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Chemical Composition of Aerosol from an E-Cigarette: A Quantitative Comparison with Cigarette Smoke

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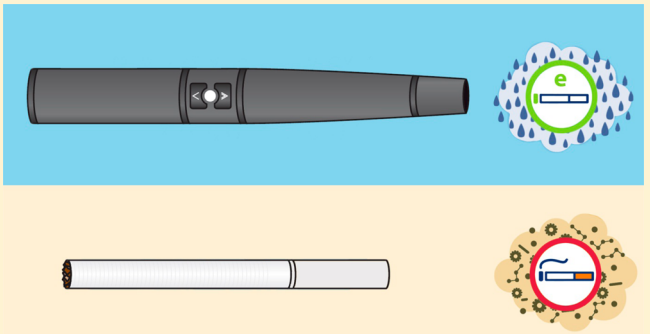
Supporting Information

ABSTRACT: There is interest in the relative toxicities of emissions from electronic cigarettes and tobacco cigarettes. Lists of cigarette smoke priority toxicants have been developed to focus regulatory initiatives. However, a comprehensive assessment of e-cigarette chemical emissions including all tobacco smoke Harmful and Potentially Harmful Constituents, and additional toxic species reportedly present in e-cigarette emissions, is lacking. We examined 150 chemical emissions from an e-cigarette (Vype ePen), a reference tobacco cigarette (Ky3R4F), and laboratory air/method blanks. All measurements were conducted by a contract research laboratory using ISO 17025 accredited methods. The data show that it is essential to conduct laboratory air/method measurements when measuring e-cigarette emissions, owing to the combination of low emissions and the associated impact of laboratory background that can lead to false-positive results and overestimates. Of the 150 measurands examined in the e-cigarette aerosol, 104 were not detected and 21 were present due to laboratory background. Of the 25 detected aerosol constituents, 9 were present at levels too low to be quantified and 16 were generated in whole or in part by the e-cigarette. These comprised major e-liquid constituents (nicotine, propylene glycol, and glycerol), recognized impurities in Pharmacopoeia-quality nicotine, and eight thermal decomposition products of propylene glycol or glycerol. By contrast, approximately 100 measurands were detected in mainstream cigarette smoke. Depending on the regulatory list considered and the puffing regime used, the emissions of toxicants identified for regulation were from 82 to >99% lower on a per-puff basis from the e-cigarette compared with those from Ky3R4F. Thus, the aerosol from the e-cigarette is compositionally less complex than cigarette smoke and contains significantly lower levels of toxicants. These data demonstrate that e-cigarettes can be developed that offer the potential for substantially reduced exposure to cigarette toxicants. Further studies are required to establish whether the potential lower consumer exposure to these toxicants will result in tangible public health benefits.



1. INTRODUCTION

The past decade has seen the rapid emergence and increasingly widespread use of electronic nicotine delivery systems (ENDS), and electronic cigarettes (e-cigarettes) in particular, as alternatives to conventional tobacco cigarettes.[1] The first modern e-cigarette design has been widely attributed to Hon Lik in the early 2000s.[2] As a result of rapid product innovation and changes in design since then, and in the past 5 years in particular, a wide range of e-cigarette designs has emerged around the world.[3] Nonetheless, most e-cigarettes comprise a battery unit providing energy to a heating coil or atomizer that generates an aerosol from a liquid ("e-liquid"). Most e-liquids tend to comprise excipients such as propylene glycol (PG), vegetable glycerol (VG), and water, as well as nicotine and flavors. Currently, e-cigarette designs can be grouped into four basic categories: small cigarette-like disposable and rechargeable designs; closed-system modular designs, comprising separate battery units and e-liquid cartridges; open-system modular designs, wherein the user adds a separately sold e-liquid to a refillable atomizer unit; and tank or box-mod systems, where the user can customize both individual components of the device and their operating conditions as well as add a choice of e-liquids.

The increasing popularity of e-cigarettes has been described as a largely consumer-led development,[4] with many tobacco cigarette users electing to trial and switch partially or totally from smoking to use of e-cigarettes. There are currently more than 10 million e-cigarette users across the world, particularly in the United States, United Kingdom, France, and wider European countries.[5−7] The predominant population of e-cigarette users comprises either ex-smokers or dual users, with relatively few never-smokers.[7,8] E-cigarettes have been recognized by some scientists as being as effective as, or more

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Figure 1. Vype ePen construction.

effective than, smoking-cessation treatments including traditional nicotine-administration cigarette surrogates such as nicotine patches and chewing gums;[8] consequently, the use of e-cigarettes among smokers is increasing.

Tobacco cigarette users face significant health risks associated with smoking: lung cancer, chronic obstructive pulmonary disorders, and heart disease are the major causes of mortality and morbidity among smokers.[9] For over 50 years, scientists have worked to establish disease mechanisms and their sources in cigarette smoke, with efforts focusing on a number of toxic chemicals in cigarette smoke.[10] The presence of over 100 harmful and potentially harmful constituents (HPHCs) of tobacco and cigarette smoke has been recognized by various scientific bodies,[11,12] and several regulatory authorities have mandated the reporting of different toxicant suites in smoke emissions from cigarettes.[10,13−16] Recently, an advisory body on Tobacco Product Regulation (TobReg) to the World Health Organisation (WHO) has proposed mandated lowering of the emission levels from cigarettes of nine toxicants: carbon monoxide, formaldehyde, acetaldehyde, acrolein, 1,3-butadiene, benzene, benzo[*a*]pyrene, *N*′-nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).[17]

E-cigarette use is regarded by many (but not all) scientists as being likely to have substantially lower levels of risk than smoking tobacco cigarettes.[18,19] Support for this position comes from *in vitro* biological studies[20,21] and the relatively simple composition of e-cigarette aerosols[22] in comparison to cigarette smoke with its thousands of constituents.[23] A growing number of studies have also investigated the emissions of some cigarette smoke toxicants from e-cigarettes, such as tobacco-specific nitrosamines,[24−27] tobacco alkaloids and nicotine decomposition products,[24,28,29] volatile organic compounds,[22,26,30−33] aromatic amines,[26] CO,[26] polycyclic aromatic hydrocarbons (PAHs),[26,32,34] phenolics,[26,27] metals,[26,30,35,36] and carbonyls.[27,30,31,37−47] Most of these studies report aerosol emission levels of toxicants that are either undetectable or a few percent of those found in cigarette smoke, and comparisons have also been made to room air.[26] However, the presence of toxicants in e-cigarette aerosols, even at comparatively low levels, suggests that e-cigarette use is not risk-free.

