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Original Investigation
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Original Investigation

Puff-Resolved Analysis and Selected Quantification of Chemicals in the Gas Phase of E-Cigarettes, Heat-Not-Burn Devices, and Conventional Cigarettes Using Single-Photon Ionization Time-of-Flight Mass Spectrometry (SPI-TOFMS): A Comparative Study

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Abstract

Introduction: A wide array of alternative nicotine delivery devices (ANDD) has been developed and they are often described as less harmful than combustible cigarettes. This work compares the chemical emissions of three ANDD in comparison to cigarette smoke. All the tested ANDD are characterized by not involving combustion of tobacco.

Aims and Methods: Single-photon ionization time-of-flight mass spectrometry (SPI-TOFMS) is coupled to a linear smoking machine, which allows a comprehensive, online analysis of the gaseous phase of the ANDD aerosol and the conventional cigarette (CC) smoke. The following devices were investigated in this study: a tobacco cigarette with a glowing piece of coal as a heating source, an electric device for heating tobacco, and a first-generation electronic cigarette. Data obtained from a standard 2R4F research cigarette are taken as a reference.

Results: The puff-by-puff profile of all products was recorded. The ANDD show a substantial reduction or complete absence of known harmful and potentially harmful substances compared with the CC. In addition, tar substances (i.e. semivolatile and low volatile aromatic and phenolic compounds) are formed to a much lower extent. Nicotine, however, is supplied in comparable amounts except for the investigated electronic cigarette.

Conclusions: The data show that consumers switching from CC to ANDD are exposed to lower concentrations of harmful and potentially harmful substances. However, toxicological and epidemiological studies must deliver conclusive results if these reduced exposures are beneficial for users.

Implications: The comparison of puff-resolved profiles of emissions from different tobacco products, traditional and alternative, may help users switch to lower emission products. Puff-resolved comparison overcomes technical changes, use modes between products and may help in their regulation.

Introduction

The development of alternative nicotine delivery devices (ANDD) is mainly driven by the severe negative health impact of conventional cigarette (CC) consumption.[1] It is well known that tobacco smoke contains high levels of toxic components like polycyclic aromatic hydrocarbons, carbonyls, other volatile organic compounds, tobacco-specific nitrosamines, and carbon monoxide.[2,3] Smoking is still the most preventable cause of premature death and causes high economic costs. In this context, it is estimated that smoking kills over 6 million people per year worldwide and costs for health care and lost productivity add up to over 1 trillion US$.[3] It has also been shown, that complication rates among smokers are higher for influenza-like diseases or in the coronavirus pandemic 2020.[4,5] Nevertheless, the CC is still the most popular nicotine delivery product.

ANDD are designed to deliver nicotine and flavor compounds with reduced amounts of toxic byproducts. It is shown that some levels of toxic components in aerosols of electronic cigarettes (ECs) are orders of magnitudes lower than in CC smoke.[6–8] However, as the aerosol of ECs and ANDD still contains harmful substances and are by no means a safe product.[9–11]

ANDD can be divided into tobacco and nontobacco heating products. The latter is known as EC. Unlike CCs, they do not contain tobacco but an electrically powered heating device that evaporates a liquid and converts it into inhalable aerosols. Liquids in ECs consist of the matrix components glycerol (propane-1,2,3-triol), propylene glycol (propane-1,2-diol), and water in different formulations with or without nicotine and with or without flavorants. Heating a liquid may lead to the generation of toxic byproducts due to thermodegra dation.[6,8–10,12] However, compared with CCs, levels of produced toxicants are generally significantly lower. It is supposed that, ECs might be a less harmful alternative for people who are addicted to smoking CCs but this is not conclusively proven.[6] Besides this, the risks of passive smoking are significantly reduced compared with CCs.[13]

The other group of ANDD is called Heat-Not-Burn (HNB) products and can be described as a device that heats tobacco or herbs. Humectants are added to HNB-cigarettes to produce a denser aerosol. Because of lower heat exposure and the absence of combustion, the HNB emissions should contain less toxic byproducts compared with the smoke of CCs.[1,11] For this reason, HNB-products are often promoted as less harmful than CCs.[14] Research on HNB-products is rare although some products have been on the market since 30 years.[3] Researchers working on HNB-products conclude that the consumption of HNB-products may entail a risk reduction for smokers, however, many are affected by conflicts of interests.[15–17]

ECs and HNB-products are competing as nicotine delivery systems. Accordingly, these ANDD need to be compared with CCs for their release patterns of toxicans. Studies of online analysis of ANDD are limited and none of these compare ECs, HNB-products and CCs with each other. Online techniques are beneficial in gaining an understanding of the puff-by-puff emission profile of these products and the advantage of direct analysis. In other techniques, sample components have to be trapped leading to loss of some volatile compounds or to secondary reactions. Breiev et al.[18] have demonstrated the potential of using the proton-transfer-reaction MS for inhaled and exhaled vapor of an e-cig. García-Gómez et al.[19] demonstrated that secondary electrospray ionization provides an interesting possibility for the online analysis of e-cigs. Surprisingly, although it is an atmospheric pressure ionization, the matrix components propylene glycol, glycerine, and water are not a problem for the analysis and are only ionized to a very small extent. However, more complex analysis systems are more susceptible to contamination due to their design, especially if besides ANDD also cigarettes are analyzed for a direct comparison.

In this study, a single-photon ionization time-of-flight mass spectrometry system (SPI-TOFMS) was used. The advantage of this technique is that it delivers online (real-time) information on the gas-phase composition in a fast and dynamically changing process like wood burning, coffee roasting, vehicle exhaust, and tobacco smoking.[20,21] A further benefit is the possibility to couple it with other analytical methods like thermogravimetric analysis[22] or gas chromatography.[23,24]

In SPI-TOFMS, the gaseous analytes are mostly ionized with 118 nm photons. At this wavelength, the absorption of only one photon is sufficient to ionize compounds with an ionization energy below 10.5 eV. This ionization technique leads to mostly intact molecular radical cations (soft ionization). Accordingly, most analytes can be easily detected by their original molecular mass without fragmentation products, which, in turn, is the case for conventional electron impact ionization. Many compounds can thus be analyzed online with subsecond time resolution. This study aims to present the possibilities of SPI-TOFMS for online characterization of ANDD and CCs as well as to give a comparative view of the investigated products on a puff-by-puff basis.

Materials and Methods

In this study, two types of different HNB smoking devices and three first-generation ECs are investigated and compared with the reference CC. The HNB-products are either electrically heated or use controlled combustion for heat generation.

In the following the investigated ANDD are briefly described (see also Figure 1A). All ANDD have been bought from the German market.

Electronical Heated Heat-Not-Burn Product

The electrically heated HNB (eHNB) selected for this study is the IQOS (System 2.4 Philip Morris Int.) with Heatsticks type "*amber*." The manufacturer recommends that the first should be taken after a warm-up of 20s.

