



Characterisation of mainstream and passive vapours emitted by selected electronic cigarettes



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ABSTRACT

Electronic cigarettes have achieved growing popularity since their introduction onto the European market. They are promoted by manufacturers as healthier alternatives to tobacco cigarettes, however debate among scientists and public health experts about their possible impact on health and indoor air quality means further research into the product is required to ensure decisions of policymakers, health care providers and consumers are based on sound science. This study investigated and characterised the impact of 'vaping' (using electronic cigarettes) on indoor environments under controlled conditions using a 30 m³ emission chamber. The study determined the composition of e-cigarette mainstream vapour in terms of propylene glycol, glycerol, carbonyls and nicotine emissions using a smoking machine with adapted smoking parameters. Two different base recipes for refill liquids, with three different amounts of nicotine each, were tested using two models of e-cigarettes. Refill liquids were analysed on their content of propylene glycol, glycerol, nicotine and qualitatively on their principal flavourings. Possible health effects of e-cigarette use are not discussed in this work. Electronic cigarettes tested in this study proved to be sources for propylene glycol, glycerol, nicotine, carbonyls and aerosol particulates. The extent of exposure differs significantly for active and passive 'vapers' (users of electronic cigarettes). Extrapolating from the average amounts of propylene glycol and glycerol condensed on the smoking machine filter pad to the resulting lung-concentration, estimated lung concentrations of 160 and 220 mg m⁻³ for propylene glycol and glycerol were obtained, respectively. Vaping refill liquids with nicotine concentrations of 9 mg mL⁻¹ led to vapour condensate nicotine amounts comparable to those of low-nicotine regular cigarettes (0.15–0.2 mg). In chamber studies, peak concentrations of 2200 µg m⁻³ for propylene glycol, 136 µg m⁻³ for glycerol and 0.6 µg m⁻³ for nicotine were reached. Carbonyls were not detected above the detection limits in chamber studies. Particles in the size range of 20 nm to 300 nm constantly increased during vaping activity and reached final peak concentrations of 7 × 10⁶ particles L⁻¹. Moreover, the tested products showed design flaws such as leakages from the cartridge reservoirs. Possible long term effects of e-cigarettes on health are not yet known. E-cigarettes, the impact of vaping on health and the composition of refill liquids require therefore further research into the product characteristics. The consumers would benefit from harmonised quality and safety improvements of e-cigarettes and refill liquids.

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Introduction

Electronic cigarettes (e-cigarettes) have become increasingly popular since their introduction onto the European market in 2005. Use in Great Britain, for example, more than doubled from 2.7% of vapers in 2010 to 6.7% in 2012 (Dockrell et al., 2013).

They are frequently advertised by manufacturers as a healthier alternative to tobacco cigarettes (Ayers et al., 2011) and a smoking cessation tool, and have become a popular substitute

for traditional tobacco because of indoor smoking restrictions on traditional tobacco cigarettes (Etter and Bullen, 2011).

Uncertainties about their impact on health and indoor air quality have caused debate among scientists and public health experts. Concerns most frequently relate to product safety in terms of product design, exposure to toxic products, potential for abuse (including dual use with tobacco products), use by young people and effectiveness in helping smokers to quit smoking tobacco cigarettes (Noel et al., 2011).

Although some studies have indicated that they are less harmful than smoking regular tobacco cigarettes (Caponetto et al., 2013; Wagener et al., 2012), e-cigarettes and refill liquids nonetheless require further research into the detail and composition of the

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products, as will be required under the newly revised Tobacco Product Directive (2014/40/EU), to ensure that the decisions of policymakers, health care providers and consumers are based on sound science (Etter et al., 2011).

Only a few studies have reported on the impact of e-cigarette vaping on indoor air quality (passive vaping). Schripp et al. (2013) found that volatile organic compounds (VOCs) and ultrafine particles (UFP) were released from an e-cigarette while actively vaping in an 8 m³ emission chamber. Schober et al. (2014) reported on VOC, particle and polycyclic aromatic hydrocarbons (PAHs), carbonyls and metals releases into a real office environment. This study also monitored the effect of vaping on FeNO release and the urinary metabolite profile. McAuley et al. (2012) compared the effects of e-cigarette vapour and cigarette smoke on indoor quality. In this study, vapours were generated using a smoking machine and were collected in a sampling bag for analysis. Fuoco et al. (2014) analysed e-cigarette generated aerosols in terms of particle number concentrations and size distribution.

Other studies have focused on safety and quality aspects of refill liquids. They reported inconsistent levels of nicotine (Goniewicz et al., 2013) and nicotine impurities (Trehy et al., 2011; Etter et al., 2013; Hutzler et al., 2014) among batches/brands. Williams et al. (2013) described the possibility of metals, or chemicals from plastics in the delivery system, leaching into the vapour before inhalation. Behar et al. (2014) identified toxicants in cinnamon-flavoured e-cigarette refill liquids.

This study proposes a systematic approach to characterise e-cigarette emissions under controlled conditions using a smoking machine with adapted smoking parameters for the generation of vapours from well characterised refill-liquids. The impact of vaping on the indoor environment was investigated introducing the generated vapours into a 30 m³ walk-in emission chamber operated under defined conditions (temperature, relative humidity and ventilation rate). The composition of e-cigarette mainstream vapours in terms of propylene glycol, glycerol, low molecular carbonyls and nicotine emissions was determined applying an adapted standardised smoking protocol for regular cigarettes. Two models of e-cigarettes were used in this study, differing primarily by the way in which refill liquids are evaporated. In order to cover the widest range possible, two very different base recipes for the refill liquid, each with three different amounts of nicotine, were used for the emission testing. Possible health effects of e-cigarette use are not discussed in this work.

Methods and materials

Table 1 summarises all measurements conducted in this study and the respective analytical methods which were applied.

Detailed description is provided in the following sections.

Electronic cigarettes used in study

Given the wide range of models and varieties of e-cigarettes available on the market, a globally representative study was not feasible. The choice of e-cigarette for this study was therefore primarily based on the most popular product type and the method by which refill liquids are evaporated. Two so called 'second generation' refillable e-cigarettes, which are available on the local market, were chosen as they belong to the most popular product type category according to a survey conducted by Dawkins et al. (2013). Differences in the way liquid is evaporated were considered choosing one type that used an atomiser (type A) and another that used a cartomiser (type B) for the evaporation of refill liquids: type A e-cigarettes (atomiser) consisted of three parts: the reservoir containing the refill liquid, the atomiser which conducted the liquid

via a wick to the atomising chamber, and the battery. Type B e-cigarettes (cartomiser) were composed of two parts: the cartomiser body (containing the filler material and a heating coil) and the battery. Both e-cigarettes resembled regular tobacco cigarettes. Type A and type B e-cigarettes were equipped with a 280mAh and a 180mAh battery, respectively. Type A e-cigarettes were button-activated and type B e-cigarettes were automatically activated by inhalation.

Detail and composition of e-cigarette refill liquids

The selection of refill liquids was made based on the same principles as the selection of e-cigarettes. A wide variety of liquids is available on the market, mainly differing in their flavouring and content of propylene glycol (PG) and glycerol. All investigations as part of this study, except those in the Qualitative determination of flavourings section, were consistently based on two different refill liquids. The flavouring chosen for both types of refill liquids was 'tobacco-like' as it is the most commonly-sold flavouring on the market (Dawkins et al., 2013). The difference between the two refill liquids was that the first ('traditional') was composed of approximately equal parts of propylene glycol (50%) and glycerol (40%) as a base (and 10% water) whereas the base of the second one ('velvet') consisted of only glycerol (80%) and water (20%). It was expected that these differences would influence the emission behaviour when vaped. Both types of refill liquids (velvet and traditional) were purchased on the internet from one of the principal e-cigarette refill liquid-selling companies in Italy with three levels of nicotine: no nicotine, 9 mg mL⁻¹ nicotine and 18 mg mL⁻¹ nicotine. All liquids were produced no more than three months before the tests took place and where stored in a cool and dark place throughout the duration of the tests.