Although the levels of toxicants in e-cigarette aerosols have commonly been reported to be a fraction of those found from tobacco cigarettes, the carbonyls formaldehyde, acetaldehyde, and acrolein are an exception to this general trend. Levels of these carbonyls can approach those from traditional tobacco cigarettes, particularly under dry-puff conditions when e-liquid transport to the atomizer is insufficient for the applied electrical power setting. Two studies that used very high e-cigarette power settings reported carbonyl emission levels higher than those found in cigarette smoke, owing to overheating of the e-liquid in the atomizer.[41,48] While the use of these high e-cigarette power settings has been criticized on the basis that they are unrepresentative of human exposure, and the attendant unpleasant off-tastes and odors generated by overheating in such dry-puff situations would restrict or prevent consumer exposure to high carbonyl emissions, the presence of carbonyls in e-cigarette emissions remains a concern.[42,43,49]

Additional compounds have been identified in the emissions of e-cigarettes that have historically received little focus in cigarette smoke toxicant prioritization exercises, such as ethylene glycol and diethylene glycol, glyoxal, methylglyoxal,[39] diacetyl and acetyl propionyl,[50,51] acetoin,[51] copper,[36] and zinc.[35]

There is emerging regulatory focus on toxicant emissions from e-cigarettes. For example, a recent data dictionary,[52] issued by the European Commission during the development and National Implementation of the 2014 EU Tobacco Products Directive, suggested the following list of emissions for product notification purposes in the EU: nicotine, ethylene glycol and diethylene glycol, formaldehyde, acetaldehyde, acrolein, crotonaldehyde, NNN, NNK, cadmium, chromium, copper, lead, nickel and arsenic, toluene, benzene, 1,3-butadiene, isoprene, diacetyl, and acetyl propionyl. In contrast, the recent FDA final deeming of regulations covering e-cigarettes did not identify any specific compounds of interest at this time.[53]

To date, no single study has attempted to comprehensively characterize the chemical composition of e-cigarettes by comparing the emissions of all known e-cigarette and tobacco cigarette priority compounds with those from a tobacco cigarette. This information is an important contribution to current interest in understanding the relative toxicities of e-cigarettes and tobacco cigarettes. To our knowledge, only three groups have examined a reasonably broad range of compounds: Flora et al.;[54] Tayyarah and Long,[26] who measured e-cigarette emissions of 55 HPHCs; and Lauterbach and Laugesen,[55,56] who examined 62 cigarette smoke emissions from an early e-cigarette design. Even these studies provide an incomplete picture of the potential chemical composition of e-cigarettes in comparison to that of tobacco cigarettes. The absence of a complete data set is an important gap that needs to be resolved. The current work addresses that gap, reporting the emission levels of 142 chemicals and 8 collated measures, covering the widest practicable range of HPHCs of cigarette smoke and key e-cigarette constituents of concern.

2. EXPERIMENTAL PROCEDURES

2.1. Test Pieces. The tobacco cigarette used in the current work was the Ky3R4F Kentucky Reference Cigarette (Center for Tobacco Reference Products, University of Kentucky, USA), designed to provide a standard test piece for scientific studies. It is a US-blended king-sized product with a cellulose acetate filter and an ISO tar yield of 9.4 mg/cigarette in 9 puffs. The composition, construction, and mainstream smoke HPHC yields from this product have been reported previously.[57,58]

The e-cigarette used was Vype ePen (Nicoventures Trading Ltd., Blackburn, UK). It is a closed-modular system (Figure 1) consisting of two modules: a rechargeable battery section and a replaceable e-liquid-containing cartridge ("cartomizer"). The device also has a removable mouthpiece and a screw connector for the cartomizer to connect to the battery section.

The battery section comprises a USB-rechargeable 650 mAh battery and an integrated circuit power controller with two voltage settings, 4 and 3.6 V, selectable by the consumer via an external twin-setting surface mounted switch. Device operation commences when the user presses either setting of the power switch, usually 1 s in advance of the

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puff being taken (1 s preheat time), with power operating within the device as long as the button is pressed, usually the length of the puff.

The disposable e-liquid cartomizers comprise a liquid tank and an atomizer. The tank is composed of an inner polypropylene liquid reservoir held within an outer polypropylene aerosol-transport tube. The liquid contained within the inner tank is fed to the atomizer through a sintered porous ceramic disk in contact with a silica transport wick. The atomizer comprises a 2.85 Ω nichrome (80% Ni/20% Cr) wire coil heater wrapped around the wick. The resistance of the nichrome wire generates heat when current is supplied by the battery; the heated wire and wick vaporize the e-liquid carried by the wick. The vapor condenses downstream of the atomizer into aerosol particles that are carried by the outer transport tube to the device user for inhalation.

A number of Vype e-liquid flavors are sold, including the "Blended Tobacco" variant examined in this study, chosen because it is the variant sold in the greatest number (data not shown). The cartomizer contained 1.58 mL of the Blended Tobacco e-liquid composed of 25% (w/w) propylene glycol containing low levels (<1%) of blended tobacco flavor, 48.14% VG, 25% water, and 1.86% nicotine. Vype ePen has an operating life of in excess of 200 puffs, depending on usage patterns, and was operated in these tests at the 3.6 V setting. The e-cigarette was developed in accordance with a detailed duty-of-care protocol,[59] which examines the device materials, liquid composition, device performance, and aerosol content.

Products were sampled from the factory at a single point in time and contained e-liquid 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.

2.2. Analysis of Emissions from the E-Cigarette Aerosol and Cigarette Smoke. Analyses were conducted by a single laboratory, Labstat International ULC (Labstat; Kitchener, Ontario, Canada), with the exception of polychlorinated dibenzodioxins/dibenzofurans and radioactive isotopes, which were subcontracted by Labstat to external laboratories.

Smoking machine parameters for the measurement of cigarette smoke constituents have been the subject of much debate.[60] The most widely used puffing conditions of a 35 cm^3 puff volume, 2 s puff duration, and 58 s interpuff interval, as defined by the International Organization for Standardization (ISO),[61] have been widely criticized for under-representing the yields of emissions in comparison to those taken by smokers while puffing. A second puffing regime has gained prominence and widespread support since 1999,[16,62] namely, the Health Canada Intense (HCI) smoking regime,[13] with a 55 cm^3 puff volume and a 2 s puff duration, with a bell-shaped puff profile, taken twice per minute, with all filter ventilation holes blocked. The increased puff volume, shorter interpuff interval, and blocked ventilation holes lead to higher smoke yields than those under the ISO conditions and, although also unlikely to represent actual human exposure, are considered by many to be more representative than ISO data. In the current study, HCI parameters were used to generate cigarette smoke emissions for subsequent toxicant measurement.

At present, there are no standardized e-cigarette puffing parameters for analytical measurements. Studies have reported the use of a wide range of puffing parameters by e-cigarette consumers, and various values have been used in machine-based aerosol chemistry measurements.[63−68] Overall, a longer puff duration has been commonly observed for e-cigarette users as compared with cigarette smokers.[69,70] Whether a single puffing regime is appropriate for all e-cigarettes remains to be demonstrated; nevertheless, urgent standardization is required to enable comparisons to be made between studies. With this in mind, the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) has published Recommended Method 81 for machine puffing e-cigarettes and uses a regime with rectangular flow profile puffs, a puff volume of 55 cm^3, and a duration of 3 s, taken twice per minute.[71] These puffing parameters were applied to the e-cigarette in the current study.

Cigarettes smoked under HCI parameters often provide between 10 and 15 puffs per cigarette.[72] Vype ePen provides in excess of 200 puffs to the consumer before the e-liquid becomes exhausted. In the measurement phase of this study, emissions data were collected on a per-cigarette basis for Ky3R4F, with the puff number recorded. For the e-cigarette, the analyses were conducted in two blocks, each with a duration of 100 puffs. Data were therefore obtained for blocks of puffs 1−100 and puffs 101−200. The reported data are based on five independent replicates of products sampled at one point in time.

