Chemical Research in Toxicology

pubs.acs.org/crt

Article

Comprehensive Chemical Characterization of the Aerosol Emissions of a Vaping Product Based on a New Technology

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Cite This: *Chem. Res. Toxicol.* 2020, 33, 789−799

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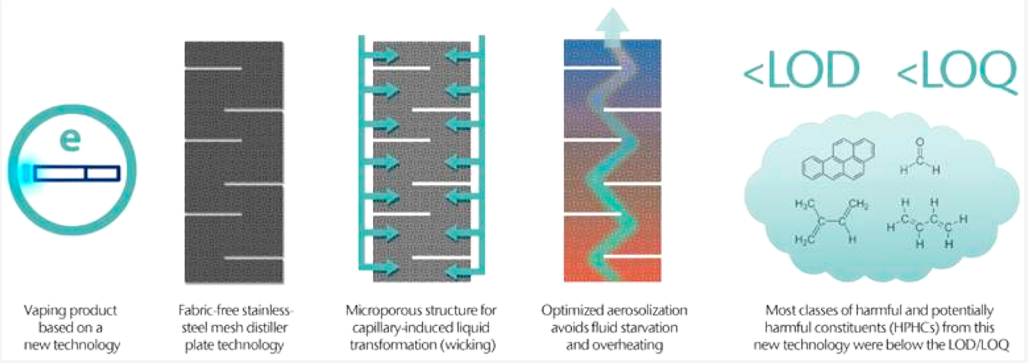

ABSTRACT: Around 10 million people in the United States and 3 million people in the United Kingdom are estimated to use vaping category products. There are some estimates that there will be 75−80 million vapers worldwide by 2020. Most of these products are based on coil-and-wick technology. Because the heating and aerosol formation are separate processes, the system can lead to dry-wicking and elevated emission of carbonyls if designed and/or manufactured poorly. Low-nicotine and low-power coil-and-wick devices have also been linked to increased exposure to formaldehyde due to compensatory behavior by users. We characterized the emissions of a vaping product which uses a fabric-free stainless-steel mesh distiller plate technology that heats and aerosolizes the e-liquid in a single process. The plate has a microporous structure for capillary-induced liquid transformation (wicking) and aerosolization that is optimized to avoid fluid starvation and overheating and improved control. Compared with emissions previously reported for a coil-and-wick nicotine vaping product (e-cigarette), most classes of harmful and potentially harmful constituents (HPHCs) from this vaping product were below the level of detection or quantification. For those that were quantifiable, this vaping product generally had lower levels of emissions than the e-cigarette, including carbonyls. Formaldehyde and methyl glyoxal levels did not differ significantly between vaping products. In this system, the single mode of liquid transfer and vapor formation permits high aerosol mass delivery but further reduces emissions of HPHCs that may be present in conventional e-cigarette aerosol, by lessening the risk of thermal breakdown of the aerosol-generating solvent mixture.

1. INTRODUCTION

Smoking is a known cause of cardiovascular disease, chronic obstructive pulmonary disease, and lung cancer.[1] With the increasing global population, the prevalence of smoking will continue to rise for the next decade or more.[2] In some countries, such as the United Kingdom, innovations in electronic nicotine delivery systems (ENDS, e-cigarettes, or vapor products) have been recognized by some public health authorities as a positive way to reduce cigarette smoking and associated disease risks.[3]

Of more than 6500 compounds in cigarette smoke[4] 158 are established as toxicants,[5] long-term exposure to which can lead to smoking-related disease. DNA damage and oxidative stress are key disease mechanisms,[6,7] but more precise actions are not yet understood. Some regulatory agencies mandate the reporting of certain harmful and potentially harmful constituents (HPHCs) from cigarette smoke[8−11] and the World Health Organization's (WHO's) advisory body on Tobacco Product Regulation (TobReg) has proposed lowering of several priority compounds.[12]

Removal of toxicants seems to be a feasible way to reduce the risk of disease. E-cigarettes and other ENDS electrically heat and aerosolize liquid matrices (e-liquid) containing glycerol, propylene glycol, and often water, nicotine, and/or flavours.[13,14] During normal use, these devices operate at temperatures up to ∼250 °C,[15] compared with 950 °C peak

Received: October 30, 2019
Published: March 3, 2020

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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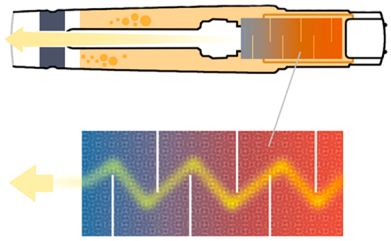
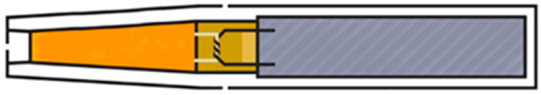
Table 1. Summary of Test Products and Their Product Codes

Product type [code]	Details/schematic	Aerosol formation mechanism	References
Test product New Vapor Product; flavor variant Twilight Tobacco code IS1.0(TT)	 10 W power, 5 mg/mL nicotine (62.6% w/w VG, 36% w/w PG, 0.43% w/w nicotine, 1% w/w water)	Capillary liquid transfer via stainless steel mesh; vaporization of the liquid by the same heated mesh	[41]
Comparator 1 Conventional e-cigarette; flavor variant Blended Tobacco code EC(BT)	 4.6 W power, 18 mg/mL nicotine (48.1% w/w VG, 25% w/w PG, 1.86% w/w nicotine, 25% w/w water)	Capillary liquid transfer via a cotton wick; vaporization of the liquid by a heated Ni/Cr wire around the wick	[17]
Comparator 2 Research reference cigarette – 1R6F code 1R6F	Blended cigarette with 8.6 mg tar under ISO 4387 machine-smoking	Pyrolysis and combustion of tobacco	[42]

temperatures reached in a burning cigarette.[16] Thus, with substantially lower heating temperatures, no combustion, and without the presence of tobacco, e-cigarette aerosol yields many fewer and much lower levels of toxicants than cigarette smoke.[3,17,18]

How HPHCs apply to e-cigarettes and the analytical methods and machine-puffing protocols used to assess them is still being determined.[19,20] We previously comprehensively characterized a closed-modular e-cigarette. Compared with cigarette smoke, this e-cigarette aerosol tested as non-mutagenic,[21] not promoting tumors[22] and showed greatly reduced cytotoxicity,[23] oxidative stress, and inflammation[24] in a series of *in vitro* tests. Of 150 compounds tested for in the emissions from this closed-modular e-cigarette, only 25 were detected at levels above air blanks and around one-third of those were below the limit of quantitation.[17] Volatile carbonyls or alkaloid-related compounds had the highest levels, including trace levels of the IARC group 1 carcinogen *N*-nitroso-nornicotine (NNN), but chromium, a constituent of the heating coil, and chrysene, a polycyclic aromatic hydrocarbon, were also quantifiable, although the source of the latter was unexplained. Toxic transition metals are known to leach from the heating coil of some coil-and-wick e-cigarettes.[25−27] Other researchers have recently concluded that e-cigarettes do not generate HPHCs that are typically derived from combustion[28] and that trace levels of nicotine-related impurities (including tobacco-specific nitrosamines) are generally due to minor impurities in pharmaceutical grade nicotine.[29] The European Tobacco Product Directive (TPD) requests notification for several compounds.[30] The U.S. Food and Drug Administration (FDA) Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems lists 31 HPHCs, 9 of which are not covered by other regulatory lists.[11] Commercial stakeholders have suggested several constituents for notification, related to formulation (*e.g.*, flavor components or breakdown products from ingredients) or substances leached from product components.[17]

