

Environmental and individual exposure to secondhand aerosol of electronic cigarettes in confined spaces: Results from the TackSHS Project

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Abstract

This study aims to investigate bystanders' exposure to secondhand electronic cigarette (e-cigarette) aerosol (SHA) in controlled conditions simulating a real-world scenario. An experiment was performed in a motor car and in a room, in which two bystanders were exposed to SHA generated during a 30-minute *ad libitum* use of one e-cigarette by an experienced user. The experiment was replicated on five consecutive days in each setting. We measured airborne fine particulate matter (PM_{2.5}) and nicotine concentrations in the near-field (1 m) and far-field (2-3 m) exposure as environmental markers of SHA. As biomarkers of exposure to SHA, we measured the concentration of nicotine, cotinine, 3'-OH-cotinine, nornicotine, tobacco-specific nitrosamines, propylene glycol, and glycerol in bystanders' saliva samples; before, during, and after the exposure period. Self-reported health symptoms related to exposure to SHA were also recorded. Results showed that the highest median PM_{2.5} concentration was recorded during the exposure period, being 21 µg/m³ in the room setting within the near-field exposure, and 16 µg/m³ in the car setting within both the near- and far-field exposure – about twofold increase compared to the background concentration. Most of concentrations of the airborne nicotine and all biomarkers were below the limit of quantification in both settings. Bystanders in both settings experienced some mild airway and ocular irritation symptoms; the most reported were dry throat, dry nose, phlegm throat, and dry eyes. Our study showed that the short-term use of an e-cigarette in confined spaces impaired the indoor air quality by increasing the level of PM_{2.5}, and caused some irritation symptoms in bystanders.

Keywords: electronic cigarette, electronic nicotine delivery systems, secondhand aerosol, passive exposure, biomarker, environmental pollution.

HIGHLIGHTS

- Short-term e-cigarette use in confined spaces more than doubled PM_{2.5} concentration
- After e-cigarette use in a room, PM_{2.5} concentration remained higher than baseline
- PM_{2.5} levels in a ventilated car was similar at different distances from user
- Airborne nicotine and biomarkers levels were very low after a 30-min e-cigarette use
- Some mild irritation symptoms were reported after exposure to e-cigarette aerosol

INTRODUCTION

The use of electronic cigarettes (e-cigarettes) is spreading worldwide and subsequent exposure to their secondhand aerosol (SHA) is becoming a matter of concern (Destailats et al., 2020). Recent studies show that exposure to SHA among non-users of e-cigarettes is not negligible, as 16% of adults from the general population in 12 European countries reported to be exposed to SHA within the past 7 days (Amalia et al., 2020), and about 37% of smokers in six European countries reported ever-exposure to SHA (Tigova et al., 2019).

Unlike secondhand tobacco smoke (SHS), SHA originates from the aerosol exhaled by an e-cigarette user only, because e-cigarettes do not produce sidestream emissions (Protano et al., 2017). Nevertheless, SHA is known to contain toxicants such as nicotine, particulate matter (PM₁, PM_{2.5}, PM₁₀), volatile organic compounds, propylene glycol (PG), glycerol, metals, tobacco-specific nitrosamines (TSNAs), and flavourings (Fernández et al., 2015; Martínez-Sánchez et al., 2019; National Academies of Sciences, Engineering, 2018; Rachel et al., 2014; van Drooge et al., 2019).

A large body of evidence has shown that some of the toxicants in SHA impair indoor air quality. Fine particulate matter (PM_{2.5}) concentration markedly increased during e-cigarette use sessions with human volunteers in settings such as a room (Schober et al., 2014; van Drooge et al., 2019; Volesky et al., 2018), home (Fernández et al., 2015), or e-cigarette conventions (Chen et al., 2018; Soule et al., 2017). Additionally, airborne nicotine concentration was found to increase after an e-cigarette use session during an experimental study in a room (Schober et al., 2014), and in an observational study in which the concentration in homes of e-cigarette users were compared to that of non-users' homes (Ballbè et al., 2014). Some of TSNAs, such as N-nitrosornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK), which are carcinogenic (Hecht, 1998; Pérez-Ortuño et al., 2016; Rivenson et al., 1988; Yuan et al., 2011) have been identified in e-cigarette aerosol (Goniewicz et al., 2014).

Although the concentrations of toxic compounds in e-cigarette aerosols are lower than those emitted from conventional cigarettes (National Academies of Sciences, Engineering, 2018), exposure to SHA may still pose harm to bystanders. Indeed, many substances in SHA are harmful to health. PM_{2.5}, for example, is known to cause cardiovascular, respiratory diseases (World Health Organization Regional Office for Europe, 2013), diabetes, and cancer (Kim et al., 2015). Exposure to airborne nicotine may cause nicotine-related diseases, such as

cardiovascular disease and impaired brain function (Ernst et al., 2001; Goriounova and Mansvelder, 2012; Qasim et al., 2017). Exposure to PG aerosols in the concentration typically found in e-cigarettes has been found to cause irritation to the eyes and throat in some individuals (National Academies of Sciences, Engineering, 2018). In an experimental study, exposure to aerosolised glycerol caused a slight local irritant effects on the respiratory tract of mice (Renne et al., 1992). Although e-cigarette use has been shown to cause inflammation in users and was recently linked to the development of respiratory diseases (Bhatta and Glantz, 2020; Palamidis et al., 2017; Schober et al., 2014; Vardavas et al., 2012), only a small number of studies have reported adverse health symptoms from exposure to SHA. Some studies found that exposure to SHA may result in a reduced respiratory function and headache, dry mouth, ocular, nasal, and airway irritation symptoms among e-cigarette non-users (Johnson et al., 2019; Tzortzi et al., 2020, 2018), and exacerbate asthma symptoms in youth with asthma (Bayly et al., 2019).

Assessing the exposure to SHA in bystanders is important, because they may be involuntarily exposed to hazardous substances from the aerosol (National Academies of Sciences, Engineering, 2018). However, previous studies on SHA exposure were based on the measurement of indoor air quality and biomarkers that were conducted by using either machine-generated aerosol, in a real-use setting but poorly controlled, or in an extreme scenario such as e-cigarette events that did not represent common use in real life. They were also largely conducted in single settings.

To address the gap, the present study, developed within the TackSHS project (Fernández et al., 2020), aimed to comprehensively investigate bystanders' short-term exposure to SHA in controlled conditions that emulate real-life scenarios by carrying out a combination of environmental and biological assessment in confined settings. Furthermore, self-reported health symptoms after SHA exposure were also explored.