Combustion Heated Heat-Not-Burn Product

The combustion heated HNB (cHNB) chosen for this study is the *Eclipse* (flavor: menthol, R.J. Reynold). In the cHNB-product, the tobacco rod is heated by hot combustion gases originating from a glowing piece of coal, which is incorporated in incombustible glass fiber at the back end. This glass fiber contains air ducts and has to be ignited before use. The *Eclipse* cigarette is not commercially available anymore but has been relaunched over the past two decades several times with different names (*Premier*, *Eclipse*, *Revo*). Due to the unique way of heat transfer to the tobacco rod, it is still of interest.[16,17,25,26] As there is no direct combustion of the tobacco but a glowing piece of coal acting as a heat source, this cigarette can be regarded as high temperature HNB. This makes it an interesting research object together with the "low temperature" eHNB.

Electronic Cigarette

The ECs tested in this study are first-generation cig-a-likes. Three different brands were investigated in this study. The EC mainly investigated in this study is the *PowerCig* (type: coffee flavor, 12 mg/

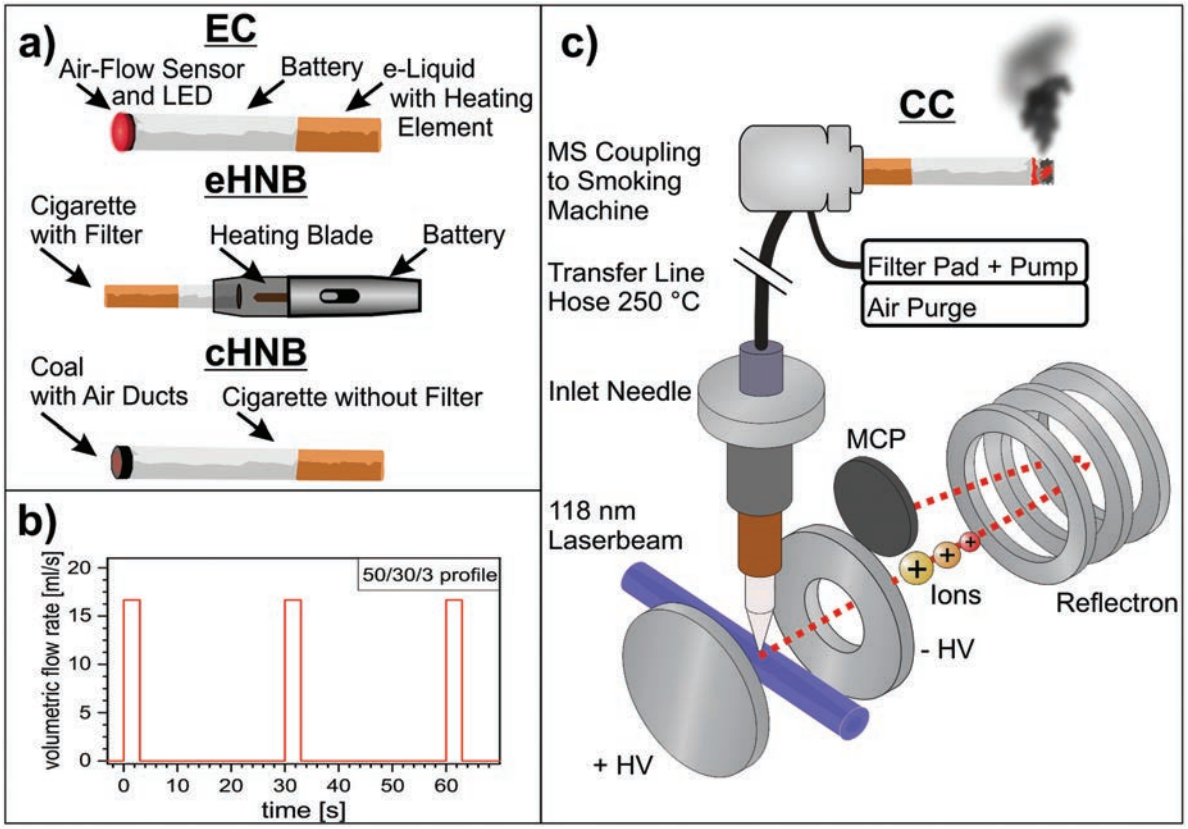


Figure 1. Materials and method scheme. (A) Schemes of the alternative nicotine delivery devices (ANDD), EC (electronic cigarette), eHNB (electrically heated Heat-Not-Burn), cHNB (combustion heated HNB). (B) The main smoking profile used in this study (50/30/3 puff volume in mL/puff interval in seconds/puff duration in seconds, ventilation holes were not blocked). (C) Smoking machine coupled with a reflectron time-of-flight photoionization mass spectrometer for online measurements of classical cigarettes (CCs) and ANDD.

mL nicotine, PowerCigs Ltd). The two other types are a low-cost EC named *Duvance* (type: vanilla flavor, 0 mg/mL nicotine, Pearl GmbH) and a brand called *Vype* (types: menthol and bold, 11.3 mg/mL nicotine and 18.6 mg/mL, CN Creative Ltd). The ECs are activated automatically with a built-in sensor that reacts to the airflow while puffing. Due to this, a rectangle-shaped puffing profile was chosen (and used for all products) instead of the bell-shaped in order to improve the response behavior of the EC.

Reference CC

As a reference, the research cigarette 2R4F (University of Kentucky) was chosen. The CCs (as well as the HNB-cigarettes) were stored at 60% relative humidity and 22°C room temperature in a desiccator before usage and were lit with a *Borgwaldt* electric lighter.

EC liquid depots and eHNB-cigarettes were bought and freshly opened. CC and cHNB-cigarettes were stored at −20°C until use.

Experimental Setup

The experimental setup features a custom-built coupling between the *Borgwaldt KC smoking machine model LM1* and the SPI-TOFMS. The MS transfer line consists of a 2 m long, deactivated, heated (250°C) metal capillary with an inner diameter of 280 µm. At the sampling point, 150°C are reached due to passive heating. The smoking machine complies with the requirements of ISO 3308 with the adjustable puffing parameters: puff time, puff volume, puff number, and the interpuff interval. Two different puff profiles were used in this study. As the ECs are equipped with an airflow sensor for activation of the coils and showing a poor response behavior for the widely used ISO 3308:2012 profile,[27] an alternative puffing profile was applied featuring 50 mL puff volume, 3 seconds puff duration, and 30 seconds puff intervals for the main comparison of ECs, HNBs, and reference CCs. This profile is illustrated in Figure 1B. For comparison also the reference CC was studied under this smoking regime in this work but data for ISO and Health Canada Intense (HCI) profile from literature are given as well (Table 1). In addition, another profile with varying puff volumes, durations, and intervals for every puff was applied for the comparison between different ECs, which is called random profile. The parameters for this profile are shown in Figure 3C.

A system using a pod filled with tobacco extract was investigated as well (Ploom, Japan Tobacco Inc.). However, as the system was not operating reproducibly and furthermore has been withdrawn from the market, the results are solely briefly discussed in Supplementary Material.

