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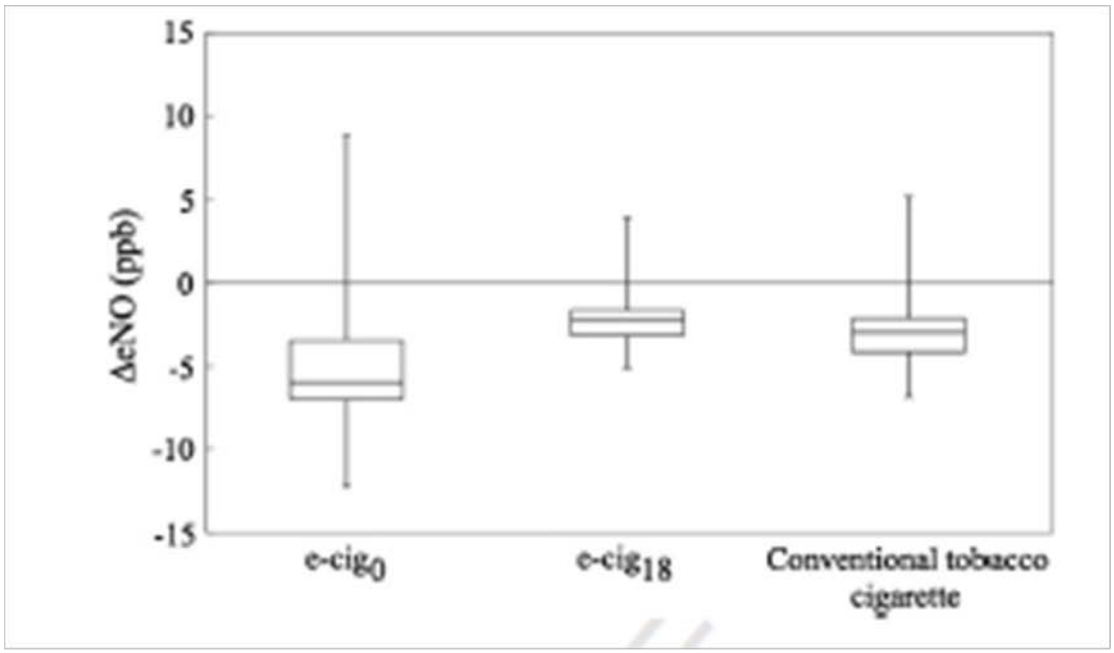
June 12, 2014

Docket No. FDA-2014-N-0189

Evidence that e-cigarette aerosol has the same effects on an important measure of lung function as cigarette smoke undermines the assumption that e-cigarettes are uniformly less risky than conventional cigarettes

The paper, "Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide," by Sara Marinia, et al, just published in *Toxicology and Applied Pharmacology* (Volume 278, Issue 1, 1 July 2014, Pages 9–15), reports important data showing that nicotine e-cigarettes, non-nicotine e-cigarettes, and conventional cigarettes all have similar effects of depression of exhaled nitric oxide (see figure from their paper).



Marini et al, http://dx.doi.org/10.1016/j.taap.2014.04.004

The authors compared three situations:

1. Smoking a conventional cigarette
2. Smoking and nicotine e-cigarette
3. Smoking a nicotine-free e-cigarette

4. Inhaling through an ecigarette without a liquid cartridge (i.e., breathing normal room air)

Conditions 1-3 all reduced exhaled nitric oxide by about the same amount. There was no effect of condition 4.

What this means is that the ultrafine particles in the e-cigarette aerosol (not the nicotine or something special about cigarette smoke aerosol) is what is causing the decrement in lung function reflected by lower exhaled nitric oxide.

This is an important finding because it shows, at least for this important biological measure of the effects of using e-cigarettes on lungs, they are no different than cigarettes and so, for this end point, do not pose less risk.

This finding, which, as the authors point out in their paper, is consistent with earlier studies, demonstrates that **the FDA must be extremely careful about assuming that e-cigarettes uniformly pose less risk than conventional cigarettes. The FDA should not make such an assumption until there is affirmative evidence to support such an assumption.**

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Toxicology and Applied Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide

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ARTICLE INFO

Article history:
Received 3 January 2014
Revised 28 March 2014
Accepted 3 April 2014
Available online xxxx

Keywords:
Exhaled nitric oxide
Electronic cigarette
Tobacco cigarette
Ultrafine particles
Alveolar and tracheobronchial particle dose
Short-term effect
Nicotine

ABSTRACT

The objective of this study was to compare the short-term respiratory effects due to the inhalation of electronic and conventional tobacco cigarette-generated mainstream aerosols through the measurement of the exhaled nitric oxide (eNO). To this purpose, twenty-five smokers were asked to smoke a conventional cigarette and to vape an electronic cigarette (with and without nicotine), and an electronic cigarette without liquid (control session). Electronic and tobacco cigarette mainstream aerosols were characterized in terms of total particle number concentrations and size distributions. On the basis of the measured total particle number concentrations and size distributions, the average particle doses deposited in alveolar and tracheobronchial regions of the lungs for a single 2-s puff were also estimated considering a subject performing resting (sitting) activity.

