Original Article

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

Running title: E-cigarette exposure in confined spaces

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Word count : 6205
Abstract word count : 204
References : 57
Numbers of figures and tables: 4
Supplementary : 0

Acknowledgements

We would like to thank all TackSHS project investigators (see the full list at the end of the manuscript) for their contributions in the overall TackSHS project, and to the three volunteers who participated in the present study.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681040. BA received the support of a fellowship from "La Caixa" Foundation (ID 100010434; Fellowship code: LCF/BQ/IN17/11620013). The Tobacco Control Research Group at ICO-IDIBELL (BA, EF, MF, OT, MB, YC) is partly supported by the Ministry of Universities and Research, Government of Catalonia (2017SGR319) and thanks CERCA Programme Generalitat de Catalunya for the institutional support to IDIBELL. EF is partly supported by the Instituto de Salud Carlos III, Government of Spain, co-funded by the European Regional Development Fund (FEDER; INT16/00211 and INT17/00103). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

No conflict of interest declared

ABSTRACT

Secondhand electronic cigarette (e-cigarette) aerosol (SHA) might impair indoor air quality and expose bystanders. This study aims to investigate exposure to SHA in controlled conditions of enclosed settings simulating real-world scenario. An experiment was performed in a car and in a room, in which SHA was generated during a 30-minute *ad libitum* use of an e-cigarette. The experiment was replicated on five consecutive days in each setting. We measured $PM_{2.5}$, airborne nicotine concentrations, and biomarkers of exposure to SHA, such as nicotine metabolites, 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. The results showed that the highest median $PM_{2.5}$ concentration was recorded during the exposure period, being 21 $\mu g/m^3$ in the room setting and 16 $\mu g/m^3$ in the car setting– about twofold increase compared to the baseline. Most concentrations of the airborne nicotine and all biomarkers were below the limit of quantification in both settings. Bystanders in both settings experienced some short-term irritation symptoms, expressed as dry throat, nose, eyes, and phlegm. In conclusion, short-term use of an e-cigarette in confined spaces increased indoor $PM_{2.5}$ level and caused some irritation symptoms in bystanders.

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

Practical Implications

- Our study demonstrates that short-term electronic cigarette (e-cigarette) use in confined spaces, a room and a car, more than doubled the $PM_{2.5}$ concentration and, in a room, the concentration remained higher than the baseline level after the e-cigarette use was stopped.
- When air ventilation was present in an enclosed space, the distance apart between e-cigarette user and bystanders used in this study did not change substantially the short-term exposure to $PM_{2.5}$.
- Although we detected very low levels of airborne nicotine and biomarkers of passive exposure to e-cigarette aerosol after a brief e-cigarette use, bystanders reported some

mild irritation symptoms, such as dry throat, eyes and nose, after the exposure to e-cigarette aerosol.

- These findings are useful to inform policy makers that e-cigarette use should be considered in indoor clean air policy given its ability to impair the indoor air quality and negatively affect bystanders.

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.[1] 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,[2] and about 37% of smokers in six European countries reported ever-exposure to SHA.[3]

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.[4] Nevertheless, many studies reported that SHA contains hazardous compounds such as nicotine, particulate matter (PM_1, $PM_{2.5}$, PM_{10}), volatile organic compounds, propylene glycol (PG), glycerol, metals, tobacco-specific nitrosamines (TSNAs), and flavourings.[5–9]

A large body of evidence has shown that some of the compounds 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,[9–11] home,[6] or e-cigarette conventions.[12,13] Additionally, airborne nicotine concentration was found to increase after an e-cigarette use session during an experimental study in a room,[10] and in an observational study in which the concentration in homes of e-cigarette users were compared to that of non-users' homes.[14] Some of TSNAs, such as N-nitrosonornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK), which are carcinogenic [15–18] have been identified in e-cigarette aerosol, although in low levels.[19]

Although the concentrations of toxic compounds in e-cigarette aerosols are lower than those emitted from conventional cigarettes,[8] 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,[20] diabetes, and cancer.[21] Exposure to nicotine may cause nicotine-related diseases, such as cardiovascular disease and impaired brain function.[22–24] 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.[8] In an experimental study, exposure to aerosolised glycerol caused a slight local irritant effects on the respiratory tract of mice.[25] Although e-cigarette use has been shown to cause inflammation in users and was recently linked to the development of respiratory diseases,[10,26–28] 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,[29–31] and exacerbate asthma symptoms in youth with asthma.[32]

Assessing the exposure to SHA in bystanders is important, because they may be involuntarily exposed to hazardous substances from the aerosol.[8] 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,[33] 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.

MATERIALS 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 vaporiser with nickel coil wire, a rechargeable battery (capacity 2600 mAh) and a cartridge containing the e-liquid (open tank).The coil was not changed throughout the experiments. The temperature of the e-cigarette used was set by the user (220^{o} C, 1 ohm, 40 watts) 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.. 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 m^2 x 2.50 m (35.2 m^3) office room in the Catalan Institute of Oncology, Barcelona. During the experiment, the user, the two non-users, and a researcher sat around a small table (60 x 120 cm); non-users sat 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 wase 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, but all participants remained in the room. The windows and the door were kept closed during the experiments, simulating a real-life situation during working hours. The room was ventilated by opening the windows for the most part of the day, before and after the experiments, and was kept unoccupied during the whole week when the experiments were not being conducted.