An important cause for the generation of volatile carbonyl compounds in e-cigarette aerosol is thermal breakdown of aerosol-generating solvent mixture used in e-liquid, due to excessive power raising the coil temperature[15] or fluid starvation causing overheating, termed "dry-wicking".[31] Carbonyl concentrations vary widely with different devices, power settings, puffing parameters, e-liquid characteristics, coil deterioration, puffing profile, aerosol collection methods and analytical protocols.[19,32−34] Flavourings have also been suggested as contributors[35,36] although experimental methods have varied[37,38] and further research is needed.

The principle of tobacco harm reduction outlined by the Institute of Medicine (US) Committee[39] is based on the replacement of high-risk tobacco products (cigarettes) with potentially reduced-risk tobacco or nicotine products (*e.g.*, e-cigarettes). There remains scope to improve e-cigarettes in terms of emission profiles, sensory performance, safety, functionality, and product design. Usage patterns also play a part, with low-nicotine and low-power coil-and-wick devices having been linked to increased formaldehyde exposure due to compensatory behavior by the user.[40] We have developed a new vaping product (IS1.0(TT)) that aerosolizes an e-liquid using a distiller plate technology.[41] The plate comprises a stainless steel wire pressed into a meshed structure with precise geometry and pore size distribution. It is fabric-free and designed to optimize surface area and bulk porosity, both of which are important for wicking, heating, and evaporating performance. Without the fabric wick and heating coil, the risk of elevated carbonyl emissions is reduced, while aerosol mass is increased. Here we report comprehensive analysis of the aerosol emissions compared with cigarette smoke and a coil-and-wick e-cigarette.

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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Table 2. Summary of Machine Puffing Regimens Used to Assess the Test Products[a]

product	regime	puff volume (mL)	puff duration (s)	puff interval (s)	ventilation blocking (%)	puff profile	ref
1R6F	HCI	55	2	30	100	bell-shaped	43
EC(BT) and IS1.0(TT)	CRM 81	55	3	30	N/A	square wave	44

[a]Abbreviations: HCI, Health Canada Intense; CRM 81, CORESTA Recommended Method number 81.

2. EXPERIMENTAL PROCEDURE

2.1. Test Products. The 1R6F Kentucky reference cigarette is designed to provide a standard test piece for scientific studies.[42] It is a U.S.-blended product with a cellulose acetate filter and an ISO tar yield of approximately 8.6 mg/cigarette. 1R6F was designed as an equivalent replacement for an earlier reference cigarette with a similar ISO tar level, the 3R4F cigarette, which was used in our previous study[16] but is no longer available.

The comparator e-cigarette EC(BT) from our previous study was Vype ePen (Nicoventures Trading Ltd., Blackburn, UK).[16] In brief, it is a closed-modular system consisting of two modules: a rechargeable battery section and a replaceable e-liquid-containing cartridge ("cartomizer"). The power output at 3.6 V is approximately 4.6 W. Device operation commences when the user presses the power switch, usually 1 s in advance of the puff being taken and for the duration of the puff. The liquid is fed to the atomizer through a sintered, porous ceramic disk in contact with a silica transport wick. The atomizer comprises a nichrome wire coil heater wrapped around the wick. The cartomizer contained 1.58 mL of Blended Tobacco e-liquid composed of 25% w/w propylene glycol (PG) containing low levels (<1% w/w) of blended tobacco flavor ingredients, 48.1%w/w vegetable glycerol (VG), 25% w/w water, and 1.86% w/w nicotine (18 mg/mL). The cartomizer has an operating life in excess of 200 puffs, depending on usage patterns, and it was operated at the 3.6 V setting.

The new product, coded IS1.0(TT), is a rechargeable two-part e-cigarette, consisting of a disposable cartridge and rechargeable device section that houses the battery cell and controlling electronics. Upon button activation, the device provides an electrical current to the heating element in the cartridge. The heating element consists of a stainless-steel mesh plate technology, which is responsible for both the wicking and subsequent heating of the e-liquid to form an aerosol. Each cartridge contains 1.95 mL of liquid. For the present analysis, the operating power was 10 W and the flavor variant was Twilight Tobacco, composed of 62.6% w/w VG, 36% w/w PG, 1% w/w water, and 0.43% w/w nicotine (5 mg/mL).

Products were sampled from the factory at a single point in time, and each e-liquid was prepared in a single batch operation. The samples were quality-control checked to ensure compliance with product specification prior to dispatch to the testing laboratory.

The test products are summarized in Table 1.

2.2. Methods for Aerosol Collection and Quantification of Aerosol Emissions. Analyses were conducted by Labstat International ULC (Kitchener, Ontario, Canada). The analytical methodology for the current and previous[16] studies is tabulated in Table S1 in the Supporting Information. Five independent replicate measurements were taken per sample, comprising randomly selected devices and cartridges, and each e-cigarette sample run was paired with a simultaneous collection of air blanks to give an estimate of laboratory background levels at that time point. The puffing parameters for machine smoking are given in Table 2. The Health Canada Intense smoking regime was chosen in preference to ISO 3308:2012. The CORESTA e-cigarette method was the working standard at the time, and has since been developed into ISO 20768:2018. Both e-cigarettes had a 1s preactivation time with activation continued for the duration of the puff.