Determination of propylene glycol, nicotine and glycerol in refill liquids

Refill liquids were diluted in isopropyl alcohol (*i*-propanol) and analysed with a gas chromatographic (GC) system coupled to a flame ionisation detector (FID). Due to strongly differing contents of nicotine and propylene glycol/glycerol, two dilutions of the refill liquids were prepared (17 times and 667 times, respectively) before injection. Diluted solutions were quantified against external calibration. A five point calibration curve was prepared in *i*-propanol. It ranged from 0.4 to 2 mg mL⁻¹ for the three investigated compounds. The gas chromatographic system used was an Agilent 6890 (Agilent Technologies, CA, USA). Chromatographic separation was achieved using a J&W Scientific capillary column (DB-624, 30 m, 0.25 mm i.d., 1.4 µm film thickness, Part-No. 122-1334) and resulted in nearly symmetric and baseline separated peaks. Injector and detector temperatures were set at 250 °C, the split ratio was 1:30, and the carrier gas flow was constant (1.5 mL min⁻¹). The oven programme used was: starting temperature 100 °C, ramp 10 °C min⁻¹ up to 260 °C, isotherm at 260 °C for 5 min (total runtime: 21 min). Detection limits for nicotine, propylene glycol and glycerol were 1.0, 2.2 and 4.0 µg mL⁻¹, respectively.

Qualitative determination of flavourings

In addition to the tobacco-like flavoured refill liquid used for all measurements in this study, a supplementary selection of other refill liquids was examined to qualitatively investigate their volatile components of these samples which might be responsible for their respective characteristic flavours. This was done by headspace analysis of non-diluted refill liquids. Approximately 1.5 g of each refill liquid was weighed in a 20 mL vial. The vial was subsequently thermostated at 100 °C for 20 min under constant agitation using a Gerstel Multi-Purpose Sampler MPS-2 Twister (Gerstel GmbH, Mühlheim, Germany). One millilitre of the headspace vapour was

Table 1
Overview of analytical parameters determined in the current study and the respective applied analytical methodologies.

Matrix	Analytical parameter	Analytical methodology	
Refill liquid	Nicotine, propylene glycol, glycerol	GC–FID	
	Flavourings	Headspace GC–MS	
	Water	Karl Fischer Titration	
Vapour	Filter Pad/Tedlar® Bag	Nicotine, propylene glycol, glycerol	Smoking Machine
			Liquid extraction of glass fibre filter
			GC–FID
		Volatile carbonyl compounds	Smoking Machine
			Tedlar® Bag
			Sep-Pak DNPH silica cartridge
Environmental chamber	Nicotine, propylene glycol, glycerol		HPLC–UV/vis
			30 m ³ walk-in chamber
			Modified Smoking Machine
	Volatile carbonyl compounds		Tenax® TA tubes
			GC–MS
			30 m ³ walk-in chamber
Aerosol particulate concentration (particles with an optical equivalent particle size <0.3 µm)		Modified Smoking Machine	
		Optical Particle Counter	
		30 m ³ walk-in chamber	
Aerosol particulate concentration (particles with an optical equivalent particle size 0.02–1 µm)		Modified Smoking Machine	
		Ultrafine Particle Counter	

finally injected into a GC–MS system (Agilent 6890 GC coupled to 5973 MS). Chromatographic separation was achieved using an HP-5MS capillary column (J&W Scientific, 30 m × 250 µm × 0.25 µm, Product Code 190915-433). Injector temperature was set at 250 °C. The oven programme started at 60 °C. This temperature was kept constant for 5 min; a 10 °C min⁻¹ gradient reached 300 °C with a final isotherm of 4 min. The most abundant (largest peak areas) compounds were identified by comparing mass-spectra of the samples with NIST library mass-spectra.

The refill liquids examined were:

- two mint-flavoured liquids, one with and one without nicotine;
- two tobacco-flavoured liquids (including those used for all measurement in this study: 'Traditional' and 'velvet')
- one liquid with a Cuban cigar flavour;
- one liquid advertised as having the taste of 'strong, dark tobacco, long lasting and sharp with dark cocoa nuances';
- one liquid advertised as possessing 'passion flavour'.

Levels of nicotine are reported in Table 2.

Determination of water content (Karl-Fischer Titration)

Water was determined in 100 µL aliquots of the undiluted refill liquids by Karl-Fischer titration. A Metrohm 870 KF Titrino Plus Karl Fischer titrator (Metrohm, Herisau, Switzerland) was used, applying the one-component technique with Hydranal Titrant Composite 5 (Sigma Aldrich, St. Louis, USA, Product Code 34805). Pure, dry methanol (Sigma Aldrich, St. Louis, USA, Product Code 34741) was used as solvent. The average content was calculated from three replicates for each refill liquid. For quality assurance, exactly 15 µL of water was added to the solvent and the recovery calculated. Recovery ranged from 98 to 100.5%.

Determination of propylene glycol, nicotine and glycerol in vapour condensate using an analytical cigarette-smoking machine

Analytical cigarette-smoking machine

A single channel Borgwaldt RM-1 Plus smoking machine (Borgwaldt KC GmbH, Hamburg, Germany) was used. Smoking

parameters and standard conditions as defined in ISO 3308 (2012) for standardised smoking of regular cigarettes were adapted in line with the findings of Farsalinos et al. (2013) who investigated e-cigarette use topography in 45 experienced e-cigarette users. The puff duration was set at 4.0 s, the puff volume at 35 mL and puff frequency at one puff every 30 s. The activation button for type A e-cigarettes was pressed one second before the puff started and for the whole duration of the puff. A bell-shaped puff profile was chosen. The puff number was set at 13 puffs corresponding to the average amount of puffs per vaping event (Farsalinos et al., 2013). The vapour condensate of 13 puffs was collected on a 44 mm glass fibre filter pad (Borgwaldt KC GmbH, Hamburg Germany, Code 8020 285 1) placed in a filter holder which is part of the smoking machine. Six filter replicates were collected and analysed for each type of e-cigarette and refill liquid.

Analysis

The glass fibre filter was transferred to an Erlenmeyer flask and was subsequently extracted with 20 mL of *i*-propanol. Full extraction was obtained after 60 min of gentle shaking. The extract was then filtered through a 0.45 µm PVDF disc filter (Millipore, Billerica MA, USA, Code SLHVX13NK). The extract was analysed by gas chromatography coupled to a flame ionisation detector (FID) and quantified against external calibration. The calibration curve was prepared in isopropanol and ranged from 3–20 µg mL⁻¹ for nicotine and 100–600 µg mL⁻¹ for propylene glycol and glycerol.

Separation by gas chromatography as described in the Determination of propylene glycol, nicotine and glycerol in refill liquids section. The detection limits for nicotine, propylene glycol and glycerol in the 20 mL filter extract solution were 1.0, 2.2 and 4.0 µg mL⁻¹, respectively.

Extraction recovery rates were determined by spiking blank glass fibre filters ($n = 10$) with known amounts of nicotine, PG and glycerol (150 µg, 1.2 mg and 1 mg, respectively) and ranged from 93–104%, 100–102% and 85–101% for propylene glycol, nicotine and glycerol, respectively.