We used a medium-size car (VW Touran, interior size approx. 10 m^3) as the second setting, in which cigarettes or e-cigarettes were never used. During the experiment there were the user (sat on the front passenger seat), the two non-users (sat 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). Once the car running on the circuit, the experiment started. The car ran continuously on 1.3 km circuit at speed up to 70 km/h during the 40-minute experiment, with the two front windows half-opened (30 cm) and the rear windows closed, simulating a real-life situation of a car's short journey. The car was ventilated 15 minutes after each experiment by running the car without passengers and let all the windows fully open.

[Figure 1 here]

In both settings, any system of heating or air conditioning during the experiment was avoided. The relative humidity during all experiments was lower than 85%. During the five-day experiment, the range of the temperature in the room experiment was 22.0º - 26.3º C, with a mean temperature of 26.6º C and an outdoor mean temperature of 27.9º C. The temperature inside the car ranged from 25.7º to 32.5º C, with a mean of 25.7º C and an outdoor mean temperature of 29.5º C. 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.[34–36] 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 ($\mu g/m^3$) 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 $\mu g/m^3$ 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 SidePak^{TM} 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.[12,30,37] 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.[33,38] 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".[18,39]

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[30] 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 $PM_{2.5}$ ($\mu g/m^3$) 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) [33] 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 $PM_{2.5}$ 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 $\mu g/m^3$; IQR: 11-88 $\mu g/m^3$), while in the car, the concentration was the same for near- and far-field (median: 16 $\mu g/m^3$; IQR near-field: 10-31 $\mu g/m^3$, IQR far-field: 10-28 $\mu g/m^3$). Additionally, the concentrations of indoor $PM_{2.5}$ in pre-exposure period in both settings and at both distances (near and far-field) were significantly lower than the outdoor $PM_{2.5}$ levels. During exposure period, the levels of all indoor $PM_{2.5}$ were significantly higher than those of outdoors.

After the user stopped using the e-cigarette in the room, $PM_{2.5}$ concentration (median: 19 $\mu g/m^3$; IQR: 11-50 $\mu g/m^3$ and 12-40 $\mu g/m^3$ 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 $\mu g/m^3$; IQR: 11-88 $\mu g/m^3$) compared to the far-field (18 $\mu g/m^3$; IQR: 9-81 $\mu g/m^3$) exposure when the e-cigarette was used, but not after its use was stopped. After e-cigarette use was stopped, indoor $PM_{2.5}$ levels at both distances returned being lower than the outdoor $PM_{2.5}$ in the room, but not in the car.

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 $\mu g/m^3$; IQR: 6-10 $\mu g/m^3$, $p<0.001$) 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 ($p=0.483$).

[Table 1 here]

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.

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 5^{th} and 4^{th} 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 here]

Biomarkers

The non-users' median concentration of saliva nicotine, cotinine, 3-OH-cotinine, nornicotine, 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 here]

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 $\mu g/m^3$) was lower than those found in similar studies

conducted in a room (mean concentrations: 246.9-289.5 µg/m^3), and in cars (mean concentrations: 75-490 µg/m^3).[11,40] However, in those studies, the exposure period lasted shorter than the present study, (6.5 minutes [11] and 20 minutes [40]), did not utilise *ad libitum* use of e-cigarette [11,40], used different e-cigarette types (cigalike, tank, and adjustable model)[11], and higher nicotine level (12 mg/ml [40] and 18 mg/ml [11]) 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.[11,41] Also, the studies from Schober et al., 2019 and Volesky et al., 2018 [11,40] 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/m^3 in room and car, whilst the reported median values were 21 and 16 µg/m^3, 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/m^3 as daily average)[42] and the United States Environmental Protection Agency Air quality index (35 µg/m^3 as daily average), [43] 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/m^3 increase in $PM_{2.5}$ exposure, even when the concentration was below the limit value of 25 µg/m^3.[44] Extensive evidence indicates that e-cigarette particles and droplets are less toxic compared to cigarette smoke. Evidence also indicates that one single e-cigarette user generates substantially lower $PM_{2.5}$ concentration compared to that of cigarettes, but the concentration markedly increases with the increase in the number of e-cigarette users.[8] However, other studies focused on the physical properties of the aerosol and its deposition in the respiratory system have found that the numbers of e-cigarette droplets doubled those of cigarettes' particles [45,46]; thus, this should be taken into consideration when assessing the potential toxicity of e-cigarette aerosol and its compounds.

E-cigarette use increases indoor $PM_{2.5}$ 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 $PM_{2.5}$ concentration during puffing period to a mean concentration level that ranged from 20 $\mu g/m^3$ to 290 $\mu g/m^3$.[9–11] In an extreme situation, a study conducted during an e-cigarette convention found that the concentration of $PM_{2.5}$ was able to reach as high as 819 $\mu g/m^3$, [12] levels that are higher than in hookah cafes and bars that allow smoking inside.[47] The increased level of indoor $PM_{2.5}$ during exposure period at both settings was also confirmed by the higher concentrations of indoor $PM_{2.5}$ than that of the outdoors, while they were lower than the outdoor measurements in the pre-exposure period.