Total particle number concentrations in the mainstream resulted equal to $3.5 \pm 0.4 \times 10^9$, $5.1 \pm 0.1 \times 10^9$, and $3.1 \pm 0.6 \times 10^9$ part. cm^{-3} for electronic cigarettes without nicotine, with nicotine, and for conventional cigarettes, respectively. The corresponding alveolar doses for a resting subject were estimated equal to 3.8×10^{10}, 5.2×10^{10} and 2.3×10^{10} particles.

The mean eNO variations measured after each smoking/vaping session were equal to 3.2 ppb, 2.7 ppb and 2.8 ppb for electronic cigarettes without nicotine, with nicotine, and for conventional cigarettes, respectively; whereas, negligible eNO changes were measured in the control session. Statistical tests performed on eNO data showed statistically significant differences between smoking/vaping sessions and the control session, thus confirming a similar effect on human airways whatever the cigarette smoked/vaped, the nicotine content, and the particle dose received.

Introduction

The adverse effects of cigarettes on human health are widely recognized: scientific studies unequivocally documented the tobacco smoke as the leading global cause of premature death and serious diseases, mainly cancer (e.g., lung, oral cavity, esophagus, larynx, pancreas, bladder, kidney), cardiovascular and chronic obstructive pulmonary diseases (COPD), myocardial infarction, and stroke (Caponnetto et al., 2012; Crawford et al., 2012; Doll et al., 2004; Fiore et al., 2008; Moolgavkar et al., 2012; World Health Organization, 2008). The harmful potential of cigarette smoking is almost entirely due to toxins and carcinogens generated from the combustion processes involved in cigarette use (Baker, 2006; Geiss and Kotzias, 2007). A comprehensive examination of the scientific literature (Smith et al., 1997) revealed that nine of the 44 chemical agents classified as "Group 1 carcinogens" by the International Agency for Research on Cancer (IARC) have been reported to occur in mainstream cigarette smoke both as vapor and particulate phases (International Agency for Research on Cancer, 2013; Smith et al., 2003). Recently, electronic nicotine delivery systems (ENDS), also known as electronic cigarettes (e-cigarettes), experienced a rapid growth in popularity as a less harmful and toxic alternative than conventional tobacco cigarettes or as a temporary method to quit smoking (Bullen et al., 2010; Etter, 2010; Etter et al., 2011; Foulds et al., 2011; McQueen et al., 2011; Polosa et al., 2011; Siegel et al., 2011). Their increasing success is also due to the possibility to be used in smoke-free places, and to the perceived, but not scientifically proved, lower toxicity with respect to traditional tobacco cigarettes (Etter and Bullen, 2011).

Electronic cigarettes are cigarette-shaped battery-powered devices made up of an electric atomizer and a replaceable cartridge containing a water-based liquid ("e-liquid"). The main components of the e-liquids are propylene glycol, glycerin, water, flavors, and a variable amount of

Abbreviations: COPD, Chronic obstructive pulmonary diseases; CPC, Condensation Particle Counter; e-cigarette, electronic cigarette; e-liquid, electronic liquid; ENDS, electronic nicotine delivery systems; eNO, Exhaled nitric oxide; FMPS, Fast Mobility Particle Sizer spectrometer; IARC, International Agency for Research on Cancer; LAMI, Laboratory of Industrial Measurements; NaCl, Sodium chloride; NOS, Nitric oxide synthase; RDTD, Rotating Disk Thermodiluter; RNA, Ribonucleic acid; TLC, Total lung capacity; UFP, Ultrafine particle; VOC, Volatile organic compound; WHO, World Health Organization.

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http://dx.doi.org/10.1016/j.taap.2014.04.004
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nicotine typically ranging from 0 to 36 mg mL^{-1} (Flouris et al., 2013). In the atomizer the e-liquid is heated and vaporized and then inhaled by the user; in particular, the atomizer is automatically turned on through an airflow sensor when user ("vaper") inhales through the mouthpiece. Thus, in e-cigarettes the tobacco combustion phenomena is replaced by vaporization of such solution, then they are claimed to provide a lower risk for vapers (Caponnetto et al., 2013; Cobb et al., 2010). However, few scientific studies aimed to characterize e-cigarettes emissions and the related health effect were performed by the scientific community.

