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Influence of puffing conditions on the carbonyl composition of e-cigarette aerosols

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ABSTRACT

Owing to their harmful effects on human health, the presence of carbonyl compounds in e-cigarette aerosols raises concerns. To date, the reported concentration levels in e-vapors vary greatly between studies and several factors that markedly influence carbonyl emission during vaping have been highlighted including the heating temperature, the power supply, the device architecture, the filling level of the tank and the main e-liquid constituents. This study investigated the impact of puffing regimen parameters on the carbonyl composition of e-cigarette aerosols with the aim of: (1) better estimating the variability of carbonyl emissions depending on puffing conditions; (2) highlighting puffing profiles that increase the exposure to carbonyls; and (3) estimating to what extent puffing topography could be implied in the variability of carbonyl concentrations reported in the current literature.

E-vapors from a single e-liquid were generated from two e-cigarette models with a smoking machine. A total of 7 different puffing regimens were used to individually study the influence of the puff volume, duration and frequency. Carbonyls were collected by DNPH cartridges and analysed by HPLC-UV. E-liquid consumption and e-vapor temperature were also monitored.

E-vapor concentrations of formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde and methylglyoxal were affected, sometimes differently, by the modification of the puffing regimen, as well as by the e-cigarette model. For example, formaldehyde concentration ranged from 20 to 255 ng/puff depending on the puffing conditions. The results of principal component analyses, applied to the concentration data sets for the 6 carbonyls, suggest that the studied parameters interact and highlight some "carbonyl-emitting" combinations of concern (e-cigarette model/puffing regimen). However, the highest concentrations measured in the present study remain far lower than those observed in conventional cigarette mainstream smoke.

This study confirms that the chosen puffing regimen contributes a part of the observed variability in the carbonyl levels reported in the scientific literature, hampering comparisons between studies and making interpretation difficult. Thus, harmonized and realistic protocols for the assessment of e-cigarette toxicity by physicochemical or experimental approaches are clearly needed.

1. Introduction

Electronic cigarettes or "e-cigs" are battery operated devices which are marketed as healthier than conventional cigarettes. Briefly, an "e-liquid", generally a mixture of propylene glycol, glycerol and flavourings supplemented or not with different concentrations of nicotine, is heated and vaporised into a liquid aerosol, also called "e-vapor", which is inhaled by the user ("vaper").

E-cig popularity and use has increased in recent years (Kasza et al., 2017; King et al., 2015). In parallel, many studies have investigated the health impact of e-cig use and particular attention has been paid to the physicochemical characterization of e-liquids (Beauval et al., 2016; Behar et al., 2014; Goniewicz et al., 2015; Han et al., 2016; Kavvalakis et al., 2015; Varlet et al., 2015), e-vapors (Beauval et al., 2017;

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Farsalinos et al., 2015; Flora et al., 2016; Goniewicz et al., 2014; Hutzler et al., 2014; Margham et al., 2016; Tayyarah and Long, 2014; Uchiyama et al., 2016; Williams et al., 2013) and indoor air quality after vaping (Czogala et al., 2014; Geiss et al., 2015; McAuley et al., 2012; Schober et al., 2013; Schripp et al., 2013). In addition to the analysis of the main e-liquid components, studies have performed the identification and quantification of several classes of toxic or potentially toxic compounds also emitted in tobacco mainstream smoke, including (but not limited to) carbon monoxide, volatile organic compounds, polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines and inorganic compounds (especially heavy metals) (Burns et al., 2008; Food and Drug Administration, 2012; Liu et al., 2011). While several reports agree that global harmfulness of e-vapors is substantially lower than that of conventional cigarette smoke (Goniewicz et al., 2014; GOV UK, 2015; Margham et al., 2016; Tayyarah and Long, 2014), e-vapors are not totally free of toxic compounds (Goniewicz et al., 2014; Kosmider et al., 2014; Margham et al., 2016; Sleiman et al., 2016). Particularly, the presence of carbonyl compounds such as formaldehyde and acetaldehyde, respectively classified in groups 1 and 2B according to the International Agency for Research on Cancer (International Agency for Research on Cancer, 2016), and acrolein which is a known irritant for mucous membranes and skin (Institut National de Recherche et de Sécurité, 2015), raises concerns. Indeed, carbonyl generation was reported to occur during vaping, potentially by oxidative (Uchiyama et al., 2016) or pyrolytic (Gillman et al., 2016) reactions of propylene glycol and glycerol, or by the thermal decomposition of flavourings (Khlystov and Samburova, 2016).

The International Organisation for Standardization and the European Committee for Standardization are currently developing standards for the determination and quantification of chemical emissions from e-cigarettes (European Committee for Standardization, 2015; International Organization for Standardization, 2016). However, no reference method has been published to date. The determination of carbonyl concentrations in e-vapors is currently widely assessed by derivatization with (2,4-dinitrophenyl)hydrazine (DNPH) used in solution in impingers or impregnated in sorbent cartridges, though some other protocols have been published (Farsalinos and Gillman, 2017). A large variability exists among reported carbonyl concentrations in e-vapors from different published studies, ranging from tens of ng/puff to hundreds of μg/puff (Table 1). Such differences were also reported in single studies (Gillman et al., 2016; Uchiyama et al., 2013). Several factors that markedly influence carbonyl generation during vaping were highlighted, such as the heating temperature (partly related to the device power supply) (Geiss et al., 2016; Gillman et al., 2016; Kosmider et al., 2014; Sleiman et al., 2016), the device architecture (Gillman et al., 2016), the filling level of the tank (Hutzler et al., 2014), the main e-liquid constituents (Kosmider et al., 2014) and the e-liquid flavouring content (Khlystov and Samburova, 2016). Considering that carbonyls are generated during the few seconds of one puff, it is likely that other factors could be involved in the aforementioned variability, especially the puff generation conditions, which frequently vary between studies (*e.g.* the puffing topography, the heating triggering, the coil material, the e-cig tilt or movement during use).

Ideally, e-vapor generation by a smoking machine should mimic real e-vaping. "Puffing regimens" are related to at least 3 key parameters for e-vapor generation by smoking machines: puff volume, puff duration and puff frequency. In studies of conventional cigarette emissions, several well-defined puffing regimens can be employed. For example, the ISO 3308:2012 (35 mL puff volume over 2 s every 60 s) and the Health Canada Intense (55 mL puff volume over 2 s every 30 s) regimens are usually compared (International Organization for Standardization, 2015) and can lead to very different results depending on targeted compounds (Eldridge et al., 2015). More specifically, carbonyl delivery per one puff was reported to be significantly affected by the modification of puff volume while other changes in puffing regimen led to more minor effects (Reilly et al., 2017). Currently, and in contrast to studies of conventional cigarettes, no defined puffing regimen has been widely adopted for e-cig analyses (Table 1). Thus, differences between studies are inevitably fraught with uncertainties that hamper data comparison and interpretation. Some effects on carbonyl emissions of puff frequency (Uchiyama et al., 2016), puff volume (Margham et al., 2016) and puff duration (Sala et al., 2017) were recently reported. However, the specific impact of the modification of puffing regimen parameters, related to e-cigarette configurations, remains to be fully established.