2.3. Test Methods. Our previous study reported the emission levels of 142 chemicals and 8 collated measures, covering a comprehensive range of HPHCs of cigarette smoke and potential constituents of concern in EC(BT).[17] We repeated this approach for IS1.0(TT) and 1R6F, excluding the following two analyte groups that were deemed irrelevant:

- Radionuclides (polonium-210, uranium-235, uranium-238), which were undetected in emissions from EC(BT) or the reference cigarette.[17]
- Chlorinated dioxins and furans (25 compounds), which were all below the limit of detection (LOD) except for Octa CDD, which was not quantified (NQ), for EC(BT) and its air blank. All of these analytes were below the limit of quantification (LOQ) for the reference cigarette except for Octa CDD, which was around seven times the estimated detection limit.[17]

We included seven further analytes (triacetin, pyrene, glycolaldehyde, isobutyraldehyde, buten-2-one, 2,3-hexanedione, and 2,3-heptanedione), which were reported by default within the suite of tests used and are mainly relevant to cigarettes, but deemed potentially relevant to e-cigarettes if present within the e-liquid ingredients. We also measured nicotine-free dry particulate matter (NFDPM), which is a gravimetric measure of particulate mass, after correcting for analytically determined nicotine and water. For e-cigarettes, this is the condensed mass of aerosol-generating liquid and is therefore not comparable to the equivalent condensed "tar" from combustible cigarettes.[45] In the ensuing period since testing, the FDA has issued a guidance document for Premarket tobacco applications for electronic nicotine delivery systems which complements and adds to the range of chemicals for consideration.[11] Of these, nine are not explicitly covered in the other lists and were not tested-for in the studies. These new candidates are all viable as flavor ingredients, which may have some bearing on their inclusion. Most are esters, but there is also an aldehyde, an alcohol, and a short chain carboxylic acid. The compounds are benzyl acetate, ethyl acetate, ethyl acetoacetate, furfural, isoamyl acetate, isobutyl acetate, menthyl acetate, *n*-butanol, and propionic acid.

2.4. Data Analysis. For many HPHCs, the e-cigarette emissions were below the method LOD or between the LOD and the LOQ. Data in this range were assigned representative values to allow numerical comparison. Using the same procedure as in our previous study[17] we assigned 15% of the LOQ for any reported mean result that was not detected (<LOD). Values between the LOD and LOQ were censored at 65% of the LOQ and termed NQ. This censoring approach cannot be applied indiscriminately because method sensitivities and aerosol collection mass (puffs per analytical determinant) vary with device type. It also stratifies results for trace compounds and influences the way in which comparisons are made; in particular, it reduces or removes variance, which reduces the accuracy of hypothesis tests or precludes them, forcing comparisons to be made by ordinal ranking. Overall, it is important to consider the differences in sensitivity and make comparisons on a case-by-case basis to avoid masking differences or introducing artificial divisions. Bearing in mind these limitations, meaningful product comparisons could still be made.

Any results that were above the LOQ provided data that were continuous variables and could therefore be compared by *t* tests. Pairs of data where one or both values were below the method LOQ were compared by ranking. Results above the LOQ were ranked higher than results below the LOQ. Results above the LOD were ranked higher than results below the LOD. In some cases, this was not possible, because some method sensitivities were not consistent across the experiments. These cases are addressed in the Results section.

Previous studies have noted similar analyte levels between e-cigarette emissions and laboratory air (air blanks).[14,17] Therefore, simultaneous air blanks were recorded for each e-cigarette sample run and samples were compared with the corresponding blank. If a sample had a result that did not differ from the blank, either by ranking or by *t* test, then the net difference from the blank was defined as zero. In

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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cases where the sample level was higher than the blank but the analyte was detected in the blank, pairwise subtraction was used to calculate a net analyte level. In cases where analyte was detected in the blank but was <LOQ, subtraction used a censored value. Standard deviations (where applicable) were combined in this process.

Comparisons between EC(BT) and IS1.0(TT) were made using these net values, which were compared by ordinal ranking or a two-sample t test, as appropriate. All two-sample t tests used Welch's correction for unequal variance and statistical significance was judged at an alpha level of 5%.

In contrast to the e-cigarette emissions, the data for the reference cigarette (1R6F) were not corrected for blank values because most analytes were substantially above the method LOQ. Blanks were acquired for some cigarette tests, where deemed relevant, and are commented on where appropriate.

During testing, each analytical sample collection had a set number of puffs, tailored to device delivery in order to avoid saturation of the collection device and to stay within the validated range of the analytical method. IS1.0(TT) produced a higher aerosol mass per puff than EC(BT) but was at risk of approaching depletion after more than 50 puffs per cartridge, whereas EC(BT) had in excess of 200 puffs. In all cases, a single emissions collection for IS1.0(TT) (50 puffs) was compared with each of the two emissions collections for EC(BT) (100 puffs each) and with a single 1R6F cigarette. We refrained from adjusting results to a per-puff basis because it can distort the influence of method sensitivity limits. Instead, we comment on the sample puff counts where appropriate. In general, the differences in effect sizes were large enough to compensate for differences in puff count without requiring any further scaling. However, where necessary and meaningful to make this comparison, we address it on a case-by-case basis.

Note that the smoke analysis methods report on a per cigarette basis (typically 8−10 puffs), but generally employ several cigarettes per collection depending on the method stipulation, again for the purpose of maximum sensitivity. This subdivision influences the reported LOD/LOQ proportionally. Where relevant, this is discussed because it influences comparison when both product categories report <LOQ.

3. RESULTS

Full emission results from the previous study on EC(BT) and 3R4F cigarette[17] and for IS1.0(TT) and the 1R6F research cigarette are tabulated in Tables S2 and S3 in the Supporting Information. Comparison of the data with air blanks and comparison between products is tabulated in Table S4 in the Supporting Information, following the approach outlined in subsection 2.4.

Figure 1 shows the emissions classification for the two e-cigarettes, EC(BT) and IS1.0(TT), grouped according to whether they were quantifiable (≥LOQ), below the detection limit (BDL), detected but not quantifiable (NQ), or detected but no different from the air blank. For both products, the majority of analytes were undetected or equivalent to the levels seen in the air blanks. We report some new test analytes for IS1.0(TT), which were mostly BDL, as can be seen in Figure 1.

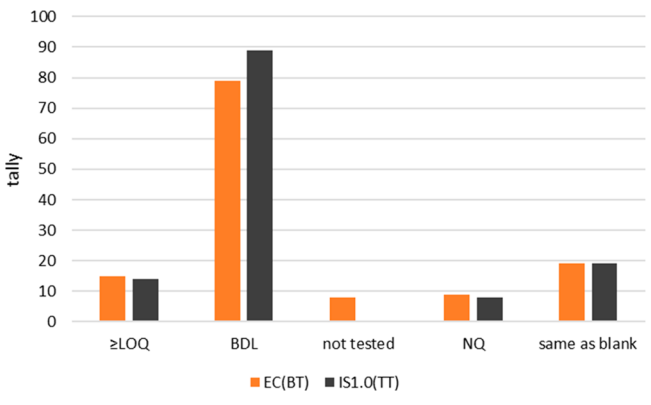

Figure 1. Summary of analyte responses in the test suite, comparing EC(BT) and IS1.0(TT).

3.1. FDA-18 and TobReg-9 Analytes. In terms of regulatory compounds of interest, the 18 priority HPHCs stipulated by the US FDA[9] termed FDA-18, and the nine priority compounds from the WHO's TobReg list[12] termed TobReg-9, overlap. The combined list, including TSNAs, volatile carbonyls, and aromatic amines, was therefore considered here. Emissions were compared between the e-cigarette and the 1R6F reference cigarette to give scale and context to the levels of toxicant reduction in e-cigarettes. The net levels of constituents discernible from the air blanks are shown in Figure 2 as a percentage (by mass) of the levels measured from a single 1R6F cigarette. Each puff block represents 100 puffs of EC(BT) or 50 puffs of IS1.0(TT). The data were not reported on a single-puff basis to avoid subdividing arbitrary limits imposed by method sensitivity. Therefore, the puff number per collection should be taken into account when making comparisons, but with due regard for censoring applied to represent analytical limits.