The increasing pattern of $PM_{2.5}$ concentration was also suggested by Figure 2, where the more puffs generated by the user, the higher the $PM_{2.5}$ concentration in the room and the car. This is consistent with the findings from a study where $PM_{2.5}$ peaks were concurrent with e-cigarette puffs made at homes of e-cigarette users [6] and another study conducted in a room.[11]

We found that $PM_{2.5}$ 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. $PM_{2.5}$ levels remained higher in the room, as also suggested by the comparison with the outdoor levels in the post-exposure period. The observed differences between room and car might be explained because the concentrations did not start dropping from the same level – $PM_{2.5}$ 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 $PM_{2.5}$ measurements.[48] Previous studies found a variation in the duration of $PM_{2.5}$ decay, from four minutes to one day after e-cigarette use stopped, depending of the peaked concentrations observed.[11,12,41] 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.[41] Thus, it is hypothesised that the setting's volume and air flow may play a role in the $PM_{2.5}$ evaporation rate. One aspect that merits a mention is the fact that e-cigarette aerosol starts evaporating within seconds, and thus there is a potential gap between the $PM_{2.5}$ concentration released by the puffing and that counted by the devices; this may result in a potential underestimation of the immediate $PM_{2.5}$ concentration exhaled by the user. Nevertheless, the $PM_{2.5}$ concentrations measured by the devices were likely to be closer to those inhaled by bystanders in real conditions as the devices were placed in typical distance of bystanders from e-cigarette user.

The variability observed between $PM_{2.5}$ 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.[11,49] 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.[49] 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.[9,10,40] 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.[50] The largest increase of airborne nicotine from e-cigarette use is in the particle phase compared to the gas phase.[9] 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.[51] 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.[10,50] Moreover, other factors determine nicotine yield from e-cigarette use, such as e-cigarette type and brand, PG/ vegetable glycerine ratio, and electrical power.[52] 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.[53] 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.[31] 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 TSNAs concentrations in

bystanders' saliva since they were below the LOQ, which was consistent with a previous study using urinary samples.[31] However, NNN and NNK were previously detected in e-cigarettes' emission,[19] 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.[7] This may reflect the effects of long or sustained exposure instead of short exposure to e-cigarette use. The unquatifiable 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.[8]

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.[30] 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.[8,21]

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.[54] 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.[4] 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, our sample included only two non-users, which made our findings on biomarkers and reported symptoms not generalisable. Nevertheless, regardless of the complexity of the study design (two

experiments one week apart, lasted 3.6 hours, replicated 5 times in consecutive days), we aimed to provide a comprehensive assessment of SHA exposure in the same individuals, avoiding potential inter-variability. Furthermore, we only tested one type of e-cigarette and e-liquid combination used by one e-cigarette user. Thus, the results of this study did not take into account different puffing topography by different users and 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.[55,56] 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.[57] Secondly, our study might not accurately estimate the actual $PM_{2.5}$ concentrations given the absence of a specific calibration factor for e-cigarette aerosol. Nevertheless, we consider it is an acceptable approach because SHA contains particles and the interpretation of the results is unlikely to change significantly, as a calibration factor would only affect the magnitude of the changes observed. Thirdly, we did not include a full control session with the same characteristics as the sessions in which the e-cigarette was used; instead, we provided a 5-minute baseline condition every day (pre-exposure period with no e-cigarette use and all participants present) for comparison as done in previous studies [11,49]. It is unlikely to observe an increase in $PM_{2.5}$ concentration because of the mobilisation of small particles from the surfaces since the participants were asked to be sat throughout the experiments. Nevertheless, if the activities without e-cigarette use in both settings generated $PM_{2.5}$, it has been taken into account by comparing the concentration in pre- vs during vs post-exposure across the five replications in the room, thus avoiding potential source of bias from the non-exposure condition.

Fourthly, we did not take into account the air exchange rate or other measures of ventilation conditions 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. As we wanted to reflect short-term exposure in real-life scenarios, we did not allow ventilation in the office room during the exposure, while in the case of the car, air exchange was allowed by a half-open window, as likely done in a typical setting in real-life conditions. Additionally, we took into account potential 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 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

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Data Availability Statement

Research data are not shared.