The retention efficiency of the filter pad for propylene glycol, glycerol and nicotine was verified by collecting exhaust air in gas sampling bags (located behind the filter pad) and subsequently

Table 2
Most abundant volatile compounds identified in the headspace of selected refill liquids.

Refill liquid identification	Nicotine content [mg mL ⁻¹] ^a	Main compounds identified in headspace (except nicotine)
Mint flavour	6	(-)-Isopulegol (CAS 89-79-2) Menthol (CAS 15356-70-4) (R)-(+)-Pulegone CAS 89-82-7 Piperitone (CAS 89-81-6) <i>trans</i> -Carane (CAS 18968-23-5) Menthyl acetate (CAS 89-48-5) <i>cis</i> -Carane (CAS 554-59-6) Menthol (CAS 15356-70-4) Dextro-Carvone (CAS 2244-16-8) rac-Linalool (CAS 22564-99-4) Anethol (CAS 104-46-1) α -Cedrene (CAS 469-61-4) 1,6-Dimethylnaphthalene (CAS 575-43-9) gamma-Murolene (CAS 30021-74-0) delta-Cadinene (CAS 483-76-1) (+)-Cuparene (CAS 16982-00-6) Cadalene (CAS 483-78-3) Valeraldehyde propylene glycol acetal (CAS 74094-60-3) Menthol (CAS 15356-70-4) <i>cis</i> -Carane (CAS 554-59-6) Ethyl maltol (CAS 4940-11-8)
Mint flavour	No nicotine	
Tobacco flavour ^b	9	
Tobacco flavour	No nicotine	
Cuban cigar flavour	No nicotine	
Advertised as 'strong, dark tobacco, long lasting and sharp with dark cocoa nuances'	9	
Advertised as 'passion flavour'	9	

^a Declared by the manufacturer.

^b Filler liquid used for all measurement in this study ('velvet' and 'traditional').

analysing it by means of Tenax[®] TA tubes, thermal desorption and GC-MS analysis. None of the investigated compounds were detected in the gas sampling bag, thus proving the quantitative retention of the investigated compounds on the filter pad.

Determination of carbonyls formed during vaping of e-cigarette using an analytical cigarette-smoking machine – Determination in gas sampling bag

The two e-cigarette models, filled with both 'traditional' and 'velvet' 9 mg mL⁻¹ nicotine refill liquids, were vaped using an analytical cigarette smoking machine in line with the smoking regime described under the Determination of propylene glycol, nicotine and glycerol in vapour condensate using an analytical cigarette-smoking machine section. A 2 L Tedlar[®] gas-sampling bag was directly connected to the exhaust port of the smoking machine. Cambridge filter pads were not removed from the smoking machine, thus letting through only gaseous compounds including volatile carbonyl compounds such as formaldehyde, acetaldehyde, acrolein and acetone. Twenty puffs plus an additional five cleaning puffs (35 mL each) were collected in the gas sampling bag. Bags were filled with nitrogen to reach a total volume of 2 L.

Two litres of air from the gas sampling bag were collected at a sampling rate of 100 mL min⁻¹ on Sep-Pak DNPH-silica cartridges (Sep-Pak DNPH-Silica cartridges, Plus Short Body 360 mg, Part No. WAT037500, Waters Corporation, Milford, USA).

The cartridges were eluted with acetonitrile into a 2 mL volumetric flask and analysed against external calibration with an HPLC system coupled to a diode array detector according to ISO 16000-3 (2011). The liquid chromatographic system (HPLC) used was an Agilent Series 1100 system (Agilent Technologies, Inc., Santa Clara, CA, USA), composed of a G1312A binary pump, a G1379A degassing device, a G1329A autosampler and a G1315B diode array detector set at a wavelength of 360 nm. Chromatographic separation was achieved using a Phenomenex Kinetex XB-C18, 100 Å, 2.6 μ m (4.6 \times 75) mm column (Phenomenex, Macclesfield, UK) and a mobile phase composed of acetonitrile and water which allowed the separation of acetone-DNPH from acrolein-DNPH running a linear gradient from 20% to 90% acetonitrile in 40 min at a flow rate of 1 mL min⁻¹. Due to the possible formation of multiple derivative peaks, the determination of acrolein, with the above described method, might underestimate the real concentration

and therefore should be considered semi-quantitative (Herrington and Hays, 2012; Clark et al., 1994).

The relative quantification limits for formaldehyde, acetaldehyde, acetone and acrolein were approximately 1 μ g m⁻³.

Chamber tests

Both e-cigarette and refill liquid types (traditional and velvet) were tested in chamber studies. Liquids containing 9 mg mL⁻¹ nicotine were used for these studies.

Chamber description

A 30 m³ walk-in environmental chamber was used in this study. The chamber allowed precise control of temperature, relative humidity and air exchange rate. The air exchange rate was set by introducing defined volumes of air per unit of time and this was verified by means of tracer gas dilution tests using sulfur hexafluoride (ASTM Standard E741, 2011). Using only stainless steel, glass and 'non-stick' poly-tetrafluoroethylene (PTFE) polymer coatings in the interior of the chamber minimised pollutant adsorption and re-emission by the inner walls. The chamber was filled with ultra-clean pre-dried and filtered air. The chamber was run at an air exchange rate of 0.5 h⁻¹ to realistically simulate an indoor environment. The temperature was set at 23 °C and the relative humidity at 50%. Homogeneity of the atmosphere inside the chamber was ensured by three internal fans.

Modification of analytical smoking machine for use in chamber studies

The aim of all the chamber studies was to determine the characteristics of e-cigarette emissions under controlled conditions (temperature, relative humidity and air exchange rate). The smoking machine was adapted to meet these requirements. Keeping the glass fibre filters in line between the e-cigarette and the piston of the smoking machine and introducing the exhaust of the smoking machine into the chamber would have altered the vapour composition. Removing the filters would have led to contamination of the piston and pneumatic valves resulting in potential pollutant carry-over. An empty gas washing bottle of 500 mL was therefore placed between the e-cigarette and the smoking machine (Fig. 1). The puff volume was calibrated where the e-cigarette was connected to the gas washing bottle. A wash bottle volume of 500 mL

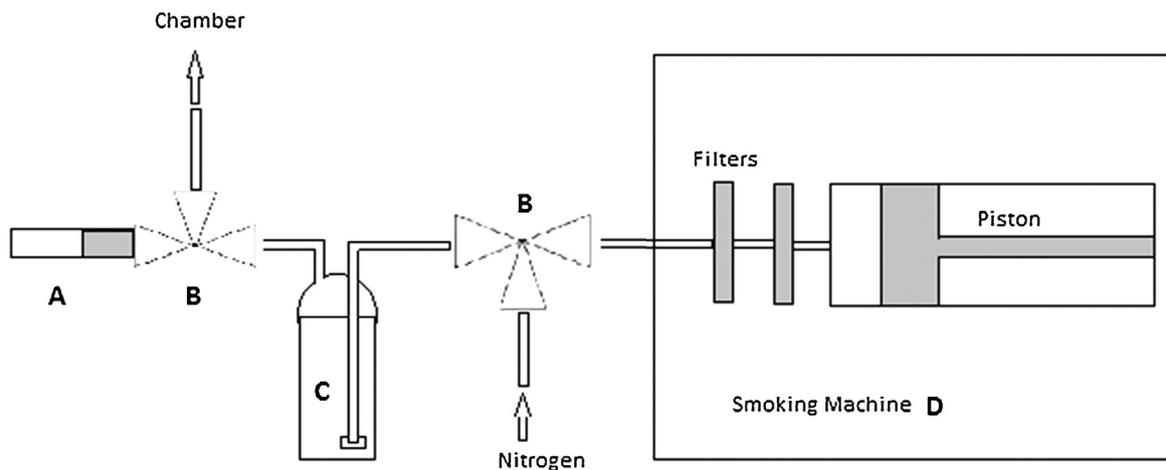


Fig. 1. Scheme of modified smoking machine setting for chamber tests. The smoking machine (D) aspirates defined volume of air. The vapour of e-cigarette (A) is trapped in wash bottle (C). Three-way valve (B) switches and a strong nitrogen flow evacuates wash bottle content into chamber.

was chosen, as it proved to contain the whole of each 35 mL puff volume without any loss. At the end of each puff, all vapour trapped inside the gas washing bottle was removed by flushing it with a strong ($>30\text{ L min}^{-1}$) flow of nitrogen for approximately 15 s and conducted into the chamber.