Mass Spectrometry

The photoionization time-of-flight mass spectrometer is based on a RFT10 mass analyzer (Stefan Kaesdorf, Germany) which separates

Table 1. Total Yields of Quantified Toxicants (in µg/Puff) for the Investigated ANDD Together With Literature Values[8,11,23,28–30]

	EC			eHNB			cHNB
µg/puff	Uchiyama	Margham	This work	Jaccard	Schaller	This work	This work
Toluene	0.04–0.11[a]	—	—	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	2.3 ± 0.5
Benzene	0.01–0.11[a]	—	—	0.05 ± 0.003	0.05 ± 0.01	—	1.6 ± 0.4
Acetone	0.3–18.7[a]	—	30.7 ± 4.9[b]	3.0 ± 0.2	2.8 ± 0.5	8.8 ± 0.5	32.5 ± 7.8[b]
Acrolein	0.2–55.8[a]	0.07 ± 0.05	1.6 ± 0.3	0.8 ± 0.06	0.8 ± 0.07	1.7 ± 0.2	15.7 ± 2.7[c]
Butadiene	0.6 ± 0.2	—	—	0.03 ± 0.003	0.03 ± 0.01	—	1.3 ± 0.1
NO	—	—	2.1 ± 0.3	1.2 ± 0.08	1.8 ± 0.7	1.0 ± 0.04	4.6 ± 0.7
	55/60/3[d]	ePen, HCI	50/30/3	THS 2.2, HCI	THS 2.2, HCI	50/30/3	50/30/3
	15 puffs	200 puffs	19 puffs	12 puffs	12 puffs	10 puffs	13 puffs
	CC						
µg/puff	Uchiyama	Margham	Jaccard	Adam	Eschner	Schaller	This work
Toluene	16.3 ± 1.8	11.0 ± 9.1	11.4 ± 1.4	9.4 ± 0.5	12.1 ± 0.7	18.8 ± 1.0	9.8 ± 1.9
Benzene	10.6 ± 0.8	6.9 ± 7.4	6.8 ± 0.7	5.4 ± 0.4	8.0 ± 0.6	9.5 ± 0.3	4.2 ± 0.8
Acetone	42.3 ± 2.4	67.2 ± 16.0	57.5 ± 3.1	29.5 ± 1.7	64.0 ± 2.9	68.8 ± 3.4	66.5 ± 13.1
Acrolein	9.8 ± 0.3	15.9 ± 3.0	13.0 ± 2.1	—	10.8 ± 1.6	18.21 ± 2.0	—[e]
Butadiene	8.0 ± 0.7	8.7 ± 5.6	8.2 ± 0.8	4.3 ± 0.2	9.7 ± 1.2	8.7 ± 1.0	3.9 ± 0.9
NO	—	47.0 ± 22.9	42.0 ± 2.4	34.4 ± 1.5	68.3 ± 5.0	47.3 ± 3.1	14.6 ± 3.8
	CM6, 55/60/3	3R4F, HCI	3R4F, HCI	2R4F, ISO	2R4F, ISO	3R4F, HCI	50/30/3
	12.3 puffs	10.x puffs	12 puffs	9 puffs	10 puffs	10.6 puffs	14 puffs

Data indicate the mean value ± SD. ANDD = alternative nicotine delivery devices, CC = conventional cigarette, cHNB = combustion heated Heat-Not-Burn, EC = electronic cigarette, eHNB = electronical heated Heat-Not-Burn.
[a]Three different brands are analyzed, min and max values are depicted.
[b]Unknown contribution of glyoxal and propanal to the signal.
[c]Unknown contribution of butene to the signal.
[d]Puffing regime, puff volume (mL)/puff interval (s)/puff duration (s).
[e]Butene shows higher contribution to the signal at 56 m/z, concentration found: (26.2 ± 5.2) µg/puff.

ions of different molecular weight by their flight-time through a field-free drift tube. After ions are generated by the absorption of vacuum-ultraviolet photons, they are accelerated in a Wiley–McLaren[31] ion source, reflected and refocused by a Mamyrin-type ion mirror to a microchannel plate detector. The ion mirror allows better focusing of isobaric compounds and enhancement of the ion drift length.[32]

Inside the ionization chamber, the gaseous analytes are ionized with 118 nm photons. These photons are generated by frequency multiplication by nonlinear optical processes of an initial 1064 nm laser beam emitted by a 10 Hz pulsed Nd:YAG (neodymium-doped yttrium aluminum garnet) laser. The SPI-TOFMS instrument is described in detail elsewhere.[33] A schematic setup of the instrument is depicted in Figure 1C.

The MS data recording and analysis was performed with the programs "Photonion-RTF-Tof-Ms Data Viewer" and "OriginPro 2018b." The external calibration of the system was carried out with a 1 ppm calibration gas standard (benzene, toluene, xylene, trimethylbenzene, in nitrogen; supplier: *Air Liquid*) after each measurement. The TOF setup with the laser ionization source and smoking machine coupling, software is commercialized by the spin-off company Photonion GmbH, Germany. This coupling with a slightly different setup is well characterized.[34]

Results and Discussion

Figure 2 illustrates an overview of the devices analyzed in this study. The spectra of HNBs and EC (PowerCig) are dominated by the signals of nicotine (162; 84 m/z) and glycerol (74; 62; 61; 60; 44 m/z). The EC also emits high amounts of propylene glycol (76; 61; 45; 44; 33 m/z). As these substances are the matrix of e-liquids, their magnitude in signal intensity is reasonable in the first column in Figure 2. Because of the soft ionization technique, mostly molecular ion signals are generated. However, especially for polyols like glycerol and propylene glycol broad fragmentation patterns are given. These substances undergo fragmentation due to the highly oxidized, flexible carbon backbone and possibility to lose stable molecules like water or formaldehyde.[35,36] SPI spectra of pure glycerol and propylene glycol together with nicotine, acetaldehyde, and acetone can be seen in Supplementary Figure S1. These spectra are also compared with conventional electron impact ionization spectra in order to demonstrate the benefits of SPI.

In the third column of Figure 2, the enlarged spectra are presented. The complexity of cigarette smoke is clearly visible due to the high number of signals. According to the literature, a large number of compounds have been identified in CC smoke,[2] from which several dozens can be detected online in real time by SPI-TOFMS. In contrast, the complexity of SPI mass spectra of the ANDD is largely reduced. A less complex spectrum is directly related to lower levels and number of gas-phase substances. This may hint to reduced emissions of harmful and potentially harmful substances by ANDD. This effect could be directly related to the lower temperatures and the absence of combustion. Hence, the levels of volatile organic compounds (VOCs, eg, nitric oxide, butadiene, acrolein, benzene) and tar should be largely reduced as well. An evaluation of the levels of these two compound classes is important for the assessment of adverse health effects.[37,38] It is known, that for ANDD the levels of VOC emissions are reduced compared with CC smoke.[39–41]

