± 23.2	15.2 ± 7.5	14.9 ± 6.0	14.1 ± 6.9	11.3 ± 5.0	12.6 ± 10.3	19.1 ± 8.9
5	23.3 ± 21.6	15.5 ± 9.0	14.3 ± 6.5	12.9 ± 6.3	10.7 ± 4.4	11.4 ± 6.0	16.8 ± 10.1
Exhaled NO Day 5 Change from Day -1							
N	15	15	15	15	15	14	14
Absolute Change	8.5 ± 10.6	4.1 ± 9.9	4.3 ± 5.0	−1.9 ± 7.1	0.1 ± 3.5	−3.1 ± 11.6	5.8 ± 9.0
p-Value	***0.0075***[a]	0.1325	***0.0053***[a]	0.3118	0.9415	0.3287	**0.0321 a**
% Change	63.4 ± 63.3	51.8 ± 108.1	45.8 ± 42.6	3.5 ± 44.5	8.4 ± 37.7	−0.7 ± 40.4	55.7 ± 79.4

Day −1 and 5 and absolute change values are presented as arithmetic mean ± SD in ppm.
% change presented as arithmetic mean ± SD.
[a] Statistically Significant.

progressive disease that gets worse over time and that patients with COPD lose lung function at a faster rate than subjects without COPD (Gross, 2005). The post-bronchodilator forced expiratory lung volume test (FEV1), which measures the volume of air that a person can force out of their lungs in 1 s, is currently one of the most widely used markers to determine the presence, severity and progression of COPD (Eberly et al., 2003; Glaab et al., 2010). The natural history of COPD is usually described with a focus on changes in the forced expiratory volume in 1 s (FEV1) over time as this allows for exploration of risk factors for an accelerated decline- and thus of developing COPD (Vestbo and Lange, 2016). Smoking cessation is viewed by many public health experts as a critical component for the prevention of COPD progression. It has been reported that FEV1 decline decreases after smoking cessation. (Vestbo and Lange, 2016).

Similarly, FVC is the maximum volume of air that can be expelled in one breath and is a determinant of the maximum volume of air that a person's lung can hold. As a respiratory function test, FVC may indicate deterioration of respiratory function prior to clinical symptoms, and can be used to diagnose the presence and severity of respiratory diseases (Tantisuwat and Thaveeratitham, 2014).

Use of the e-cigarettes for five days under the various study conditions did not lead to negative respiratory health outcomes. The pulmonary function test results associated with the current study showed small, but non-statistically significant improvements in FVC and FEV1 measurements in most user groups. These spirometry findings are consistent with the results of other e-cigarette studies which have demonstrated a lack of significant effect on airflow obstruction or lung function, as measured by FEV1 or FVC, following short-term e-cigarette use (Flouris et al., 2013; Callahan-Lyon, 2014). Moreover, in the previously discussed longer-term studies, significant positive changes have also been observed in forced expiratory flow after 1-year in smokers that either quit or reduced their tobacco cigarette use by switching to e-cigarettes (Cibella et al., 2016; Polosa, 2015).

4.3. Impacts of observed exhaled CO and NO findings

Prior studies have indicated that CO may contribute to cardiovascular disease (Zevin et al., 2001; Papathanasiou et al., 2014) with CO and NO serving as biomarkers of airway diseases (Taylor et al., 2006). Smokers characteristically exhale higher CO (Deveci et al., 2004) and lower NO (Kharitonov et al., 1995; Malinovschi et al., 2006) than non-smokers. Some researchers have reported that increased CO levels are correlated to lower FEV1% predicted scores and to accelerated decline in lung function (Fabricius et al., 2007).

The study findings associated with exhaled breath biomarkers in the cessation and exclusive use groups were consistent with other research findings associated with reductions in exhaled CO and increases in NO following smoking cessation (Jarvis, 1980; Ripoll et al., 2012; West et al., 2005; Hogman et al., 2002; Robbins et al., 1997; Malinovschi et al., 2006; Chambers et al., 1998) and switching to e-cigarettes (Vansickel and Eissenberg, 2013; Goniewicz et al., 2016; Farsalinos and Polosa, 2014); both of which may be indicative of immediate and future physiological benefits.

4.4. Impacts of tolerability and AE findings

Overall, the e-cigarettes used in this study were generally well tolerated under exclusive and dual use conditions. The most frequently reported AEs were headache, cough and dry throat. These findings are consistent with other studies and surveys which report similar AEs for e-cigarettes, indicating a lack of serious AEs associated with e-cigarette product use (Farsalinos and Polosa, 2014; McRobbie et al., 2014; Callahan-Lyon, 2014). Importantly, the self-limiting effects are also comparable to FDA-approved oral NRT drug products (Callahan-Lyon, 2016; Farsalinos and Polosa, 2014; Walele et al., 2016a,b).

5. Conclusions

The results of this study demonstrate that reducing conventional cigarette smoking led to small, but not always statistically