The vaping protocol for all chamber experiments consisted of six series of 13 puffs separated by 5 min intervals, resulting in a total vaping time of approximately 65–70 min. Vaping conditions (puff volume, intermission, puff profile) were as described under the Analytical cigarette-smoking machine section.

Sampling and determination of propylene glycol, nicotine and glycerol in chamber air

Nicotine, glycerol and propylene glycol were sampled on Tenax[®] TA filled stainless steel tubes for use with Perkin Elmer TurboMatrix thermal desorbers (Sigma-Aldrich, St. Louis, USA; Perkin Elmer thermal desorption tubes, Product code 25055). Two litres of chamber air were sampled at a flow rate of 100 mL min^{-1} . A control (blank) sample was taken before starting to vape. During the vaping process, three consecutive 20-min samples were collected, covering the whole duration of the vaping process. For type B e-cigarettes the investigation period was extended to a total sampling time of 180 min, equivalent to nine consecutive samples of 20 min each. This allowed for observation of how emitted compounds decayed after the vaping process stopped. Tubes were thermally desorbed and analysed with GC-MS in line with ISO method 16017-1 (ISO 16017-1, 2000). The gas chromatographic system used was an Agilent 7890A (Agilent Technologies, Santa Clara, CA, USA) equipped with a model 5975C mass selective detector. The thermal desorption system used was a Perkin Elmer Turbo Matrix 650 (Perkin Elmer, Waltham, USA). Chromatographic separation was achieved using a J&W Scientific capillary column (DB-624, 30 m, 0.25 mm i.d., 1.4 μm film thickness). Analytical calibration was achieved by means of a spiking liquid injected onto conditioned sorbent tubes. The detection limit for propylene glycol, nicotine and glycerol were 1.0, 0.6 and $2.0\text{ }\mu\text{g m}^{-3}$, respectively. During the thermal desorption process nicotine was found to partially undergo thermal decomposition to β -nicotyrine. The portion of decomposed nicotine was below 5% and therefore deemed to be negligible.

Sampling and determination of carbonyls in chamber air

A control sample of approximately 100 L at a flow of 1.5 L min^{-1} before starting the vaping procedure and a sample covering the complete vaping duration of approximately 65–70 min (Volume: approx. 100 L, Flow: 1.5 L min^{-1}) were trapped on Sep-Pak

DNPH-silica cartridges (Sep-Pak DNPH-Silica cartridges, Plus Short Body 360 mg, Part No. WAT037500, Waters Corporation, Milford, USA). Cartridges were eluted and analysed as described in the Determination of carbonyls formed during vaping of e-cigarette using an analytical cigarette-smoking machine – Determination in gas sampling bag” section. Due to the possible formation of multiple derivative peaks, the determination of acrolein with the above described method might underestimate the real concentration and thus should be considered semi-quantitative (Herrington and Hays, 2012; Clark et al., 1994).

Detection limits were 0.03, 0.04 and $0.05\text{ }\mu\text{g m}^{-3}$ for formaldehyde, acetaldehyde, and acetone and acrolein, respectively.

Measurement of aerosol particulate concentrations

Two types of real-time laser photometers (Optical Particle Counters, OPCs) were used for all particulate measurements conducted in this study. Particles with an optical equivalent particle size range of $>0.3\text{ }\mu\text{m}$ were measured using a GRIMM model 1.108 portable aerosol spectrometer (GRIMM Aerosol Technik GmbH & Co. KG, Ainring, Germany) and particles with an optical equivalent particle size range of $0.02\text{--}1\text{ }\mu\text{m}$ were measured using a P-Trak[®] Ultrafine Particle Counter (TSI Model 8525, TSI Incorporated, Shoreview, USA), an instrument based on the condensation particle counting technique using isopropyl alcohol. Both instruments were calibrated by the manufacturers against certified calibration standards: P-Trak against the PortaCount Bench 1 calibration standard and GRIMM against Arizona Test Dust (ISO 12103-1, 1997). Data collection frequency was one point per minute. Measurement units were number of particles per millilitre of air (particles per cubic centimetre). Chamber air was aspirated by the instrument through an antistatic tube approximately 1 m long.

Results and discussion

Characterisation of e-cigarette refill liquids

Determination of propylene glycol, nicotine, glycerol and water in refill liquids

On the accompanying leaflet provided by the manufacturer, proportions of propylene glycol and glycerol were declared as being 46% and 45% for ‘traditional’ and 83% glycerol for ‘velvet’ refill liquids. Values measured in this study (Table 3) are very close to the declared values. The similarity of concentrations for propylene glycol and glycerol between refill liquid bottles with differing

Table 3
Nicotine, propylene glycol, glycerol and water contents determined for investigated refill liquids. The lower row in each cell represents the percentage amount in the liquid.

Liquid identification	Nicotine [mg mL ⁻¹]	Propylene glycol [mg mL ⁻¹]	Glycerol [mg mL ⁻¹]	Water content [%]
Replicates (n=)	5	5	5	3
Traditional (0 mg mL ⁻¹)	<DL ^a	533.9 ± 5.5 (45.5 ± 0.6)%	496.2 ± 8.7 (42.4 ± 0.88)%	10.3 ± 0.05
Traditional (9 mg mL ⁻¹)	10.3 ± 0.1 (0.88 ± 0.01)%	538.7 ± 15.3 (45.1 ± 0.85)%	490.1 ± 19.8 (41.0 ± 1.4)%	9.5 ± 0.55
Traditional (18 mg mL ⁻¹)	20.8 ± 0.1 (1.78 ± 0.02)%	530.7 ± 11.3 (45.5 ± 0.8)%	469.5 ± 7.1 (40.2 ± 0.7)%	11.4 ± 0.11
Velvet (0 mg mL ⁻¹)	<DL ^a	<DL ^a	962.6 ± 17.5 (80.2 ± 1.7)%	25.9 ± 0.22
Velvet (9 mg mL ⁻¹)	10.2 ± 0.11 (0.83 ± 0.008)%	<DL ^a	965.4 ± 3.9 (79.3 ± 0.93)%	19.8 ± 1.1
Velvet (18 mg mL ⁻¹)	19.5 ± 0.28 (1.59 ± 0.03)%	<DL ^a	966.8 ± 14.3 (79.7 ± 1.3)%	16.8 ± 0.09

^a Below the limit of detection.

nicotine contents suggests a manufacturing process that adds different amounts of nicotine to a pre-mixed base of propylene glycol and glycerol. Nicotine contents determined in this study systematically exceed the values declared on the liquid refill bottles by approximately 10%. In practice, however, this seems to be non-critical in terms of increased inhalation of nicotine while vaping. Liquids declared as nicotine-free were proven to contain no nicotine above the detection limit.