E-cigarette aerosol emission characterization

Schripp et al. (2013) examined the possible emission of volatile organic compounds (VOCs) as well as fine and ultrafine particles (UFPs, particle smaller than 100 nm in diameter) of e-cigarettes testing them in a chamber. An increase in both particle and VOC concentrations was detected during the tests. In particular, the e-cigarette-generated aerosols showed a bimodal size distribution peaking at 60 and 100 nm.

Zhang et al. (2013) investigated *in vitro* the particle size distributions of electronic and conventional cigarette aerosols. The particle number distribution measured for e-cigarette aerosols was found similar to that of conventional tobacco cigarettes in the range of 100–600 nm. Moreover, the authors applied a lung deposition model to estimate the deposition in the different respiratory tracts: they predicted 7%–18% alveolar delivery, 9%–19% venous delivery, mostly in the head, and 73%–80% losses by exhalation.

Fuoco et al. (2014) performed an experimental campaign to characterize e-cigarette-generated particles from a dimensional point of view. The effect of different operating parameter such as type of e-cigarette, flavor, nicotine content, and puffing time on particle number concentration and size distribution in the mainstream aerosol of e-cigarettes was also evaluated. They recognized e-liquid nicotine content and puffing time as the major influential parameters on particle emission.

Respiratory symptoms due to e-cigarettes

Preliminary results evaluating the acute effect of electronic cigarette vaping on pulmonary functions were obtained by Gennimata et al. (2012). They considered 24 smokers (11 with normal spirometry, and 13 with COPD and asthma) and 8 never-smokers founding that vaping an e-cigarette for 10 minutes causes a significant increase in airway resistance. Vakali et al. (2012) studied 37 subjects (15 smokers with normal spirometry, 13 smokers with chronic airway obstruction, and 9 never-smokers) during their normal vaping activity: they recognized that the participants reported cough (65%), sore throat (68%), irritation in eyes (24%), difference in taste (78%), dizziness (24%) and feeling of satisfaction (51%) after vaping a single e-cigarette for 10 minutes. In addition, a statistically significant increase in heart rate, expired CO, and decrease in SpO_2 were also noticed after vaping.

An interesting method to evaluate the possible airway inflammation is the non-invasive measure of the exhaled nitric oxide (eNO) which is an inflammation marker (Haussermann et al., 2013; Kharitonov and Barnes, 2006; Leung and Sin, 2013; Saito et al., 2004). As example, high eNO values were measured in people affected by several airway diseases such as asthma and house dust mite (Ashutosh, 2000; Bahna, 2012; Buonanno et al., 2013; Fuoco et al., 2014). On the contrary, low levels of eNO were detected in cystic fibrosis (Balfour-Lynn et al., 1996; Dotsch et al., 1996; Grasemann et al., 1997, 1998; Lundberg et al., 1996), HIV infection (Loveless et al., 1997), and pulmonary hypertension (Cremona et al., 1994; Ozkan et al., 2001; Riley et al., 1997).

Previous studies associated the conventional cigarette smoke exposure to chronically reduced levels of exhaled nitric oxide (Kharitonov et al., 1995; Malinovschi et al., 2006; Persson et al., 1994; Schilling et al., 1994; Su et al., 1998) even if not definitive explanation were provided to clearly support which is the mechanism regulating the eNO reduction due to cigarette smoking (Min and Min, 2014). A possible hypothesis for the reduction in eNO is that cigarette smoke negatively affects constitutive NO synthase (NOS) activity. As example, Su et al. (1998) observed that the exposure to cigarette smoke reduced the presence of endothelial NOS and endothelial NOS messenger Ribonucleic acid (RNA) in the pulmonary artery endothelial cells of pigs. eNO reduction due to nicotine use seems also to be associated to both an increased consumption of NO in the airways likely happening in the transformation of NO to peroxynitrite (Helen et al., 2000; Iho et al., 2003; Malinovschi et al., 2006) and an inactivation of NO by oxidants in cigarettes or toxin-induced damage to NO-producing epithelial cells (Persson et al., 1994; Rengasamy and Johns, 1993; Yates et al., 2001).