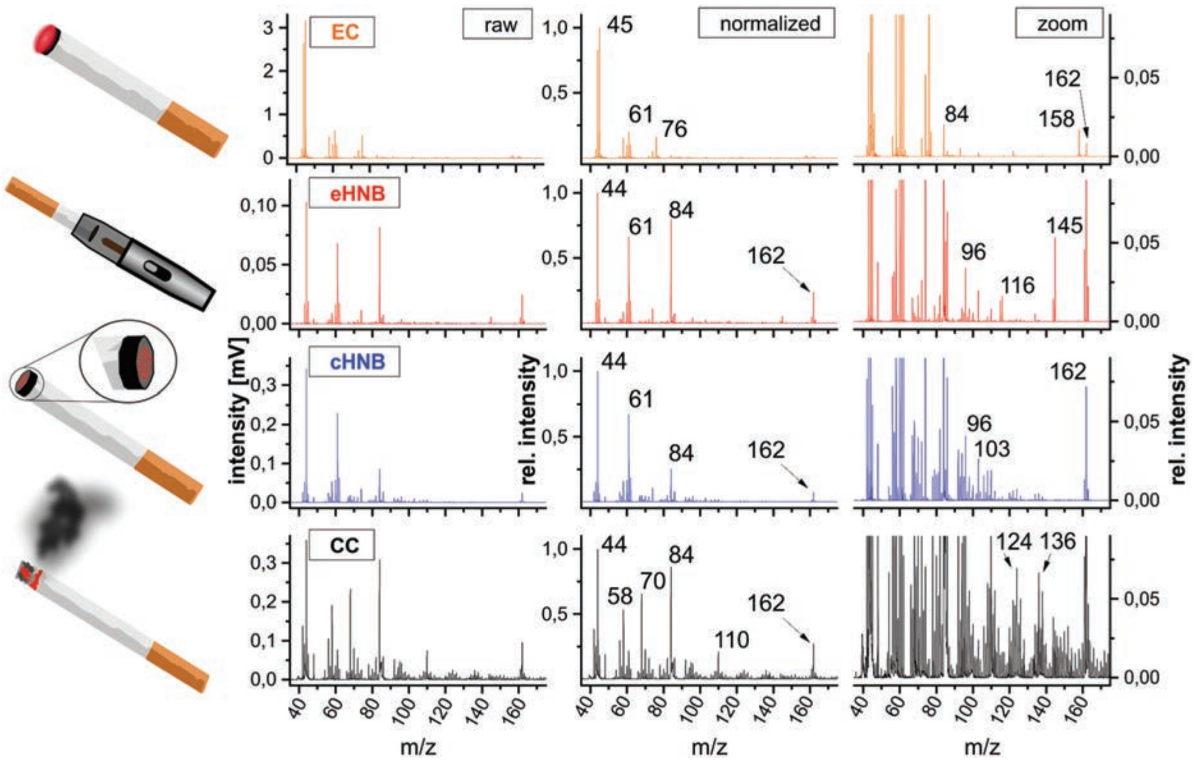


Figure 2. SPI mass spectra of several smoking devices, which are visualized as unnormalized raw spectra (first column, please note the different *y*-axis), normalized to their highest signal (second column), and zoomed normalized spectra (third column). All mass spectra represent the average over all puffs. The depicted SPI mass spectra indicate that the gas phases of ANDD contain fewer substances, mostly in lower concentrations compared with CCs. ANDD = alternative nicotine delivery devices, CC = conventional cigarette, SPI = single-photon ionization.

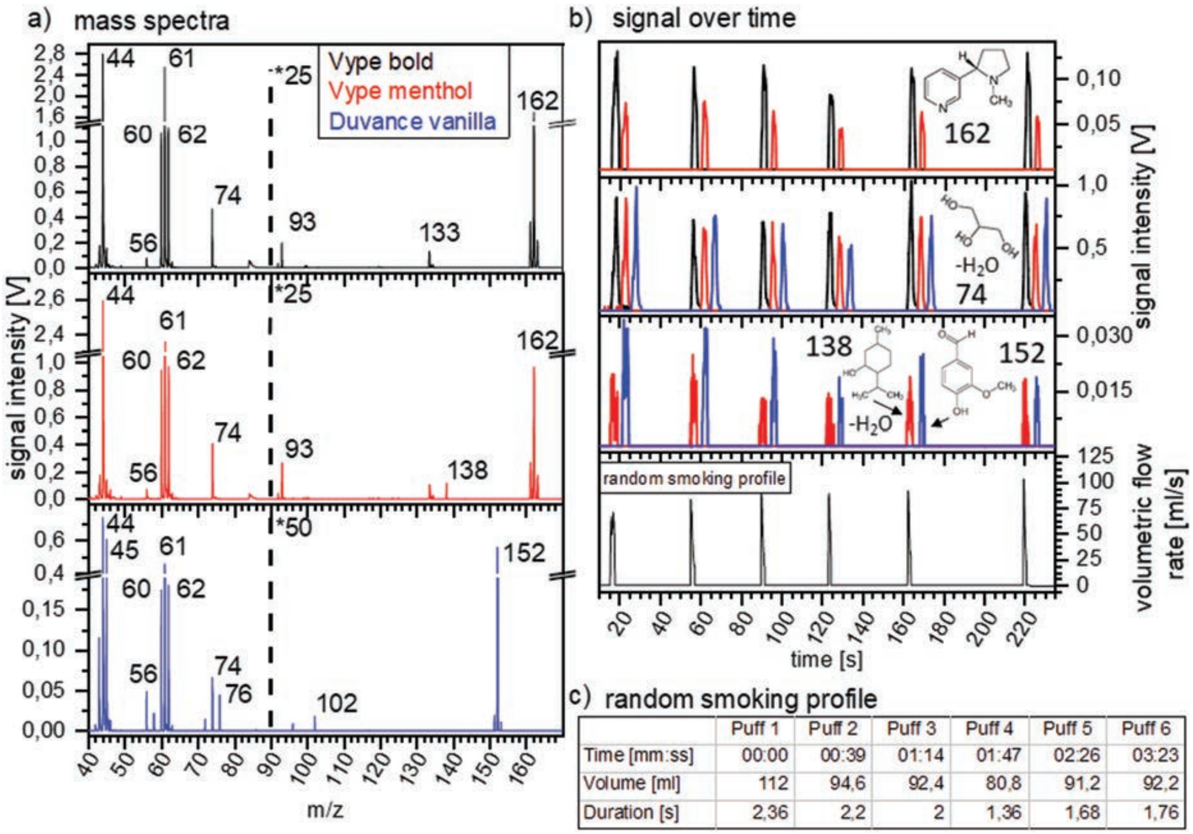
For tar, there are contradictory results. When measured under ISO 4387:2000 tar levels of CCs and ANDD are similar,[39,41] but considering Figure 2, there should exist discrepancy in tar levels, as there is also a big discrepancy in the gas-phase composition. The solution for this inconsistency lies in the high amounts of humectants, which are determined as tar according to the ISO method. The tar fraction in CCs is a high boiling, highly aromatic biomass pyrolysis condensate, containing highly toxic and cariogenic compounds such as the polycyclic aromatic hydrocarbons and tobacco-specific nitrosamine. The nature of tar composition differs widely for CCs and ANDD.[42,43] A more detailed discussion on VOC and tar levels is given in Supplementary Figure S2 together with a qualitative comparison of VOC and tar levels for CCs and ANDD without applying ISO 4387:2000 and the humectants discrepancy issue.