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REFERENCES

1. Destaillats H, Singer B, Salthammer T. Does vaping affect indoor air quality? *Indoor Air*. 2020;30(5):793-794. doi:10.1111/INA.12663

2. Amalia B, Liu X, Lugo A, et al. Exposure to secondhand aerosol of electronic cigarettes in indoor settings in 12 European countries: data from the TackSHS survey. *Tob Control*. 2021;30(1):49-56. doi:10.1136/tobaccocontrol-2019-055376

3. Tigova O, Amalia B, Castellano Y, et al. Secondhand exposure to e-cigarette aerosols among smokers: A cross-sectional study in six European countries of the EUREST-PLUS ITC Europe Surveys. *Tob Induc Dis*. 2019;16(2). doi:10.18332/tid/99117

4. Protano C, Manigrasso M, Avino P, Vitali M. Second-hand smoke generated by combustion and electronic smoking devices used in real scenarios: Ultrafine particle pollution and age-related dose assessment. *Environ Int*. 2017;107(July):190-195. doi:10.1016/j.envint.2017.07.014

5. Rachel G, Neal B, A. GS. E-Cigarettes. *Circulation*. 2014;129(19):1972-1986. doi:10.1161/CIRCULATIONAHA.114.007667

6. Fernández E, Ballbè M, Sureda X, Fu M, Saltó E, Martínez-Sánchez JM. Particulate Matter from Electronic Cigarettes and Conventional Cigarettes: a Systematic Review and Observational Study. *Curr Environ Heal Reports*. 2015;2(4):423-429. doi:10.1007/s40572-015-0072-x

7. Martínez-Sánchez JM, Ballbè M, Pérez-Ortuño R, et al. Secondhand exposure to aerosol from electronic cigarettes: pilot study of assessment of tobacco-specific nitrosamine (NNAL) in urine. *Gac Sanit*. 2019;33(6):575-578. doi:10.1016/j.gaceta.2018.07.016

8. National Academies of Sciences, Engineering and M. *Public Health Consequences of E-Cigarettes*. Vol 178. (Stratton K, Kwan LY, Eaton DL, eds.). National Academies Press; 2018. doi:10.17226/24952

9. van Drooge BL, Marco E, Perez N, Grimalt JO. Influence of electronic cigarette vaping on the composition of indoor organic pollutants, particles, and exhaled breath of bystanders. *Environ Sci Pollut Res*. 2019;26(5):4654-4666. doi:10.1007/s11356-018-3975-x

10. Schober W, Szendrei K, Matzen W, et al. Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. *Int J Hyg Environ Health*. 2014;217(6):628-637. doi:10.1016/j.ijheh.2013.11.003

11. Volesky KD, Maki A, Scherf C, et al. The influence of three e-cigarette models on indoor fine and ultrafine particulate matter concentrations under real-world conditions. *Environ Pollut*. 2018;243:882-889. doi:10.1016/j.envpol.2018.08.069

12. Soule EK, Maloney SF, Spindle TR, Rudy AK, Hiler MM, Cobb CO. Electronic cigarette use and indoor air quality in a natural setting. *Tob Control*. 2017;26(1):109-112. doi:10.1136/tobaccocontrol-2015-052772

13. Chen R, Aherrera A, Isichei C, et al. Assessment of indoor air quality at an electronic cigarette (Vaping) convention. *J Expo Sci Environ Epidemiol*. 2018;28(6):522-529. doi:10.1038/s41370-017-0005-x

14. Ballbè M, Martínez-Sánchez JM, Sureda X, et al. Cigarettes vs. e-cigarettes: Passive exposure at home measured by means of airborne marker and biomarkers. *Environ Res*. 2014;135:76-80. doi:10.1016/J.ENVRES.2014.09.005

15. Rivenson A, Hoffmann D, Prokopczyk B, Amin S, Hecht SS. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived N-nitrosamines. *Cancer Res*. 1988;48(23):6912-6917. Accessed December 8, 2019. http://www.ncbi.nlm.nih.gov/pubmed/3180100

16. Yuan J-M, Knezevich AD, Wang R, Gao Y-T, Hecht SS, Stepanov I. Urinary levels of the tobacco-specific carcinogen N'-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. *Carcinogenesis*. 2011;32(9):1366-1371. doi:10.1093/carcin/bgr125

17. Hecht SS. Biochemistry, Biology, and Carcinogenicity of Tobacco-Specific N -Nitrosamines. *Chem Res Toxicol*. 1998;11(6):559-603. doi:10.1021/tx980005y

18. Pérez-Ortuño R, Martínez-Sánchez JM, Fu M, et al. Assessment of tobacco specific nitrosamines (TSNAs) in oral fluid as biomarkers of cancer risk: A population-based study. *Environ Res*. 2016;151:635-641. doi:10.1016/j.envres.2016.08.036

19. Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23(2):133-139. doi:10.1136/tobaccocontrol-2012-050859

20. World Health Organization Regional Office for Europe. *Health Effects of Particulate Matter*. World Health Organization; 2013. Accessed August 14, 2019. http://www.euro.who.int/__data/assets/pdf_file/0006/189051/Health-effects-of-particulate-matter-final-Eng.pdf

21. Kim K-H, Kabir E, Kabir S. A review on the human health impact of airborne

particulate matter. *Environ Int*. 2015;74:136-143. doi:10.1016/j.envint.2014.10.005

22. Goriounova NA, Mansvelder HD. Short- and Long-Term Consequences of Nicotine Exposure during Adolescence for Prefrontal Cortex Neuronal Network Function. *Cold Spring Harb Perspect Med*. 2012;2(12):a012120-a012120. doi:10.1101/cshperspect.a012120