MATERIAL AND METHODS

Study Design

We performed an experimental study in two confined settings, a room and a car, in which two bystanders were exposed to aerosol produced from short-term e-cigarette use. This study was

performed with volunteers in the course of one week in each setting, firstly in the room and, after 10 days, in the car. The study was conducted in July and August 2019.

Participants

We enrolled two healthy non-users of e-cigarettes or any other tobacco/nicotine product (the “non-users”) and one healthy experienced e-cigarette user (the “user”). Participants were recruited through a database of previous studies and personal contacts of the research team. All participants agreed to participate and received a monetary compensation for their participation.

The inclusion criteria for the non-users were: to be an adult (18 years old and above), never user of e-cigarettes or have stopped using them for more than six months, never user of any tobacco or nicotine product or have quit for more than six months, and not being regularly exposed to SHS or SHA in any setting. For the user, the inclusion criteria were to be an adult (18 years old and above), daily e-cigarette user (at least during the past two months prior to the study), and not being a user of any tobacco/other nicotine product (at least two months prior to the study). The exclusion criteria for all participants were: pregnancy or breastfeeding, ongoing or recent illness (less than four weeks prior to the study), acute or chronic condition or disease (e.g., diabetes, asthma, chronic obstructive pulmonary disease, hypertension), and consumption of any type of medication (less than two weeks before the study).

Characteristics of the volunteers recruited were as follows: non-users were one female and one male; aged 40 and 49, respectively; both Caucasians; the user was a 59-year-old Caucasian female, who had used e-cigarette daily for 3.5 years by the time of the study.

The user was asked to use her own e-cigarette and e-liquid during the study, to reproduce her typical e-cigarette use. The e-cigarette was Eleaf iStick TC40W, this is a ‘Mod’ e-cigarette consisting of a vapouriser, a rechargeable battery and a cartridge containing the e-liquid (open tank). The voltage, power, and temperature of the e-cigarette used was set by the user (2600 mAh, 1 ohm) and maintained constant across experiments. The e-liquid (60 ml) contained nicotine (3 mg/ml), PG and glycerol (50:50 ratio), and was cinnamon cookies flavoured (Atmos Lab brand). The same e-cigarette and e-liquid were used during all replicates of the study.

Experiment Conditions

The study aimed to simulate a real-world exposure to SHA by the use of one e-cigarette in a room and in a car. The experiment was replicated five times, on five consecutive days (Monday-Friday) in each setting. After each daily replicate, all participants were not allowed to use e-cigarette or be exposed to SHA or SHS for three hours after the experiment. The room and the car were ventilated and were not used until the next replicate. To ensure no biological marker of exposure remained in the body of non-users, we made a ten-day washout window between experiments in both settings.

We first conducted the experiment in a 14.08 m² x 2.50 m (35.2 m³) office room in the Catalan Institute of Oncology, Barcelona. During the experiment, the user, the two non-users, and a researcher seated around a small table (60 x 120 cm); non-users were seated approximately one metre from the user. The overall experiment lasted 40 minutes which was stratified into three parts (Figure 1). The first part included five-minute baseline measurements, where the user was not allowed to use the e-cigarette. The second part consisted of 30 minutes of exposure to SHA generated by *ad libitum* use of the e-cigarette by the user; the number of puffs per minute were recorded during this period. The third part of the experiment included five minutes of post-exposure measurements when the user stopped using the e-cigarette. The room's windows and the door were kept closed during the experiment.

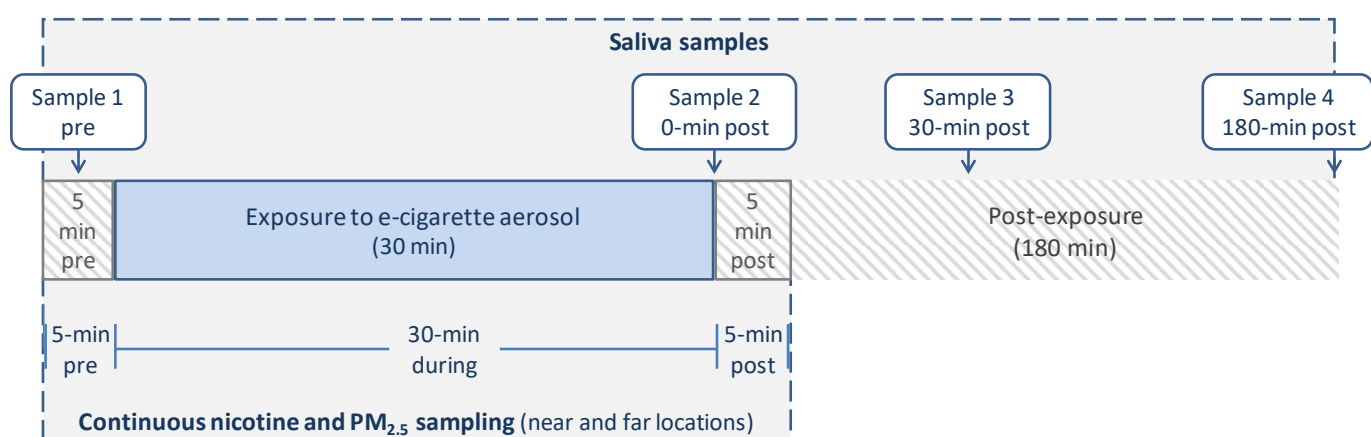


Figure 1. Sequence of environmental and biological exposure measurements conducted in a car and in a room. TackSHS Study, 2019

We used a medium-size car (VW Touran, interior size approx. 10 m³) as the second setting. During the experiment there were the user (seated on the front passenger seat), the two non-users (seated on the rear seats), the driver, and one researcher on the rear seat. The overall experiment lasted 40 minutes which consisted of the same three parts as in the room (Figure 1). The car ran continuously on an urban 1.3 km circuit at speed up to 70 km/h during the experiment, with the two front windows half-opened (30 cm) and the rear windows closed.

In both settings, any system of heating or air conditioning during the experiment was avoided. All experiments were done in conditions with a relative humidity lower than 85%. During the five-day experiment, the range of the temperature in the room experiment was 22.0° - 26.3° C and the mean temperature was 26.6° and 27.9° C for indoor and outdoor areas, respectively. The temperature of the car ranged from 25.7° to 32.5° C and the mean temperature was 25.7° and 29.5° C for indoor and outdoor areas, respectively. The outdoor temperature and relative humidity were checked against an official weather report website (www.meteo.cat).