With the aim of: (1) better estimating the variability of carbonyl emissions depending on puffing conditions; (2) highlighting puffing profiles that increase the exposure to carbonyls; and (3) estimating to what extent puffing topography could be implied in the variability of carbonyl concentrations observed in the current literature, this work investigated whether puffing regimen parameters influence carbonyl emission from 2 e-cig models using 1 e-liquid and smoking machine-assisted procedures.

2. Materials and methods

2.1. E-cigarettes and e-liquids

The experiments were performed using two models of e-cig obtained from a French manufacturer[1] (NHOSS® brand).

The second generation "Lounge" model is equipped with 2.8 Ω nichrome top-coil and 4.6 W power supply. The heating was triggered by suction and a dedicated USB connected power supply replaced the original battery. Aiming to highlight the impact of puffing regimens on carbonyl emissions, with minimal confusing factors, only one heating coil unit was used for all the experiments.

The third generation "Mod box TC" model, used with the "Air Tank" clearomiser, is equipped with 0.5 Ω kanthal bottom-coil and configurable power supplies ranging from 7 to 50 W. An operator activated the heating pushing and maintaining the pressbutton 1 s before each puff and during the whole puff duration. Two batteries were alternated to maintain a high level of charge during all the experiments. In order to avoid an overheated environment for e-liquids, 3 tanks were alternated (1 per 1 puff series). However, only 1 heating coil was kept for all the experiments. For this study, the Mod box TC power supply was fixed to 18 and 30 W, which correspond to low and middle range power supplies recommended by the manufacturer for suitable experience regarding the aforementioned e-cig configuration.

All the experiments were performed with nicotine containing chlorophyll mint flavoured e-liquid (obtained from the same French manufacturer as for the e-cig devices) provided in 20 mL plastic bottles and labelled as follows: propylene glycol < 65%; glycerol < 35%; food flavourings; 16 mg/mL nicotine.

2.2. E-vapor generation

E-vapors were generated with a Vitrocell® VC1 smoking machine (Vitrocell, Waldkirch, Germany). The CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) square approach was used as the reference puffing regimen with 55 mL puff volume over 3 s, 1 puff every 30 s (Tayyarah, 2015). In addition, modified puffing regimens were selected according to the current literature and are summarized in Table 2. Briefly, each parameter was independently decreased and increased as follows: 35 and 100 mL puff volume ("PV-" and "PV+", respectively), 2 and 6 s puff duration ("PD-" and "PD+", respectively), 1 puff every 60 and 14 s ("PF-" and "PF+", respectively).

Clearomisers were filled before each vaping session and e-liquid consumption was monitored by weighting clearomisers before and after

[1] Innova SAS, Bondues, France.

Table 1
Summary of ranges of published values for carbonyl emissions along with protocol details.

Reference	E-cig	E-liquid	Puffing regimen[a]	Collection device	Puff number per collection	Studied parameter(s)	Approximate range of reported concentration (ng/puff)						Other reported compounds
							FA	AA	AO	AC	PA	MG	
(Uchiyama et al., 2013)	363 e-cigs	–	55/2/2	DNPH + Hydroquinone cartridges	10	–	< 72 - 14300	< 17 - 11550	–	< 61 - 4015	< 39 - 4565	< 88 - 2090	glyoxal
(Hutzler et al., 2014)	1 e-cig (prefilled[b])	1 e-cig/brand associated e-liquid	55/3/2	DNPH solution filled impinger	10 to 60	E-liquid level in cartridge	100–5000	100–8000	–	< 100 - 3500	< 100 - 1100	–	–
(Goniewicz et al., 2014)	12 e-cig (prefilled)	12 e-cig/brand associated e-liquids	70/1.8/6	DNPH cartridge	150	–	21–374	13–91	< 0.1	< 0.1–279	< 0.07	–	o-methylbenzealdehyde
(Kosmider et al., 2014)	1 e-cig (refillable)	10 commercial + 3 self-made e-liquids	70/1.8/3.5	DNPH cartridge	30	Main e-liquid components battery voltage	< 2 - 1800	< 1 - 282	< 2 - 506	< 2	< 1.3	–	crotonaldehyde benzaldehyde isovaleraldehyde m-methylbenzealdehyde
(Tayyarah and Long, 2014)	5 e-cigs (prefilled)	5 e-cig/brand associated e-liquids	55/-/2	DNPH solution filled impinger	99	–	< 350	< 70 - 320	–	< 30 - 190	< 30 - 110	–	–
(Farsalinos et al., 2015)	2 prepared e-cigs (refillable)	1 e-liquid	–	DNPH solution filled impinger	60	Power level	< 25 - 34500	< 75 - 20600	< 92 - 2250	< 17 - 21000	–	–	–
(Geiss et al., 2015)	2 e-cigs (refillable)	2 e-liquids	35/4/2	Tedlar bag + DNPH cartridge	20	E-cig type main e-liquid components	19.6–23.5	8.1–39.9	2.7–8.8	0.5–13.5	0.9–4.9	–	–
(El-Hellani et al., 2016)[c]	27 e-cigs (prefilled and refillable)	27 e-cig/brand associated e-liquids	100/4/6	DNPH cartridge	15	–	58–505	45–2120	71–344	< 13 - 139	< 14 - 291	–	–
(Flora et al., 2016)	4 e-cigs (prefilled)	E-cig/brand associated e-liquid(s)	55/4/2	DNPH solution filled impinger	20	–	90–330	< 710	–	< 360	–	–	–
(Geiss et al., 2016)	1 e-cig (refillable)	1 e-liquid	50/3/3	DNPH cartridge	10	Power level temperature	24–1560	13–350	–	≤2.5	–	–	–
(Gillman et al., 2016)	5 e-cigs (refillable)	1 self-made e-liquid	55/4/2	DNPH solution filled impinger	25	E-cig design power level	< 22 - 99200	< 22 - 81440	–	< 22 - 23720	–	–	–
(Khlystov and Samburova, 2016)[c]	3 e-cigs (prefilled and refillable)	17 e-liquids	40/4/2	DNPH cartridge	2	Flavouring e-liquid content	< 9 - 49500	< 7 - 27700	–	< 10 - 2720	< 13 - 4180	–	benzaldehyde m-methylbenzealdehyde
(Margham et al., 2016)	1 e-cig (prefilled)	1 e-cig/brand associated e-liquid	55/3/2 35/3/2 80/3/2 110/3/2 140/3/2	DNPH solution filled impinger	100	Puff volume	121–123	104–107	60–86	61–79	< 67	–	methyl ethyl ketone

(continued on next page)

Table 1 (*continued*)