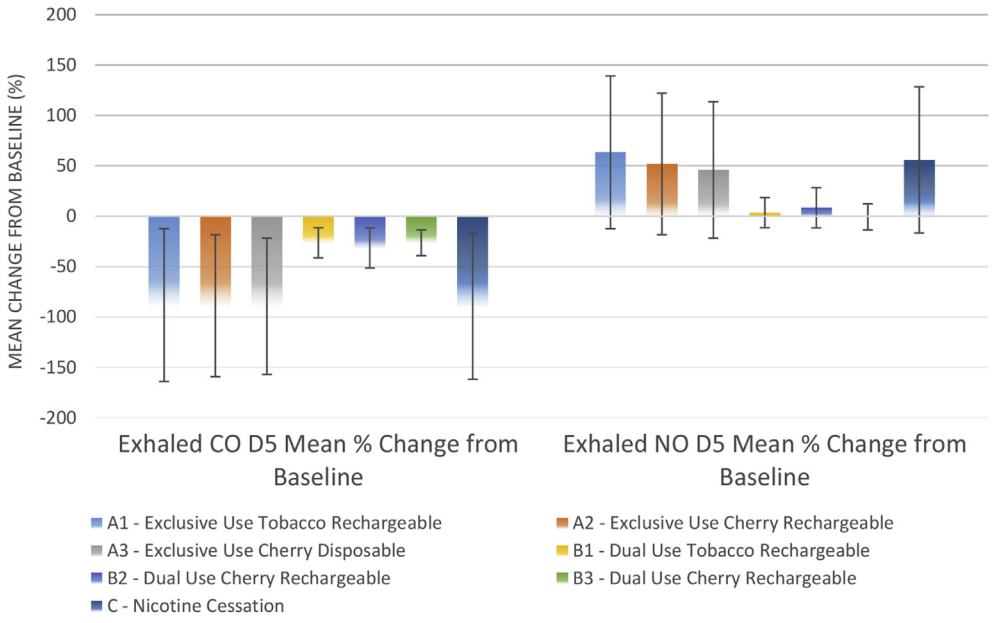

Fig. 5. Summary of exhaled CO and NO changes from baseline by use group from baseline to day 5.

Table 16
Summary of product use-emergent adverse events.

Group	Number (%) Subjects Reporting AEs	Number of AEs Reported
A1 (N = 15)	7 (47%)	15
A2 (N = 15)	5 (33%)	13
A3 (N = 15)	4 (27%)	9
B1 (N = 15)	6 (40%)	10
B2 (N = 15)	2 (13%)	4
B3 (N = 15)	7 (47%)	19
C (N = 15)	1 (7%)	2
Total (N = 105)	32 (30%)	72

A1 = Exclusive tobacco flavor rechargeable e-cigarette.
A2 = Exclusive cherry flavor rechargeable e-cigarette.
A3 = Exclusive cherry flavor disposable e-cigarette.
B1 = Dual tobacco flavor rechargeable e-cigarette and usual brand combustible cigarette.
B2 = Dual cherry flavor rechargeable e-cigarette and usual brand combustible cigarette.
B3 = Dual cherry flavor disposable e-cigarette and usual brand combustible cigarette.
C = Nicotine cessation.

significant improvements in cardiovascular and pulmonary function in individuals who exclusively used electronic cigarettes or ceased using tobacco and nicotine products over a period of five days.

Measurements of key physiological parameters associated with cardiovascular physiology (systolic and diastolic blood pressure and heart rate), pulmonary function (FVC, FEV1, and exhaled CO and NO) and adverse events in adult smokers that quit smoking or reduced the number cigarettes smoked by switching to e-cigarettes over a period of five days did not lead to higher blood pressure or heart rate values, negative respiratory health outcomes or serious adverse health events.

The findings of this study are consistent with, and further augment, the existing evidence associated with the beneficial effects of switching from smoking to e-cigarettes that have been reported in prior studies evaluating the short and long-term effects of e-cigarette use on the cardiovascular and pulmonary function endpoints. Furthermore, our study also confirms the finding of other clinical studies which have observed that the reductions in HPHCs such as COHb and exhaled CO in smokers who quit smoking and switch to e-cigarettes have positive effects on cardiovascular and respiratory function.

Finally, the results of this study provide additional data to address a deficit in scientific knowledge related to the physiological impacts associated with switching from conventional tobacco smoking to the exclusive use of e-cigarettes or the dual-use of e-cigarettes and conventional tobacco cigarettes in adult smokers. In general, our findings suggest that the short-term use of e-cigarettes does not result in any serious adverse effects and that there are potential cardiovascular and pulmonary function benefits associated with switching from conventional cigarettes to e-cigarette products. This may be due to a reduction in exposure to HPHCs, which are believed to be contributors to smoking-related disease risks.

The main limitation of this study is that it was only a short-term (5-day) trial looking at a few, select, cardiovascular and pulmonary parameters associated with a single product type (i.e., closed system e-cigarettes). Moreover, the relatively short-term duration of the study may have been the reason that some of the observed differences between groups were not found to be statistically significant (e.g., differences in morning and evening SBP and DBP values from the Day 5 morning to evening). Longer-term studies may be more appropriate for measuring the outcomes associated with e-cigarette product use and these physiological parameters. Nevertheless, the study contributes to a growing body of scientific research in this field. Longer-term studies assessing biomarkers of effect linked to inflammatory and oxidative stress endpoints may be more informative for assessing potential long-term effects of e-cigarettes. They may also provide physiological relevance of reduced exposure to HPHCs comparing exclusive e-cigarette users with dual users. Information from longer-term e-cigarette product tolerability and adverse event surveillance studies may also be informative. Work in these areas is planned.

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Abbreviations

AE	adverse event
BoE	biomarkers of exposure
BP	blood pressure
CC	conventional cigarettes
COHb	carboxyhemoglobin
COPD	chronic obstructive pulmonary disease
CPD	cigarettes per day
CVD	cardiovascular disease
DBP	diastolic blood pressure
FTND	Fagerström test for cigarette dependence
FVC	forced expiratory volume
FEF25-75%	forced expiratory flow from 25% to 75% of vital capacity
FEV1	forced expiratory volume in one second
HR	heart rate
HPHC	harmful or potentially harmful constituents
Mg	milligram
mm Hg	millimeters mercury
NNK	nicotine-derived nitrosamine ketone
NRT	nicotine replacement therapy
PG	propylene glycol
NO	nitric oxide
SBP	systolic blood pressure
WISDM	Wisconsin inventory of smoking dependence motives

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.05.002.

Transparency document

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