2.3. Measurement Methods. The methods used by the analysis laboratory are summarized in Supporting Information Table S1. In total, 27 different analytical methods were used to quantify the emissions of 150 measurands, including 142 analytes and 8 collated values, in the mainstream emissions from the e-cigarette, Ky3R4F, and air/method blanks. The methods used were largely based on Health Canada methods for cigarette smoke analysis, with additional methods developed by Labstat for the other HPHCs and e-cigarette compounds of interest. The methods were adapted for use with e-cigarettes where necessary. The operation of the methods is accredited to ISO/IEC 17025:2005[73] for all reported constituents of mainstream tobacco smoke and e-cigarette aerosols, except where noted in Supporting Information Table S1. No analytical methods were identified for the analysis of three HPHCs, NSAR, coumarin, and aflatoxin, in smoke or aerosol. The FDA TPSAC 2010 draft list implies that these substances and *N*-nitrosomorpholine are not expected to be present in smoke, only in tobacco;[74] therefore, these compounds were not included in the study.

2.4. Data Treatment for Comparison Between Test Pieces. As noted above, tobacco cigarettes are consumed in approximately 10 puffs when machine smoked, whereas e-cigarettes may continue to function for several hundred puffs. Consequently, the current data are presented both "as measured" (i.e., per-stick for Ky3R4F or per-100 puff block for the e-cigarette) and also on a per-puff basis by dividing the reported values by the number of puffs taken during the measurement. The calculated per-puff values allow a direct comparison of emissions between products and facilitate scaling by daily puff consumption values to provide a daily toxicant exposure assessment. Percentage differences between the emissions from the two devices are calculated on a per-puff basis.

This approach seems to be a reasonable basis for comparison because, on average, cigarette smokers smoke between 15 and 20 cigarettes per day,[7] leading to a total cigarette puff count approaching 200 per day. Consumption measurements with e-cigarettes suggest that 150−250 puffs are taken each day by the average vaper.[67,75] Extending per-puff comparisons to provide daily exposure estimates is challenging because Ky3R4F is a research cigarette rather than a commercial product; in addition, the intake/uptake efficiency of toxicants from either test piece is unclear at present.

2.5. Inclusion of Limit of Detection/Quantification Values. For many measurands, the emissions were below the limit of detection (LOD) and/or limit of quantification (LOQ). To enable the percentage difference between ePen and Ky3R4F to be calculated for as many constituents from each toxicant subset as possible, <LOD and <LOQ values were imputed as follows. For data <LOD, the value was calculated as one-half of the analytical method's reported LOD:

$$\text{calculated value} = \frac{\text{reported LOD}}{2}$$

For data <LOQ but >LOD, the value was calculated as the midpoint between the reported LOD and LOQ of the analytical method:

$$\text{calculated value} = \text{reported LOD} + \left(\frac{\text{reported LOQ} - \text{reported LOD}}{2}\right)$$

In cases where the e-cigarette and Ky3R4F reference cigarette emissions were both <LOD or <LOQ, the measurand was omitted from the percentage difference calculation.

2.6. Estimation of Ky3R4F Smoke Yields under ISO Conditions. Our study focused on measurement of Ky3R4F smoke yields under the HCI regime. When comparing smoke yields from Ky3R4F to the e-cigarette aerosol emissions, it was considered of interest to also conduct comparisons with Ky3R4F mainstream smoke

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Table 1. Emissions Measured from ePen at Quantifiable Levels[a]

		ePen and air/method blank LOD and LOQ per collection		ePen					air/method blank					Kentucky reference 3R4F smoke measurements					
				puffs 1−100		puffs 101−200			puffs 1−100		puffs 101−200			LOD and LOQ per collection			per cigarette emission		
smoke constituent	toxicant list	LOD	LOQ	mean	SD	mean	SD	ePen per puff	mean	SD	mean	SD	blank per puff	LOD	LOQ	number of puffs	mean	SD	3R4F per puff
formaldehyde, μg	T, F18, BCV, FEL	0.722	2.41	12.1	4.8	12.3	4.9	0.122	6.59	0.31	6.79	0.4	0.067	0.4	1.2	10.8	94.9	6.2	8.79
acetaldehyde, μg	T, F18, BCV, FEL	1.95	6.49	10.4	2.7	10.7	2.9	0.106	NQ	NQ	NQ	NQ	0.042	0.97	3.24	10.8	1732	43	160.4
acrolein, μg	T, F18, BCV, FEL	1.43	4.75	6.11	4.94	7.9	5.56	0.070	BDL	BDL	BDL	BDL	0.007	0.71	2.38	10.8	172	3	15.94
allyl alcohol, ng	ECIG	67.65	226.5	464	134	609	282	5.365	BDL	BDL	BDL	BDL	0.338	67.65	226.5	10.4	16978	644	1.63×10^{3}
glyoxal, μg	ECIG	0.126	0.42	4.81	10.1	6.45	7.6	0.056	NQ	NQ	0.44	0.17	0.004	0.63	2.1	10.6	20.44	2.66	1.93
methyl glyoxal, μg	ECIG	0.077	0.256	4.59	2.7	4.62	1.92	0.046	0.29	0.12	NQ	NQ	0.002	0.38	1.28	10.6	18.2	0.68	1.72
glycerol, mg	ECIG	0.072	0.24	153	18.3	162.7	13	1.579	NQ	NQ	NQ	NQ	0.002	0.02	0.08	10.5	2.05	0.12	1.95×10^{-1}
propylene glycol, mg	ECIG	0.012	0.04	66.7	8.61	75.06	6.22	0.709	NQ	NQ	NQ	NQ	0.000	0	0.01	10.5	0.03	0	2.92×10^{-3}
chrysene, ng	FEL	0.23	0.78	0.88	0.35	1.23	0.33	0.011	NQ	NQ	BDL	BDL	0.003	0.08	0.26	10.3	36.79	3.59	3.57
nicotine, mg	F18, FEL	0.007	0.022	3.57	1.10	2.75	0.981	0.032	BDL	BDL	BDL	BDL	0.00004	0.002	0.007	10.8	1.84	0.077	0.170
myosmine, ng	ECIG	318	1060	2664	526	2810	455	27.37	BDL	BDL	BDL	BDL	1.590	64	212	11.1	9809	701	883.7
cotinine, ng	ECIG	76.3	254	1123	145	1044	148	10.835	BDL	BDL	BDL	BDL	0.382	15	51	11.1	50861	1912	4582
NNN, ng	T, F18, BCV	0.492	1.641	5.16	0.56	5.61	1.03	0.054	NQ	NQ	1.83	0.51	0.014	0.2	0.66	10.6	264.67	22.2	24.97
chromium, ng	BCV, FEL	13.54	45.13	50.4	13.9	NQ	NQ	0.399	NQ	NQ	NQ	NQ	0.293	1.35	4.51	10.9	NQ	NQ	0.27
Compounds Not Higher than Air/Method Blank in Original Study but Measured Higher from ePen than Air/Method Blank during Puff Volume Study (Table 4)																			
acetone, μg	BCV, FEL	1.69	5.64	5.95	3.29	8.56	2.98	0.073	10	0.7	11.1	0.9	0.106	0.8	2.8	10.8	726	16	67.21
butyraldehyde	BCV	1.62	5.41	BDL	BDL	BDL	BDL	0.008	BDL	BDL	BDL	BDL	0.008	0.812	2.71	10.8	92.5	4.80	8.56

[a]Abbreviations: NNN, *N*-nitrosonornicotine; BCV, British Columbia List; ECIG, chemicals reported in e-cigarette emissions; F18, FDA current reporting list; FEL, full FDA established lists of HPHCs; BDL, below detection limit; LOD, limit of detection; LOQ, limit of quantification; NQ, not quantifiable.