Vardavas et al. (2012) evaluated the impact of e-cigarettes on lung function measuring the fraction of exhaled nitric oxide in healthy adult smokers. They detected that vaping for 5 min was sufficient to increase the lung flow resistance as well as to decrease the eNO concentrations. Besides, they showed effects similar to those detected during tobacco smoking. Conversely, the authors did not recognize the same airway effects on the control subjects that used e-cigarettes without cartridges. A main limitation of the aforementioned studies is the lack of a direct comparison between electronic and standard tobacco cigarettes (Caponnetto et al., 2013). To this purpose, Flouris et al. (2013) compared the acute and short term effects of e-cigarettes with respect to the active and passive tobacco cigarette smoking on serum cotinine and lung function in 15 smokers and 15 never-smokers. Their results suggested that, when the same nicotine dosage was considered, e-cigarettes generated smaller changes in lung function compared to tobacco cigarettes. To properly perform the comparison, they used a survey method to calculate the number of puffs needed to deliver equivalent nicotine to each participant's preferred tobacco cigarette brand. The e-liquid used for this experiment had a nicotine concentration of 11 mg mL^{-1}, which can be considered an average nicotine concentration for e-cigarette.

Currently, to the authors' knowledge, no data for e-cigarette without nicotine were provided in order to test whether the changes in lung function are due to the presence of nicotine itself or other e-liquid components.

Aims of the work

The aim of the present study was to compare the short-term effects of electronic and tobacco cigarettes on the fraction of exhaled nitric oxide. To this purpose 25 volunteers were asked: a) to smoke conventional tobacco cigarettes, nicotine-free e-cigarettes, and e-cigarettes with nicotine, and b) to undergo eNO tests before and after smoking to evaluate possible eNO variations. The mainstream aerosol generated by electronic and conventional tobacco cigarettes was characterized in terms of particle number concentration and size distribution. Moreover, particle deposited doses for a single 2-s puff were evaluated for all the cigarettes under investigations, and then related to the eNO data.

Material and methods

Mainstream aerosol characterization: experimental apparatus

Cigarette-generated mainstream aerosol characterization was performed at the European Accredited Laboratory of Industrial Measurements (LAMI) of the University of Cassino and Southern Lazio, Italy, where thermo-hygrometric conditions were continuously monitored, in order to guarantee temperature and relative humidity values equal to 20 ± 1 °C and 50 ± 10%, respectively.

A rechargeable e-cigarette model made up of a tank system was used (major details are reported in Fuoco et al. (2014)). A tobacco flavor e-liquid was considered in the experimental campaign. The authors point out that the flavor was recognized as a negligible influential parameter in e-cigarette particle emission (Fuoco et al., 2014). Two

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different nicotine concentration levels were tested: zero (e-cig$_0$, 0 mg mL^{-1}) and high (e-cig$_{18}$, 18 mg mL^{-1}). As regard the conventional tobacco cigarettes, cigarettes with a nicotine concentration equal to 0.8 mg per cigarette were tested since subjects selected for the study usually smoke cigarettes with similar nicotine concentration.

Measurements of total particle number concentrations and size distributions were carried out for each cigarette under investigation through a Condensation Particle Counter (CPC 3775, TSI Inc.) and a Fast Mobility Particle Sizer spectrometer (FMPS 3091, TSI Inc.), respectively. The CPC 3775 measures the total particle number concentration down to 4 nm in diameter with a one-second-time resolution up to a maximum concentration of 10^7 part. cm^{-3}. It was calibrated before the experimental campaign at the LAMI through comparison with a TSI 3068B Aerosol Electrometer using NaCl particles generated using a Submicrometer Aerosol Generator (TSI 3940) (Stabile et al., 2013). The FMPS 3091 measures particle size distributions in the range 5.6-560 nm through the electrical mobility technique. Particle classification and counting are performed simultaneously by means of several aerosol electrometers able to count particles of different sizes with a 1-s time resolution (Johnson et al., 2004).

Due to the possible high particle number concentration expected in the electronic and conventional cigarette mainstream aerosol under investigation, the aerosol was diluted before entering the measuring section (Fig. 1). To this end, a thermodilution system (two-step dilution), made up of a Rotating Disk Thermodiluter, RDTD (model 379020, Matter Engineering AG; Hueglin et al. (1997)) and a thermal conditioner (model 379030, Matter Engineering AG; (Burtscher, 2005), was used. It is able to ensure a proper sample conditioning during the measurement of size distributions and total concentrations of particles emitted by the cigarettes as hereinafter described.