Nicotine levels are linked to formulation content and so are excluded from Figure 2. Fifty puffs from IS1.0(TT) provided 95.5% (SE 2.73) of the nicotine delivered by the reference cigarette. The equivalent datum for EC(BT) puffs 1−100 was 190.0% (SE 26.24) and for puffs 101−200 was 146.4% (SE 23.36). Of the 18 analytes in the combined group, only 7 were present at levels above the blank. Two of these were at trace levels and poorly differentiated from the blanks. Three were volatile carbonyls, and two were alkaloid-related compounds. Nicotine delivery was similar on a per-puff basis between the two e-cigarettes. The other measurable HPHCs were all lower in emissions from IS1.0(TT) than from 1R6F. The three carbonyls were lowest in IS1.0(TT) emissions. This effect was more pronounced than would be explained by the difference in the puff numbers per collection.

The level of formaldehyde was not significantly different from the air-blank for either EC(BT) sample block (p = 0.06) at the chosen level of alpha risk. Given that formaldehyde is a known component of e-cigarette emissions and known to be variable under different puffing regimes,[36] we chose to view it as a genuine feature of EC(BT) emissions. Notably the background (air blank) levels for both EC(BT) sample sets were roughly half the level of the test samples and had lower variance (blank 1: mean, 6.59 μg; SD, 0.31; blank 2: mean, 6.79 μg; SD, 0.40). The levels of the other carbonyls (acrolein and acetaldehyde) were no higher for IS1.0(TT) than for the air blank.

The nitrosamine NNK which was detected at trace levels from EC(BT) was absent from IS1.0(TT) emissions. The levels of trace alkaloids and related impurities are likely to be due to formulation ingredients; therefore, this finding is not attributed to differences in device performance.

Several e-cigarette samples had trace levels of aromatic amines, at the limits of sensitivity. IS1.0(TT) had a 1-aminonaphthalene level of NQ, and one puff block from EC(BT) had a similar level of 4-aminobiphenyl. Closer inspection of this class of analytes showed that the levels fluctuated around the lower analytical limits, and the proportion of samples with detectable levels was the same in

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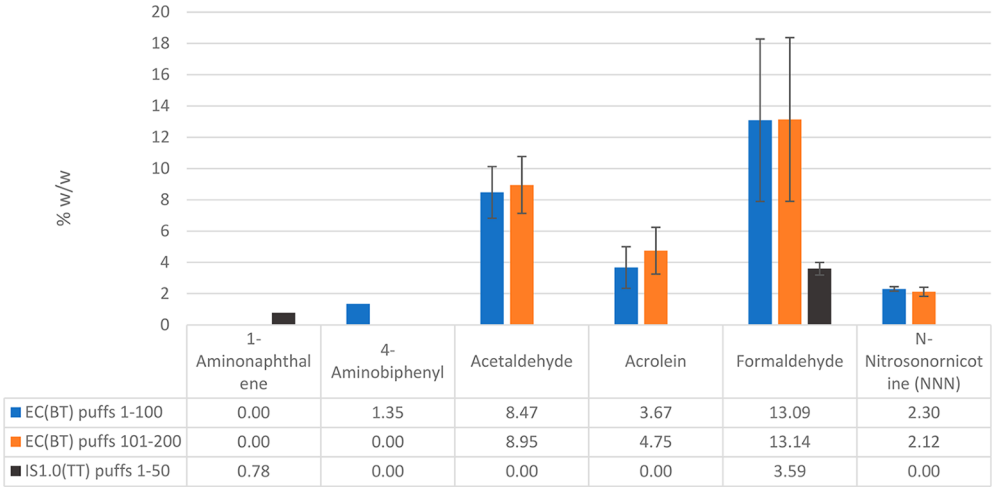

Figure 2. TobReg-9/FDA-18 e-cigarette analytes that were discernible from the air blank. Values were expressed as a percentage ratio (by contained weight) of each e-cigarette collection to one 1R6F control cigarette. Error bars indicate 1 SE (n = 5), and the absence of error bars indicates left-censored values. Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.

Table 3. Classification of Results for Aromatic Amines, Comparing Air Blanks to Test Samples[a]

	IS1.0 (TT)		EC (BT) puff block 1		EC (BT) puff block 2	
analyte	test	blank	test	blank	test	blank
1-aminonaphthalene	NQ	BDL	BDL	BDL	BDL	BDL
3-aminobiphenyl	Q	NQ	NQ	NQ	BDL	NQ
2,6-dimethylaniline	NQ	BDL	BDL	BDL	BDL	BDL
2-aminonaphthalene	BDL	NQ	NQ	NQ	NQ	NQ
4-aminobiphenyl	NQ	NQ	NQ	BDL	BDL	NQ
o-anisidine	BDL	BDL	BDL	BDL	BDL	BDL
o-toluidine	Q	NQ	Q	Q	Q	Q
benzidine	BDL	BDL	BDL	BDL	BDL	BDL

[a]Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs. Abbreviations: BDL, below detection limit; NQ, detected below limit of quantitation; Q, quantifiable.

Table 4. Comparison of Quantitative (≥ LOQ) TobReg-9/FDA-18 Emissions between E-cigarette Types on a Per-Collection Basis[a]

	net difference between test sample and blank						
	IS1.0(TT) puffs 1−50		EC(BT) puffs 1−100d		EC(BT) puffs 101−200		
analyte	mean	SD	mean	SD	mean	SD	comparison between IS1.0(TT) and EC(BT)
formaldehyde (μg)	1.50	0.35	5.48	4.84	5.50	4.87	2-sample t test: p = 0.14 DF = 4 vs puff block 1; p = 0.11 DF = 4 vs puff block 2
nicotine (mg)	1.80	0.11	3.57	1.10	2.75	0.98	2-sample t test: p = *0.02*, DF = 4 vs puff block 1; p = *0.01*, DF = 4 vs puff block 2

[a]Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.

the blanks as in the e-cigarette test runs (Table 3). Although not conclusive, it suggests that the trace levels of aromatic amines were randomly distributed (Pearson χ^2 = 0.000, DF = 1, p > 0.99). Individual replicate values for all aromatic amines measured for IS1.0(TT) and its blanks are plotted in Figure S1 in the Supporting Information; with each value scaled by its LOQ to facilitate comparison among values, *i.e.*, scaled according to analytical method sensitivity. For all analytes, there were air blank measurements with equivalent levels to the test samples. We therefore conclude that the method sensitivity and environmental background levels combined to prevent meaningful measurement of aromatic amines in EC(BT) and IS1.0(TT) emissions, indicating extreme low levels or absence of these analytes. This conclusion is supported by independent findings for other e-cigarettes.[28]

For two analytes, nicotine and formaldehyde, both IS1.0-(TT) and EC(BT) had net values > LOQ, which were formally compared using two-sample t tests (Table 4). The difference between IS1.0(TT) and EC(BT) was not significant for formaldehyde, where the net results for EC(BT) were especially variable (coefficient of variation, 88%). Therefore, although the formaldehyde emissions (per sample block) seemed different between the two products, the difference was not statistically significant, based on five replicates.