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Table 2. Emissions Identified from ePen at Levels Too Low to Quantify[a]

				ePen					air/method blank					Kentucky reference 3R4F smoke measurements					
		ePen and method blank LOD and LOQ per collection		puffs 1−100		puffs 101−200			puffs 1−100		puffs 101−200			LOD and LOQ per collection			per cigarette emission		
smoke constituent	toxicant list	LOD	LOQ	mean	SD	mean	SD	ePen per puff	mean	SD	mean	SD	air blank per puff	LOD	LOQ	number of puffs	mean	SD	3R4F per puff
propionaldehyde, μg	BCV, FEL	2	6.67	BDL	BDL	NQ	NQ	2.67×10^{-2}	BDL	BDL	BDL	BDL	1.00×10^{-2}	1	3.34	10.8	133	5	12.3
menthol, mg	ECIG	0.012	0.041	NQ	NQ	NQ	NQ	2.65×10^{-4}	NQ	NQ	BDL	BDL	1.63×10^{-4}	0	0.01	10.5	BDL	BDL	1.94×10^{-4}
diacetyl, μg	ECIG	0.087	0.29	NQ	NQ	NQ	NQ	1.89×10^{-3}	BDL	BDL	BDL	BDL	4.35×10^{-4}	0.44	1.45	10.6	266.52	27.9	2.51×10^{1}
anatabine, ng	ECIG	252	841	NQ	NQ	NQ	NQ	5.47	BDL	BDL	BDL	BDL	1.26	50.4	168	11.1	30071	1131	2709.07
anabasine, ng	FEL	431	1440	NQ	NQ	NQ	NQ	9.36	BDL	BDL	BDL	BDL	2.16	86.2	288	11.1	9364	450	843.56
β-nicotyrine, ng	ECIG	234	779	NQ	NQ	NQ	NQ	5.07	BDL	BDL	BDL	BDL	1.17	47	156	11.1	9790	149	882.01
NDMA, ng	FEL	1.78	5.93	BDL	BDL	NQ	NQ	2.37×10^{-2}	BDL	BDL	BDL	BDL	8.89×10^{-3}	0.59	1.98	10.5	8.46	2.58	0.81
nickel, ng	BCV, FEL	28.48	94.92	NQ	NQ	NQ	NQ	6.17×10^{-1}	NQ	NQ	BDL	BDL	3.80×10^{-1}	2.85	9.49	10.9	NQ	NQ	0.57
Compound BDL in Original Study but Measured Higher from ePen than Air/Method Blank in Some Experiments of Puff Volume Study (Table 4)																			
propylene oxide, ng	FEL	156	520	BDL	BDL	BDL	BDL	0.78	BDL	BDL	BDL	BDL	0.78	15.6	52	10.5	1.1×10^{3}	90.7	103

[a]Abbreviations: NDMA, *N*-nitrosodimethylamine; BCV, British Columbia List; ECIG, chemicals reported in e-cigarette emissions; F18, FDA current reporting list; FEL, full FDA established lists of HPHCs; BDL, below detection limit; LOD, limit of detection; LOQ, limit of quantification; NQ, not quantifiable.

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Table 3. Compounds Showing No Significant Difference between ePen and Air/Method Blank Emissions[a]

Smoke constituent	Toxicant list	ePen and air/method blank LOD & LOQ per collection		ePen					Air/Method Blank					Kentucky Reference 3R4F smoke measurements					
				Puffs 1-100		Puffs 101-200		ePen per puff	Puffs 1-100		Puffs 101-200		Blank per puff	LOD & LOQ per collection		Number of puffs	Per cigarette emission		3R4F per puff
		LOD	LOQ	Mean	SD	Mean	SD		Mean	SD	Mean	SD		LOD	LOQ		Mean	SD	
2-butanone, µg	BCV, FEL	2.2	7.33	NQ	NQ	9.08	7.07	0.069	14.7	2.1	16.8	3.6	0.158	1.1	3.7	10.8	202	7	18.71
Ethylene glycol, mg	ECIG	0.003	0.008	NQ	NQ	NQ	NQ	5.47E-05	NQ	NQ	NQ	NQ	5.47E-05	0	0	10.5	0.03	0	2.88E-03
CO, mg	T, F18, BCV, FEL	0.48	1.59	4.74	0	6.75	0.28	0.057	4.74	0.23	6.69	0.27	0.057	0.16	0.53	10.8	29.63	1.46	2.74
Toluene, µg	F18, BCV, FEL	0.61	2.04	NQ	NQ	2.64	0.22	0.020	NQ	NQ	2.62	0.2	0.020	0.06	0.2	10.5	115.9	9.12	11.04
Naphthalene, ng	FEL	0.5	1.68	5.01	1.2	5.87	0.84	0.054	5.97	2.9	4.62	0.79	0.053	0.17	0.56	10.3	1005	125.5	97.57
Styrene, µg	BCV, FEL	0.117	0.388	0.518	0.401	NQ	NQ	0.004	1.05	0.41	NQ	NQ	0.007	0.04	0.13	10.2	17.4	1.7	1.7
o-Toluidine, ng	FEL	0.1	0.34	0.6	0.26	0.53	0.12	0.006	0.41	0.05	0.47	0.19	0.004	1.00E-02	3.40E-02	10.6	115.3	4.97	1.09E+01
2-Aminonaphthalene, ng	F18, BCV, FEL	0.04	0.12	NQ	NQ	NQ	NQ	7.73E-04	NQ	NQ	NQ	NQ	7.73E-04	4.00E-03	1.20E-02	10.6	12.47	0.48	1.18
3-Aminobiphenyl, ng	BCV	0.01	0.04	NQ	NQ	BDL	BDL	1.60E-04	NQ	NQ	NQ	NQ	2.60E-04	1.00E-03	4.00E-03	10.6	2.91	0.76	2.74E-01
4-Aminobiphenyl, ng	F18, BCV, FEL	0.01	0.05	NQ	NQ	BDL	BDL	1.91E-04	BDL	BDL	NQ	NQ	1.91E-04	1.00E-03	5.00E-03	10.6	2.14	0.5	2.01E-01
NAT, ng	BCV	0.97564	3.25214	BDL	BDL	NQ	NQ	1.30E-02	NQ	NQ	NQ	NQ	2.11E-02	0.39	1.3	10.6	269.07	18.21	25.38
NNK, ng	T, F18, BCV, FEL	0.75275	2.50916	BDL	BDL	NQ	NQ	1.00E-02	BDL	BDL	NQ	NQ	1.63E-02	0.3	1	10.6	282.67	24.17	26.67
NDBA, ng	FEL	1.66	5.53	17.15	14.85	NQ	NQ	0.104	22.84	20.3	BDL	BDL	0.118	0.55	1.84	10.5	BDL	BDL	0.03
NPYR, ng	FEL	1.98	6.6	NQ	NQ	11.57	7.14	0.079	NQ	NQ	10.36	6.94	0.073	0.66	2.2	10.5	18.54	8.38	1.77
NDELA, ng	FEL	0.42	1.42	7.95	3.77	14.5	2.85	0.112	6.68	5.65	13.01	5.55	0.098	0.14	0.47	10.5	0.48	0.64	0.05
Arsenic, ng	BCV, FEL	8.79	29.29	NQ	NQ	NQ	NQ	1.90E-01	NQ	NQ	NQ	NQ	1.90E-01	0.88	2.93	10.9	9.57	0.25	0.88
Copper, ng	ECIG	24.6	82.01	202.1	205.8	176	83.89	1.8905	90.75	37.43	96.61	33.49	0.937	2.46	8.2	10.9	24.67	3.1	2.26
Zinc, ng	ECIG	176.8	589.2	1209	611.4	1259	203.8	12.34	1276	777.2	1343.1	1364.4	13.10	17.7	58.9	10.9	257.29	36.92	23.6
Iron, ng	ECIG	56.31	187.7	472	106	362	121	4.17	423	309	382	261	4.025	5.63	18.8	10.9	34.51	13.9	3.17
Pyridine, µg	BCV	0.27	0.899	NQ	NQ	NQ	NQ	5.85E-03	NQ	NQ	NQ	NQ	5.85E-03	0.09	0.3	10.2	32.2	2.6	3.16