23. Ernst M, Matochik JA, Heishman SJ, et al. Effect of nicotine on brain activation during performance of a working memory task. *PNAS April*. 2001;10(8):4728-4733. Accessed August 12, 2019. www.pnas.orgcgidoi10.1073pnas.061369098

24. Qasim H, Karim ZA, Rivera JO, Khasawneh FT, Alshbool FZ. Impact of electronic cigarettes on the cardiovascular system. *J Am Heart Assoc*. 2017;6(9). doi:10.1161/JAHA.117.006353

25. Renne RA, Wehner AP, Greenspan BJ, et al. 2-Week and 13-Week Inhalation Studies of Aerosolized Glycerol in Rats. *Inhal Toxicol*. 1992;4(2):95-111. doi:10.3109/08958379209145307

26. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK. Short-term Pulmonary Effects of Using an Electronic Cigarette. *Chest*. 2012;141(6):1400-1406. doi:10.1378/chest.11-2443

27. Bhatta DN, Glantz SA. Association of E-Cigarette Use With Respiratory Disease Among Adults: A Longitudinal Analysis. *Am J Prev Med*. 2020;58(2):182-190. doi:10.1016/j.amepre.2019.07.028

28. Palamidas A, Tsikrika S, Katsaounou P, et al. Acute effects of short term use of e-cigarettes on airways physiology and respiratory symptoms in smokers with and without airways obstructive diseases and in healthy non smokers. *Tob Prev Cessat*. 2017;3(March). doi:10.18332/tpc/67799

29. Tzortzi A, Teloniatis S, Matiampa G, et al. Passive Exposure to E-cigarette Emissions: Immediate Respiratory Effects. *Tob Prev Cessat*. 2018;4(May):1-8. doi:10.18332/tpc/89977

30. Tzortzi A, Teloniatis S, Matiampa G, et al. Passive exposure of non-smokers to E-Cigarette aerosols: Sensory irritation, timing and association with volatile organic compounds. *Environ Res*. 2020;182(2020):108963. doi:10.1016/j.envres.2019.108963

31. Johnson JM, Naeher LP, Yu X, et al. A biomonitoring assessment of secondhand exposures to electronic cigarette emissions. *Int J Hyg Environ Health*. 2019;222(5):816-823. doi:10.1016/j.ijheh.2019.04.013

32. Bayly JE, Bernat D, Porter L, Choi K. Secondhand Exposure to Aerosols From Electronic Nicotine Delivery Systems and Asthma Exacerbations Among Youth With Asthma. *Chest*. 2019;155(1):88-93. doi:10.1016/j.chest.2018.10.005

33. Fernández E, López MJ, Gallus S, et al. Tackling second-hand exposure to tobacco smoke and aerosols of electronic cigarettes: the TackSHS project protocol. *Gac Sanit*. 2020;34(1):77-82. doi:10.1016/j.gaceta.2019.07.002

34. Fu M, Fernández E, Martínez-Sánchez JM, et al. Second-hand smoke exposure in indoor and outdoor areas of cafés and restaurants: Need for extending smoking regulation outdoors? *Environ Res*. 2016;148:421-428. doi:10.1016/j.envres.2016.04.024

35. López MJ, Fernández E, Gorini G, et al. Exposure to Secondhand Smoke in Terraces and Other Outdoor Areas of Hospitality Venues in Eight European Countries. Amre D, ed. *PLoS One*. 2012;7(8):e42130. doi:10.1371/journal.pone.0042130

36. Hammond SK, Leaderer BP, Roche AC, Schenker M. Collection and analysis of Nicotine as a marker for environmental tobacco smoke. *Atmos Environ*. 1987;21(2):457-462. doi:10.1016/0004-6981(87)90027-8

37. Czogala J, Goniewicz ML, Fidelus B, Zielinska-Danch W, Travers MJ, Sobczak A. Secondhand Exposure to Vapors From Electronic Cigarettes. *Nicotine Tob Res*. 2014;16(6):655-662. doi:10.1093/ntr/ntt203

38. Ruprecht AA, Invernizzi G, Dautzenberg B, et al. Mass Calibration and Relative Humidity Compensation Requirements for Optical Portable Particulate Matter Monitors: The IMPASHS (Impact of Smoke-free Policies in Eu Member States) Wp2 Preliminary Results. *Epidemiology*. 2011;22:S206. doi:10.1097/01.ede.0000392314.24613.c6

39. Pérez-Ortuño R, Martínez-Sánchez JM, Fernández E, Pascual JA. High-throughput wide dynamic range procedure for the simultaneous quantification of nicotine and cotinine in multiple biological matrices using hydrophilic interaction liquid

chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2015;407(28):8463-8473. doi:10.1007/s00216-015-8993-7

40. Schober W, Fembacher L, Frenzen A, Fromme H. Passive exposure to pollutants from conventional cigarettes and new electronic smoking devices (IQOS, e-cigarette) in passenger cars. *Int J Hyg Environ Health*. 2019;222(3):486-493. doi:10.1016/j.ijheh.2019.01.003