In summary, the TobReg-9 and FDA-18 priority compounds offer an overview of key HPHCs and cover important classes of compounds to consider in e-cigarette emissions. With the exception of nicotine, multipuff sample collections from the e-cigarettes contained much lower levels of HPHCs as compared with a single conventional cigarette. In addition, key volatile carbonyls were lower for IS1.0(TT) than for EC(BT),

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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Table 5. Comparison of Other Quantitative (≥ LOQ) Emissions between E-cigarette Types on a Per-Collection Basis[a]

	net difference between test sample and blank						
	IS1.0(TT)		EC(BT) puffs 1−100		EC(BT) puffs 101−200d		
analyte	mean	SD	mean	SD	mean	SD	comparison between IS1.0(TT) and EC(BT): 2-sample *t* test
methyl glyoxal (μg)	1.09	0.77	4.30	2.71	4.45	1.92	$p = 0.06$ DF = 4, vs puff block 1 $p = 0.02$ DF = 5 vs puff block 2
cotinine (ng)	58.58	44.26	1122.89	145.29	1044.19	147.70	$p < 0.001$ DF = 4 vs puff block 1 $p < 0.001$ DF = 4 vs puff block 2
glycerol (mg)	334.82	20.72	152.69	18.26	162.53	13.00	$p < 0.001$ DF = 7 vs puff block 1 $p < 0.001$ DF = 6 vs puff block 2
propylene glycol (mg)	142.27	7.78	66.69	8.61	75.03	6.22	$p < 0.001$ DF = 7 vs puff block 1 $p < 0.001$ DF = 7 vs puff block 2

[a]Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.

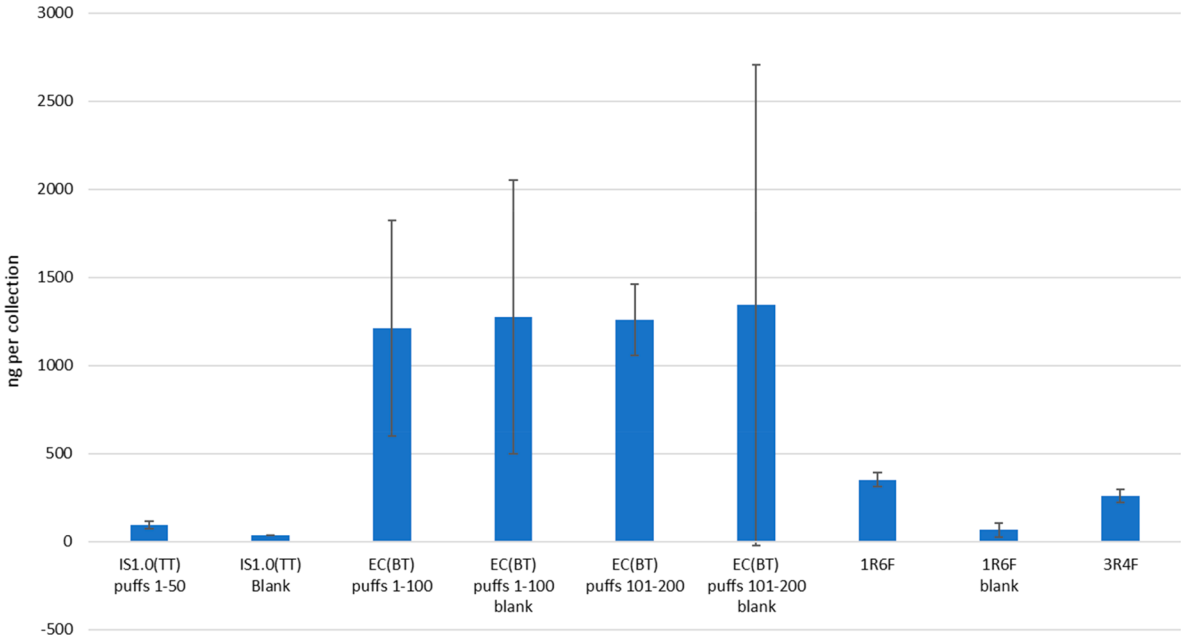

Figure 3. Comparison of zinc values demonstrating fluctuating levels in the air blanks. Error bars indicate 1 SD ($n = 5$). Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.

although the result for formaldehyde was not statistically significant based on a sample size of five.

3.2. EU TPD Data Dictionary Analytes. The current EU TPD Data Dictionary lists 22 chemicals of interest for e-cigarette products.[30] In addition to the chemicals discussed above, it highlights a further 10 HPHCs, which can be divided into three classes. Acetyl propionyl and diacetyl are semi-volatile carbonyls that can be used as flavor ingredients.[46] Arsenic, cadmium, chromium, copper, lead, and nickel are toxic heavy metals that may potentially be released from the heating element.[26,47] Diethylene glycol and ethylene glycol are solvents that have been detected in some e-liquids.[48]

Overall, the emissions from IS1.0(TT) were no higher than the blank for any of these analytes. The levels from EC(BT), as described previously,[17] were higher than the blank for nickel and chromium.

3.3. Analytes Potentially Relevant to E-Cigarettes. Emissions were tested for 16 additional compounds considered relevant to e-cigarettes but not covered by the above regulatory lists. These chemicals were the metals iron, tin, and zinc;[26,27] the short-chain glycol ingredients propylene glycol and glycerol; their derivatives glycidol, allyl alcohol[49,50] glyoxal, and methyl glyoxal;[19] the C4 dicarbonyl acetoin;[46] the alkaloid-related compounds anatabine, cotinine, myosmine, nicotine-*N*-oxide, and β-nicotyrine;[17,51] and the sensory cooling agent menthol.[52] Of these, glycerol and propylene glycol make up a large proportion of the e-liquid[53] and NFDPM.