Reference	E-cig	E-liquid	Puffing regimen[a]	Collection device	Puff number per collection	Studied parameter(s)	Approximate range of reported concentration (ng/puff)						Other reported compounds
							FA	AA	AO	AC	PA	MG	
(Sleiman et al., 2016)[c]	2 e-cigs (refillable)	3 e-liquids	50/5/2.4	DNPH cartridge	1 to 5	E-cig type e-cig conditioning e-cig aging power level temperature main e-liquid components	10660–342220	1173–135468	153–10011	328–71426	51–22720	–	crotonaldehyde butyraldehyde benzaldehyde valeraldehyde p-methylbenzealdehyde, hexaldehyde methyl ethyl ketone metacrolein
(Uchiyama et al., 2016)	10 e-cigs (prefilled and refillable)	1 e-liquid for all refillable e-cigs and 2 prefilled cartridges	55/2/2 55/2/1 55/2/4	Cambridge filter pad + CX-572 cartridge	1 to 30	Puff number battery voltage puff frequency	< 70 - 110000	< 100 - 100000	< 50 - 6400	< 210 - 15000	< 50 - 8900	< 60 - 33000	glyoxal
(Beauval et al., 2017)	1 e-cig (refillable)	6 e-liquids	55/3/2	DNPH cartridge	96	–	20.4–81.4	8.8–52.8	–	< 2.6–116.1	–	–	–
(Farsalinos et al., 2017a)	2 e-cigs (refillable)	2 e-liquids	50/5/2 50/4/2	DNPH solution filled impinger	50	Battery voltage	90–29817	0.3–15093	–	35–4365	–	–	–
(Farsalinos et al., 2017b)	1 e-cig (refillable)	1 e-liquid	60/4/2	DNPH solution filled impinger	50	Battery voltage	340–71820	–	–	–	–	–	–
(Ogunwale et al., 2017)	2 e-cigs (prefilled and refillable)	4 e-cig/brand associated e-liquids + 6 commercial e-liquids	91/4/2	Tedlar bag + Silicon microreactor with a coating phase of AMAH[d]	10	E-cig type power supply puff volume puff duration	18–81981	15–53210	129–80872	2–1621	≤57 - 1792	–	butyraldehyde
(Sala et al., 2017)	2 e-cigs (refillable)	1 e-liquid	70/2/5 70/5/5 70/10/5	Glass vial + SPME[e] technique with on-fiber derivatization	5	Puff duration	392 µg/mL[f]	272 µg/mL[f]	–	2.15 µg/mL[f]	–	–	–

Values were selected regardless conditions of use (*e.g.* power supply, voltage) and were extrapolated into the good unit according to available data. "-": unavailable data. [a] Puffing regimens are described as follows: puff volume (mL)/puff duration (s)/puff frequency (min^{-1}). [b] "Prefilled" refers to prefilled disposable e-cigs and prefilled replaceable cartridges. [c] E-vapors were produced by custom-designed devices. [d] AMAH: 4-(2-aminooxyethyl)-morpholin-4-ium chloride. [e] SPME: solid-phase microextraction. [f] Results were reported in "µg/mL of liquid in vapor", extrapolation to ng/puff is not possible.

Table 2
Description of puffing regimen parameters.

Puffing regimen	Abbreviation	Puff volume (mL)	Puff duration (s)	Puff frequency (min^{-1})
CORESTA[a] (initial and final)	IC and FC	55	3	2
Puff volume +	PV+	100	3	2
Puff volume -	PV-	35	3	2
Puff duration +	PD+	55	6	2
Puff duration -	PD-	55	2	2
Puff frequency +	PF+	55	3	4.3
Puff frequency -	PF-	55	3	1

[a] (Tayyarah, 2015).

each sampling. Before daily experiments, 40 conditioning puffs were performed on the e-cig.

2.3. E-vapor collection

Carbonyl compounds were collected from 3 independent and consecutive collections of 20 puffs using 2 specific silica cartridges coated with DNPH (Sep-Pak XPoSure Plus Short Cartridge 350 mg, Waters, Guyancourt, France), placed in series. Cartridges were connected to the outlet of the smoking machine exhaust so that no obstacle was positioned between the suction system and the e-cigarette. Thus, no pressure drop phenomenon nor air flow modification could occur during the experiments. The second cartridge was used to evaluate the collection efficiency. The smoking machine exhausted each puff sample over 10 s, thus, the collecting flow ranged from 0.21 to 0.60 L/min for 35 and 100 mL puff volumes, respectively.

The alteration of c-cigarette components after repeated use (a charred coil for example) may affect vaporizing conditions and hence the composition of the e-vapor (Gillman et al., 2016). This e-cig "aging" over 480 puffs was monitored by performing and comparing the results of CORESTA reference puff collections at the beginning (initial CORESTA or "IC") and at the end (final CORESTA or "FC") of the experiments. Moreover, collections for each e-cig model/configuration were performed over a single day in order to minimize intra-model variability.

Before each daily experiment, a thorough cleaning of the Vitrocell® pumping system was performed with ethanol. Laboratory air quality and machine cleanliness were evaluated by performing experimental blank collections from 60 laboratory air puffs without any e-cig connected to the smoking machine. Daily experimental blank values were subtracted from those of respective e-vapor samples.

2.4. Analytical method

DNPH cartridges were desorbed with 3 mL of acetonitrile. The cartridge dead volume was taken into account by weighting the eluted acetonitrile. Twenty µL were injected into a high performance liquid chromatographic system (Waters 2695) coupled to an ultraviolet detector (Waters 2487). Chromatographic separation was achieved on a Pinnacle Ultra C18 250 mm × 4.6 mm x 5 µm (Restek, Lisses, France). The oven temperature was set at 40 °C and the mobile phase was a gradient mixture of acetonitrile, tetrahydrofuran and water with a constant flow of 1.5 mL/min over 27 min. Acquisition was performed at 365 nm wavelength.

The method allows the detection and quantification of formaldehyde (FA), acetaldehyde (AA), acetone (AO), acrolein (AC), propionaldehyde (PA), methyl vinyl ketone, crotonaldehyde, methyl ethyl ketone, butyraldehyde, benzaldehyde, glyoxal, isovaleraldehyde, methylglyoxal (MG), 2,5-dimethylbenzaldehyde and hexaldehyde with limits of detection (LOD) in acetonitrile from 3 to 5 µg/L (or from 0.45 to 0.75 ng/puff considering a theoretical maximum elution volume of 3 mL and 20 puff series). Mean carbonyl mass per cartridge obtained after elution of non-exposed cartridges, from the same batch, were subtracted in data work up.

2.5. E-vapor temperature monitoring

The temperature of e-vapor, at the outlet of the e-cig drip tip, was monitored by a NTC 3950 thermistor coupled with an Arduino UNO microcontroller board (www.arduino.cc) at 10 Hz. In order not to alter puff generation during carbonyl collection, temperature measurements were performed in separate experiments, though under the same conditions.

2.6. Statistical analysis

Principal component analysis (PCA) and hierarchical ascendant classification (HAC) were assessed on carbonyl concentrations in e-vapor using the FactoMineR Package on R (www.R-project.org) (Lê et al., 2008).