Mainstream aerosol characterization: aerosol sampling

Cigarette-generated aerosol measurements were performed considering 2-s puffs. In particular, three puff profiles made up of four consecutive puffs with a 30-s inter puff interval were performed for each test. A time-controlled switch valve was used to generate the puff profiles: in the closed position only room air was sampled; whereas during opening time the 2-s puff was performed. Batteries of the e-cigarettes were fully charged prior to performing experiments. The first puff was considered a "warm up" puff as it could lead to possible measurement errors during the e-cigarette tests, as also reported in Ingebrethsen et al. (2012). Puffs were performed connecting the aerosol sampling line to the cigarette mouthpiece itself, then only the mainstream aerosol was tested (Fig. 1). Before entering the measurement section (CPC or FMPS), the aerosol was flown to the thermodilution system in order to prevent measurement artifacts likely happening in the sampling process (Burtscher, 2005; Hueglin et al., 1997). In particular, the thermodilution system drew the mainstream aerosol from the cigarette's mouthpiece at a fixed flow rate of 1 L min^{-1}. Flow rates were checked through the Flow meter TSI 4410. The thermodilution system temperature was set at 37 °C in order to simulate the respiratory apparatus. After the thermodilution process, the aerosol was flown to the CPC (aerosol flow rate of 1.5 L min^{-1}) or the FMPS (aerosol flow rate of 10 L min^{-1}) depending on whether particle number concentrations or size distributions were measured. Thus, dilution ratios equal to 1:1000 and 1:880 were chosen for CPC and FMPS, respectively, to avoid over-range measurements.

Since the long path experienced by the aerosol before entering the CPC or FMPS, a diffusion loss correction was applied to estimate the particle losses onto the inner surface of the connecting tubing. In particular, the method proposed in Gormley and Kennedy (1949) was applied; further details about diffusion loss correction evaluation are reported by Buonanno et al. (2011). Despite the 5.6-560 nm FMPS measurement range, particle distribution were cut off down to 14 nm. In fact, Fuoco et al. (2014) and Ingebrethsen et al. (2012) demonstrated an artifact in measuring particle size distributions of mainstream e-cigarette aerosol in the lower diameter range (a 10-nm fake mode). Particle number concentration and size distribution data reported in the Results section represent the average of the peaks (maximum concentrations) measured for the three puffs (in fact, the first puff was excluded as discussed above) during the three puff profiles.

Data reported in the Results section are expressed as average ± standard deviation. Comparisons between the particle number concentration data measured in the mainstream aerosols of the cigarettes under investigation were performed using the paired Student's t test with an accepted level of significance of 99% ($p \leq 0.01$). All the tested data were previously checked for normality, in order to evaluate the pertinence of the Student's t test, through the Shapiro-Wilk test.

Deposited particle dose evaluation

Alveolar and tracheobronchial deposited particle dose due to electronic and conventional tobacco cigarette smoking were estimated multiplying the 2-s average total particle number concentration, measured through the CPC 3775 during the puffs, by the volume involved in a puff and by the deposition fractions characteristics of the alveolar and tracheobronchial regions of the human lungs. The volume puffed through

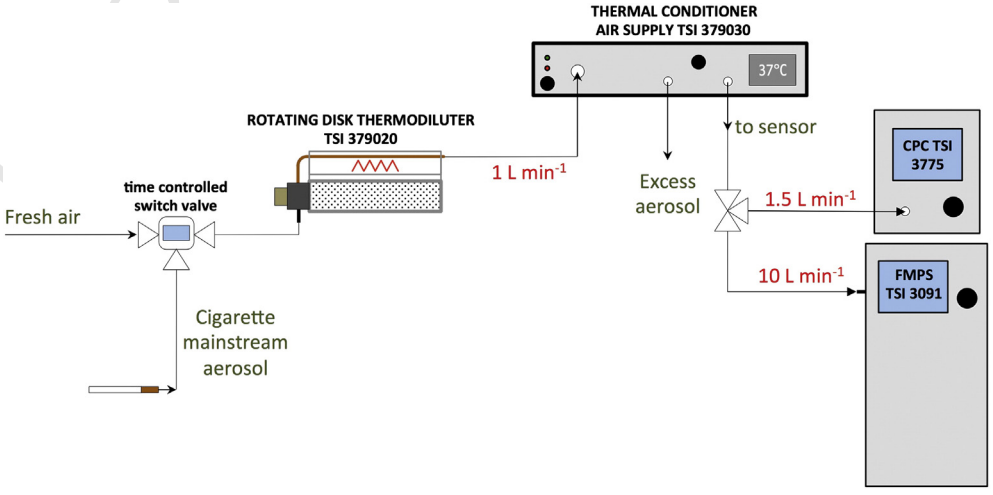

Fig. 1. Detailed scheme of the experimental apparatus used to characterize the cigarette-generated mainstream aerosols.

the cigarette was chosen on the basis of a review of the scientific literature. In particular, the surgeon general report on health consequences of smoking and nicotine addiction (US Department of Health and Human Services, 1988) documented puff volumes in the range 21–66 cm^3. The median puff volume value, 42.5 cm^3, was considered. Particle deposition fractions in alveolar and tracheobronchial regions were evaluated applying the ICRP dosimetry model (International Commission on Radiological Protection, 1994). The fractional deposition data for subjects in resting (sitting) activity were taken into account. Since the study population under investigation was made up of both men and women, the inter-gender average fractional deposition data were calculated. Further details on deposited particle dose evaluation were reported in Buonanno et al., 2011. Statistical comparisons between the particle dose data were performed using the abovementioned procedure used in particle number concentration data statistical analysis.