The combination of photoionization with time-of-flight mass spectrometry also allows a high time resolution. With this technique it is possible to show puff-by-puff emission profiles of individual components such as flavor additives or nicotine as well as the emission of humectants.

Figure 3 shows a comparison of two brands and three e-liquids. The spectra in Figure 3 illustrate that the investigated e-liquids mainly consist of humectants and are partially flavored and/or contain nicotine. All visible signals below 100 m/z can be assigned to the humectants (see Supplementary Figure S1 for spectra of pure glycerol and propylene glycol). As mentioned before, polyols are highly affected by photofragmentation. Glycerol does not appear at its molecular mass at 92 m/z, moreover the $M+H^+$ adduct is visible at 93 m/z. This is unusual for vacuum ionization and likely stems from an ionized glycerol dimer or water adduct. The first two spectra in Figure 3A are derived from the same brand. The e-liquids differ in nicotine content (18.6 and 11.3 mg/mL) and the e-liquid of the middle spectrum (in red), additionally contains menthol. The bottom spectrum (in blue) in Figure 3A is from a different brand. This e-liquid is nicotine-free and depicted to be vanilla flavored. Therefore, the signal at 152 m/z can be assigned to vanillin. Additionally, propylene glycol can be identified at 76; 45 m/z. In Figure 3B, the potential of the online system is demonstrated by monitoring highly dynamic signals over time. Three signals were chosen corresponding to nicotine (top), glycerol (second graph), and the flavor compounds menthol and vanillin (third graph). In the bottom graph of Figure 3B, the applied random smoking profile is shown with its basic parameters in Figure 3C. The signals in Figure 3B are time-shifted from each other by 5 seconds for better visualization. It can be seen that single puffs are well separated from each other with little or no baseline noise on the specific mass signals. The difference in nicotine content is also well perceptible.

The random smoking profile was tested in order to mimic a more realistic smoking behavior and to identify differences in the puff-by-puff behavior,[44] which, however, were not significant here.

In Figure 4, the puff-by-puff profiles are shown for several relevant substances in a qualitative manner. For some substances, the relative photoionization cross-sections are known, which enables a semiquantitative characterization without applying calibrations standards. This approach was detailed elsewhere.[23,45]



	Puff 1	Puff 2	Puff 3	Puff 4	Puff 5	Puff 6
Time [mm:ss]	00:00	00:39	01:14	01:47	02:26	03:23
Volume [ml]	112	94,6	92,4	80,8	91,2	92,2
Duration [s]	2,36	2,2	2	1,36	1,68	1,76

Figure 3. Comparison of different first-generation ECs. (A) SPI mass spectra of ECs with different flavors of two different brands. The number at the dashed line indicates the zoom factor used beyond 90 m/z. Mass spectra are averaged over the second puff of four replicates. (B) Puff-by-puff profiles of some characteristic m/z: 162 nicotine, 152 vanillin, 138 menthol (-water), and 74 glycerol (-water). The time profiles of the menthol and vanilla flavored ECs are time-shifted for 5 or 10 seconds, respectively, for a better comparison. (C) Random smoking profile for the measurements. EC = electronic cigarette, SPI = single-photon ionization.

Delivery of nicotine is one of the main purposes for the investigated products. As illustrated in Figure 4, the HNB-products reach similar nicotine levels (about two to three times lower) with respect to CC. EC nicotine output is remarkably lower (about an order of magnitude).

Large differences can also be seen in the puff-by-puff profiles. ECs give a rather constant puff profile over several puffs and for all gas-phase substances. In contrast, CCs show a well-known puff-by-puff increase for most of all substances[46] and generally provide the highest amounts. The puff-by-puff increase of nicotine in CC over many puffs is believed to be an important aspect for addiction and smoking behavior.[47] The unusual decreasing release of nicotine in the last puffs of the CCs is caused by the used profile and the complete smoking of the cigarette up to the filter. The eHNB and cHNB puff profiles are influenced by a rather slow tobacco heating and suppressed reevaporation due to the absence of a moving source of heat. The working principles of ANDD differ from each other, especially for the HNB-products. However, it is likely that the heating program of the electrical HNB systems are set in a way that the puff-by-puff increase of nicotine of CC is resembled. This is also observed in data from another form of eHNB, utilizing heated tobacco-extract capsules, as discussed in Supplementary Figures S4 and S5.

For a complex matrix as cigarette smoke, more than one compound can be associated to a specific mass signal. For example, in CC smoke, 56 m/z represents nearly exclusively butene[23] but for the ANDD with electrical heating, no butene emissions are reported in literature. If the content of humectant becomes higher in the product, the more likely acrolein causes the signal at 56 m/z. Glycerol and propylene glycol are highly oxidized molecules and are prone to be affected by alpha-bond cleavages or loss of water. Even if the complexity of the spectra of ANDD is lower, their interpretation should be carefully done due to the ionization induced fragmentation of the humectants. As a consequence, the amounts of compounds such as acrolein and acetone are also lower than they appear in the SPI mass spectra. A method for separating thermal- und photofragmentation is shown in Supplementary Figure S3. This method has to be carefully checked with larger datasets and have not been used for the data presented in this work. Acrolein is considered to be the most toxic component in CC smoke while the most relevant carcinogenic substance is butadiene.[23,48] For the investigated electrical heated ANDD, acrolein (even with contributions to isobaric compounds and fragmentation) is drastically reduced with respect to CC while butadiene is usually not detected in ECs and eHNB-cigarettes with the exception of the work of Uchiyama et al.[28] for one EC (limit of detection in the low ppb range).[33] For cHNB no clear statement can be given, as some butene formation might be possible. Note that formation of acrolein in EC depends on the heat flux into the liquid. Thus, different generation EC devices or different designs can emit