Measurements

Environmental measurements

We monitored gas-phase nicotine using nicotine samplers of 37 mm in diameter containing a filter treated with sodium bisulphate as performed in previous studies (Fu et al., 2016; Hammond et al., 1987; López et al., 2012). We used active sampling with nicotine samplers attached to air pumps (SKC SideKick© 224-52MTX) set at a constant flow rate of 3 L/min. The Air pumps were calibrated before and after monitoring using a gas flow calibrator Bios Defender 510M (Mesa Labs company). We sampled airborne nicotine for each of the three parts of the experiment separately. In total, 60 air samples were analysed for the determination of nicotine concentration (µg/m³) at the laboratory of the Public Health Agency of Barcelona by gas chromatography-mass spectrometry. For every 20 nicotine samples, one blank filter that had not been exposed was analysed for control purposes. We quantified the time-weighted average nicotine concentration by dividing the amount of nicotine extracted from the filter by the volume of air sampled (estimated flow rate multiplied by the minutes the filter had been exposed). This procedure has a limit of quantification (LOQ) of 5 ng per filter, which is equivalent to 0.06 µg/m³ of nicotine per 30 minutes of exposure. For values that were under the LOQ, we assigned half of this LOQ's value when

they were not more than 20% of data in the category of analysis; otherwise, we presented them as “<LOQ”.

Besides airborne nicotine, we measured real-time airborne mass of PM_{2.5} concentration at 1-sec interval with two aerosol monitors (TSI SidePakTM AM510). We also used a third monitor to simultaneously measure outdoor PM_{2.5} concentration as background information. Given the absence of standard calibration factors for e-cigarette aerosol, we applied individual SHS gravimetric calibration factors to each of the three devices, as done in other studies (Czogala et al., 2014; Soule et al., 2017; Tzortzi et al., 2020). These k-factors were obtained in individual experiments with a reference instrument (Met One Instruments BAM 1020) that automatically measures and records ambient particulate mass concentration levels using the principle of beta ray attenuation (Fernández et al., 2020; Ruprecht et al., 2011). The individual k-factors obtained for each monitor were 0.353, 0.367, and 0.393. PM_{2.5} data were downloaded to a local computer afterwards from the monitors’ internal memory for further analyses.

Airborne nicotine and PM_{2.5} were measured simultaneously for each of the three parts of the experiment separately in both settings (Figure 1). For indoor measurement, two nicotine air pumps and two PM_{2.5} monitors were used in each setting. We ensured that all devices were placed in a location where the air was adequately circulating. In the room, one nicotine sampler and one PM_{2.5} monitor were placed on a table, about one metre from the user, where all participants sat around (near-field), and the other nicotine sampler and PM_{2.5} monitor on another table, at about three metres away from the user (far-field). In the car, one nicotine sampler and one PM_{2.5} monitor were fixed at the back of the headrest of the driver’s seat, about one metre from the user (near-field). For the far-field measurements in the car, we placed the second nicotine sampler and PM_{2.5} monitor about two metres away from the user, on the headrest of the rear seat, so as to simulate a child’s exposure from an adult using an e-cigarette in the car.

Biological measurements

Saliva samples were collected from the two non-users four times in each daily replicate in both settings (Figure 1): once pre-exposure (just before starting the exposure) and three times after the exposure period finished (0-min, 30-min and 180-min post-exposure), leading to a total of 80 saliva samples. Samples were prepared into two aliquots for storage at -20° C in a freezer in the laboratory at ICO-IDIBELL. All samples were sent in dry ice to the laboratory

at IMIM-Hospital del Mar Medical Research Institute for analyses by liquid chromatography-tandem mass spectrometry to determine the concentration of nicotine (LOQ: 0.50 ng/mL), cotinine (LOQ: 0.05 ng/mL), 3'-OH-cotinine (LOQ: 0.040 ng/mL), nornicotine (LOQ: 0.10 ng/mL), tobacco-specific nitrosamines (NNN, NNK, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) with LOQ of 1.0, 2.0 and 0.50 pg/mL, respectively), PG (1,2-PG and 1,3-PG with LOQ for both: 3.0 nmol/mL), and glycerol (LOQ: 10.0 nmol/mL). We assigned half of the LOQ values for biomarker concentrations that were lower than their LOQ if they were not more than 20% of data in the category of analysis; otherwise, we presented them as "<LOQ" (Pérez-Ortuño et al., 2016, 2015).

Observational measurements

Puff frequency

The volunteer used the e-cigarette *ad libitum*. The number of puffs produced each minute by the user were recorded by a researcher in a register sheet during the 30-min exposure period.

Self-reported health symptoms

Participants were asked to answer a brief questionnaire (Tzortzi et al., 2020) to identify potential health symptoms associated to their exposure to SHA during its completion. The questionnaire was self-administered during the pre-exposure period and also at 0-min, 30-min and 180-min post-exposure, concurrently with the collection of saliva samples. The questionnaire included symptoms of irritation relating to ocular system (itchiness, burning, watery eyes and dryness), nasal system (nasal drip, itchiness, dryness, sneezing and stuffiness), and throat – respiratory system (dryness, soreness, cough, phlegm, and breathlessness) as well as general complaints (headache, nausea, and fatigue). For each symptom in the questionnaire, participants indicated the intensity level of the symptoms they perceived as none (score 0), little (score 1), moderate (score 2), strong (score 3), and very strong (score 4).

General information

An *ad hoc* questionnaire was filled in by the participants at the enrolment time to gather information about sociodemographics, smoking status, e-cigarette use patterns and their usual exposure to SHS and SHA. Also, prior to each experimental session, the participants were

asked to fill in a specific form to report if there had been exposure to SHS or SHA in different settings, the day before that experimental session.

Statistical analysis

We estimated the median concentration of airborne nicotine and PM_{2.5} (µg/m³) before, during, and after exposure periods in each setting across the five replicates (day 1-5) of the experiment. Median test was performed to obtain *p-values* for the difference of estimates of the near- and far-field exposure and in different periods (i.e., pre- vs. during exposure; during vs. post-exposure; pre- vs. post-exposure) of PM_{2.5}. *P-values* for the difference between PM_{2.5} concentration in indoor (near- and far-field exposure, in both settings) and outdoor were also calculated. In case more than 20% of airborne nicotine values were under the LOQ in a category of analysis we assigned it as “<LOQ”. The number of puffs across time of the exposure period were plotted against PM_{2.5} concentration.