Smoke constituent	ePen							Air/method blank							3R4F smoke measurements					
	EDL1 & EDL2 per collection		Puffs 1-100		Puffs 101-200		ePen per puff	EDL1 & EDL2 per collection		Puffs 1-100		Puffs 101-200		Air blank per puff	EDL	Number of puffs	Kentucky Reference 3R4F		3R4F per puff	TEQ
	EDL1	EDL2	Mean	SD	Mean	SD		EDL1	EDL2	Mean	SD	Mean	SD				Mean	SD		
Chlorinated dioxins and furans																				
Octa CDD, pg	2	2.1	NQ	NQ	BDL	BDL	1.53E-02	1.89	2.12	NQ	NQ	BDL	BDL	1.48E-02	2.18	10.8	14.73	1.67	1.36	0.00041

[a]Abbreviations:; NAT, *N*-nitrosoanatabine; NNK, nicotine-derived nitrosamine ketone;.NDBA, *N*-nitrosodi-*n*-butylamine; NPYR, *N*-nitrosopyrrolidine; NDELA, *N*-nitrosodiethanolamine; BCV, British Columbia List; ECIG, chemicals reported in e-cigarette emissions; F18, FDA current reporting list; FEL, full FDA established lists of HPHCs; BDL, below detection limit; LOD, limit of detection; LOQ, limit of quantification; EDL, estimated detection limit; NQ, not quantifiable.

emissions conducted under ISO conditions, as these would provide a lower comparative set of toxicant levels with which to compare the e-cigarette emissions. To achieve this comparison, we used the data of Roemer et al.,[58] who reported the emissions of a number of cigarette smoke toxicants from Ky3R4F under both ISO and HCI smoking regimes, and provided ratios for the yields measured under the two regimes. Roemer et al. identified smoke yields that were, on average, approximately 2.75 times higher under HCI smoking conditions than those under the ISO regime. For a small number of toxicants, the ratio was higher or lower than the average ratio. Data from Eldridge et al. were also used to identify a typical puff number of 8 puffs for 3R4F under ISO smoking conditions.[81]

From the data measured in the present study for all 150 measurands, equivalent emissions under the ISO regime were estimated on a per-puff basis for Ky3R4F using our measured HCI values, the HCI/ISO ratio for each compound from Roemer et al. (where no value was available the mean value of 2.75 was used), and the respective puff numbers for Ky3R4F under HCI and ISO.

2.7. Air/Method Blank Measurements. Tayyarah and Long[26] noted that e-cigarette emissions of some HPHCs approach those of background air. Consequently, the 142 target constituents were measured in the laboratory background at the same time as the e-cigarette emissions. This facilitated an assessment of HPHC background contamination within the air, analytical equipment, and reagents used to measure the e-cigarette emissions, thereby distinguishing between potential background contaminants and actual e-cigarette emissions.

Background air/method blank measurements were performed by collecting blocks of 100 puffs of laboratory air without any e-cigarette present on the puffing machine and by analyzing the puffs with the same equipment and reagents used for the e-cigarette emission analysis. An equivalent control step was not practicable for the tobacco cigarette measurements because cigarettes release relatively high emissions of toxicants into the sidestream (room) air while they burn; therefore, obtaining a simultaneous relevant measure of room air/method contamination is not straightforward.

To minimize the potential for the e-cigarette measurements to be contaminated by background from tobacco cigarette smoking, the e-cigarette emissions were generated by using puffing machines in a separate laboratory from that used for smoking tobacco cigarettes, with segregated air-handling systems. Because Ky3R4F and the e-cigarette were puffed in different rooms with different puffing machines, different air/method background levels might be expected. However, laboratory background is less of a concern for tobacco cigarettes than for e-cigarettes because toxicant levels are significantly higher in tobacco smoke, and the 10-fold fewer puffs taken on tobacco cigarettes might reduce contamination commensurately. The use of air/method background values is discussed in detail below.

3. RESULTS

All of the emissions data for the 150 measurands are presented in Supporting Information Tables S2−S11. The study encompassed all but three HPHCs, as well as compounds previously associated with e-cigarettes. Supporting Information Tables S2−S11 provide separate LODs and LOQs for the analytical methods used for the e-cigarette and air experiments and the LODs and LOQs operating for the cigarette smoke measurement experiments. Different values operate in many cases owing to the substantially different numbers of puffs taken

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on the different test pieces. In all cases, the data for the e-cigarette are presented as two 100-puff blocks, covering puffs 1−100 and puffs 101−200. Air/method blank data are also presented in Supporting Information Tables S2−S11, also in two separate blocks equivalent to puffs 1−100 and 101−200 taken at exactly the same time as the e-cigarette puffs. Tables 1 and 2 summarize the 25 compounds detected at some level in the emissions from the e-cigarette, Table 3 summarizes the compounds showing no significant difference from the air/method blank values, and Table 4 presents the results of the puff volume experiment. The data are discussed by compound group below.

3.1. Oxygen-Containing Toxicants. *3.1.1. Carbon and Nitrogen Oxides (Supporting Information Table S2).* Carbon monoxide was detected in the emissions of ePen (Table 3); this was unexpected because CO is a combustion-generated product and combustion does not occur in e-cigarettes. However, effectively identical levels of CO were detected in the room air/method analyses, indicating that the measured CO is an air/method contaminant rather than an e-cigarette generated emission. The background levels were 98% lower than the levels from Ky3R4F. Other combustion gases, NO and NOx, were not detected in the e-cigarette emissions or room air, but they were present in Ky3R4F mainstream smoke (MSS; ePen levels >99% lower per-puff versus Ky3R4F).