The levels of glycol components were considerably higher in emissions from IS1.0(TT) than in those from EC(BT), despite the disparity in puff count (Table 5). This is a characteristic of the product design of IS1.0(TT), which is intended to deliver a large mass of aerosol, which is composed mainly of glycerol and propylene glycol and is largely independent of nicotine concentration. Menthol is a sensory ingredient and was not a component of the e-liquid in either test product. Its level was NQ in one sample for EC(BT) (puff block 1) as previously reported[17] and ND in all other samples and air blanks. In particular, the zinc data illustrate the susceptibility to spot contamination in metals analysis. It was detected at quantifiable levels in every sample except for the blank for IS1.0(TT). Summary data in Figure 3 show that the zinc levels were lowest for IS1.0(TT), but this was masked by fluctuating levels in the blanks. We therefore conclude that zinc cannot be

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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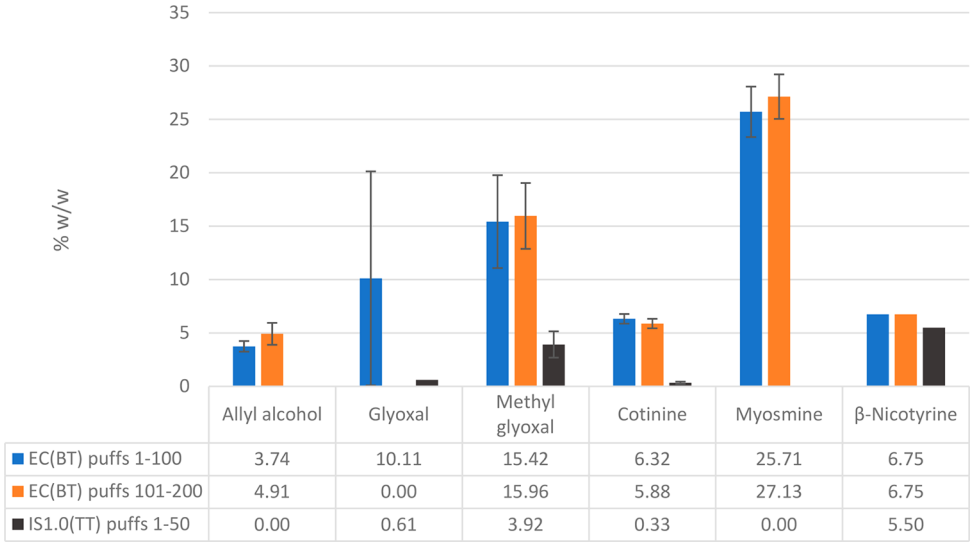
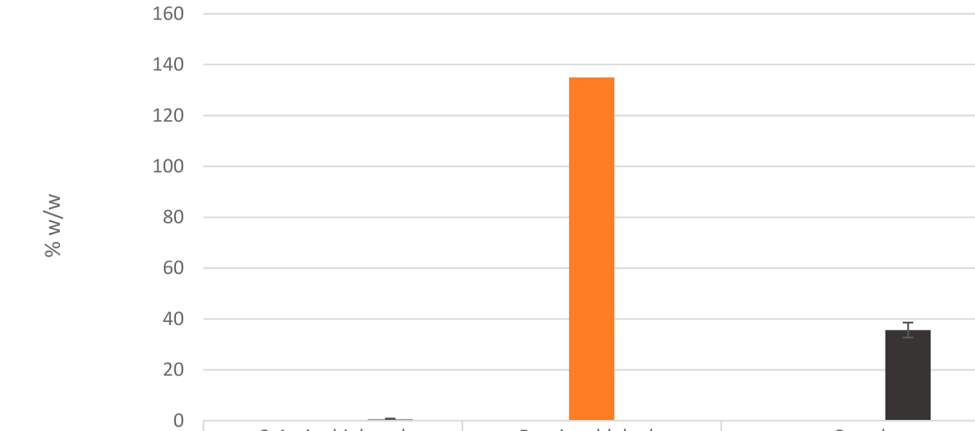


	Allyl alcohol	Glyoxal	Methyl glyoxal	Cotinine	Myosmine	β-Nicotyrine
EC(BT) puffs 1-100	3.74	10.11	15.42	6.32	25.71	6.75
EC(BT) puffs 101-200	4.91	0.00	15.96	5.88	27.13	6.75
IS1.0(TT) puffs 1-50	0.00	0.61	3.92	0.33	0.00	5.50

Figure 4. Nonregulatory analytes in e-cigarettes that were discernible from the air blank. Values are expressed as a percentage ratio (by contained weight) of each e-cigarette collection to one 1R6F control cigarette. Data for menthol, PG, and VG are discussed separately in the text. Error bars indicate 1 SE (n = 5), and absence of error bars indicates left-censored values. Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.



	3-Aminobiphenyl	Propionaldehyde	o-Cresol
EC(BT) puffs 1-100	0.00	0.00	0.00
EC(BT) puffs 101-200	0.00	134.95	0.00
IS1.0(TT) puffs 1-50	0.49	0.00	35.65

Figure 5. Health Canada test analytes in e-cigarettes emissions that were discernible from the air blank. Values were expressed as a percentage ratio (by contained weight) of each e-cigarette collection to one 1R6F control cigarette. Analytes were from the Health Canada test schedule but not covered by the FD A-18 or the EU TPD data dictionary. Error bars indicate 1 SE (n = 5), and absence of error bars indicates left-censored values. Each collection for EC(BT) was 100 puffs. Each collection for IS1.0(TT) was 50 puffs.

compared at these levels using the analytical method employed.

Six other analytes were detected at levels higher than the blanks in either IS1.0(TT) or EC(BT) (Figure 4). Of these, the three alkaloids are known impurities in formulation and expected to be unrelated to device performance: β-nicotyrine was NQ in all e-cigarettes emissions; cotinine was lower in IS1.0(TT) but had a high level in the corresponding blank, which influenced the result; and myosmine was clearly higher in EC(BT). We believe that the fluctuation in trace alkaloids is attributable to batchwise variation in nicotine purity. Although allyl alcohol was measured from EC(BT),[17] it was not detected from IS1.0(TT).

Methyl glyoxal and glyoxal are oxidation products from propylene glycol[49] and are commonly seen in e-cigarette emissions. The average levels of both were lower in IS1.0(TT) than in EC(BT) emissions, although the difference in methyl glyoxal between IS1.0(TT) and puff block 1 of EC(BT) was borderline significant (Table 5). The differences between IS1.0(TT) and puff block 2 of EC(BT) were compared on a per-puff basis, because both measurements were >LOQ. When scaled per 100 puffs, the mean result for IS1.0(TT) was not significantly different to that for EC(BT) (p = 0.08). Volatile carbonyls give high dispersion between replicates, which makes it difficult to make unambiguous comparisons. It is possible that with higher numbers of replicates, the trends would be more conclusive.

In summary, among the 16 analytes with specific relevance to e-cigarettes, other than aerosol-generating solvent mixture and menthol, 3 alkaloid-related compounds were present and

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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attributed to formulation and 3 solvent breakdown products were detected. Two of these were present at lower levels or were not detected in IS1.0(TT) emissions.