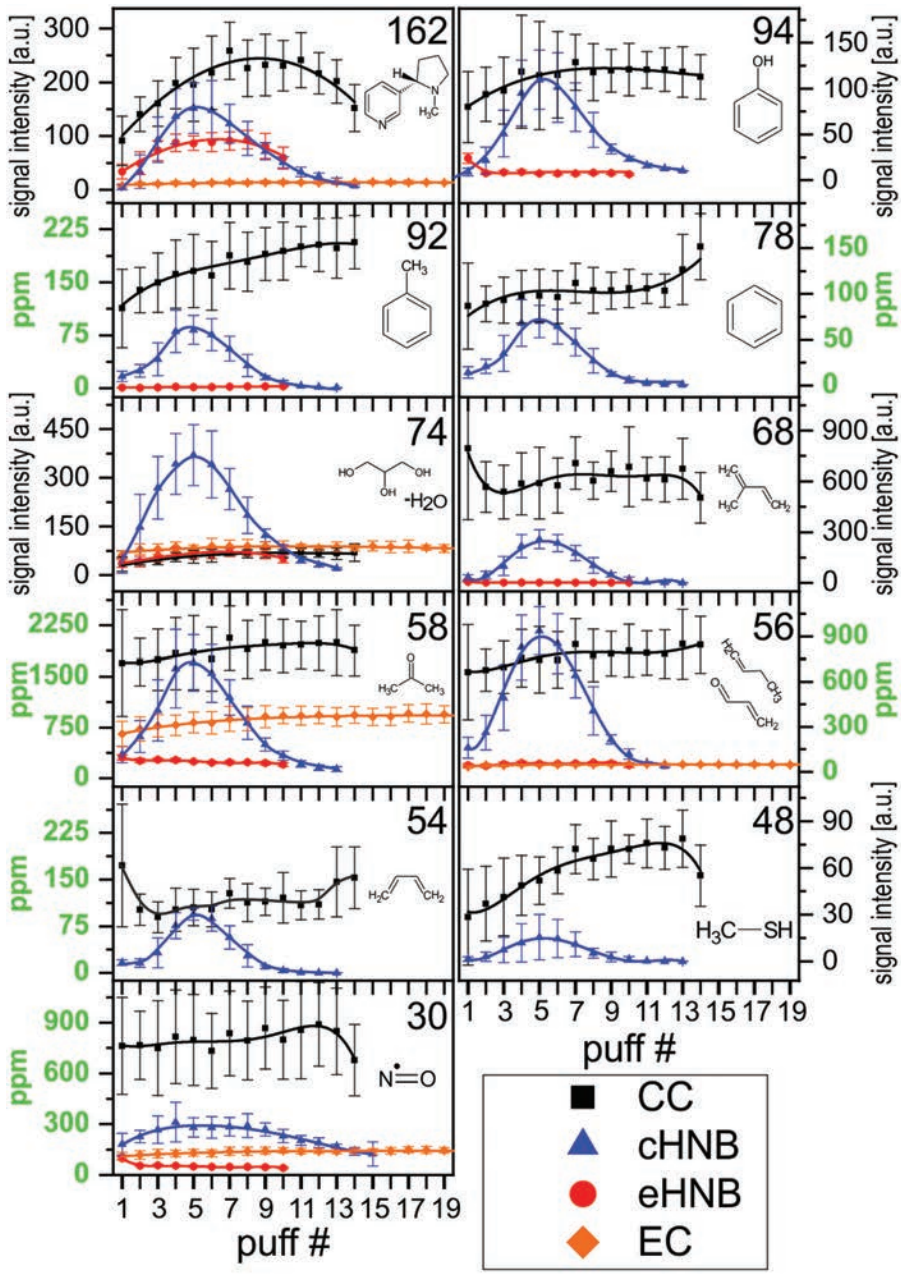


Figure 4. Puff-by-puff profiles of the investigated ANDD. Signals are integrated for each puff. Lines are only for visualization. Concentrations in ppm are calculated using SPI cross-sections from the literature.[45] Mass signals correspond predominately to 162 m/z nicotine, 94 m/z phenol, 92 m/z toluene, 78 m/z benzene, 74 m/z glycerol–water, 68 m/z isoprene, 58 m/z acetone, 56 m/z acrolein (for EC and HNB-cigarette) and butene (for CC), 54 m/z butadiene, 48 m/z methanethiol, 30 m/z nitric oxide. For the EC no dry burns were observed. CC and eHNB are measured seven times, EC five times, and cHNB four times. ANDD = alternative nicotine delivery devices, CC = conventional cigarette, cHNB = combustion heated Heat-Not-Burn, EC = electronic cigarette, eHNB = electronical heated Heat-Not-Burn, HNB = Heat-Not-Burn, SPI = single-photon ionization.

much higher acrolein concentrations, but mainly less with respect to CC.[49] In Table 1, the quantified compounds of this work together with a literature overview are summarized.

The amount of heat transferred to the tobacco of cHNB-cigarettes is between the values for CCs and eHNBs. Due to the glowing piece of coal at the front, the temperature gradient within the tobacco rod should be severe. This explains the occurrence of typical biomass pyrolysis products like phenol,[50] toluene, and butadiene.[51] It is known that the highest concentrations of these substances are found in the high temperature zones of CCs[21] and, therefore, these markers should be produced from the tobacco directly located behind the glowing coal piece. Formation of toxicants is strongly temperature dependent.[52,53] Levels of the previously mentioned toxicants and many others like furan, methanethiol, or nitric oxide are often close to or even below the detection limit (eHNB and EC), whereas cHNB-cigarettes show relatively high signals. The results indicate that several toxic components are significantly reduced in ANDD.

In summary, as shown in Table 1. Instead of reporting all findings on a per consumable basis, a per-average-puff basis was chosen. The levels of CC and eHNB toxicants are in good agreement with literature values, but differences can be observed for nitric oxide in CCs and acetone in eHNBs. For the EC, acetone and nitric oxide seem to be overestimated. For CC measurements, ventilation holes were not blocked, which could explain the difference for nitric oxide. The discrepancies for the eHNB and EC are probably caused by photofragmentation of the humectants and the occurrences of isobaric compounds at specific mass signals are of greater impact. The signal at 58 m/z can be assigned to acetone, glyoxal, and allyl alcohol with unknown contributions. Due to the high amount of humectants, this effect is more pronounced in the EC. The direct sampling method leads also to an explanation for higher concentrations of carbonyles as loss through indirect sampling and secondary reactions is largely prevented. This can also lead to changes in the concentrations of reactive compounds. The relatively high amount of nitric oxide for the EC remains unsolved. Maybe the occurrence of isobaric thermal- or photofragments is the main contributor, but the most probable isobaric compound—formaldehyde—is not ionized by SPI due to an ionization potential of 10.88 eV.[54] For a quantification of all isobaric compounds further investigations with an additional fast GC coupling could be very beneficial due to the nominal mass resolution of the system.[23]

A further limitation of the study clearly is the comparison with only one puffing regime. It is well known for CC, that a change in puffing regimes gives a nonlinear change in the emission of hazardous substances.[23] This is also true for HNB-products and over long terms for ECs.[8,55] It is well known, that users puffing regime changes with the type of nicotine delivery product.[56] This was demonstrated with the compensatory behavior of light cigarettes users.[57] For ECs and HNB-products, users puffing regimes are also different from to CC regimes.[56]