2.7. Optimization of sampling procedure

The determination of carbonyl emissions in e-vapors is widely assessed by DNPH-derivatization methods using DNPH solution filled impingers and impregnated silica cartridges (Bansal and Kim, 2016). However, these materials were not fully validated for this specific matrix which differs from air or smoke in many regards (Farsalinos and Gillman, 2017).

In this study, the number of puffs for each collection was set so as to both collect representative and realistic samples and avoid analytical limitations. Preliminary experiments showed there to be strong saturation phenomena with large puff numbers (up to 96 puffs), leading to the collection of up to 73% of the total mass of an analyte in the second DNPH cartridge for the Mod box TC model (data not shown). In fact, cartridges were full of a condensate of e-vapor which affected the collection efficiency and reproducibility of carbonyl retention in cartridges. Finally, 20 puffs was found to be the best compromise with satisfying collection efficiency, reproducibility and relevant e-cig durations of use, from 4.7 to 20 min depending on puff frequency.

3. Results and discussion

E-liquid consumption during vaping, e-vapor temperature at the outlet of the e-cig drip tip and carbonyl concentrations in e-vapors were measured for each puffing regimen set on the smoking machine. The CORESTA profile was chosen as the basal condition and was modified, according to puffing regimens already reported in the literature, in order to study the impact of puff volume, duration and frequency on carbonyl emission and generation.

3.1. E-liquid consumption

E-liquid consumption during vaping sessions is the first and most easily recordable parameter permitting evaluation of experimental reproducibility. Whatever the conditions, the standard deviations of 3 independent measurements of e-liquid consumption during puff series suggest the experiment reproducibility to be satisfying (Fig. 1). Comparing initial *vs* final CORESTA collections results revealed no, or barely noticeable, differences between the beginning and the end of the vaping sessions, suggesting that the whole day experiment (480 puffs) may not cause significant device aging. Briefly, puffs from the Lounge model consumed less e-liquid than those from the Mod box TC model, while a higher power supply increased consumption. However, the interpretation of power supply must be related to the e-cig configuration: preliminary tests showed no generation of e-vapor while setting 7 W on the Mod box TC. These results are in accordance with the findings of

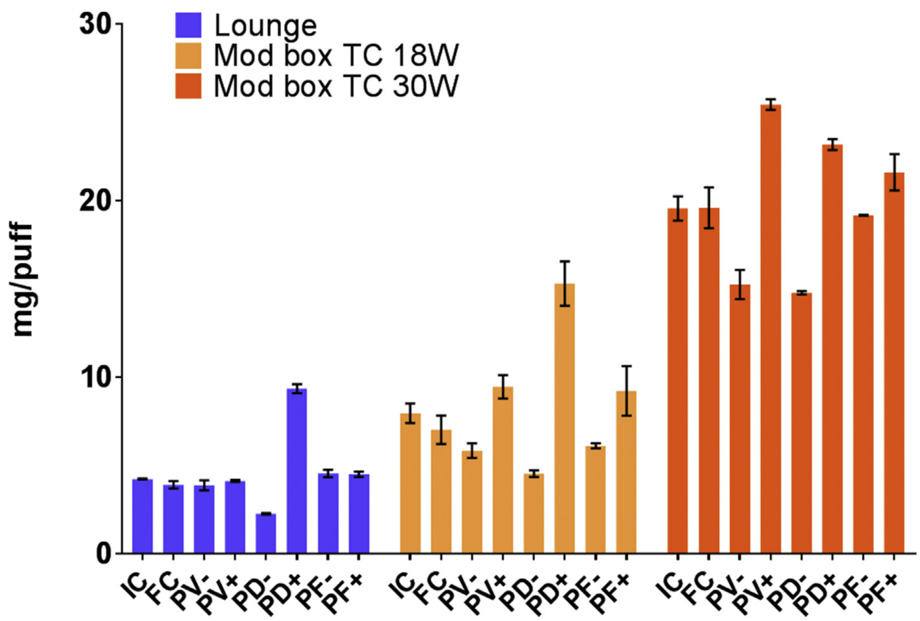

Fig. 1. E-liquid consumption during 20 puffs vaping sessions expressed in mg of e-liquid per 1 puff.

Gillman et al. (2016).

Regarding the Lounge model, puff duration appears to be the main factor influencing e-liquid consumption: the longer the puff, the greater the consumption (Fig. 1). The same trends were observed with the Mod box TC 18 W, though lower fluctuations were also measured varying puff volume and frequency. Increasing power supply up to 30 W strongly increased e-liquid consumption while fluctuation trends mainly remained comparable with the Mod box TC 18 W. However, increasing puff duration with 30 W did not lead to the greatest e-liquid consumption as for other conditions. This could be due to the saturation of e-liquid availability at the coil with such long puff duration.

Considering that the mass of e-liquid vaporised to generate one puff may vary by up to an order of magnitude depending on puff generation conditions (Fig. 1), results expressed as mass/puff cannot provide sufficient information for data interpretation and should be complemented with data expressed as mass/consumed amount of e-liquid. While ng/puff data represent more particularly the mass of compounds emitted during use and provide an estimate of the consumer exposure, ng/mg of consumed e-liquid data emphasises the capacity of the e-cig device to generate compounds in the conditions set. To date, relatively few data on carbonyl emission have been reported as mass/consumed amount of e-liquid, or mass/mass of total particulate matter, and even fewer have been published with both units (Farsalinos et al., 2017a, 2017b; Gillman et al., 2016; Khlystov and Samburova, 2016; Sala et al., 2017; Sleiman et al., 2016). Considering the goals of the present study, both units appear relevant for interpretation.

3.2. E-vapor temperature

High heating coil temperatures during vaporisation (usually assimilated with high power supplies) were cited several times as one of the principle factors causing carbonyl generation during vaping – the transformation of propylene glycol and glycerol being, to date, the most frequently mentioned mechanism (Geiss et al., 2016; Gillman et al., 2016; Kosmider et al., 2014; Sleiman et al., 2016). Geiss et al. reported that higher carbonyl concentrations were correlated with higher coil temperatures (up to 300 °C), depending on conditions (Geiss et al., 2016). Moreover, simulating e-cig vaporisation conditions using a dedicated system, Wang et al. reported the generation of FA, AA and AC from propylene glycol and/or glycerol under precisely controlled temperatures (≥215 °C) (Wang et al., 2017).

In the current study, temperature was measured at the outlet of the e-cig drip tip over a series of 20 puffs (Fig. S1) which could reflect on one hand the vaporisation temperature, though indirectly, and on the other hand the e-vapor temperature experienced by the user.

For the Lounge and the Mob box TC 18W, temperature profiles were globally similar (Fig. 2). Consistently, temperature profiles could be split into 2 phases: the first 5 puffs were characterised by an increase in temperature up to a relative plateau, and this remained for the following 15 puffs. Puff duration clearly affected e-vapor temperature, especially when increased (up to 100 °C with 6 s puff duration), as well as high puff frequency (up to 70 °C with 4.3 puff/s), whereas other conditions remained relatively comparable to the initial CORESTA collection. Temperatures measured with the Mod box TC 30 W were markedly higher compared with the Lounge and the Mod box TC 18 W models (from 65 to 115 °C at the plateau).