41. Zhao J, Nelson J, Dada O, Pyrgiotakis G, Kavouras IG, Demokritou P. Assessing electronic cigarette emissions: linking physico-chemical properties to product brand, e-liquid flavoring additives, operational voltage and user puffing patterns. *Inhal Toxicol*. 2018;30(2):78-88. doi:10.1080/08958378.2018.1450462

42. World Health Organization. WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide: Global update 2005. Published online 2005:1-21. doi:10.1016/0004-6981(88)90109-6

43. US EPA. The National Ambient Air Quality Standards for Particle Matter: Revised Air Quality Standards for Particle Pollution and Updates to the Air Quality Index (AQI). *Environ Prot Agency*. Published online 2012:1-5. http://www.epa.gov/pm/2012/decfsstandards.pdf

44. Beelen R, Raaschou-Nielsen O, Stafoggia M, et al. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. *Lancet (London, England)*. 2014;383(9919):785-795. doi:10.1016/S0140-6736(13)62158-3

45. Pichelstorfer L, Hofmann W, Winkler-Heil R, Yurteri CU, McAughey J. Simulation of aerosol dynamics and deposition of combustible and electronic cigarette aerosols in the human respiratory tract. *J Aerosol Sci*. 2016;99:125-132. doi:10.1016/j.jaerosci.2016.01.017

46. Manigrasso M, Buonanno G, Fuoco FC, Stabile L, Avino P. Aerosol deposition doses in the human respiratory tree of electronic cigarette smokers. *Environ Pollut*. 2015;196:257-267. doi:10.1016/j.envpol.2014.10.013

47. Cobb CO, Vansickel AR, Blank MD, Jentink K, Travers MJ, Eissenberg T. Indoor air quality in Virginia waterpipe cafés. *Tob Control*. 2013;22(5):338-343.

doi:10.1136/tobaccocontrol-2011-050350

48. Vardavas CI. Environmental tobacco smoke exposure in motor vehicles: a preliminary study. *Tob Control*. 2006;15(5):415-415. doi:10.1136/tc.2006.017459

49. Zhao T, Nguyen C, Lin C, et al. Characteristics of secondhand electronic cigarette aerosols from active human use. *Aerosol Sci Technol*. 2017;51(12):1368-1376. doi:10.1080/02786826.2017.1355548

50. Logue JM, Sleiman M, Montesinos VN, et al. Emissions from electronic cigarettes: assessing vapers' intake of toxic compounds, secondhand exposures, and the associated health impacts. *Environ Sci Technol*. 2017;51(16):9271-9279. doi:10.1021/acs.est.7b00710

51. Dawkins L, Turner J, Roberts A, Soar K. 'Vaping' profiles and preferences: an online survey of electronic cigarette users. *Addiction*. 2013;108(6):1115-1125. doi:10.1111/add.12150

52. EL-Hellani A, Salman R, El-Hage R, et al. Nicotine and Carbonyl Emissions From Popular Electronic Cigarette Products: Correlation to Liquid Composition and Design Characteristics. *Nicotine Tob Res*. Published online October 7, 2016:ntw280. doi:10.1093/ntr/ntw280

53. Melstrom P, Sosnoff C, Koszowski B, et al. Systemic absorption of nicotine following acute secondhand exposure to electronic cigarette aerosol in a realistic social setting. *Int J Hyg Environ Health*. 2018;221(5):816-822. doi:10.1016/j.ijheh.2018.05.003

54. Drehmer JE, Nabi-Burza E, Hipple Walters B, et al. Parental Smoking and E-cigarette Use in Homes and Cars. *Pediatrics*. 2019;143(4):e20183249. doi:10.1542/peds.2018-3249

55. Williams R. The rise of disposable JUUL-type e-cigarette devices. *Tob Control*. Published online December 5, 2019:tobaccocontrol-2019-055379. doi:10.1136/tobaccocontrol-2019-055379

56. Fadus MC, Smith TT, Squeglia LM. The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. *Drug Alcohol Depend*. 2019;201:85-93. doi:10.1016/j.drugalcdep.2019.04.011

57. Zare S, Nemati M, Zheng Y. A systematic review of consumer preference for e-

cigarette attributes: Flavor, nicotine strength, and type. Cormet-Boyaka E, ed. *PLoS One*. 2018;13(3):e0194145. doi:10.1371/journal.pone.0194145

TABLES

Table 1. Median concentration and its corresponding interquartile range (IQR) of $PM_{2.5}$ (both expressed in $\mu g/m^3$) 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[†]	P-value[‡]	P-value[§]
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[¶]	<0.001	<0.001	0.729			
P-value[††]	<0.001	<0.001	<0.001			
P-value[‡‡]	<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¶	0.001	0.474	0.483			
P-value††	<0.001	<0.001	<0.001			
P-value‡‡	<0.001	<0.001	<0.001			