Exhaled nitric oxide measurement: study population and experiment design

In order to evaluate possible eNO responses due to the inhalation of cigarette-generated mainstream aerosols, 25 healthy (i.e. non atopic) smokers (14 men, 11 women), aged 19 to 49 (28 ± 9 years) were considered. Subjects under investigation usually smoke 12 ± 7 conventional tobacco cigarette per day with a pack-year index equal to 6; they smoke cigarettes of different brands with a typical nicotine concentration of 0.8 mg $cigarette^{-1}$. Respiratory status of the test-case population was considered. In particular, we excluded subjects a) reporting any past or current respiratory disease, b) experiencing upper respiratory tract infection within the past 6 weeks, c) taking any chronic medication or antibiotics. The research protocol was approved by the Administrative Board of the University of Cassino and Southern Lazio; moreover, all the subjects provided written informed consent to participate in the study.

Each participant attended four smoking/vaping sessions in the following order: i) a control session, ii) a tobacco cigarette smoking session, iii) an e-cigarette without nicotine (e-cig_0) vaping session, and iv) an e-cigarette with nicotine vaping session (e-cig_{18}, nicotine concentration of 18 mg mL^{-1}). In the control session smokers were asked to use the e-cigarette without the e-cigarette cartridge. In the tobacco cigarette smoking session, smokers were asked to smoke a tobacco cigarette that they usually smoke. In the e-cigarette vaping sessions, smokers were asked to vape the e-cigarettes for 5 min. As general indication, the participants were asked vaping as they usually would have smoked thus not providing constrain concerning puff and inter-puff lengths. Smoking/vaping sessions were performed in a 150 m^3 room of the LAMI. After each test the room air was refreshed through the mechanical ventilation system able to provide an air exchange rate of 0.3 h^{-1}. The four sessions were separated at least by a 1-day wash out period. Smoking/vaping sessions were performed individually. Participants were asked to abstain from smoking, eating, drinking any kind of beverages, as well as extreme physical activity for at least 1 hour before the test. Exhaled NO tests were performed during the morning roughly 9.00 a.m.-12.00 p.m.

Inter-groups comparison between each smoking/vaping session were performed using the paired Student's t test with an accepted level of significance of 99% ($p \leq 0.01$). All the tested data were previously checked for normality in order to realize the pertinence of the Student's t test: the Shapiro-Wilk test was applied.

Exhaled nitric oxide measurement: method and apparatus

Exhaled NO was measured before and immediately after each smoking session using an electrochemical analyzer (NObreath®, Bedfont Scientific Ltd., Rochester, Kent, UK) that measures eNO in the range 1–300 ppb. According to the American Thoracic Society guidelines (ATS/ERS, 2005) participants were instructed to inhale ambient air (in a sitting position) over 2–3 seconds near their total lung capacity (TLC) and then exhale for 16 s through a mouthpiece into the instrument at a constant flow rate of 50 mL s^{-1}. Subjects were aided to keep a constant exhalation flow rate by means of an eye level flow indicator. Three eNO measurements were performed for each test: mean values were used in the calculations.

Results

Mainstream aerosol characterization and particle doses

Average particle number concentration peaks (for 2-s puff) in the mainstream aerosol of the cigarettes under investigation are reported in Table 1. Concentration peaks equal to $3.5 \pm 0.4 \times 10^9$, $5.1 \pm 0.1 \times 10^9$, and $3.1 \pm 0.6 \times 10^9$ were measured for nicotine-free e-cig, nicotine-containing e-cig, and conventional tobacco cigarette, respectively. Student's t test results showed statistically significant differences between the aerosol concentrations produced by the nicotine-containing electronic cigarette (e-cig_{18}) and the concentrations emitted from the electronic cigarette without nicotine (e-cig_0) and conventional cigarette. In particular, for the e-cig_{18} was found a concentration about 1.5 times greater than the other ones.

In Fig. 2 average particle number distributions (for 2-s puff) corresponding to the concentration peaks measured in the mainstream aerosols, produced by electronic and conventional cigarettes, are reported. Particle number distribution data were normalized to the total particle number concentrations measured by the CPC 3775. Distributions were unimodal, in particular, average modes equal to 107 nm, 143 nm and 165 nm were measured for e-cig_0, e-cig_{18} and conventional tobacco cigarettes, respectively.