3.1.2. Carbonyl Emissions (Supporting Information Table S3). All of the measured carbonyl emissions from the e-cigarette were considerably lower (98.6−99.9%) than those from Ky3R4F, whether on a per-collection or per-puff basis. Emissions of butyraldehyde, crotonaldehyde, and, in one puff block, propionaldehyde were not detected from the e-cigarette. In contrast, emissions of acetone, 2-butanone, and, in one puff block, propionaldehyde were detected for the e-cigarette, but they were at lower levels than those measured for the air/method blank, suggesting their presence in the e-cigarette emissions may be artifactual rather than due to the e-cigarette (Table 3); this is discussed further in Section 3.5.

Emissions of formaldehyde, acetaldehyde, and acrolein were also quantified in the e-cigarette (Table 1). For formaldehyde and acetaldehyde, however, the levels measured in the air/method blank were approximately one-half of those measured in the e-cigarette aerosol, demonstrating a significant but not exclusive contribution from room air. Acrolein was not detected in the air/method blank samples; therefore, the measured emissions from ePen, albeit much lower than those from Ky3R4F, were generated by the e-cigarette.

3.1.3. Dicarbonyls (Supporting Information Table S4). Glyoxal and methyl glyoxal were quantified in both e-cigarette emissions and in Ky3R4F MSS at levels substantially higher than in the corresponding air/method blanks (Table 1). The emissions from the e-cigarette were 97% lower on a per-puff basis as compared with Ky3R4F.

Diacetyl was detected but not quantifiable in the e-cigarette emissions (Table 2) and was not detected in the air/method blank, but it was quantified at substantially higher levels in Ky3R4F MSS. The levels of diacetyl were 99.9% lower on a per-puff basis in the e-cigarette emissions than in Ky3R4F MSS. The homologue acetyl propionyl was not detected in the e-cigarette emissions or in the air/method blanks; however, it was quantified in Ky3R4F MSS at substantially higher levels (Supporting Information Table S4). The levels in the e-cigarette aerosol were estimated to be at least 99.9% lower on a per-puff basis as compared with those in Ky3R4F smoke. Neither diacetyl nor acetyl propionyl is an ingredient included in Vype products; therefore, their presence in these aerosol measurements is unlikely.

3.1.4. Alcohols and Polyalcohols (Supporting Information Table S4). Menthol was detected at unquantifiable levels in the e-cigarette emissions and in one puff block of the air/method blanks; it was not detected in Ky3R4F emissions (Table 2). Allyl alcohol was quantified at higher levels in emissions from the e-cigarette than in the air/method blank, but its level was >99% lower than Ky3R4F on a per-puff basis (Table 1).

Acetoin was not detected in any of the samples analyzed. Ethylene glycol was detected but not quantifiable in either the e-cigarette emissions or the air/method blank, but it was quantified in Ky3R4F smoke (Table 2). On a per-puff basis, the e-cigarette emissions of ethylene glycol were >98% lower compared with those of Ky3R4F MSS. Diethylene glycol was not detected in ePen emissions or the air/method blank, and it was not quantifiable in Ky3R4F smoke. Both propylene glycol and glycerol were measured at higher per-puff levels in the e-cigarette emissions than in Ky3R4F MSS.

3.1.5. Phenols (Supporting Information Table S3). None of the measured phenols was detected in the e-cigarette aerosol or air/method blanks. In contrast, Ky3R4F generated measurable levels of all phenols except caffeic acid (which was not detected). Consequently, the levels of phenolics were >99% lower on a per-puff basis from the e-cigarette than from the Ky3R4F tobacco cigarette.

3.1.6. Oxygen Heterocycles (Supporting Information Tables S2 and S5). Ethylene oxide, propylene oxide, furan, benzo{*b*}furan, and glycidol were all detected in the MSS from Ky3R4F, but none was detected in the e-cigarette emissions or air/method blanks. Per-puff emissions of these compounds were >99% lower from the e-cigarette than from Ky3R4F.

3.1.7. Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (Supporting Information Table S6). Seven chlorinated dibenzodioxins and 10 chlorinated dibenzofurans were examined in the emissions from the e-cigarette, air/method blanks, and Ky3R4F. Very few of these compounds were observed in these measurements: most were below the LOD for all three matrices. For the e-cigarette, octa-CDD was detected at an unquantifiable level in one puff block, but because the air/method blank also registered unquantifiable levels in the same puff block, we concluded that this signal was a background measurement rather than an e-cigarette emission (Table 3). The emissions from Ky3R4F showed unquantifiable levels for 1,2,3,4,6,7,8-hepta-CDD and a quantifiable level of octa-CDD.

3.2. Hydrocarbons. *3.2.1. Volatile Hydrocarbons (Supporting Information Table S2).* 1,3-Butadiene, isoprene, and the aromatic hydrocarbons benzene and ethylbenzene were not detectable in the e-cigarette emissions or room air, but they were detected in Ky3R4F smoke (ePen levels >99% lower versus Ky3R4F).

Styrene and toluene were quantified in Ky3R4F MSS and in one e-cigarette puff block but not in the other; the levels were >99% lower from the e-cigarette than from Ky3R4F. They were also found in the matching air/method blanks at levels effectively identical to those of the e-cigarette (Table 3). On that basis, we conclude that they are present as analytical background and not as ePen-generated toxicants.

3.2.2. Polycyclic Aromatic Hydrocarbons (Supporting Information Table S7). The e-cigarette aerosol, laboratory air, and Ky3R4F MSS were examined for the possible presence

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Table 4. Emissions of Toxicants under Different Machine Puff Volumes[a]

	Vype ePen blended tobacco regular strength eCaps (100 puffs)								air blank (100 puffs)								modified reagent blanks	
	35 mL, 30 s, 3 s		80 mL, 30 s, 3 s		110 mL, 30 s, 3 s		140 mL, 30 s, 3 s		35 mL, 30 s, 3 s		80 mL, 30 s, 3 s		110 mL, 30 s, 3 s		140 mL, 30 s, 3 s			
aerosol constituent	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Selected Carbonyls																		
formaldehyde, μg	18.9	14.4	17.3	2	14.9	1.7	14.6	1.5	8.61	0.27	13.1	1.7	12.8	1.3	12.5	0.3	8.2	0.25
acetaldehyde, μg	22.1	13.3	14.7	1.7	12.6	0.7	12.7	0.8	10.8	0	8.93	0.18	8.61	0.4	8.81	0.21	11	0.1
acetone, μg	6.95	1.44	6.31	0.22	6.84	1.37	8.01	1.17	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
acrolein, μg	15	13.5	11.2	2.7	6.72	2.42	5.86	2.43	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
propionaldehyde, μg	NQ	NQ	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
crotonaldehyde, μg	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
methyl ethyl ketone, μg	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
butyraldehyde, μg	6.34	0.73	7.68	0.21	8.5	1.43	10.4	2.1	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Semivolatiles																		
pyridine, μg	BDL	BDL	BDL	BDL	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
quinoline, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
styrene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
nitrobenzene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
benzo(b)furan, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Tar, Nicotine, and Carbon Monoxide																		
CO, mg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NA	NA
Volatiles																		
1,3-butadiene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
isoprene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
acrylonitrile, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
benzene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
toluene, μg	NQ	NQ	3.26	0.09	4.22	0.17	NQ	NQ	NQ	NQ	3.19	0.06	3.89	0.1	BDL	BDL	NQ	NQ
ethylbenzene, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
ethylene oxide, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
vinyl chloride, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
propylene oxide, ng	NQ	NQ	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
furan, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
vinyl acetate, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
nitromethane, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Aromatic Amines																		
1-aminonaphthalene, ng	NQ	NQ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NQ	NQ	BDL	BDL	NQ	NQ	BDL	BDL
2-aminonaphthalene, ng	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
3-aminobiphenyl, ng	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	0.042	0.017	BDL	BDL	NQ	NQ	NQ	NQ	NQ	NQ
4-aminobiphenyl, ng	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	BDL	BDL	NQ	NQ	BDL	BDL	BDL	BDL
2,6-dimethylaniline, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
benzidine, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
o-anisidine, ng	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	NQ	NQ	NQ	NQ	BDL	BDL
o-toluidine, ng	0.457	0.225	0.547	0.283	0.62	0.141	0.743	0.085	0.48	0.05	0.361	0.242	0.6	0.312	0.57	0.114	NQ	NQ