† p-value for pre- vs. during exposure

‡ p-value for during vs. post-exposure

§ p-value for pre- vs. post-exposure

¶ p-value for near-field vs. far-field

†† p-value for near-field vs. outdoor

‡‡ p-value for far-field vs. outdoor

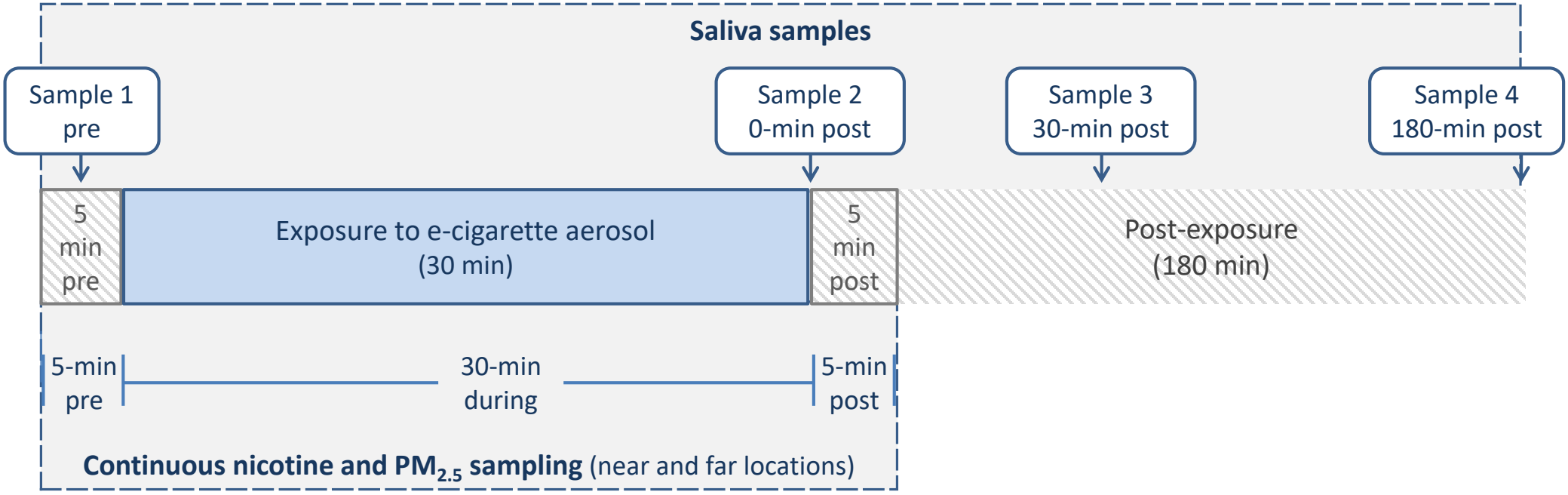
FIGURE LEGENDS

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

Figure 2. The time course of PM2.5 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.

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.



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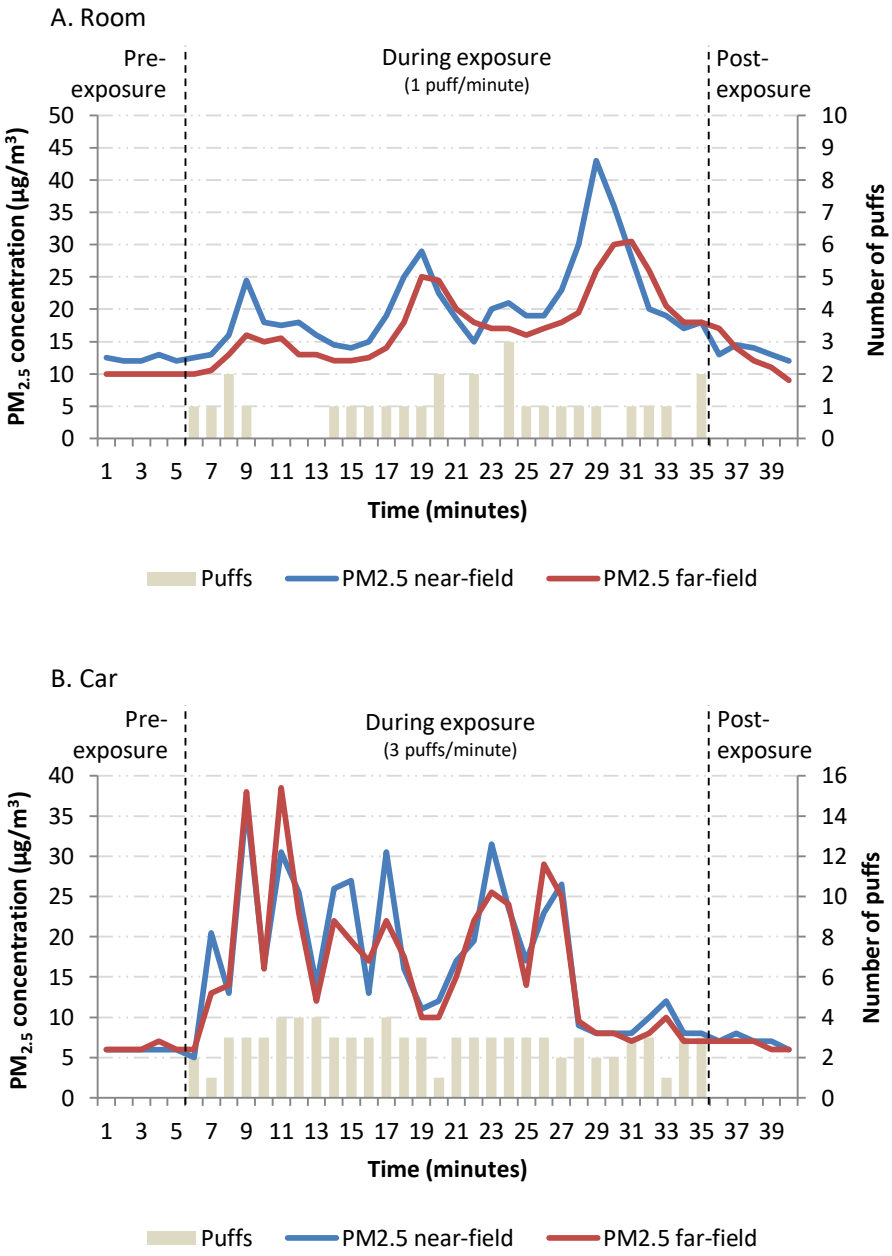