3.4. Health Canada-Specific Test Analytes. In addition to those already discussed, the Health Canada analytical methods for the quantification of mainstream smoke HPHCs[10] list a further 22 analytes: specifically, 3-aminobiphenyl (an aromatic amine), butyraldehyde, acetone, and propionaldehyde (all volatile carbonyls); eugenol (a flavor additive), hydroquinone, resorcinol, catechol, ortho-, meta- and para-cresol, and phenol (phenolics, which are usually associated with thermal decomposition of lignin in plant material); *N*-nitrosoanabasine and *N*-nitrosoanatabine (tobacco-specific nitrosamines); NO, NOx, and HCN (predominately gaseous compounds); pyridine, quinoline, and styrene (semivolatile compounds); the composite measurement of tar (NFDPM); and mercury (a toxic heavy metal).

"Tar" or NFDPM is relevant to cigarette smoke and was not previously reported for EC(BT). It is a measure of the total condensed aerosol mass, after correction for water and nicotine, and arose historically as a way of discriminating between high and low tar cigarette products. For e-cigarettes, the aerosol is formed from evaporation and condensation of a supplied liquid, with very little chemical change occurring. In the context of e-cigarettes, the term relates to a high proportion of the aerosol mass and may be useful as a sensorial benchmark. The NFDPM was determined for IS1.0(TT) in this study, and is reported here. The result was high, as expected due to the amount of glycerol and propylene glycol present in the e-liquid. Fifty puffs from IS1.0(TT) yielded the same corrected aerosol mass as approximately 16 cigarettes.

Among the other 21 Health Canada analytes, only three were detected in either IS1.0(TT) or EC(BT) at levels higher than those in the blank (Figure 5). The presence of *o*-cresol from IS1.0(TT) was unexpected and unexplained. The trace level of 3-aminobiphenyl from IS1.0(TT) was probably due to fluctuating background levels, which showed a similar range of values. The difference in propionaldehyde between EC(BT) and 1R6F appeared pronounced, but the values should be considered in terms of the measurement scales. The level of propionaldehyde in EC(BT) emissions was NQ, whereas that from cigarette smoke was BDL; therefore, the level from EC(BT) was formally higher. However, the e-cigarette and combustible methods had different sensitivities and are not directly comparable, although the calculated ratio is directionally correct. Propionaldehyde was not detected in emissions from IS1.0(TT).

In summary, among the additional compounds from Health Canada's cigarette smoke testing suite, only NFDPM and three other compounds were detected in the e-cigarettes. Of these, cresol was unexpected and is provisionally viewed as a contaminant, subject to further testing. 3-Aminobiphenyl was <LOQ and showed a similar range of values in the air blank. Propionaldehyde, which was previously reported at trace levels in EC(BT) emissions, is linked to the breakdown of solvent and was absent from IS1.0(TT) emissions.

3.5. FDA Established List of Analytes. In addition to the above analytes, a further 54 HPHCs have been identified by the FDA.[8] These have been discussed previously.[17] Of these, 10 were detected in either IS1.0(TT) or EC(BT) at levels above those in the air blanks.

Several of these analytes were not numerically comparable between the e-cigarettes and the cigarette, owing to large differences in method sensitivity. The volatile nitrosamine *N*-nitrosodimethylamine (NDMA) was previously reported as NQ in EC(BT) (versus BDL in the blank), but it was not detected in emissions from IS1.0(TT) or the cigarette. The sensitivity between the methods varied by approximately a factor of 5; therefore, it was not possible to make a direct comparison between the cigarette and the e-cigarettes, but the assigned level in EC(BT) was formally ~ 20-fold higher than the assigned level in the cigarette, although both were <LOQ.

The aromatic amines 2,6-dimethylaniline and *o*-toluidine were detected in samples and blanks at trace levels, as discussed above and summarized in Table 3 and Figure S1 in the Supporting Information. The levels in the test products are considered impossible to resolve from background levels using the current methods.

Nornicotine is a minor alkaloid and is present in the formulation as a trace impurity of nicotine. Traces of this alkaloid were present in IS1.0(TT) emissions at 4.4% w/w relative to levels in cigarette smoke.

The remaining analytes were the polycyclic aromatic hydrocarbons (PAH) benz(*j*)aceanthrylene, benzo(*a*)-anthracene, chrysene, naphthalene, benzo(*b*)fluoranthene, and benzo(*k*)fluoranthene. These were detected sporadically at trace levels in emissions from both e-cigarettes and in air blanks. The levels in blanks were higher for the IS1.0(TT) study than for the EC(BT) study (tabulated in Table S4 in the Supporting Information). Individual replicate values for PAH levels in IS1.0(TT) and the blanks, with each analyte scaled by its LOQ, are plotted to facilitate comparison among the series in Figure S2 in the Supporting Information. The range of levels in background air were comparable to those in the emissions from IS1.0(TT). It was therefore considered that the measurement of polycyclic aromatic hydrocarbons is not possible in the presence of trace environmental contamination in laboratory air, chiefly due to the extreme low levels (or absence) of these analytes in the emissions from e-cigarettes.

In summary, of the additional analytes on the FDA established list of HPHCs, most were either undetected or not possible to detect in the presence of environmental background levels in laboratory air. The exception was nornicotine, which is a known minor alkaloid impurity in the e-liquid formulation.

4. DISCUSSION

We have comprehensively characterized the emissions of a vaping product, IS1.0(TT), in comparison to both a reference cigarette, 1R6F, and previously published data on an e-cigarette, EC(BT). To help with assessing the emissions data, the analytes were grouped by their inclusion on lists of potential toxicants proposed by leading public health and regulatory bodies such as the WHO's TobReg and the FDA.

Drawing on the results from this and our previous study of the 18 analytes (including nicotine) in the combined TobReg-9/FDA-18 group, only 7 were present at levels above the air blank in emissions from either IS1.0(TT) or EC(BT). Two of these were at trace levels with a similar range of values in the corresponding air blanks, and one was related to a known trace impurity from alkaloids. The remaining three compounds were carbonyls derived from breakdown of the aerosol-generating solvent mixture. Of these, only formaldehyde was emitted from

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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IS1.0(TT) and was present at a low level (1.50 μg per 50 puffs, SD 0.35, n = 5).

Of the 10 additional analytes from the EU TPD Data Dictionary, none were detected at levels above the blank for IS1.0(TT). In comparison, EC(BT) emissions contained measurable amounts of nickel and chromium, probably associated with the nichrome heating coil. We tested for 16 additional compounds as potentially relevant e-cigarette analytes, of which two were components of the aerosol generating solvent mixture (PG and VG) and were measured at the expected levels. Two compounds were sensory ingredients that were not added to either e-liquid. One of these, menthol, was previously observed at trace levels from EC(BT), but neither was detected from IS1.0(TT). Five analytes were alkaloid-related, three of which were recorded at various levels and attributed to formulation. Three analytes were metals, whose levels were indistinguishable from those of air blanks, highlighting the issue of environmental contamination. Four analytes were breakdown products of the aerosol-generating solvent mixture: glyoxal, methylglyoxal, and allyl alcohol were previously reported at low levels in emissions from EC(BT). The dicarbonyls glyoxal and methylglyoxal were also detected in IS1.0(TT) emissions: the level of glyoxal was lower than that from EC(BT), but methyl glyoxal levels were statistically indistinguishable (alpha level 0.05) when adjusted (adversely) to a per-puff basis.