We estimated the median concentration of each biomarker pre-exposure, 0-min , 30-min and 180-min post-exposure in each setting across the five replicates of the experiment. Similar to airborne nicotine, we only calculated the median concentration of a category whose more than 20% of its values were higher than the LOQ.

The total number of symptoms reported by non-users was calculated and grouped according to the experiment period (pre-exposure, 0-min , 30-min and 180-min post-exposure) in each replicate and setting. The top three most frequent symptoms reported by the non-users were identified and explored for their intensity level.

In all analyses, the significance level was set at *p-value* < 0.05. The analyses were performed with STATA 14.0.

Ethical Issues

The Ethics & Research Committee of the Bellvitge University Hospital approved the overall project (TackSHS Project, PR341/15) (Fernández et al., 2020) as well as this specific study (PR217/19), that has also been registered at www.clinicaltrials.gov (NCT04140617). All participants and researchers taking part in the data collection were properly informed about the potential harms of exposure to SHA, and all of them provided written consent.

RESULTS

Environmental markers

The overall median concentrations of airborne PM_{2.5} over the five replicates in both settings are summarised in Table 1. In the room setting, PM_{2.5} concentration during and after the exposure period were significantly higher than baseline concentrations, while in the car, this occurred only during the exposure period.

The highest median PM_{2.5} concentrations in the room and the car were identified during the exposure period – about twofold the baseline median concentrations in both settings. During exposure, the highest concentration in the room was at near-field exposure (median: 21 µg/m³; IQR: 11-88 µg/m³), while in the car, the concentration was the same for near- and far-field (median: 16 µg/m³; IQR near-field: 10-31 µg/m³, IQR far-field: 10-28 µg/m³).

After the user stopped using the e-cigarette in the room, PM_{2.5} concentration (median: 19 µg/m³; IQR: 11-50 µg/m³ and 12-40 µg/m³ at near- and far-field exposure, respectively) did not fall significantly from the concentration during the exposure period (p=0.398 for the comparison at near-field exposure and p=0.280 for the comparison at far-field exposure), and remained significantly higher than the corresponding pre-exposure levels. A significantly higher median PM_{2.5} concentration was also found at near-field (21 µg/m³; IQR: 11-88 µg/m³) compared to the far-field (18 µg/m³; IQR: 9-81 µg/m³) exposure when the e-cigarette was used, but not after its use was stopped.

The median concentrations of PM_{2.5} after exposure session in the car at both distances dropped significantly to half the concentration measured during the exposure period. After the puffing ceased, PM_{2.5} concentration at near-field exposure remained at a higher level (8 µg/m³; IQR: 6-10 µg/m³, p<0.001) compared to the pre-exposure level. The median concentration of PM_{2.5} at near- and far-field exposure was similar in both periods, during (p=0.474) and after exposure (p=0.483).

For airborne nicotine, the majority of the median concentrations were below the LOQ, and, thus, we were unable to estimate the differences of the nicotine concentration in pre-, during, and post-exposure periods, and between near- vs. far-field measurements.

Table 1. Median concentration and its corresponding interquartile range (IQR) of PM_{2.5} (both expressed in µg/m³) measured at near-field (1 metre) and far-field (2-3 metres) distance from an e-cigarette user, and in outdoors before, during and after exposure from e-cigarette use in room and car settings across 5 replications. TackSHS Study, 2019

	Pre-exposure (IQR)	During exposure (IQR)	Post-exposure (IQR)	P-value ^a	P-value ^b	P-value ^c
Room						
Near-field	8 (6-11)	21 (11-88)	19 (11-50)	<0.001	0.398	<0.001
Far-field	7 (6-9)	18 (9-81)	19 (12-40)	<0.001	0.280	<0.001
Outdoors	17 (14-25)	11 (9-12)	10 (9-11)	<0.001	<0.001	<0.001
P-value ^d	<0.001	<0.001	0.729			
P-value ^e	<0.001	<0.001	<0.001			
P-value ^f	<0.001	<0.001	<0.001			
Car						
Near-field	7 (6-10)	16 (10-31)	8 (6-10)	<0.001	<0.001	<0.001
Far-field	7 (6-11)	16 (10-28)	8 (6-11)	<0.001	<0.001	0.553
Outdoors	17 (14-25)	11 (9-12)	10 (9-11)	<0.001	<0.001	<0.001
P-value ^d	0.001	0.474	0.483			