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Table 4. continued

aerosol constituent	Vype ePen blended tobacco regular strength eCaps (100 puffs)								air blank (100 puffs)								modified reagent blanks	
	35 mL, 30 s, 3 s		80 mL, 30 s, 3 s		110 mL, 30 s, 3 s		140 mL, 30 s, 3 s		35 mL, 30 s, 3 s		80 mL, 30 s, 3 s		110 mL, 30 s, 3 s		140 mL, 30 s, 3 s			
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Acrylamide and Acetamide																		
acetamide, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
acrylamide, μg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

[a]Abbreviations: BDL, below detection limit; LOD, limit of detection; LOQ, limit of quantification; NA, not applicable; NQ, not quantifiable.

of 16 PAHs. Two PAHs, dibenz[*a*,*h*]pyrene and dibenz[*a*,*l*]-pyrene, were not detected in any sample. Dibenz[*a*,*e*]pyrene was not detected in the e-cigarette emissions or the air/method blank, and it was detected but not quantified in Ky3R4F MSS (e-cigarette levels 93% lower versus Ky3R4F). Eleven PAHs were not detected in the e-cigarette or the air/method blanks, but they were detected in Ky3R4F emissions (e-cigarette levels 94−99.9% lower versus Ky3R4F). Naphthalene and chrysene were quantified at low levels in both puff blocks of the e-cigarette aerosol and were also identified in the air/method blank. For naphthalene, the air/method blank value was almost identical to the e-cigarette emissions value (Table 3), suggesting that it was present in ePen aerosol due to contamination. Chrysene also had a measurable level in one block of the air/method blank, but not both, suggesting some formation in the e-cigarette aerosol (Table 1). However, both naphthalene and chrysene were quantified at much higher levels in Ky3R4F emissions than in the e-cigarette aerosol (ePen levels both >99.7% lower versus Ky3R4F).

3.2.3. Substituted Hydrocarbons (Supporting Information Table S2). Nitromethane, 2-nitropropane, vinyl acetate, and vinyl chloride were detected in Ky3R4F MSS but not in ePen emissions or air/method blanks. Levels from the e-cigarette were >99% lower than those from Ky3R4F. In contrast, nitrobenzene was not detected in any of the samples analyzed.

3.3. Nitrogenous Species. *3.3.1. Volatile Nitrogenous Species (Supporting Information Table S2).* NO, NOx, ammonia, and acrylonitrile were quantified in Ky3R4F MSS, but they were not detected in the e-cigarette emissions (>99% lower versus Ky3R4F) or laboratory air. Hydrazine was not detected in the ePen, air/method blank samples, or Ky3R4F MSS.

3.3.2. Amines, Amides, and Azines (Supporting Information Table S5). Ethyl carbamate was not detected in any of the samples. Acetamide, acrylamide, and quinoline were not detected in the e-cigarette emissions or in the air/method blanks. Pyridine was detected but not quantified in the e-cigarette and laboratory air (Table 3). The comparability of the e-cigarette and air/method blank values indicated that pyridine is a laboratory background contaminant rather than an ePen-generated toxicant. All four compounds were present in the e-cigarette aerosol at levels >99% lower than those from Ky3R4F MSS.

3.3.3. Aromatic and Aliphatic Amines (Supporting Information Table S8). Three aliphatic amines (Glu-P-1, Glu-P-2, and PhIP) were not detected in any sample. Three aromatic amines (1-aminonaphthalene, *o*-anisidine, and 2,6-dimenthylaniline) and five aliphatic amines (IQ, Trp-P-2, AaC, Trp-P-1, and MeAac) were not detected in the e-cigarette aerosol or the air/method blank, but they were detected in Ky3R4F MSS (ePen levels >99% lower versus Ky3R4F).

Three aromatic amines (2-aminonaphthalene, 3-amino-biphenyl, and 4-aminobiphenyl) were detected at comparable levels, but not quantified, in the e-cigarette and air/method blank samples (Table 3). One aromatic amine, *o*-toluidine, had quantifiable levels in the e-cigarette emissions and the air/method blank that were not statistically different. These observations suggest that the source of these compounds in the e-cigarette aerosol is laboratory contamination. The levels observed in ePen aerosol were >99% lower than those in Ky3R4F MSS.

3.3.4. Nicotine and Related Compounds (Supporting Information Table S9). Nicotine yields were higher from the

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e-cigarette than from Ky3R4F on a per puff-block basis, but they were significantly lower on a per-puff basis (Table 1). Nicotine-related impurities might be expected at low levels in e-cigarette emissions[76] because they are permitted impurities in European Pharmacopeia standard nicotine.[77] Anabasine, anatabine, β-nicotyrine, cotinine, myosmine, nicotine-*N*-oxide, and nornicotine are allowed individually at levels up to 0.3%, together with 0.1% of unspecified impurities, as long as the total impurity level does not exceed 0.8%.[28]

Our analysis showed the presence of most of these impurities in the e-cigarette aerosol. The levels of myosmine and cotinine were quantifiable in the e-cigarette emissions but not in the air/method blank samples (Table 1). Anatabine, anabasine, and β-nicotyrine were detected but not quantifiable in the e-cigarette emissions, and they were not found in the air/method blank samples (Table 2). In contrast, nornicotine and nicotine-*N*-oxide were not detected in either the e-cigarette or air/method blank samples. All of these compounds were quantified at much higher levels in the MSS from Ky3R4F: the per-puff emissions from the e-cigarette were all at least 97% lower than those from Ky3R4F. These observations confirm that some impurities in pharmaceutical-grade nicotine may be present in e-cigarette emissions; nonetheless, the levels of these nicotine-related impurities were significantly lower than those in Ky3R4F cigarette smoke.