The French national organisation for standardization suggests that e-vapor temperatures exceeding 60 °C may not be expected by the user (Associaton Française de Normalisation, 2016). Hence, this parameter could be considered as an indicator of the experiment relevance and realism. Considering the plateaux values for the Lounge and the Mod box TC 18 W, most conditions tested here led to e-vapor temperatures ≤60 °C, though these are higher than previously reported temperatures (Sleiman et al., 2016). Noticeably higher temperatures were measured with long puff duration and, to a lesser extent, with high puff frequency. The relevance of such puffing regimens could be questionable, however. While long puff durations (greater than 6 s) have already been reported in human puff topography studies (Hua et al., 2013; Robinson et al., 2015), the puff volumes associated with them were much higher than the 55 mL used in our protocol (up to 304 mL – Robinson et al., 2015). Puff frequencies have been reported as being between approximately 20 and 50 s (Cunningham et al., 2016; Robinson et al., 2015), highlighting that intensive e-cig use is also naturally observed and remains a realistic condition to study.

Temperatures observed for the Mod box TC 30 W were markedly high, seemingly higher than in real conditions. Indeed, 30 W is in the middle range of power supplies recommended by the manufacturer and is used by consumers. The Mod box TC and the associated Air tank clearomiser are among the latest generation devices, very different from the first-generation e-cigs (called "cig-a-likes") used in the first scientific studies, and it is likely that the puffing parameters used in this study are not fully suitable for all e-cig devices, especially the newest

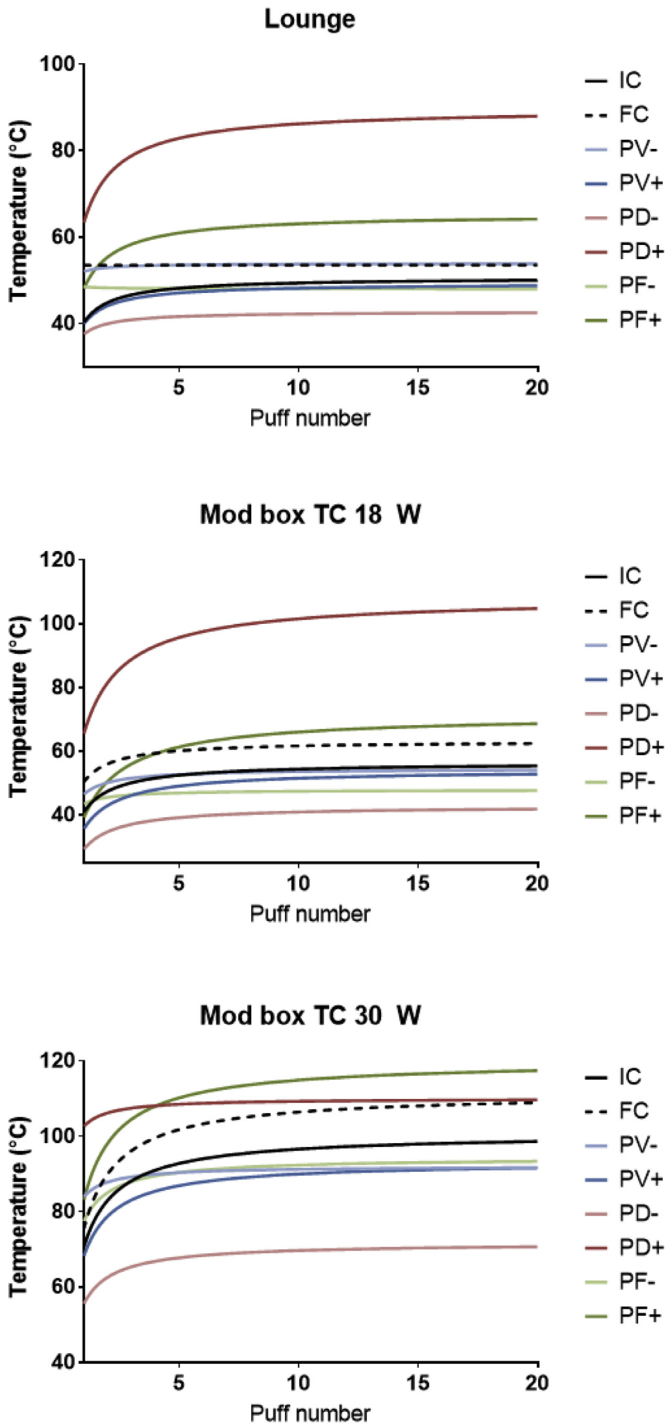

Fig. 2. E-vapor temperature measured over 20 puffs vaping sessions expressed in °C. Curves were constructed by the interpolation of the maximum temperatures reached during each puff, while the temperature measured during inter-puff intervals was not taken into account. “IC”: initial CORESTA. “FC”: final CORESTA. “PV-”: decreased puff volume. “PV + ”: increased puff volume. “PD-”: decreased puff duration. “PD + ”: increased puff duration. “PF-”: decreased puff frequency. “PF + ”: increased puff frequency. IC and FC experiments were respectively performed at the beginning and at the end of the experiment series.

ones. The same observation was made by Cunningham et al. who reported in real life observations significant differences in puff volume and puff frequency between 2 different e-cig models: a rechargeable “cig-a-like” and a larger button-activated device (Cunningham et al., 2016). Further investigations characterising puffing topography with such new generation devices and their applicability to smoking machine protocols should be carried out. In the current study, and based on our measurements of the e-vapor temperature, the Mod box TC 30 W cannot be considered as a realistic condition. However, all other parameters were set to be in accordance with normal e-cig use. Considering the actual purpose of our study, and compared with data from the literature for which the realism of conditions used is not systematically proven (Farsalinos et al., 2017a, 2017b), this experiment was nevertheless deemed sufficiently pertinent to be retained.

3.3. Carbonyl emission/generation

Carbonyl generation has been reported to occur during vaping by different mechanisms (Gillman et al., 2016; Khlystov and Samburova, 2016; Uchiyama et al., 2016) and variable concentration levels, sometimes differing by several orders of magnitude, have been reported in scientific literature (Table 1). Considering the large variability of tested devices, conditions of use, and methods of collection and analysis, it is unclear to what extent these values are comparable and can thereafter be used to assess user exposure.