P-value ^e	<0.001	<0.001	<0.001
P-value ^f	<0.001	<0.001	<0.001

^a p-value for pre- vs. during exposure

^b p-value for during vs. post-exposure

^c p-value for pre- vs. post-exposure

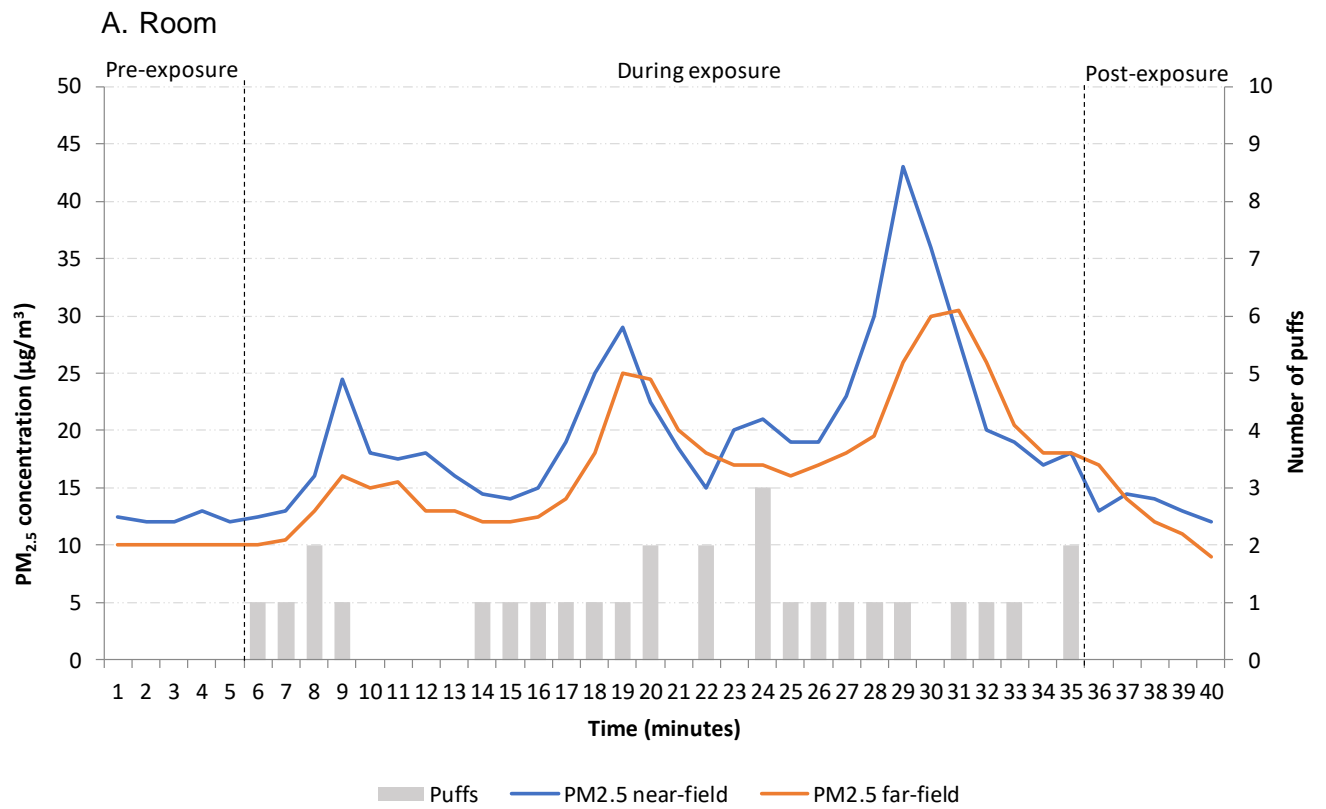
^d p-value for near-field vs. far-field

^e p-value for near-field vs. outdoor

^f p-value for far-field vs. outdoor

The distribution of real-time PM_{2.5} concentration during a whole experimental session at near- and far-field exposure is shown in Figure 2 derived through particles monitoring before (first 5 minutes), during (30 minutes) and after exposure period (last 5 minutes) in the 5th and 4th day of the 5-day replication for room (Panel A) and car (Panel B), respectively. The graphs show that the trend of PM_{2.5} concentration follows the variation in the number of puffs produced by the user (indicated with bars).

The total number of puffs per 30-minute exposure period across the five-day replication ranged from 28 to 42 in the room and from 51 to 84 in the car. As illustrated in Figure 2, PM_{2.5} concentration at near- and far-field exposure increased immediately as the first puff was made in the room (Panel A) and in the car (Panel B) and quickly decreased after the puffing stopped. In the room, the peak values for near- and far-field reached about four and three times, respectively, higher than pre-exposure concentration. PM_{2.5} concentration lasted 1-5 minutes to reach its peak concentration when the e-cigarette was used. A similar trend occurs in the car where the highest number of puffs per minute (4 puffs) was followed by the highest peak value of PM_{2.5} concentration at near- and far-field exposure. Also, the peak concentration during the exposure period was seven-fold higher than the baseline concentration at both distances. The time-lag for PM_{2.5} concentration to reach its peak after a given puff in the car setting was about 0-1 minutes; shorter than in the room.



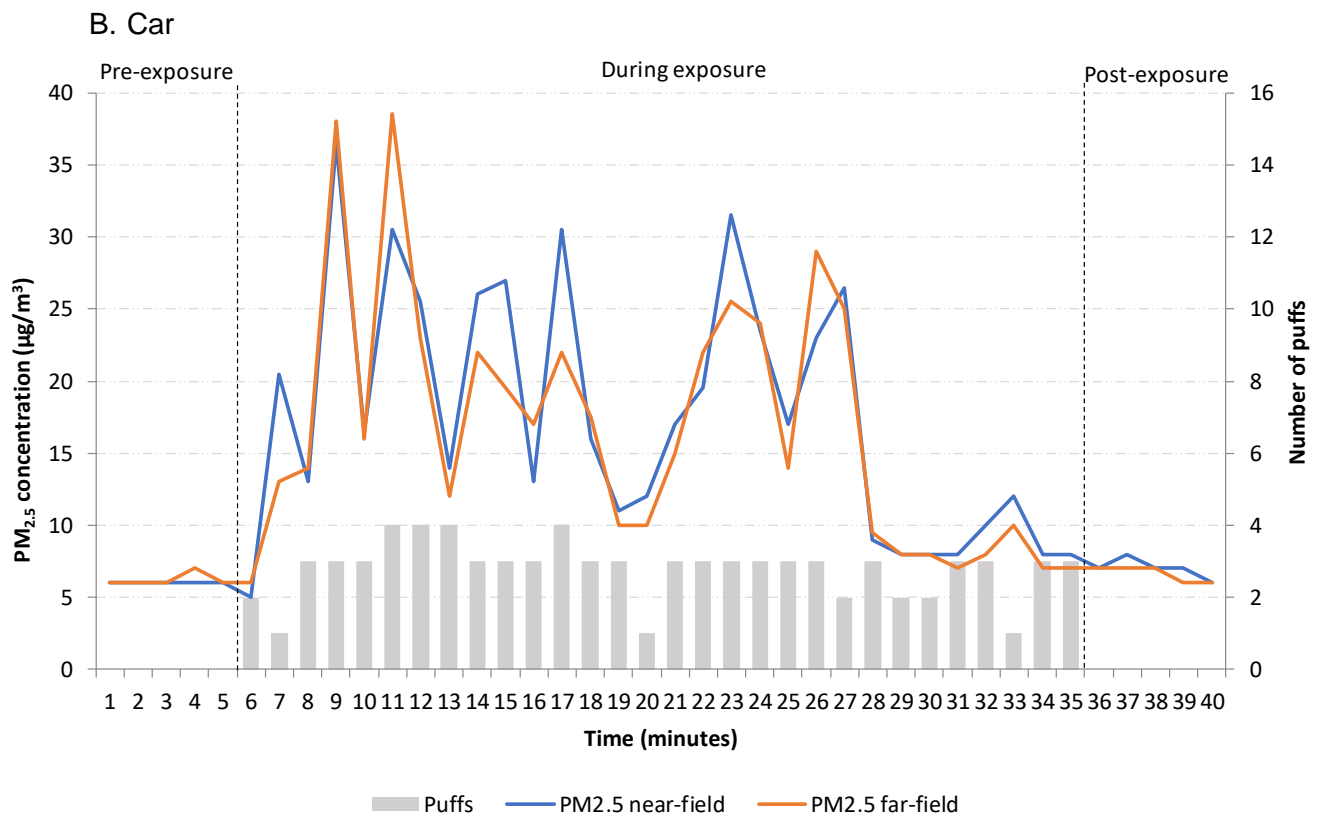


Figure 2. The time course of PM_{2.5} concentration at near- (1 metre) and far- (2-3 metres) field exposure in day 5 of room (Panel A) and day 4 of car experiment session (Panel B) related to the number of puffs performed by an e-cigarette user. The exposure period lasted 30 minutes (between minutes 5 and 35 in the graphs). The three parts of the experiment are indicated by vertical dashed lines. TackSHS Study, 2019.