Conclusion

Online soft ionization mass spectrometry is an excellent tool for the chemical characterization of highly dynamic processes. In this context, SPI-TOFMS enables the direct monitoring of gaseous compounds of CC and ANDD on a puff-by-puff basis. This was applied to comprehensively analyze and compare CC and ANDD products. Puff-by-puff profiles of the investigated products' aerosol constituents highly differ among the tested products. This is attributed to by the temperatures applied to the tobacco products and the different principles of aerosol generation. ANDD have a highly reduced amount of substances in the gas phase compared with CCs. In the order cHNB, eHNB, and EC, levels of adverse health effects related substances like benzene, toluene, acetone, acrolein, butadiene, and many others are highly reduced or not present. Nevertheless, nicotine is still present in similar amounts in HNB-cigarettes. The analyzed EC delivers lower amounts of nicotine in single puffs but, on the other hand, can be consumed over a much longer time. Modern ECs have the potential to provide the same or even higher amounts of nicotine compared with CCs.[58] The absence of higher molecular mass compounds should have direct influence on ANDD and CC aerosol composition. Consequently, users of ANDD are exposed to significantly lower amounts of harmful and potentially harmful substances. A problem of the SPI-TOFMS application is the extremely high abundance of humectants in the vapor of ANDD. Although SPI is much softer than conventional electron impact ionization, the generation of photofragmentation products of the humectants complicates the quantification of specific compounds especially for ECs. However, if in future application of the technique, the contributions of photofragmentations are known, compounds such as acrolein, glyoxal, acetone, acetaldehyde, and other relevant carbonyl compounds in ECs could be easily and quickly investigated on a quantitative basis. The puff-resolved analysis will allow to monitor varying emissions by technical changes of existing devices, including tempering of commercial devices, new developments in this field and in the regulation of products. Furthermore, this technique can be beneficial for monitoring the puff-resolved release of legal and illegal psychoactive compounds, eg, Joints or Vaporizers containing cannabis.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

None declared.

References

1. Patskan G, Reininghaus W. Toxicological evaluation of an electrically heated cigarette. Part 1: Overview of technical concepts and summary of findings. *J Appl Toxicol.* 2003;23(5):323–328.
2. Perfetti TA, Rodgman A. The complexity of tobacco and tobacco smoke. *Beitr Tabakforsch/Contrib Tobacco Res.* 2011;24(5):1793.
3. U.S. National Cancer Institute and World Health Organization. *The Economics of Tobacco and Tobacco Control. 21*. NIH Publication No. 16-CA-8029A; 2016. https://cancercontrol.cancer.gov/brp/tcrb/monographs/monograph-21. Accessed February 8, 2017.
4. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* 2020;18(382):1708–1720.
5. Kark JD, Lebiush M. Smoking and epidemic influenza-like illness in female military recruits: a brief survey. *Am J Public Health.* 1981;71(5):530–532.
6. Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control.* 2014;23(2):133–139.
7. Kim HJ, Shin HS. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography-tandem mass spectrometry. *J Chromatogr A.* 2013;1291(24):48–55.
8. Margham J, McAdam K, Forster M, et al. Chemical composition of aerosol from an e-cigarette: a quantitative comparison with cigarette smoke. *Chem Res Toxicol.* 2016;29(10):1662–1678.
9. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J, Luch A. Chemical hazards present in liquids and vapors of electronic cigarettes. *Arch Toxicol.* 2014;88(7):1295–1308.
10. Kosmider L, Sobczak A, Prokopowicz A, et al. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. *Thorax.* 2016;71(4):376–377.
11. Schaller J-P, Keller D, Poget L, et al. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. *Regul Toxicol Pharmacol.* 2016;81(suppl 2):S27–S47.