The sum of the main components reported in Table 3 ranged from 98 to 106%. Differences from 100% can be explained by analytical variation and undetermined amounts of fragrance.

Results presented in this study were all obtained using refill liquids from the same batch, produced no more than three months before analysis. Analysis conducted on earlier batches, however, showed differences in composition. Analysis of a 'traditional' refill liquid with a declared nicotine level of 9 mg mL⁻¹ that was purchased approximately three months earlier than that used for all other studies, resulted in determined levels of 9.3 mg mL⁻¹ of nicotine, 324 mg mL⁻¹ of propylene glycol and 605 mg mL⁻¹ of glycerol. Evident differences in concentrations for propylene glycol and glycerol were observed, with glycerol being the main component in the older batch. Analysis conducted on a refill bottle of the same type purchased approximately one year earlier showed similar results, except that the water content was approximately 5% higher compared to the most recent batch which could be explained by the hygroscopic nature of glycerol.

In conclusion, notable differences in component concentrations were detected between different batches of the same type of refill liquid.

Table 4
Absolute amounts of nicotine, propylene glycol and glycerol trapped on filter (13 puffs). Values correspond to the average of six replicates (n = 6) and the respective standard deviation.

Traditional						
Declared conc. of nicotine	Nicotine [µg/filter]		Propylene glycol [mg/filter]		Glycerol [mg/filter]	
	e-cig Type A	e-cig Type B	e-cig Type A	e-cig Type B	e-cig Type A	e-cig Type B
0 mg mL ⁻¹	<DL ^a	<DL ^a	11.5 ± 0.8	12.9 ± 0.8	14.3 ± 1.4	18.2 ± 2.3
9 mg mL ⁻¹	0.21 ± 0.03	0.11 ± 0.01	10.7 ± 1.4	12.8 ± 0.8	19.5 ± 3.8	16.7 ± 2.7
18 mg mL ⁻¹	0.42 ± 0.02	0.46 ± 0.02	12.1 ± 0.7	12.7 ± 0.6	18.6 ± 1.0	18.3 ± 1.0
Velvet						
Declared conc. of nicotine	Nicotine [µg/filter]		Propylene glycol [mg/filter]		Glycerol [mg/filter]	
	e-cig Type A	e-cig Type B	e-cig Type A	e-cig Type B	e-cig Type A	e-cig Type B
0 mg mL ⁻¹	<DL ^a	<DL ^a	<DL ^a	<DL ^a	40.0 ± 4.9	21.0 ± 0.8
9 mg mL ⁻¹	0.25 ± 0.02	0.16 ± 0.02	<DL ^a	<DL ^a	45.2 ± 3.5	37.1 ± 3.6
18 mg mL ⁻¹	0.42 ± 0.03	0.30 ± 0.02	<DL ^a	<DL ^a	33.0 ± 2.0	22.5 ± 1.4

^a Below the limit of detection.

Qualitative determination of flavourings

Table 2 lists the most abundant compounds identified in the headspace vapour of the refill liquids investigated in this study under the given conditions. Most substances are flavouring agents and terpenes typically used for flavouring food. The substance 1,6-dimethylnaphthalene, for example, has the flavour of smoked food and is sold as liquid smoke flavouring. Companies manufacturing refill liquids do indeed sometimes also sell food flavourings. Other than the first and the fourth liquid in Table 2, all other liquids seem to contain only a few major components responsible for flavour.

Determination of propylene glycol, nicotine and glycerol in vapour condensate using an analytical cigarette-smoking machine

The use of a smoking machine aimed at generating and measuring cigarette emissions under defined vaping conditions, obtaining thus comparable values between the two e-cigarette types investigated in this study. Currently no standard smoking regime for e-cigarettes is available. Settings have therefore been adapted in line with the findings of Farsalinos et al. (2013) as described in the Analytical cigarette-smoking machine section. Results obtained during these measurements are shown in Table 4. Unexpectedly, the amount of nicotine found on the filter pads and therefore present in the vapour condensate was not always proportionate to the concentrations of nicotine in the vaped refill liquids. Results do not allow any trend, such as a difference between the two types of e-cigarettes or between the two types of refill liquids vaped, to be clearly identified. In general, it seems that emissions are

comparable between both e-cigarette types and the tested refill liquids. The difference in results may be explained by the difficulty in keeping emissions of e-cigarettes constant, despite trying to minimise any variance through use of a smoking machine. The supply of refill liquid to the atomising chamber in type A e-cigarettes or to the heating coil in type B e-cigarettes sometimes varied because of the formation of bubbles or diminishing quantities of liquid.

Results do however show that vaping refill liquids with nicotine concentrations of 9 mg mL^{-1} is in line with what is believed to be the equivalent of smoking one single regular tobacco cigarette (13 puffs, 4.0 s intermission) results in vapour condensate nicotine amounts of around 0.15–0.20 mg. In terms of inhalation of nicotine this is comparable with smoking a low-nicotine regular tobacco cigarette (Charles et al., 2011) under standard smoking conditions as defined under ISO 3308 (2012). Vaping refill liquids containing 18 mg mL^{-1} (under the conditions set in the current study) can be compared to smoking a 0.5 mg nicotine regular tobacco cigarette.

Amounts of propylene glycol condensed on the filter pad were very similar for both types of e-cigarette. The ratio of propylene glycol to glycerol is approximately 1:1, reflecting the proportions of these two compounds in the refill liquid and indicating that both components are evaporated with the same efficiency. Results obtained when vaping the ‘velvet’ refill liquid, which contains only glycerol, indicate a more efficient heating – and therefore evaporation – process in type A e-cigarettes, as amounts of glycerol on the filter pad are higher.

Extrapolating in a simplified way from the average amounts of propylene glycol and glycerol condensed on the filter pad to one puff only and the resulting concentration in the lungs (assuming 5 L of lung volume) for each puff, results in estimated lung concentrations of 160 and 220 mg m^{-3} for propylene glycol and glycerol, respectively when vaping the ‘traditional’ refill liquid and 460 mg m^{-3} of glycerol when vaping the ‘velvet’ refill liquid. Wieslander et al. (2001) reported acute ocular and upper airway irritation in non-asthmatic subjects after short exposure to propylene glycol mist from artificial smoke generators. Discussing possible health effects of the consumption of e-cigarettes is, however, beyond the scope of this work.

Determination of carbonyls formed during vaping of e-cigarettes using an analytical cigarette-smoking machine – Determination in gas sampling bag

Aldehydes are of particular interest because of their potential adverse impact on human health. Formaldehyde was classified as a group 1 carcinogen for humans by the International Agency for Research on Cancer (IARC, 2006). Acrolein is listed as a hazardous air pollutant by the US Environmental Protection Agency (USEPA, 2003) and has been shown to cause inflammation, cytotoxicity in the airways, and increased mucus production (Bein and Leikauf, 2011; Moretto et al., 2012). Acetaldehyde has been classified (IARC, 1999) as being a possible carcinogen for humans (Group 2B). In this study, formaldehyde, acetaldehyde, acetone, acrolein and low amounts of propanal were found to be emitted during the vaping process of both e-cigarette types. The respective concentrations of these carbonyls are shown in Table 5. Concentrations reported for acrolein are semi-quantitative, as the possible formation of multiple derivative peaks might underestimate the real concentration (Herrington and Hays, 2012; Clark et al., 1994).