Biomarkers

The non-users' median concentration of saliva nicotine, cotinine, 3-OH-cotinine, nor nicotine, NNN, NNK, NNAL, 1,2-PG, 1,3-PG, and glycerol before, during, and after the exposure period in the room and car settings were mostly below the LOQ. Eight out of 10 values of the cotinine concentration at 0-min post-exposure in the room were higher than its LOQ (0.050 ng/mL), ranging from 0.051 to 0.093 ng/mL with a median of 0.071 ng/mL (IQR: 0.054-0.087 ng/mL).

Short-term health symptoms

Figure 3 shows the total number of short-term symptoms reported by each non-user before (pre-exposure), right after (0-min post-exposure), 30 minutes (30-min post-exposure) and 3 hours (180-min post-exposure) after the exposure period ended across the five replicates in

each setting. The highest combined number of all symptoms reported by both non-users occurred on the first day in each setting, reporting 14 and 9 symptoms in the room, and 13 and 8 symptoms in the car for non-user 1 and 2, respectively. In the room (Figure 3, Panel A), the highest number of symptoms was mainly reported right after the exposure period (0-min post-exposure) except for day 4, where the non-user 1 had more symptoms later (30-min post-exposure). Some symptoms were still reported at 30 minutes and 180 minutes after exposure. The three most reported symptoms in the room by both non-users were dry throat, dry nose, and phlegm in the throat, with mild intensity (average score 1 in the 0-4 range). In the car (Figure 3, Panel B), most symptoms were also reported just after the exposure ended (0-min post-exposure), and few symptoms remained until 180 minutes after exposure period. Dry throat, dry nose, and dry eyes were the three most frequently reported symptoms by the non-users. Both non-users experienced a mild intensity (average score 1) for the three symptoms from immediately (0-min post-exposure) until 180 minutes after exposure.

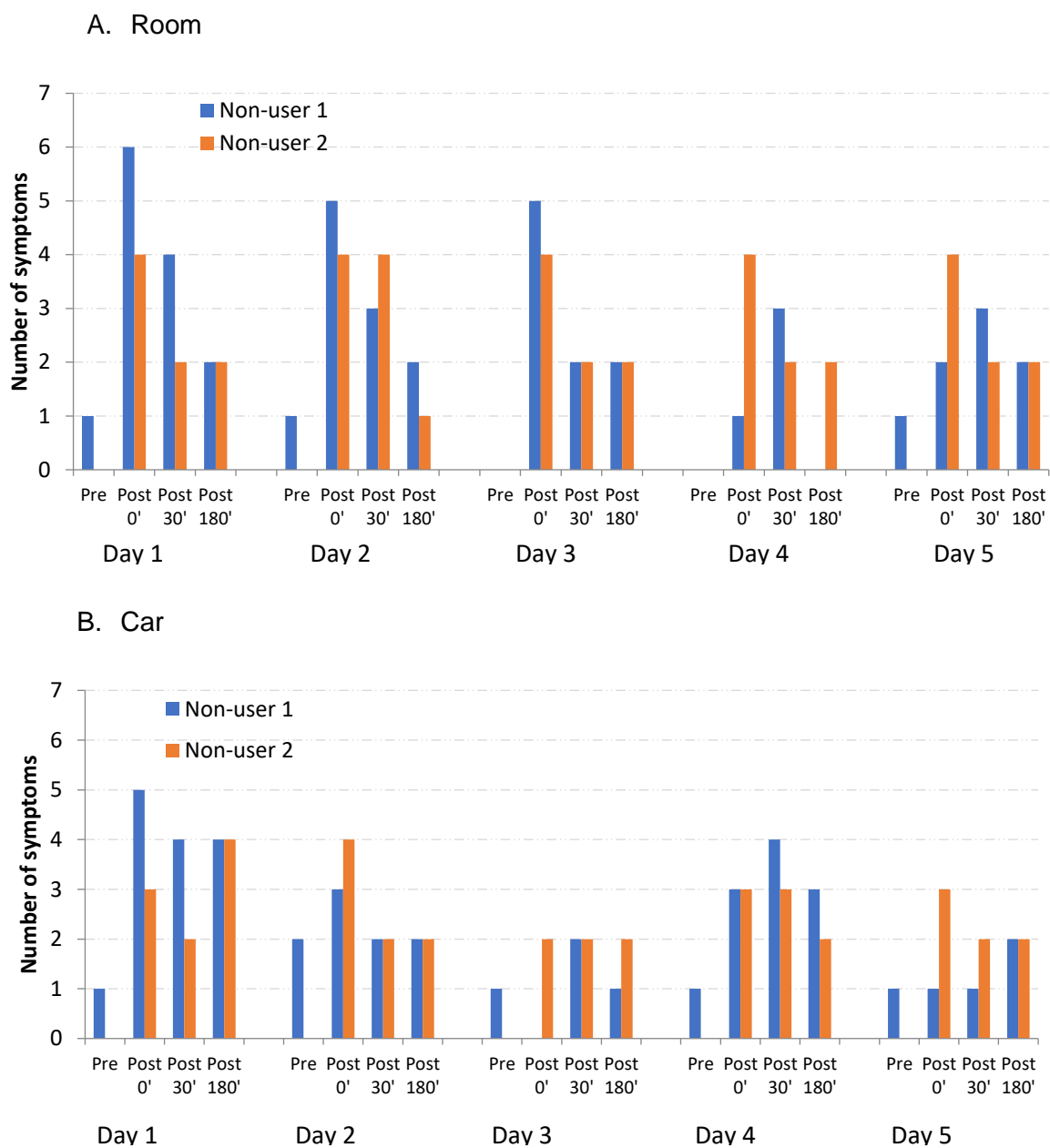


Figure 3. Number of short-term health symptoms reported by the two non-users exposed to secondhand aerosol from e-cigarettes at different time of the experiment across 5-day replications in room (Panel A) and car settings (Panel B). TackSHS study, 2019.