In our experimental conditions, FA, AA, AO, AC, PA and MG were quantified in almost all samples whereas the levels of the other targeted carbonyl compounds were not different from those of the experimental blanks. The concentrations of FA, AA, AO, AC, PA and MG are reported in ng/puff and ng/mg of consumed e-liquid in Fig. 3. Regarding the whole experiments, initial and final CORESTA results were comparable for almost all conditions, compounds and units. Only some slight variations were observed with FA and MG, so device aging was considered negligible. A maximum of 2.2% of the total collected mass of AA, AC and PA was quantified in the second DNPH cartridge, whatever the e-cig or the puffing regimen used, suggesting the collection of these compounds to be nearly total. However, the collection efficiency of FA, AO and MG was differently affected depending on experimental conditions, especially the e-cig model: the average proportions of the carbonyl mass collected on the second cartridge for the Lounge, the 18 W and the 30 W Mod box TC models were of 19.6, 5.5 and 6.4% for FA, of 15.7, 1.9 and 0.7% for AO and of 31.1, 11.2 and 8.2% for MG, respectively. Thus, we observed in these conditions some cartridge breakthrough even with ng/puff concentrations that are far lower than some already reported carbonyl levels (Table 1). Considering that the mass of consumed e-liquid during vaping was generally lower with the Lounge model compared to Mod Box TC models (Fig. 1), these results contrast with the aforementioned observation that the mass of vaporised e-liquid passing through sampling cartridges decreases their collection efficiency. Hence, under the present conditions, it is possible that collection efficiency was affected by the physical parameters of the e-vapors, including their temperature, their particle size or their gas/particulate partition.

Although the analysis of FA, AO and MG is not fully quantitative, especially for the Lounge model, the values were obtained from a relevant and reproducible protocol. In the same way, while the confidence of AC results is limited to the performance of sorbent cartridges (Ho et al., 2011), the concentrations remain comparable with each other.

As evoked previously, data interpretation differs depending on the unit. Considering all conditions, e-vapor concentrations ranged from 20 to 255 ng/puff for FA, from 29 to 364 ng/puff for AA, from 4.4 to 28 ng/puff for AO, from “not detected” to 40 ng/puff for AC, from 1.0 to 32 ng/puff for PA and from 4.5 to 141 ng/puff for MG. These concentrations are in the lower panel of the actually reported emission levels (Table 1), below 1 µg/puff and far lower than already reported tens or hundreds of µg/puff (Gillman et al., 2016; Khlystov and Samburova, 2016; Sleiman et al., 2016; Uchiyama et al., 2016). They are also far lower than the levels measured in conventional cigarette mainstream smoke (Eldridge et al., 2015; Reilly et al., 2017). In a general manner, carbonyl concentrations in e-vapors were higher with

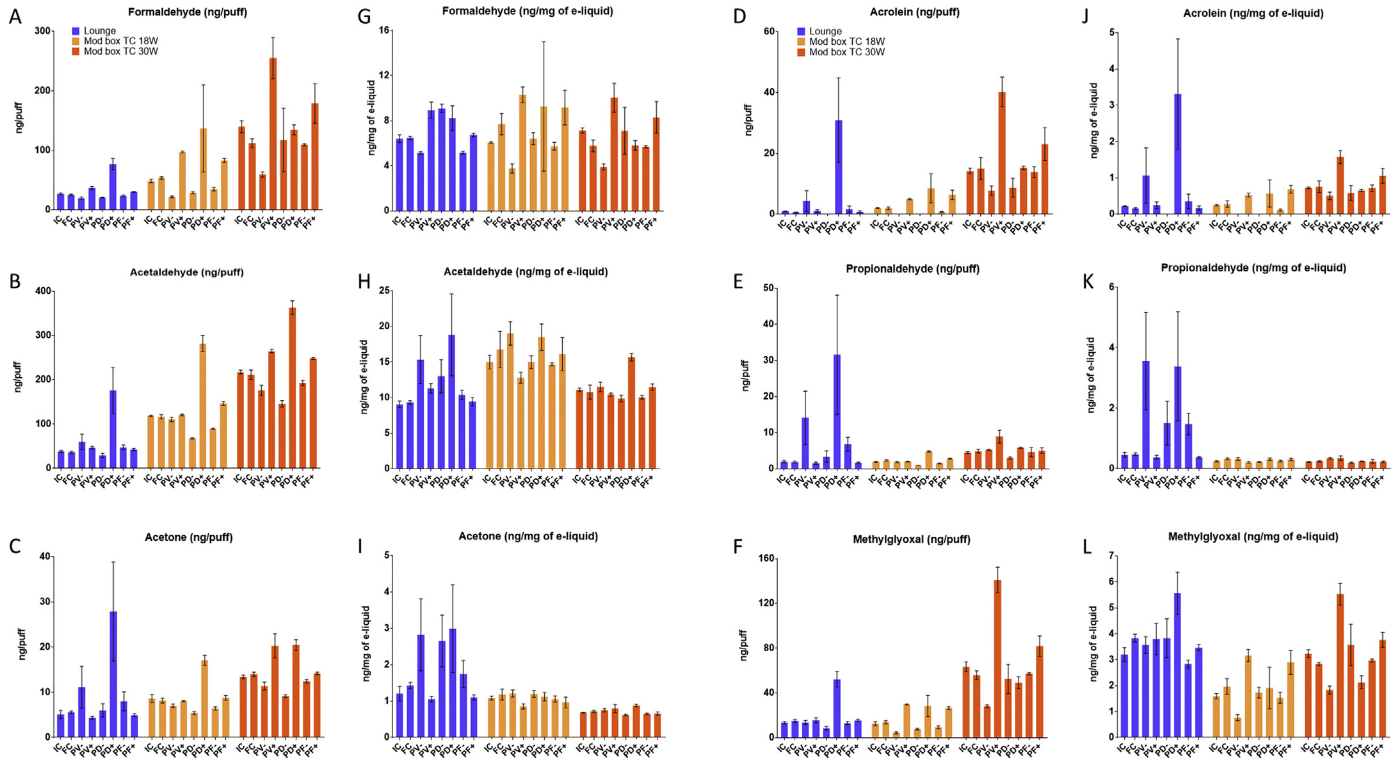

Fig. 3. Carbonyl emissions depending on the puffing regimen used, expressed in ng/puff (A–F) and in ng/mg of consumed e-liquid (G–L). Bars represent mean ± standard deviation of 3 independent and consecutive collections of 20 puffs. "IC": initial CORESTA. "FC": final CORESTA. "PV-": decreased puff volume. "PV + ": increased puff volume. "PD-": decreased puff duration. "PD + ": increased puff duration. "PF-": decreased puff frequency. "PF + ": increased puff frequency. IC and FC experiments were respectively performed at the beginning and at the end of the experiment series.

the Mod Box TC than with the Lounge and higher with 30W than with 18 W, which is in accordance with published data (Farsalinos et al., 2015; Geiss et al., 2016; Gillman et al., 2016; Kosmider et al., 2014; Uchiyama et al., 2016, 2013). However, these trends were sometimes corrected regarding ng/mg results (Fig. 3), which confirms the necessity to communicate e-liquid consumption data. Concretely, rates of carbonyl generation as a function of e-liquid consumption were in a smaller range than concentrations per puff: from 3.8 to 10 ng/mg for FA, from 9.1 to 19 ng/mg for AA, from 0.6 to 3.0 ng/mg for AO, from "not detected" to 3.3 ng/mg for AC, from 0.2 to 3.6 ng/mg for PA and from 0.8 to 5.6 ng/mg for MG. Thus, while it is obvious that e-liquid consumption influences e-vapor carbonyl concentration, the generation of some carbonyl compounds are related to puffing conditions.