E-cigarettes vaped using the ‘velvet’ refill liquid resulted in higher emissions of carbonyls in general and acrolein and acetaldehyde in particular. A type A e-cigarette vaped using the ‘velvet’ refill liquid resulted in 4.4 times higher emissions of acrolein and 1.6 times higher emissions of acetaldehyde than when using the ‘traditional’ refill liquid. Vaping a type B e-cigarette using the ‘velvet’

Table 5
Carbonyl concentrations determined in gas sampling bags and extrapolation to absolute emitted amount of respective carbonyl per puff.

	Formaldehyde		Acetaldehyde		Acetone		Acrolein ^a		Propanal	
	Conc. in gas sampling bag [$\mu\text{g m}^{-3}$]	Mass per puff [ng]	Conc. in gas sampling bag [$\mu\text{g m}^{-3}$]	Mass per puff [ng]	Conc. in gas sampling bag [$\mu\text{g m}^{-3}$]	Mass per puff [ng]	Conc. in gas sampling bag [$\mu\text{g m}^{-3}$]	Mass per puff [ng]	Conc. in gas sampling bag [$\mu\text{g m}^{-3}$]	Mass per puff [ng]
E-cigarette type A, traditional ^b	202	20.2	235	23.5	73	7.3	13	1.3	49	4.9
E-cigarette type A, velvet ^b	235	23.5	387	38.7	88	8.8	57	5.7	27	2.7
E-cigarette type B, traditional ^b	196	19.6	81	8.1	27	2.7	5	0.5	9	0.9
E-cigarette type B, velvet ^b	227	22.7	399	39.9	77	7.7	135	13.5	24	2.4

^a Semi-quantitative determination.

^b Refill liquids containing 9 mg mL^{-1} nicotine.

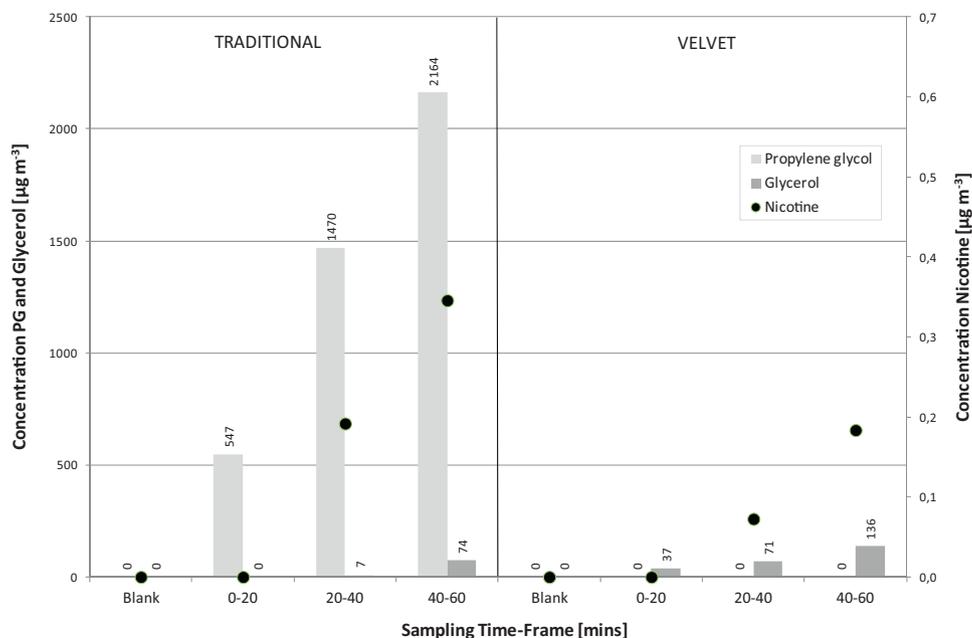


Fig. 2. Concentrations of propylene glycol, glycerol and nicotine measured in chamber air while vaping e-cigarette type A with 'traditional' (left) and 'velvet' (right) 9 mg mL⁻¹ nicotine containing refill liquids.

refill liquid resulted in 27 times higher emissions of acrolein and 4.9 times higher emissions of acetaldehyde than when using the 'traditional' refill liquid. Differences between the two types of e-cigarette vaping the same type of refill liquid are less evident. However, type A e-cigarettes vaped using the 'traditional' refill liquid generally emit higher amounts of carbonyls except formaldehyde than for type B e-cigarettes. The same comparison using 'velvet' as the refill liquid only reveals a difference in the amount of emitted acrolein, which was higher for e-cigarette type B. Extrapolating from the absolute amount of emitted carbonyls while vaping (13 consecutive puffs, the equivalent of one regular tobacco cigarette) to the corresponding concentration in a hypothetical real indoor environment of 60 m³ of volume, would yield an approximate concentration ranging from 0.004 to 0.005 µg m⁻³ formaldehyde, 0.0002 to 0.003 µg m⁻³ acrolein and 0.002 to 0.009 µg m⁻³ acetaldehyde.

Typically detected indoor concentrations range from 2 to 170 µg m⁻³ for formaldehyde (Raw et al., 2004; Gilbert et al., 2005; Liu et al., 2006; Geiss et al., 2011; Kim et al., 2013), from 7 to 80 µg m⁻³ for acetaldehyde (Gilbert et al., 2005; Liu et al., 2006; Geiss et al., 2011; Kim et al., 2013) and from 0.1 to 4.9 µg m⁻³ for acrolein (Gilbert et al., 2005; Liu et al., 2006). For indoor formaldehyde concentrations, the World Health Organization (WHO, 2010) has set a maximum guideline at 100 µg m⁻³ defined for 30-min short term average exposure.

Comparable studies undertaken in the past detected formaldehyde, acetaldehyde and acrolein in aerosols of e-cigarettes, although at considerably lower levels compared to regular cigarette smoke (Goniewicz et al., 2014; Laugesen, 2008; Ohta et al., 2011). Goniewicz et al. (2014) attributed the formation of formaldehyde and acrolein to the heating of glycerol. The current study supports this hypothesis. Studies conducted in the past (Ohta et al., 2011; Paschke et al., 2002) suggested that the formation of formaldehyde, acetaldehyde and methylglyoxal in the e-cigarette was due to the oxidation of propylene glycol during contact with the heating coil. This study found higher emissions of acetone in the glycerol-based refill liquid compared to the refill liquid based on a mixture of glycerol and propylene glycol.

In conclusion, the current study demonstrates that the additional amount of carbonyls contributed into the atmosphere by

vaping under the given conditions can be deemed to be negligible when compared to levels of the same substances typically found indoors.

Consideration should however be given to the fact that vapour directly inhaled by vapers contains double the concentration measured in the gas sampling bags (dilution with nitrogen is approximately 1:1); e.g. for formaldehyde the concentration would approximately be 400 µg m⁻³. This concentration considerably exceeds the guideline value set by the WHO defined for 30-min short term average exposure as preventing sensory irritation in the general population.

Chamber studies

Sampling and determination of propylene glycol, nicotine and glycerol in chamber air

Figs. 2 (type A e-cigarettes) and 3 (type B e-cigarettes) illustrate concentrations of propylene glycol, glycerol and nicotine measured in the chamber while vaping both types of e-cigarettes filled with both refill liquids, respectively.