No symptoms were reported by the non-users at the time where the bars are not present in the graph.

DISCUSSION

This study evaluated exposure to SHA by measuring the concentration of airborne markers, biomarkers, and self-reported short-term health symptoms in bystanders while an e-cigarette was used in a room and in a car, simulating real-world conditions.

The highest median PM_{2.5} concentration during e-cigarette use across the five days replication found in the present study (21 µg/m³) was lower than those found in similar studies conducted in a room (mean concentrations: 246.9-289.5 µg/m³), and in cars (mean concentrations: 75-490 µg/m³) (Schober et al., 2019; Volesky et al., 2018). However, in those studies, the exposure period lasted shorter than the present study, [6.5 minutes (Volesky et al., 2018) and 20 minutes (Schober et al., 2019)], did not utilise *ad libitum* use of e-cigarette (Schober et al., 2019; Volesky et al., 2018), used different e-cigarette types (cigalike, tank, and adjustable model) (Volesky et al., 2018), and higher nicotine level [12 mg/ml (Schober et al., 2019) and 18 mg/ml (Volesky et al., 2018)] than in our study (3 mg/ml). Previous studies suggested that variations in the concentration compounds of e-cigarette aerosol, including PM_{2.5}, might be accounted to user puffing pattern (duration and frequency) as well as to e-cigarette brand, type, voltage and flavour additive (Volesky et al., 2018; Zhao et al., 2018). Also, the studies from Schober et al., 2019 and Volesky et al., 2018 measured the PM_{2.5} load by reporting the mean concentration of PM_{2.5}, instead of median concentration as used in the present study, which might lead to a higher, but biased, estimation of PM_{2.5} concentrations due to their non-normal data distribution. We used the median as point of estimates for the PM_{2.5} concentration due to extremely skewed distribution of our data. For example, the mean PM_{2.5} concentrations during exposure for the near-field exposure were 104 and 35 µg/m³ in room and car, whilst the reported median values were 21 and 16 µg/m³, respectively.

Although the highest median PM_{2.5} concentration in our study did not exceed the outdoor guidance level of World Health Organization air quality standard (25 µg/m³ as daily average)(World Health Organization, 2005) and the United States Environmental Protection Agency Air quality index (35 µg/m³ as daily average) (US EPA, 2012), the concentration we found is not negligible and illustrates that fine particulate concentrations approximately double when bystanders spend time in typical indoor environments where one e-cigarette user is present. A multi-country pooling of 22 European cohorts found that there was a significant increase in the hazard ratio for natural-cause mortality for each 5 µg/m³ increase in PM_{2.5}

exposure, even when the concentration was below the limit value of $25 \mu\text{g}/\text{m}^3$ (Beelen et al., 2014).

E-cigarette use increases indoor $\text{PM}_{2.5}$ concentration, as shown by a significantly higher concentration during e-cigarette use (vs. pre-exposure) at both near- and far-field exposure. This trend was in line with previous experimental studies which showed an increase of $\text{PM}_{2.5}$ concentration during puffing period to a mean concentration level that ranged from $20 \mu\text{g}/\text{m}^3$ to $290 \mu\text{g}/\text{m}^3$ (Schober et al., 2014; van Drooge et al., 2019; Volesky et al., 2018). In an extreme situation, a study conducted during an e-cigarette convention found that the concentration of $\text{PM}_{2.5}$ was able to reach as high as $819 \mu\text{g}/\text{m}^3$ (Soule et al., 2017), levels that are higher than in hookah cafes and bars that allow smoking inside (Cobb et al., 2013).

The increasing pattern of $\text{PM}_{2.5}$ concentration was also suggested by Figure 2, where the more puffs generated by the user, the higher the $\text{PM}_{2.5}$ concentration in the room and the car. This is consistent with the findings from a study where $\text{PM}_{2.5}$ peaks were concurrent with e-cigarette puffs made at homes of e-cigarette users (Fernández et al., 2015) and another study conducted in a room (Volesky et al., 2018).

We found that $\text{PM}_{2.5}$ concentration, at both distances in the room, did not return to the baseline level five minutes after the e-cigarette use ceased, while at a far-field exposure in the car, the concentrations significantly decreased from that registered during the puffing period, dropping to the baseline level. The observed differences between room and car might be explained because the concentrations did not start dropping from the same level – $\text{PM}_{2.5}$ concentrations during the exposure period were higher in the room than in the car – and because the car, unlike the room, had half-open windows while moving allowing ventilation, which has been shown to impact $\text{PM}_{2.5}$ measurements (Vardavas, 2006). Previous studies found a variation in the duration of $\text{PM}_{2.5}$ decay, from four minutes to one day after e-cigarette use stopped, depending of the peaked concentrations observed (Soule et al., 2017; Volesky et al., 2018; Zhao et al., 2018). The diversity in the rate of decay may be affected by the dilution, evaporation of the e-cigarette emission, and the ambient partial pressure of the emission (Zhao et al., 2018). Thus, it is hypothesised that the setting's volume and air flow may play a role in the $\text{PM}_{2.5}$ evaporation rate.

The variability observed between $\text{PM}_{2.5}$ concentrations at near- and far-field exposures during e-cigarette use periods in the room indicates that the distance between e-cigarette user and bystander does matter in short periods of exposure when there is not any system to dissipate

the particles such as a fan or other ventilation methods. Nevertheless, the distance became an unimportant factor if air ventilation is present, as we found in the car. Previous evidence shows that the further the distance $PM_{2.5}$ was measured from the e-cigarette user, the lower the $PM_{2.5}$ concentration measured (Volesky et al., 2018; Zhao et al., 2017). At a further distance, the particles in the aerosol are less detected because of the nature of the particles which are volatile and that are less able to travel far from the user without ventilation systems (Zhao et al., 2017). It is worth to note that this finding does not have implications with regard to the safe distance for SHA exposure.