On the basis of the measured total particle number concentrations, the average alveolar/tracheobronchial deposited particle doses for a puff volume of 42.5 cm^3 and a puff length of 2 s were estimated, for a typical resting subject, equal to $3.8 \pm 0.3 \times 10^{10}/1.4 \pm 0.1 \times 10^{10}$, $5.2 \pm 0.4 \times 10^{10}/1.9 \pm 0.2 \times 10^{10}$ and $2.3 \pm 0.2 \times 10^{10}/7.5 \pm 0.1 \times 10^9$ particles for e-cig_0, e-cig_{18}, and conventional tobacco cigarette, respectively. Therefore, vaping nicotine-containing e-cig causes statistically higher doses deposited in subjects' lungs.

Exhaled nitric oxide

The baseline eNO levels of the subjects considered in the study and their pulmonary changes related to the use of the three test cigarettes are reported in Table 2. After smoking/vaping sessions, smokers/vapers reported a decrease in eNO concentrations with respect to their baseline values. In particular, the average decreases in eNO concentrations were 3.2 ± 8.4 ppb, 2.2 ± 5.8 ppb, and 2.8 ± 5.3 ppb for e-cig_0, e-cig_{18}, and conventional tobacco cigarette smoking/vaping session, respectively. On the contrary, in the control session negligible changes in eNO concentrations were detected (−0.1 ppb). In Fig. 3 the statistics of eNO variations (minimum, first quartile, median, third quartile, and maximum value), evaluated as the difference between the eNO values measured after and before vaping/smoking, are also reported for each smoking session (e-cig_0, e-cig_{18} and conventional tobacco cigarettes) including all the participants.

Table 1
Average total particle number concentration peak (for 2-s puff) measured in the mainstream aerosol from e-cigarette and conventional tobacco cigarette tested.

Type of cigarette	Average total particle number concentration (part. cm^{-3})
e-cig_0	$3.5 \pm 0.4 \times 10^9$
e-cig_{18}	$5.1 \pm 0.1 \times 10^9$
Conventional cigarette	$3.1 \pm 0.6 \times 10^9$

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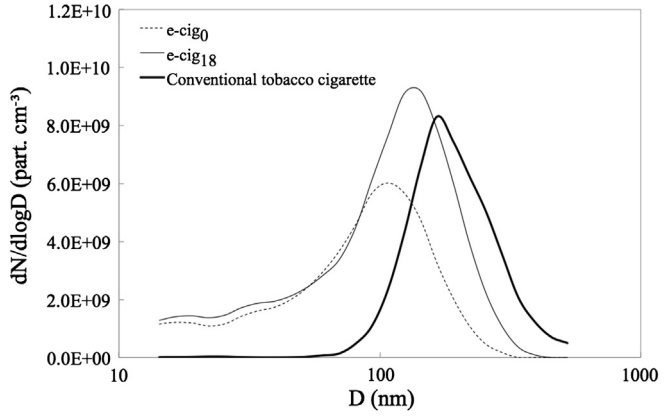

Fig. 2. Particle number distributions, averaged across the concentration peaks, of cigarette-generated mainstream aerosols measured through the FMPS 3091. Distributions concerning both electronic and conventional tobacco cigarettes are shown. Distributions were normalized to corresponding total particle number concentrations measured by the CPC 3775.

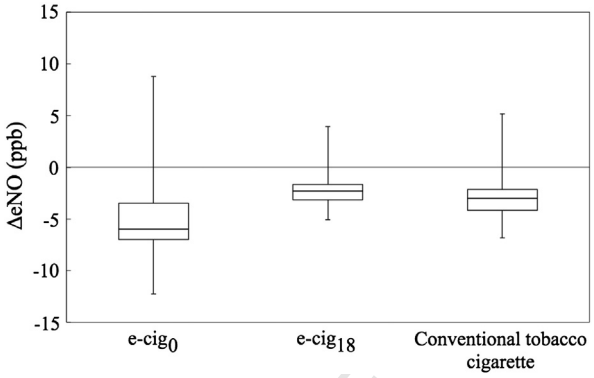

Fig. 3. Statistics of the eNO variations due to the three vaping/smoking sessions under investigation for all the participants. Box-plots report median, 1st (Q1) and 3rd (Q3) quartile, minimum and maximum values. Upper (U) and lower (L) whiskers were evaluated as $U = Q_3 + 1.5\times(Q_3\text{-}Q_1)$ and $L = Q_1\text{-}1.5\times(Q_3\text{-}Q_1)$, respectively. Measurement data higher than the "upper whisker" or lower than the "lower whisker" were considered outliers, they are not showed here.

Student's t-test, carried out in order to perform inter-groups comparison between each smoking/vaping sessions, demonstrated that eNO variations measured during the three smoking/vaping sessions were statistically different ($p < 0.01$) with respect to the control session: p values were measured equal to 0.007, 0.007, and 0.002, for e-cig$_0$, e-cig$_{18}$ and conventional tobacco cigarette smoking/vaping sessions, respectively, when compared to the control session (Table 2). On the contrary, the three smoking/vaping sessions caused eNO variations not statistically different among themselves.