Of the further 22 analytes introduced from the Health Canada test schedule, only NFDPM and three others were seen in emissions from either e-cigarette. The aromatic amine 3-aminobiphenyl was detected at trace levels in IS1.0(TT) emissions, but had a similar range of values in its air blank. Propionaldehyde, a solvent breakdown product, had been recorded at trace levels from EC(BT) but was absent from IS1.0(TT). A quantity of *o*-cresol was recorded in emissions from IS1.0(TT), which was unexplained.

The established list of chemicals and chemical compounds identified by the FDA as HPHCs in tobacco products and tobacco smoke provided 54 more test analytes. Other than nornicotine (an alkaloid present in e-liquids), these were all either absent or present at levels that were impossible to distinguish from levels in the corresponding blanks. The recently published FDA guidance document for premarket tobacco applications for electronic nicotine delivery systems includes nine chemicals that are not covered in this work.[11] These new candidates are all viable as flavor ingredients: six are esters, one is an aldehyde, one is an alcohol, and one is a short chain carboxylic acid.

Overall, the difference between the emissions from the e-cigarettes and those from the reference cigarette was characterized by a substantial reduction, or absence, of most HPHCs despite the fact that the puff count and aerosol delivery per collection were far higher for the e-cigarettes. The reductions observed in this study have to be substantiated with human in-use measurements.

One limitation of this study is that the power and e-liquid compositions were not exactly matched, when comparing device types. This was unavoidable due to physical differences in device technology. However, we believe the trends in analyte delivery are strong enough to be distinct. When comparing the e-cigarettes, it is apparent that EC(BT), which uses a coil and wick for aerosol formation and fluid transfer, shows slightly higher toxicant levels, albeit still typically much lower than those found in cigarette smoke, due in part to the lack of synergy between the two processes. In contrast, IS1.0(TT) has a single mode of liquid transfer and vapor formation, which is designed to facilitate aerosol delivery without fluid starvation or consequent overheating. The net result is that the delivery of propylene glycol and glycerol was higher for IS1.0(TT), but most carbonyls were lower or absent. Formaldehyde and methyl glyoxal trended towards lower levels in IS1.0(TT) emissions, but the difference was not statistically significant in all cases.

Other than intended ingredients, such as nicotine solvents and flavours, or impurities, such as cotinine, myosmine, β-nicotyrine, and nornicotine, the key e-cigarette HPHCs identified here and in our previous study[17] were NNN, acetaldehyde, acrolein, formaldehyde, allyl alcohol, glyoxal, methylglyoxal, propionaldehyde, nickel, and chromium (and potentially *o*-cresol). With the exception of *o*-cresol, which is provisionally assumed to be a contaminant, these HPHCs are breakdown products of the aerosol-generating solvent mixture, metals, or an *N*-nitroso alkaloid derivative. The majority of these were absent or substantially lower in IS1.0(TT) emissions than in EC(BT) emissions. In particular, it seems that production of the volatile carbonyls was controlled in IS1.0(TT), despite an increase in device power. Alongside their potential health risks, volatile carbonyls are known to impart undesirable sensorial properties.[15,31] This sensory aspect was prioritized during the design process leading to IS1.0(TT), along with features affecting air flow and the aerosol condensation pathway, in order to deliver a product platform that would compete with cigarettes for established smokers. The results presented here demonstrate that technological innovation can improve the performance of e-cigarettes and at the same time reduce the emission of harmful substances. This study does not address whether the innovation might further reduce any health risks associated with vaping compared to conventional e-cigarettes, and both this new innovation and conventional e-cigarettes have much fewer and lower levels of toxicants compared to cigarette smoke.

These findings for HPHCs are generally consistent with the current knowledge on e-cigarettes. However, the results in previously published studies have seen widely varying results, possibly due to uncontrolled factors such as e-liquid composition, flavor ingredients, maturation during storage, varied device types and power deliveries, wicking and coil materials, and testing and measurement procedures, as well as the approach used to report results.

Further development in analytical methods and their standardization would help to differentiate among products, along with a consistent approach to statistical comparison, especially when the emission levels are close to the test environment levels. The methods used for aerosol capture and analysis, as well as suitable puffing regimes, should all be evaluated to allow meaningful comparison of the results obtained from different studies. A standardized reference e-cigarette would also be very useful. For the time being, provisional comparisons can be made with careful consideration of product types, puffing parameters, and emissions.

From a manufacturing point of view, innovation in product design combined with rigorous product stewardship should be in place and actively enforced during the development of e-cigarettes in order to minimize potential hazards and risks from the products.[54] Product manufacturing quality is also critical in ensuring consistency and stability during the full life cycle of

https://dx.doi.org/10.1021/acs.chemrestox.9b00442
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the product, helping to reduce variability across time for consumers and regulatory testing alike.

5. CONCLUSIONS

We have described a vaping product, coded IS1.0(TT), and characterized it in terms of its aerosol emissions. IS1.0(TT) contains a fabric-free, stainless steel mesh plate, which functions both to wick the e-liquid and to heat it to form an aerosol. This single aerosolization action replaces the common nichrome coil and cotton wick assembly found in the vast majority of e-cigarettes sold today. The porosity of the steel plate and its electrical resistance works in synergy to allow a larger aerosol mass to be delivered per puff with reduced risks of thermal breakdown of key aerosol agents and flavor ingredients.

The emission results showed that IS1.0(TT) and EC(BT) had substantially reduced levels of toxicants in their emissions as compared with 1R6F cigarette smoke. Furthermore, the yields of thermal degradants (in particular volatile carbonyls) in the vapor of IS1.0(TT) were lower than those in EC(BT) vapor. The reduced degradants in IS1.0(TT) aerosol should help to maintain better flavor delivery for improved sensory performance. Current studies are assessing the toxicology of IS1.0(TT) aerosol using a range of toxicological approaches, as well as clinical studies examining exposure to tobacco smoke toxicants when smokers switch to IS1.0(TT) or stop using any tobacco or nicotine products. The findings will be reported in future papers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.9b00442.

Analytical methodology for the current study and for the previous study; numerical results from the previous and current studies; comparison between products; individual replicate values for all aromatic amines measured for IS1.0(TT) and its blanks; individual replicate values for PAH levels in IS1.0(TT) and the blanks (PDF)

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Funding

Work was funded by British American Tobacco (BAT). All authors are either employees of BAT or paid consultants.

Notes

The authors declare no competing financial interest.

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https://dx.doi.org/10.1021/acs.chemrestox.9b00442
Chem. Res. Toxicol. 2020, 33, 789−799