Considering puffing regimens, increasing puff volume, duration and frequency could be expected to increase carbonyl-emissions, though we found this was not systematically the case and it depended upon the compound and the e-cig model (Fig. 3). Interestingly, increasing puff duration and frequency displayed high temperatures during vaping sessions compared with other conditions (Fig. 2). Ogunwale et al. reported comparable trends with puff volume and puff duration, using a 1st generation e-cig, for FA, AA and AC (Ogunwale et al., 2017). In the present study, while carbonyl emission from the Lounge model was mainly affected by long puff duration, those from the Mod Box TC model were affected by all 3 conditions. Noticeably, higher carbonyl emissions in ng/puff were not systematically associated with the highest ng/mg generation rates. E-vapor concentrations of AC, PA, and to a lesser extent AO, were greatly increased with low puff volume and short puff duration using the Lounge model and were associated with high generation rates. Margham et al. also reported relatively greater concentrations of AA and AC with low puff volumes using an e-cig technologically closer to the Lounge than to the Mod Box TC, though with high variability at 35 mL puff volume (Margham et al., 2016). Our results, marked by relatively high instability, contrast with the related repeatable e-liquid consumption (Fig. 1) and suggest that e-liquid consumption is not the major parameter influencing the emission of these compounds. As well, temperature is unlikely to be a major influencing parameter: the highest and most variable concentrations observed for AC, PA and AO were not systematically associated with the highest e-vapor temperatures (Fig. 2). In summary, it appeared that several factors, including the puffing regimen, the heating temperature and the e-cig model, affect the emission/generation of the measured carbonyl compounds and that these factors likely interact.

In order to visually assess specific puffing conditions regarding their measured carbonyl emissions and potentially highlight the most important emitting conditions, PCA was applied to the carbonyl concentration data sets (in both ng/puff and ng/mg for the 6 identified carbonyls). PCA reduces the dimensionality of the data set into a new set of variables, named principle components (PC), and does not require previous knowledge of the data set. For each set, 2 PC (PC1 and PC2) which could explain 92% and 74% of the total variance respectively (Figs. S2 and S3), were identified. The resulting score plots and loading plots are presented in Figs. 4 and 5. The data set was separated by hierarchical cluster analysis (HCA) into clusters which are highlighted with different colours in Figs. 4 and 5.

Considering ng/puff concentrations, all the 6 carbonyl concentrations are positively correlated with PC1 while PC2 seems to differentiate 2 groups of compounds: PA, AO and AC *versus* FA, AA and MG. However, PC2 cannot be considered as highly discriminant for most of the compounds, except for PA. Briefly, the tested conditions are mainly positioned on the PC1 axis according to their carbonyl e-vapor concentrations. Most of the tested conditions are grouped in 2 clusters. While cluster 1 mostly contains Lounge and Mod Box TC 18 W conditions, thus the equivalent of the "less emitting" conditions, most of Mod Box TC 30 W conditions are grouped in cluster 2 which is mainly shifted according to PC1 axis. The two final clusters constitute 2 single conditions: high puff volume with Mod Box TC 30 W and long puff duration

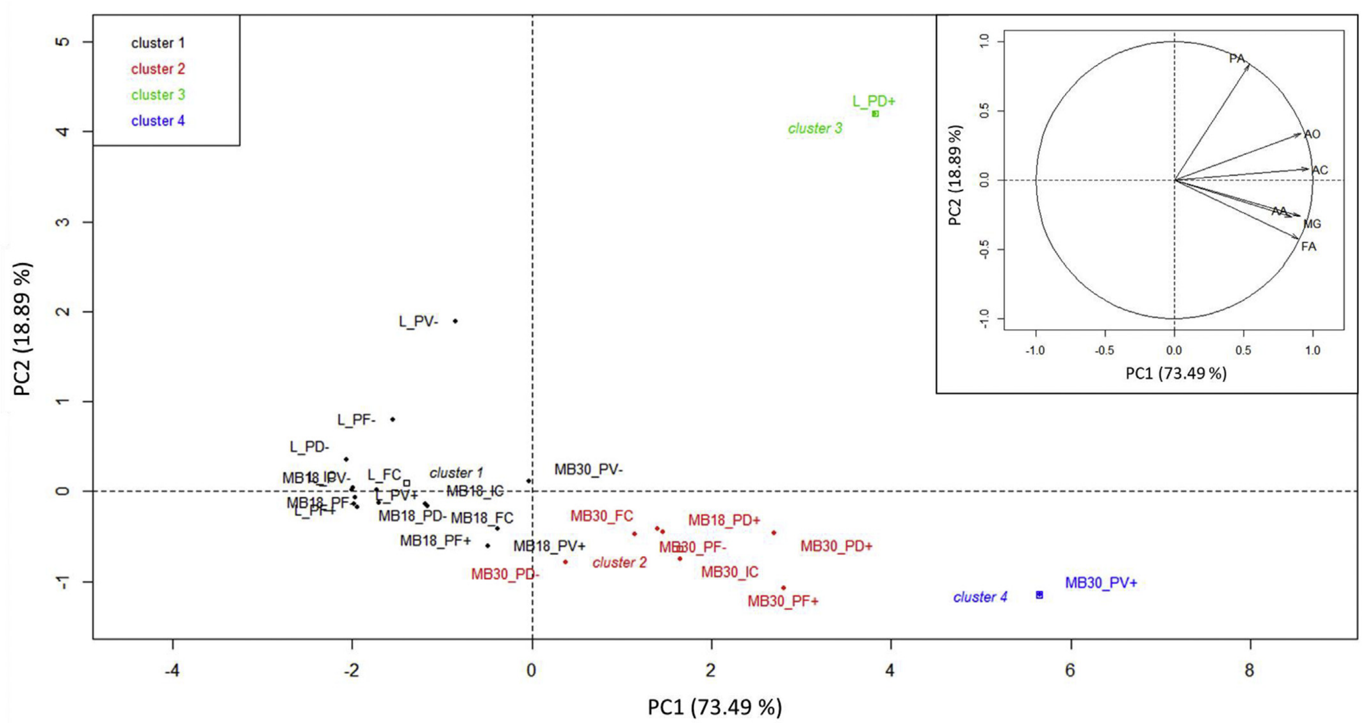

Fig. 4. Score plots, loading plots and clustering results of PCA and HCA for carbonyl e-vapor concentrations (ng/puff) depending on vaping conditions. "FA": formaldehyde, "AA": acetaldehyde, "AO": acetone, "AC": acrolein, "PA": propionaldehyde, "MG": methylglyoxal, "L": lounge, "MB18": mod box 18 W, "MB30": mod box 30 W, "IC": initial CORESTA. "FC": final CORESTA. "PV-": decreased puff volume. "PV+": increased puff volume. "PD-": decreased puff duration. "PD+": increased puff duration. "PF-": decreased puff frequency. "PF+": increased puff frequency. IC and FC experiments were respectively performed at the beginning and at the end of the experiment series.