Unlike the present study, previous experiments showed an increased concentration of airborne nicotine during e-cigarette use period. However, these studies had longer periods of e-cigarette use, from 2 to 12 hours, involved more than one user at a time, and did not employ *ad libitum* use (Schober et al., 2019, 2014; van Drooge et al., 2019). The unquantifiable concentration of airborne nicotine in this study may be because the method only captured the nicotine in the gas phase, not particle phase, thus, underestimating the chemicals present (Logue et al., 2017). The largest increase of airborne nicotine from e-cigarette use is in the particle phase compared to the gas phase (van Drooge et al., 2019). Additionally, the e-cigarette user in our study used a relatively low concentration of nicotine in the e-liquid (3 mg/ml) compared to the typical concentration (18 mg/ml) used by users found in 33 countries (Dawkins et al., 2013). We did not modify the concentration as we wanted to preserve participant typical patterns of use. Previous studies reported that the higher the nicotine concentrations in the e-liquid the higher the indoor air nicotine concentration (Logue et al., 2017; Schober et al., 2014). Moreover, other factors determine nicotine yield from e-cigarette use, such as e-cigarette type and brand, PG/ vegetable glycerine ratio, and electrical power (EL-Hellani et al., 2016). Although the e-cigarette used in this study was a Mod type, the user did not change the setting to follow her typical pattern of use.

Although the present study found that most biomarkers were below the LOQ, a previous study found a systemic absorption of nicotine by detecting a significant rise of saliva cotinine in non-users after two hours of exposure to SHA with three e-cigarette users at the same time in the same room (Melstrom et al., 2018). In another study, saliva cotinine also increased up to 12-fold after six-hour exposure, but the concentration was also very low (range: 0.030-0.017 ng/mL), peaking at four hours after the e-cigarette use period stopped (Johnson et al., 2019). Thus, a shorter exposure period and lower e-cigarette user density might be accountable for the samples under the LOQ in the current study. We were also unable to

measure the trend of TSNA concentrations in bystanders' saliva since they were below the LOQ, which was consistent with a previous study using urinary samples (Johnson et al., 2019). However, NNN and NNK were previously detected in e-cigarettes' emission (Goniewicz et al., 2014), and NNAL has been found in the urine of people living with e-cigarette users at a concentration significantly higher than those living with non-users and non-smokers (Martínez-Sánchez et al., 2019). This may reflect the effects of long or sustained exposure instead of short exposure to e-cigarette use. The unquantifiable salivary PG and glycerol concentration in our study might be due to the unclear relation between both biomarkers in the saliva to the e-cigarettes exposure as previous studies used plasma sample to identify the biomarkers (National Academies of Sciences, Engineering, 2018).

The four most reported short-term symptoms by non-users were dry throat, dry nose, dry eyes, and phlegm in the throat. Ocular, nasal, and throat-respiratory irritation complaints were also increasingly reported after exposure to SHA in a room in a previous experiment with 40 volunteers, with the last ones persisting even until 30-min post-exposure (Tzortzi et al., 2020). The study also found that the reported nasal and throat-respiratory symptoms were significantly associated with volatile organic compound concentrations present in the SHA. However, PM_{2.5}, PG, and glycerol may also partly play a role in generating the irritation symptoms, as these constituents are known to provoke eyes and airway irritation symptoms (Kim et al., 2015; National Academies of Sciences, Engineering, 2018).

Although elevation of biomarkers was unable to be detected in the current study, the participants reported short-term health symptoms during and even after the exposure period, suggesting that exposure to SHA is associated with some adverse health effects in bystanders. This raises concern for vulnerable groups like children, elderly, and people with respiratory diseases in a long term and intense exposure, especially for children, since our far-field exposure in the car resembles a child's exposure in the back seat, but parents tend to perceive e-cigarette use in enclosed spaces as safe for their children (Drehmer et al., 2019). Moreover, infants are at the highest risk among other age groups because they receive the highest doses per kg body weight of e-cigarette aerosol (Protano et al., 2017). The discrepancy between the level of biomarkers and frequency of short-term health symptoms found in this study may indicate that future studies should evaluate the relevant biomarkers that correspond to such symptoms. Given the small number of non-users in this study, the symptoms they reported, however, may also reflect individual sensitivity to SHA. Thus, our results should be interpreted with caution.

There are some limitations in this study that should be noted. Firstly, we only tested one type of e-cigarette and e-liquid combination. Thus, the results of this study might underestimate the exposure to SHA from other types or models of e-cigarette in the current market, that are continuously developing, and becoming more popular, especially among youth, like pod and disposable e-cigarettes (Fadus et al., 2019; Williams, 2019). Nevertheless, e-cigarettes with the tank system, like the one we used in the present study, are more likely to be used by experienced users (Zare et al., 2018). Secondly, our study might not accurately estimate the actual PM_{2.5} concentrations given the absence of the specific calibration factor for e-cigarette aerosol. Thirdly, we did not take into account the air exchange rate in the analysis that might affect the concentration of airborne markers. However, we measured them in two confined settings at near- and far-field exposure to control the potential effect of the distance from the user. In the case of the car, air exchange was allowed by a half-open window, as likely done in a typical setting like this in real-life conditions. Additionally, we took into account variability across days by conducting five-day replicates in each setting. Lastly, this study measured short-term exposure to SHA; chronic exposure might have a different outcome. However, it is likely that longer-term exposure might result in worse indoor air quality and adverse health effects.

Despite the above limitations, we assessed SHA exposure by using environmental and biological measurements concurrently with short-term health symptoms evaluation from the same subjects; thus, it captures comprehensive dimensions of the passive exposure to e-cigarette aerosol. By maintaining similar conditions across the five replicates, we ensured the repeatability of the experiment and, hence, controlled the potential systematic errors which sometimes present in observational studies. Additionally, the arrangement of the settings (half-open windows for the car and closed windows for the room) and the involvement of an actual exclusive user puffing *ad libitum* were simulating real-world e-cigarette use conditions.

CONCLUSION

Our study showed that a short-term e-cigarette use increases PM_{2.5} concentration in a room and a car, while the concentrations of airborne nicotine and biomarkers of passive exposure to e-cigarette aerosol were remained very low. The distance apart between e-cigarette user and bystanders that we used did not alter short-term exposure to PM_{2.5} significantly when air

ventilation was present in a confined space. Bystanders reported a mild level of eye and airway irritation symptoms after short-term exposure to SHA.

Authors contribution: EF, SS, LC, and MJL had the original idea for the study; EF, MF, OT, MB, and BA contributed to the finalisation of the study protocol and questionnaire; NC was responsible for airborne nicotine analyses; JAP and RPO were responsible for the analyses of biomarkers; BA and YC carried out the statistical analysis with the supervision of MF; BA wrote the first draft of the article in collaboration with MF, OT, MB, YC, and EF; SS, LC, CV, MJL, NC, RPO, and JAP made substantial contributions to interpretation of results; all the authors contributed to manuscript preparation and approved its final version prior to submission. EF is the guarantor.

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