As expected, peak concentrations for all three compounds were reached after the final series of puffs. Peak concentrations for propylene glycol were approximately 2200 µg m⁻³ for type A e-cigarettes and 1400 µg m⁻³ for type B e-cigarettes vaped with the 'traditional' refill liquid. As propylene glycol is absent from the 'velvet' refill liquid, it was not detected while vaping this type of liquid. Although present in approximately the same concentration as propylene glycol in the 'traditional' refill liquid, glycerol was detected in significantly lower concentrations in chamber air, reaching peak concentrations ranging from 60 to 136 µg m⁻³. Higher concentrations of glycerol were also measured while vaping a type A e-cigarette for both types of refill liquids, compared to type B. The highest nicotine concentrations ranged between 0.2 and 0.6 µg m⁻³. Comparison between e-cigarette types is difficult for nicotine, because of the relatively low concentrations detected.

The generally higher emissions of propylene glycol and glycerol from a type A e-cigarette may be attributed to the different way of vaporising the refill liquid. In a type A e-cigarette, a wick conducts the liquid to the atomising chamber where the liquid is evaporated,

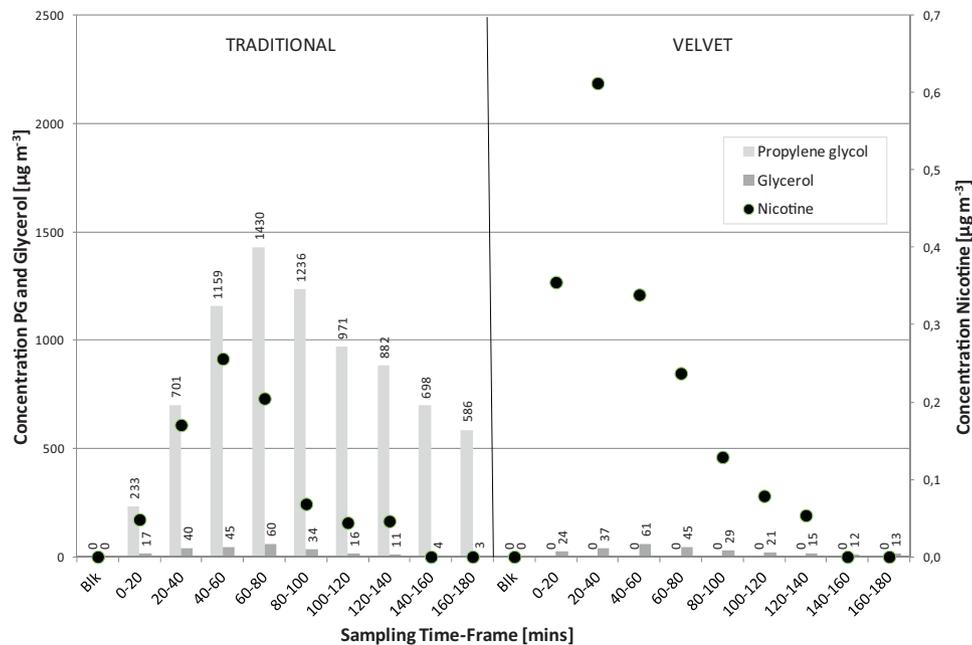


Fig. 3. Concentrations of propylene glycol, glycerol and nicotine measured in chamber air while vaping e-cigarette type B with 'traditional' (left) and 'velvet' (right) 9 mg mL⁻¹ nicotine containing refill liquids.

whereas in a type B e-cigarette, a refill material is imbued with liquid which is vaporised by a heating coil located at the end of the cartomiser body facing the battery. The vaporising efficiency in type A e-cigarettes seems to be higher.

Only a small amount of comparable data is available in existing literature. Schripp et al. (2013) used a volunteer vaper in their work who sat in an 8 m⁻³ chamber with air exchange set to 0.3 h⁻¹ taking six puffs at intervals of 60 s. Three litres of air from the chamber were trapped on Tenax tubes. No propylene glycol above the detection limit was detected in that study. As the visible formation of vapour during the vaping process clearly indicated the emission of vaporised propylene glycol, the authors discussed short usage and sink effects (sorption on internal test chamber surfaces) as possible reasons. Schober et al. (2014) studied e-cigarette emissions in a 18 m² (45 m³) furnished office room in which three volunteers vaped liquids with (22 mg mL⁻¹) and without nicotine over a defined 2 h period. The air exchange rate in the office was approximately 0.5 h⁻¹. Time weighted average concentrations of approximately 199 µg m⁻³, 73 µg m⁻³ and 2.2 µg m⁻³ were found for propylene glycol, glycerol and nicotine respectively. Taking into account any differences in the experimental setup, results are in good agreement with those measured in this study.

It is outside the scope of this study to assess toxicologically the safety of compounds emitted during the vaping process. However with the current experimental set-up and using the vaping devices and refill liquids described under the "Electronic cigarettes used in study" and "Detail and composition of e-cigarette refill liquids" section, relatively high concentrations of propylene glycol and glycerol could be quantified in the air of the chamber tests. The chamber test conditions applied in this study reflect ideal conditions, with minimised sink effects, thus allowing the precise determination of emissions from the tested products. The measured concentrations may therefore differ from 'real environment' studies where sink effects are possibly stronger. Consideration should moreover be given to the fact that in this study concentrations are measured in the air of a 30 m³ chamber, simulating passive inhalation. Active vapers of e-cigarettes are exposed to much higher concentrations when inhaling the vapours. Possible short- and long-term health effects should be further explored.

Sampling and determination of carbonyls in chamber air

Carbonyls were not detected above detection limits in any of the chamber tests. The concentration of carbonyls found in gas sampling bags directly connected to the smoking machine (see the Determination of carbonyls formed during vaping of e-cigarettes using an analytical cigarette-smoking machine – Determination in gas sampling bag section) – and therefore free from any major dilution – showed that the extrapolated concentration to a 30 m³ chamber would be very close to or below the relative detection limits. Therefore, as discussed in the Determination of carbonyls formed during vaping of e-cigarettes using an analytical cigarette-smoking machine – Determination in gas sampling bag section, the additional carbonyl contribution from vaping (under the current test conditions) into the atmosphere can be deemed to be negligible when compared to levels of the same substances typically found indoors.

Aerosol characterisation

Fig. 4A–D illustrates the formation of particles while vaping both types of refill liquids with both types of e-cigarettes. Values on the y-axis correspond to the concentration measured inside the 30 m³ chamber while vaping e-cigarettes according to the procedure described under the Modification of analytical smoking machine for use in chamber studies section.

Three main conclusions can be drawn from the results:

- aerosol in the size range 20 nm to 300 nm does constantly increase in all four cases (using both refill liquids with both e-cigarette types) and reaches similar final peak concentrations of around 7 million particles L⁻¹;
- concentrations of particles larger than 300 nm immediately drop to almost baseline levels after each vaping series of 13 puffs;
- when vaping the 'velvet' glycerol-only refill liquid the portion of particles larger than 300 nm is higher compared to vaping 'traditional' refill liquid, and concentrations drop less drastically after each series of 13 puffs.