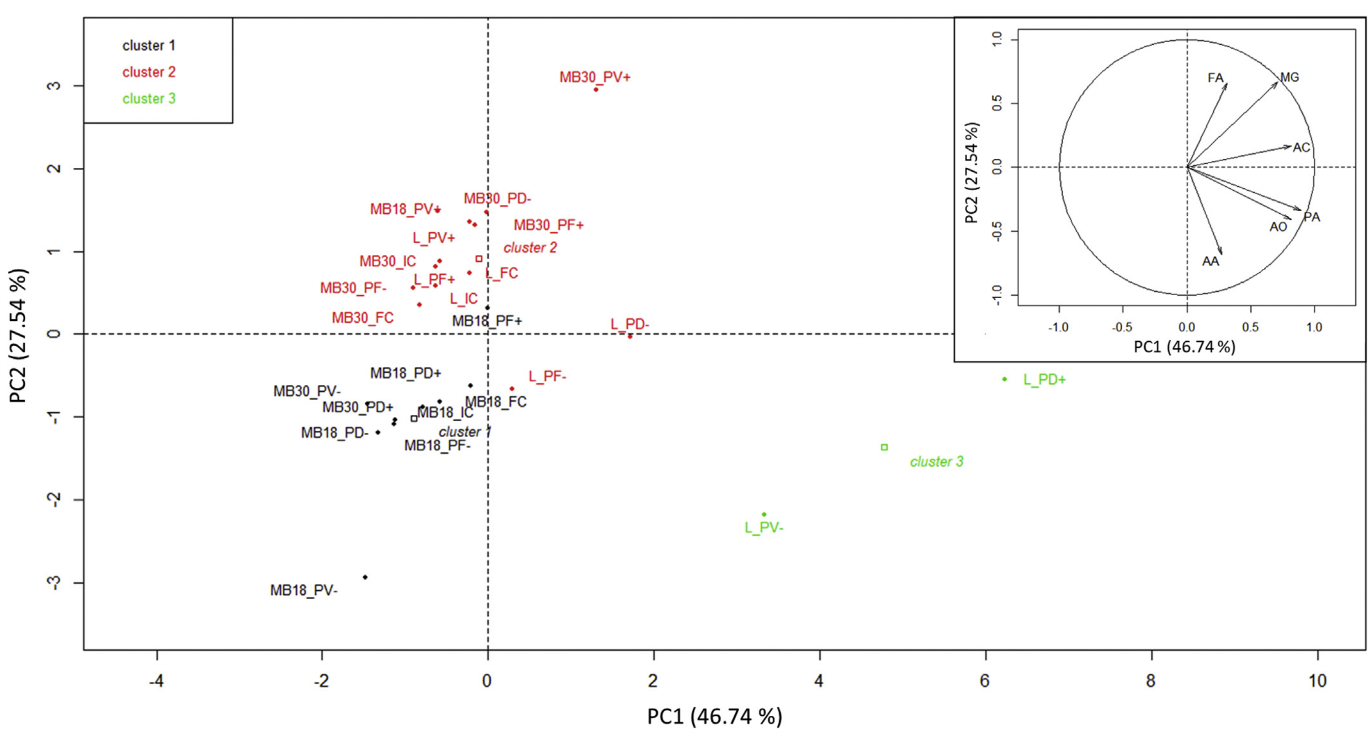

Fig. 5. Score plots, loading plots and clustering results of PCA and HCA for carbonyl e-vapor concentrations (ng/mg) depending on vaping conditions. "FA": formaldehyde, "AA": acetaldehyde, "AO": acetone, "AC": acrolein, "PA": propionaldehyde, "MG": methylglyoxal, "L": lounge, "MB18": mod box 18 W, "MB30": mod box 30 W, "IC": initial CORESTA. "FC": final CORESTA. "PV-": decreased puff volume. "PV+": increased puff volume. "PD-": decreased puff duration. "PD+": increased puff duration. "PF-": decreased puff frequency. "PF+": increased puff frequency. IC and FC experiments were respectively performed at the beginning and at the end of the experiment series.

with Lounge, which are positively correlated with PC1 and both PCs, respectively. PC1 may be associated to power supply, e-vapor temperature, e-liquid consumption or their combination, though a clear conclusion cannot be drawn.

By contrast, ng/mg PCA shows that the generation rates of AO, AC, PA and MG are mainly related to PC1, while those of FA and AA are mainly related to PC2. Once again, most of the conditions are grouped into 2 clusters and 2 of them are highlighted into a third cluster: low puff volume and long puff duration with Lounge, which are positively correlated with PC1. These 2 conditions especially led to higher generation rates of AO, AC and PA compared with other conditions, suggesting that a favourable element may be involved. Indeed, they are characterised by low air flow during vaping, though this parameter does not seem to affect carbonyl generation with the Mod Box TC e-cig. The PCA suggests that the conditions leading to FA generation are those that generate less AA, and conversely. Regarding raw results (Fig. 3), differences remain small and some exceptions exist, like low puff frequency with Lounge and long puff duration with Mod Box TC 18 W.

Finally, although definitive conclusions from this study are limited, the PCA confirms the interaction between several vaping conditions and highlights some combinations of concern, especially high puff volume with Mod Box TC 30 W and both high and low puff volumes with Lounge.

Importantly, the observations made in the present study apply to the devices and e-liquids used and do not pretend to predict to what extent other e-cig devices and e-liquids, which may emit different amounts of carbonyls, would be affected using comparable conditions. To go further, e-liquid carbonyl concentrations could be studied to estimate potential e-liquid contamination and better discriminate generation from emission of carbonyl compounds during vaping. However, this latter point was beyond the scope of this study.

4. Conclusion

The results of the present study demonstrate that the modification of puffing conditions leads to significant variations in the carbonyl composition of e-cigarette aerosols. While the formaldehyde, acetone and methylglyoxal measured concentrations might be underestimated, especially for the 2nd generation e-cigarette model, these variations were observed with concentration levels in the lower panel of the literature data, far lower than those of conventional cigarette mainstream smoke. However, in addition to the large range of available products on the market, of the analytical techniques used and of the different types of e-vapor generation methods, these results support that the puffing regimen also contributes to the observed variability in carbonyl levels reported in the scientific literature, and that their comparison and interpretation could thus be limited. Harmonized protocols to be used in physicochemical or experimental studies are clearly needed and should be adapted to the e-cig technologies under investigation in order to mimic as closely as possible the probable range of real conditions. However, some conditions of concern can be highlighted and should not be neglected regarding risk assessment. Many other vaping conditions including the heating triggering, the e-cig tilt or the device aging still need to be investigated with respect to their impact on the carbonyl composition of e-cigarette aerosols, with the ultimate goal of better characterising and preventing e-cigarette-related health hazards.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2018.08.015.

Tables computing the different puffing regimen used and the results of e-liquid consumption (mg/puff), carbonyl emission (ng/puff) and carbonyl generation rate (ng/mg of consumed e-liquid) are available in supplementary data (Tables S1 and S2), along with an example of the temperature measurement over a 20 puffs series (Fig. S1) and the PCA bar plots (Figs. S2 and S3).

Conflicts of interest

None.

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