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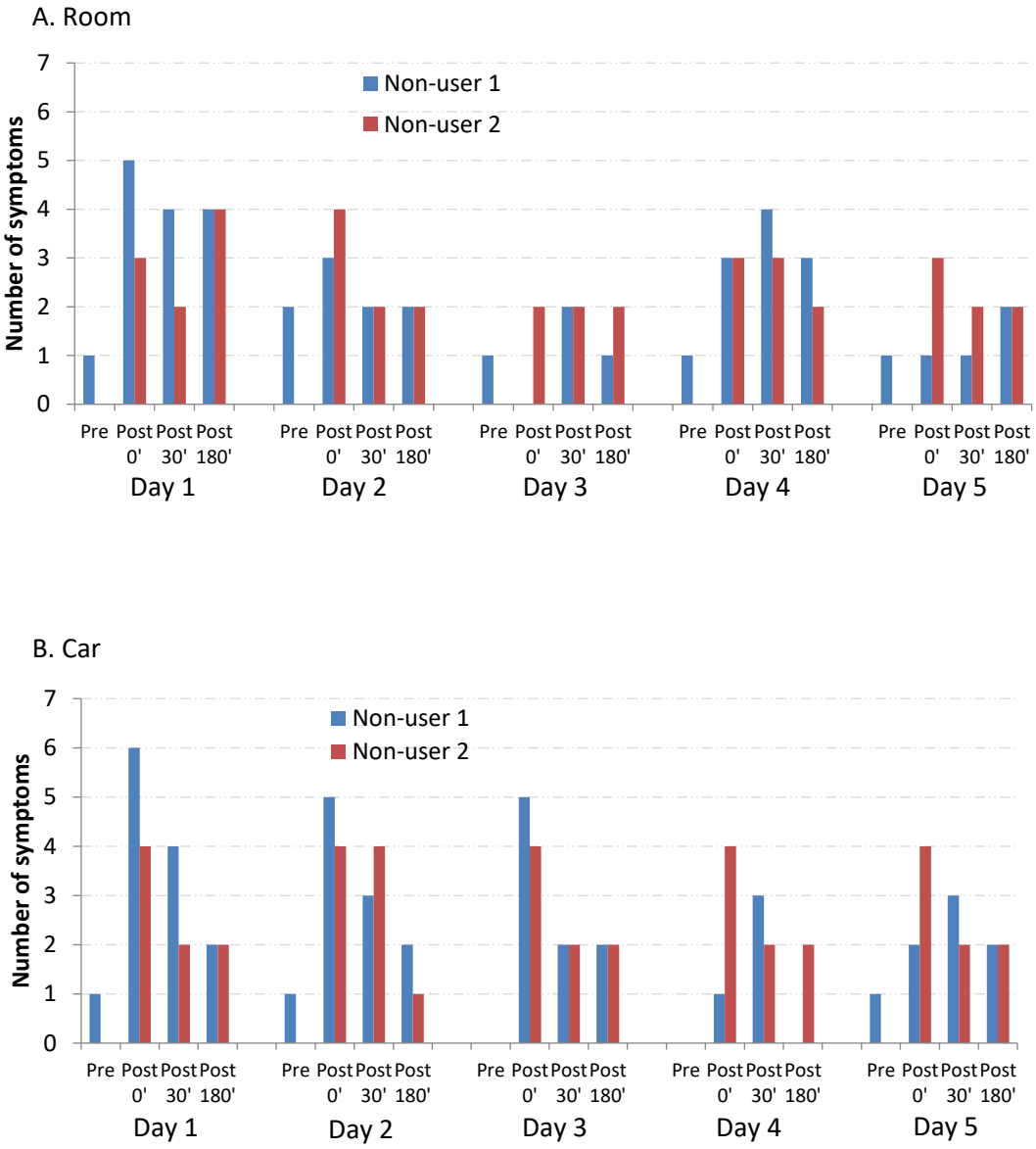

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