12. Uchiyama S, Ohta K, Inaba Y, Kunugita N. Determination of carbonyl compounds generated from the E-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. *Anal Sci.* 2013;29(12):1219–1222.
13. McAuley TR, Hopke PK, Zhao J, Babaian S. Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. *Inhal Toxicol.* 2012;24(12):850–857.
14. Glantz SA. Heated tobacco products: the example of IQOS. *Tob Control.* 2018;27(suppl 1):s1–s6.
15. Tricker AR, Schorp MK, Urban HJ, et al. Comparison of environmental tobacco smoke (ETS) concentrations generated by an electrically heated cigarette smoking system and a conventional cigarette. *Inhal Toxicol.* 2009;21(1):62–77.
16. Phillips B, Veljkovic E, Peck MJ, et al. A 7-month cigarette smoke inhalation study in C57BL/6 mice demonstrates reduced lung inflammation and emphysema following smoking cessation or aerosol exposure from a prototypic modified risk tobacco product. *Food Chem Toxicol.* 2015;80:328–345.
17. Foy JW, Bombick BR, Bombick DW, Doolittle DJ, Mosberg AT, Swauger JE. A comparison of *in vitro* toxicities of cigarette smoke condensate from Eclipse cigarettes and four commercially available ultra low-"tar" cigarettes. *Food Chem Toxicol.* 2004;42(2):237–243.
18. Breiev K, Burseg KM, O'Connell G, et al. An online method for the analysis of volatile organic compounds in electronic cigarette aerosol based on proton transfer reaction mass spectrometry. *Rapid Commun Mass Spectrom.* 2016;30(6):691–697.
19. García-Gómez D, Gaisl T, Barrios-Collado C, Vidal-de-Miguel G, Kohler M, Zenobi R. Real-time chemical analysis of E-cigarette aerosols by means of secondary electrospray ionization mass spectrometry. *Chemistry.* 2016;22(7):2452–2457.
20. Streibel T, Zimmermann R. Resonance-enhanced multiphoton ionization mass spectrometry (REMPI-MS): applications for process analysis. *Annu Rev Anal Chem (Palo Alto Calif).* 2014;7(1):361–381.
21. Cui H, Ehlert S, Xie F, et al. Integration of time and spatially resolved in-situ temperature and pressure measurements with soft ionisation mass spectrometry inside a burning superslim cigarette. *J Anal Appl Pyrol.* 2018;135:310–318.
22. Hölzer J, Fischer M, Gröger T, et al. Hyphenation of thermogravimetry and soft single photon ionization–ion trap mass spectrometry (TG–SPI–ITMS) for evolved gas analysis. *J Therm Anal Calorim.* 2014;116(3):1471–1479.
23. Eschner MS, Selmani I, Gröger TM, Zimmermann R. Online comprehensive two-dimensional characterization of puff-by-puff resolved cigarette smoke by hyphenation of fast gas chromatography to single-photon ionization time-of-flight mass spectrometry: quantification of hazardous volatile organic compounds. *Anal Chem.* 2011;83(17):6619–6627.
24. Reda AA, Czech H, Schnelle-Kreis J, et al. Analysis of gas-phase carbonyl compounds in emissions from modern wood combustion appliances: influence of wood type and combustion appliance. *Energy Fuels.* 2015;29(6):3897–3907.
25. Kogel U, Schlage WK, Martin F, et al. A 28-day rat inhalation study with an integrated molecular toxicology endpoint demonstrates reduced exposure effects for a prototypic modified risk tobacco product compared with conventional cigarettes. *Food Chem Toxicol.* 2014;68:204–217.
26. Lüdicke F, Haziza C, Weitkunat R, Magnette J. Evaluation of biomarkers of exposure in smokers switching to a carbon-heated tobacco product: a controlled, randomized, open-label 5-day exposure study. *Nicotine Tob Res.* 2016;18(7):1606–1613.
27. International Organization for Standardization. *ISO 3308:2012 Routine Analytical Cigarette-Smoking Machine—Definitions and Standard Conditions.* 5th ed. 2012. 65.160 Tobacco, tobacco products and related equipment. Geneva, Switzerland: ISO Central Secretariat; Published 2012-10.
28. Uchiyama S, Noguchi M, Sato A, Ishitsuka M, Inaba Y, Kunugita N. Determination of thermal decomposition products generated from E-cigarettes. *Chem Res Toxicol.* 2020;33(2):576–583.
29. Jaccard G, Tafin Djoko D, Moennikes O, Jeannet C, Kondylis A, Belushkin M. Comparative assessment of HPHC yields in the Tobacco Heating System THS2.2 and commercial cigarettes. *Regul Toxicol Pharmacol.* 2017;90:1–8.
30. Adam T, Mitschke S, Streibel T, Baker RR, Zimmermann R. Quantitative puff-by-puff-resolved characterization of selected toxic compounds in cigarette mainstream smoke. *Chem Res Toxicol.* 2006;19(4):511–520.
31. Wiley WC, McLaren IH. Time-of-flight mass spectrometer with improved resolution. *Rev Sci Instrum.* 1955;26(12):1150–1157.
32. Gross JH. *Mass Spectrometry: A Textbook.* 2nd ed. Berlin, Heidelberg: Springer-Verlag; 2011.
33. Mitschke S, Adam T, Streibel T, Baker RR, Zimmermann R. Application of time-of-flight mass spectrometry with laser-based photoionization methods for time-resolved on-line analysis of mainstream cigarette smoke. *Anal Chem.* 2005;77(8):2288–2296.
34. Hawke J, Errington G, von Frowein MB. Evaluation of an online, real-time, soft-photon ionisation time-of-flight mass spectrometer for mainstream tobacco smoke analysis. *BMC Chem.* 2019;13(1):135.
35. Bell F, Ruan QN, Golan A, et al. Dissociative photoionization of glycerol and its dimer occurs predominantly via a ternary hydrogen-bridged ion-molecule complex. *J Am Chem Soc.* 2013;135(38):14229–14239.
36. Laino T, Tuma C, Moor P, Martin E, Stolz S, Curioni A. Mechanisms of propylene glycol and triacetin pyrolysis. *J Phys Chem A.* 2012;116(18):4602–4609.
37. Meyers TJ, Chang SC, Chang PY, et al. Case-control study of cumulative cigarette tar exposure and lung and upper aerodigestive tract cancers. *Int J Cancer.* 2017;140(9):2040–2050.
38. Nakayama T, Kaneko M, Kodama M. Volatile gas components contribute to cigarette smoke-induced DNA single strand breaks in cultured human cells. *Agric Biol Chem.* 2014;50(12):3219–3220.
39. Bekki K, Inaba Y, Uchiyama S, Kunugita N. Comparison of chemicals in mainstream smoke in heat-not-burn tobacco and combustion cigarettes. *J UOEH.* 2017;39(3):201–207.
40. Kim KH, Kabir E, Jahan SA. Review of electronic cigarettes as tobacco cigarette substitutes: their potential human health impact. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* 2016;34(4):262–275.
41. Li X, Luo Y, Jiang X, et al. Chemical analysis and simulated pyrolysis of Tobacco Heating System 2.2 compared to conventional cigarettes. *Nicotine Tob Res.* 2019;21(1):111–118.
42. Anderson C, Majeste A, Hanus J, Wang S. E-cigarette aerosol exposure induces reactive oxygen species, DNA damage, and cell death in vascular endothelial cells. *Toxicol Sci.* 2016;154(2):332–340.
43. Misra M, Leverette RD, Cooper BT, Bennett MB, Brown SE. Comparative *in vitro* toxicity profile of electronic and tobacco cigarettes, smokeless tobacco and nicotine replacement therapy products: e-liquids, extracts and collected aerosols. *Int J Environ Res Public Health.* 2014;11(11):11325–11347.
44. McAdam K, Warrington A, Hughes A, et al. Use of social media to establish vapers puffing behaviour: findings and implications for laboratory evaluation of e-cigarette emissions. *Regul Toxicol Pharmacol.* 2019;107:104423.
45. Adam T, Zimmermann R. Determination of single photon ionization cross sections for quantitative analysis of complex organic mixtures. *Anal Bioanal Chem.* 2007;389(6):1941–1951.
46. Adam T, Mitschke S, Streibel T, Baker RR, Zimmermann R. Puff-by-puff resolved characterisation of cigarette mainstream smoke by single photon ionisation (SPI)-time-of-flight mass spectrometry (TOFMS): comparison of the 2R4F research cigarette and pure Burley, Virginia, Oriental and Maryland tobacco cigarettes. *Anal Chim Acta.* 2006;572(2):219–229.
47. Collins CC, Epstein DH, Parzynski CS, Zimmerman D, Moolchan ET, Heishman SJ. Puffing behavior during the smoking of a single cigarette in tobacco-dependent adolescents. *Nicotine Tob Res.* 2010;12(2):164–167.
48. Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tob Control.* 2003;12(4):424–430.

49. Son Y, Bhattarai C, Samburova V, Khlystov A. Carbonyls and carbon monoxide emissions from electronic cigarettes affected by device type and use patterns. *Int J Environ Res Public Health*. 2020;17(8):2767.
50. Czégény Z, Blazsó M, Várhegyi G, Jakab E, Liu C, Nappi L. Formation of selected toxicants from tobacco under different pyrolysis conditions. *J Anal Appl Pyrol.* 2009;85(1–2):47–53.
51. Piadé JJ, Wajrock S, Jaccard G, Janeke G. Formation of mainstream cigarette smoke constituents prioritized by the World Health Organization—yield patterns observed in market surveys, clustering and inverse correlations. *Food Chem Toxicol.* 2013;55:329–347.
52. Fischer M, Wohlfahrt S, Saraji-Bozorgzad M, et al. Thermal analysis/evolved gas analysis using single photon ionization. *J Therm Anal Calorim.* 2013;113(3):1667–1673.
53. Müller B-H. Degradation kinetics of tobacco—survey by fast thermogravimetry analysis. *J Anal Appl Pyrol.* 1993;25:273–283.
54. National Institute of Standards and Technology. *Formaldehyde*. https://webbook.nist.gov/cgi/. Accessed January 16, 2020.
55. McAdam K, Davis P, Ashmore L, et al. Influence of machine-based puffing parameters on aerosol and smoke emissions from next generation nicotine inhalation products. *Regul Toxicol Pharmacol.* 2019;101:156–165.
56. Jones J, Slayford S, Gray A, Brick K, Prasad K, Proctor C. A cross-category puffing topography, mouth level exposure and consumption study among Italian users of tobacco and nicotine products. *Sci Rep.* 2020;10(1):12.
57. Hecht SS, Murphy SE, Carmella SG, et al. Similar uptake of lung carcinogens by smokers of regular, light, and ultralight cigarettes. *Cancer Epidemiol Biomarkers Prev.* 2005;14(3):693–698.
58. Voos N, Goniewicz ML, Eissenberg T. What is the nicotine delivery profile of electronic cigarettes? *Expert Opin Drug Deliv.* 2019;16(11):1193–1203.