Particles emitted from e-cigarettes are assumed to be formed from supersaturated propylene glycol and glycerol (Schripp et al.,

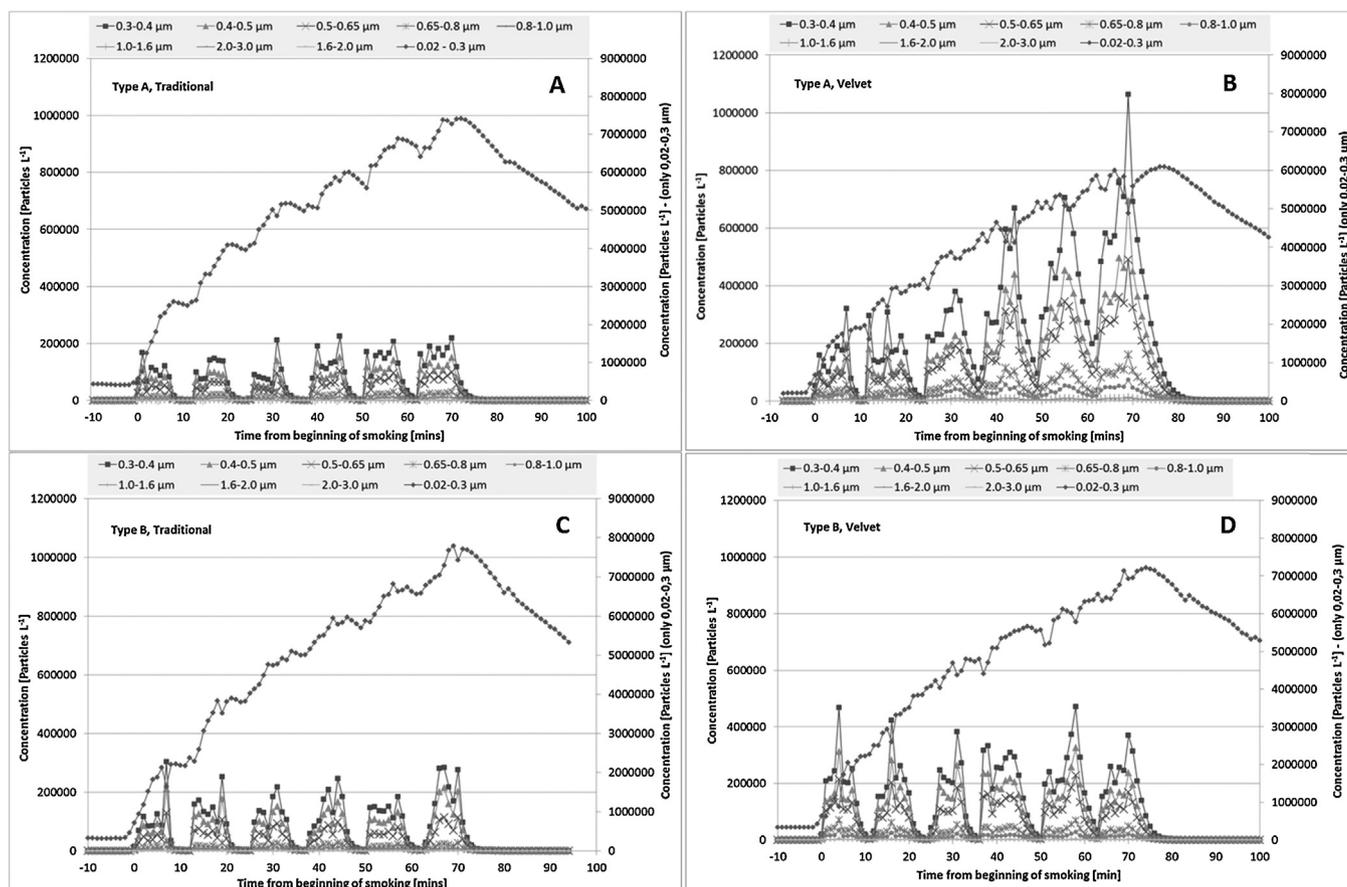


Fig. 4. (A)–(D) Aerosol concentration of various sizes formed while vaping both refill liquids (each containing 9 mg mL^{-1} nicotine) with both types of e-cigarette in chamber test.

2013). Owing to their high vapour pressures larger particles are assumed to evaporate and in parallel deposit on the chamber walls (sink effect). These assumptions serve as a basis to explain the sharp falls in concentration of particles $>300 \text{ nm}$ across the vaping series. Glycerol has a lower vapour pressure compared to propylene glycol, which is the reason for higher concentration of larger particles when vaping the 'velvet' refill liquids. The incomplete evaporation between one series and the other leads to an accumulation of larger particles. This can be clearly seen in Fig. 4B.

Diapouli et al. (2011) analysed particulate matter number concentrations in typical residential microenvironments in the centre of Athens and on average found $20,000 \text{ particles mL}^{-1}$ corresponding to 20 million particles per litre for the size range $10\text{--}400 \text{ nm}$. Chamber peak concentrations detected in this study in the similar size range of $20\text{--}300 \text{ nm}$ would therefore add approximately 35% of particles to what can be assumed to be a typical indoor concentration in a big city. Differences in physico-chemical properties (such as solubility) of secondary formed propylene glycol and glycerol droplets, such as those formed during the vaping process, and solid/poorly soluble particles, such as those found in urban areas, have however to be given adequate consideration when evaluating toxicological effects.

Considerations on the manufacture of tested e-cigarettes

In addition to the chemical aspect of refill liquid and emissions characteristics, e-cigarettes should also be evaluated on the quality of their construction. E-cigarettes investigated in this study were found to have deficiencies, particularly during the re-filling process.

During this process the user inevitably comes into direct contact with the undiluted refill liquid. For type A e-cigarettes, the contact is primarily with the users' skin, whereas the re-filling process for type B e-cigarettes means that liquid frequently comes into contact with lips. This was perceived to be unpleasant, and potentially relevant from a toxicological point of view. This finding is supported by Trtchounian and Talbot (2011), who found that the fluids in cartridge reservoirs leak out of most brands and that there are difficulties in assembling and disassembling e-cigarettes without coming into contact with the refill liquid.

General considerations

The wide variety of refill liquids and e-cigarettes on the market and the fast rate of innovation in this sector make a widely-applicable safety assessment difficult. For example, when vaping the same refill liquids used in this study, more powerful batteries in new products might lead to higher emission rates of nicotine and other compounds. Comparing the chemical emissions of various e-cigarettes models by applying standardised smoking regimes using smoking machines has limitations as e-cigarette vapers seem to change their way of vaping depending on the model of e-cigarette. Therefore, overheating – with its consequent formation of undesired compounds – would not be possible with human vaping, whereas laboratory testing could only slightly account for this. In practice, this means that every single e-cigarette model would need a 'personalised' vaping regime for a correct assessment of chemical emissions. Further studies are required to understand whether standardised machine-vaping regimes can be generally

applied to e-cigarettes and under which conditions in terms of puff-duration, puff-volume, puff-frequency and number of puffs per vaping-series.

Conclusion

Electronic cigarettes tested in this study proved to be sources of propylene glycol, glycerol, nicotine, carbonyls and aerosol particulates. The extent to which people could be passively exposed to these depends on the ventilation rate, room size, indoor climate, room equipment and number of e-cigarettes in use. In addition to exposure to toxicants, consideration must also be given to the generally perceived air quality in microenvironments where vaping is permitted (independently of its toxicity). Sensory assessment of the acceptability of air quality or odour intensity by a human panel could answer this question and should be further explored.

In addition to considering exposure to second-hand vapour, this study shows that active vapers inhale relatively high concentrations of propylene glycol, glycerol, aerosol particulates and certain carbonyls. This exposure might require further toxicological evaluation.

Possible long term effects of e-cigarettes health are not yet known. E-cigarettes, the impact of vaping on health and the composition of refill liquids require therefore further research into the product characteristics. For the benefit of consumers, quality and safety requirements of e-cigarettes and refill liquids should be harmonised.

Conflict of interest statement

Authors declare that they have no conflict of interest. They have never received products for research purposes from, never undertaken research for and never received funding from the e-cigarette, tobacco or pharmaceutical industries.

The content of this work reflects the views of the authors and does not necessarily represent an official position of the European Commission.

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