Discussion

The findings of the present paper show that, from a dimensional point of view, the mainstream aerosols generated by electronic cigarettes are similar to those typical of conventional cigarettes since analogous particle number distributions were measured. Anyway, measurements of particle number concentrations showed that the amount of particles emitted strongly varies as function of the nicotine content. Nicotine-free electronic cigarettes were recognized producing a lower number of particles with respect to nicotine-containing e-cigarettes and similar to the conventional tobacco cigarette. Therefore, deposited particle dose evaluation allowed to detect that a statistically significant higher particle dose is deposited in subjects' respiratory regions vaping through a 2-s puff nicotine-containing e-cig (5.2×10^{10} particles in the alveolar region) with respect to nicotine-free e-cig (3.8×10^{10} particles in the alveolar region) and conventional tobacco cigarettes (2.3×10^{10} particles in the alveolar region). The authors emphasize that the average total particle dose due to a single 2-s puff from a nicotine-containing e-cigarette correspond to 24% of the typical alveolar daily dose of a not smoking Italian adult (2.2×10^{11} particles) and it's higher than the one received by an Australian adult (3.3×10^{10} particles), as estimated by Buonanno et al. (2011, 2012) in their studies where activity pattern data were combined to aerosol size distribution and total concentration data in all the resided microenvironments.

As regards the short-term respiratory effects, the authors observed that using electronic cigarettes, both with and without nicotine, leads to an immediate reduction of exhaled nitric oxide in smokers. These effects were similar to those caused by traditional tobacco cigarettes (Malinovschi et al., 2006; Min and Min, 2014). Analogous results were detected by Vardavas et al. (2012) in their investigation on the respiratory effects of the electronic cigarette on 30 healthy smokers. In particular, they observed an eNO decrease of 2.14 ppb ($p = 0.005$) within the vaper population under investigation, whereas no variations were measured in the control group who used e-cigarettes without e-liquid. However, they did not consider the e-cigarette nicotine content effects.

The results of the present paper provide additional information since a possible effect of the nicotine content on lung function was excluded on the basis of the similar eNO variations obtained testing both e-liquids with and without nicotine. Summarizing, a statistically similar eNO response was measured even if: i) statistically different doses were provided, ii) different cigarettes were tested, and iii) different nicotine levels in e-liquids were considered. Therefore, the present study allows to state that e-cigarettes are not safer than tobacco cigarettes when effects related to eNO reduction are considered. No further conclusions aiming to detect the parameter mainly affecting the lung function during e-cigarette vaping can be carried out on the basis of the methodology here considered. The authors can just speculate that a key parameter could be the propylene glycol. The main function of propylene glycol in e-cigarettes is the production of the vapor. Some studies described that repeated exposure to inhalations of theatrical smoke, which contains propylene glycol, was associated with acute cough and dry throat (Moline et al., 2000) and decrease in lung function (Varughese et al., 2005; Wieslander et al., 2001). Along with propylene glycol, electronic cigarettes frequently also contain glycerine for aerosol production. It is generally considered non-hazardous and has low oral toxicity, although McCauley et al. (2012) reported that glycerine-based oils from the aerosol of electronic cigarettes can cause lipoid pneumonia.

Table 2
Average eNO data measured before and after smoking/vaping sessions for all the study population. p values evaluated through the paired Student's t test are reported to compare e-cig$_0$, e-cig$_{18}$, and conventional tobacco cigarette smoking/vaping sessions to the control session.

Session	Number of smokers/vapers	Average eNO measured before smoking/vaping session (ppb)	Average eNO measured after smoking/vaping session (ppb)	Average eNO difference due to the smoking/vaping session (ppb)	p values
e-cig$_0$	25	9.5	6.3	−3.2	0.007
e-cig$_{18}$	25	9.4	7.2	−2.2	0.007
Conventional cigarette	25	8.7	5.9	−2.8	0.002
Control	25	9.4	9.3	−0.1	

Please cite this article as: Marini, S., et al., Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.04.004

The authors, once again, point out that on the basis of the current literature no definitive results can be provided to clearly evidence the main parameter affecting lung function during e-cigarette vaping.

Conflict of interest

The authors declare no conflicts of interest.

Uncited reference

General, 1988

Acknowledgments

The authors would like to thank all the volunteers involved in the experiments.

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Please cite this article as: Marini, S., et al., Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.04.004

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Please cite this article as: Marini, S., et al., Short-term effects of electronic and tobacco cigarettes on exhaled nitric oxide, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.04.004