3.3.5. Nitrosamines (Supporting Information Table S10). The IARC Group 1 carcinogen NNN was quantified in the emissions from the e-cigarette and at lower levels in the air/method blank samples (Table 1). Per-puff emissions from the e-cigarette were 99.8% lower than those from Ky3R4F. The likely source of NNN is an impurity in the Pharmacopoeia-standard nicotine used in the e-cigarette.[78]

The other IARC Group 1 carcinogen NNK was either not detected or not quantifiable in the e-cigarette and air/method blank samples (Table 3): the measurements from the first and second puff blocks were equivalent between the e-cigarette and air/method blank samples; consequently, it is not possible to determine conclusively that the e-cigarette contributed to the measured NNK emissions. E-cigarette emissions of *N*-nitrosoanatabine (NAT) were detected but not quantifiable in one puff block and were not detectable in the second puff block; by contrast, the air/method blanks gave unquantifiable but detectable levels in both puff blocks, suggesting an analytical artifact (Table 3). *N*-Nitrosoanabasine (NAB) was not detectable in either puff block from the e-cigarette or air/method blank. All four tobacco-specific nitrosamines were quantified in Ky3R4F MSS at substantially higher levels: per-puff emissions of NNK, NAT, and NAB were 99.9% lower in the e-cigarette aerosol than for Ky3R4F.

Measurements of volatile nitrosamines (VNAs) provided a comparatively complex picture. Ten VNAs were measured, six of which were not detected in the e-cigarette aerosol or air/method blanks. Three VNAs, *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosodiethanolamine (NDELA), were detected and quantified in emissions from Ky3R4F. NDMA was detected but not quantified in one puff block from the e-cigarette but not in the other block or the corresponding air/method blanks, suggesting possible low-level emissions from the e-cigarette (Table 2). NDELA was quantified in all e-cigarette and air/method blank puff blocks, although the measured values for the e-cigarette and the background levels were not statistically different from each other (Table 3). Similarly, *N*-nitrosodi-*n*-butylamine (NDBA) and NPYR were detected in both puff blocks, but they were quantifiable in only one puff block for both the e-cigarette aerosol and the air/method blanks. For both of these VNAs, levels from the air/method blanks were not significantly different from those from the e-cigarette, suggesting analytical artifacts may be the source of these compounds. Per-puff emissions from the e-cigarette and air/method blanks were substantially lower in comparison to those from Ky3R4F smoke, other than emissions of NDBA and NDELA, which were higher for the per-puff air/method blank measurements and e-cigarette aerosol.

3.4. Metals and Radionuclides (Supporting Information Table S11). Mercury, cadmium, lead, selenium, cobalt, beryllium, tin, uranium-235, uranium-238, and polonium-210 were not detectable in either the e-cigarette aerosol or air/method blank samples. Analysis of Ky3R4F MSS showed that cobalt, beryllium, tin, and uranium isotope emissions were undetectable, and chromium, nickel, and selenium emissions were not quantifiable.

Arsenic was detected but not quantifiable in the e-cigarette and air/method blank samples, but it was quantifiable in Ky3R4F MSS (Table 2). Arsenic emissions from the e-cigarette and air/method blanks were estimated to be 78% lower than those from Ky3R4F. Nickel emissions from the e-cigarette and Ky3R4F were detected but not quantifiable, whereas nickel was not detected in one puff block of the air/method blank sample and not quantifiable in the other (Table 2). This suggests the possible presence of nickel in the e-cigarette emissions, albeit at very low levels.

Zinc, iron, and copper were identified and quantified in all three samples (Table 3). Zinc emissions from the e-cigarette were comparable to those from air/blank samples, which were approximately one-half (on a per-puff basis) of those quantified from Ky3R4F MSS. Iron emissions from the e-cigarette and air/method blank were neither significantly different from each other or from Ky3R4F MSS on a per-puff basis. Similarly, although higher mean levels of copper were found in the e-cigarette emissions than in the air/method blank sample, the very high variance between replicates meant that these differences were not significant at the 95% confidence level and were comparable on a per-puff basis to Ky3R4F MSS.

Chromium emissions were quantified only in the emissions from one e-cigarette puff block; the other puff block, both air/method blanks, and Ky3R4F MSS showed detectable but not quantifiable chromium emissions (Table 1). Notably, the level measured for the first e-cigarette puff block was very close to the LOQ (within 5%), and replicates were variable (coefficient of variance, 60%); therefore, it is difficult to establish whether the measured e-cigarette chromium emissions are significantly different from those of the air/method blank. However, it is also worth noting that all air/method blank replicates were below the LOQ, whereas the levels of 2−4 of the five replicates of the two puff blocks of the e-cigarette were above the LOQ. Therefore, it seems possible that the chromium emission from the e-cigarette may be higher than that from the air/method blank and possibly higher than from Ky3R4F MSS on a per-puff basis. If the e-cigarette emission levels are higher than the air/method blank, then the toxicological significance of this is difficult to establish. Chromium may exist in up to three distinct chemical states, elemental Cr(0) or as Cr(III) and Cr(VI) compounds, with exposure to Cr(VI) posing the greatest toxicological concern. Cr(VI) is highly reactive, and although it is stable in oxidizing environments, it is unstable in

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reducing or redox neutral environments. Recent chromium speciation studies found no evidence for the presence of Cr(VI) in cigarette smoke,[79] but there is no comparable information currently available for e-cigarette aerosols. Consequently, this is an area that requires further investigation to fully understand.

3.5. Contribution and Significance of Air/Method Blank Contaminants to E-Cigarette Emissions. A clear finding emerging from the above data was the importance of conducting air/method blank experiments to understand the presence of some constituents found in e-cigarette emission measurements, reinforcing the conclusions of Tayyarah and Long.[26] To further understand the sources of external contamination in low level e-cigarette constituent measurements, a series of ePen and air/method blank constituent measurements was conducted in which different puff volumes (35, 80, 110, and 140 cm^3) were used at a fixed puff duration of 3 s and a puffing frequency of twice per minute. The analytes measured were the carbonyls, semivolatile compounds, CO, volatile constituents, and aromatic amines.

The premise behind these experiments was to distinguish the possible contributions of laboratory air and other sources of contamination to the measured yields of the e-cigarette toxicants; if laboratory air made a significant contribution to the measured e-cigarette yields, then increasing the puff volume would increase the quantity of any contaminants trapped from the air and hence higher emissions would be seen from the air/method blank and e-cigarette experiments. If, however, other aspects of the methodology and reagents were responsible for the observed air/method blank levels, then the levels would not change with the 4-fold range in air volumes. The possibility of reagent contamination has been raised by Flora et al.,[54] who reported the presence of formaldehyde in the 2,4-dinitrophenylhydrazine analytical reagent used in e-cigarette carbonyl measurements. Hence, an additional experiment was conducted for each constituent measurement in which the reagents were measured on a blank sample in which no puffs were taken, and the results were compared to the air/method blanks and e-cigarette emissions (Table 4).

The CO yield in all of these experiments was below the LOD, in sharp contrast to the value of 4.74 mg/100 puffs observed for both air/method blanks and e-cigarette emissions in the original measurements (Supporting Information Table S2). However, the observations confirm the hypothesis of air contamination as the source of the ePen CO emissions, given the absence of e-cigarette CO emissions when CO is absent in the laboratory air and the presence at identical levels when it is present. The presence and absence of CO in these measurements is most likely due to transient environmental factors.

Similarly, styrene and pyridine yields were below the LOD in all but one (pyridine, 100 mL puff volume experiment) of these experiments, in contrast to the 0.52 and 1.05 μg/100 puffs of styrene measured for e-cigarette and air/method blanks and the not quantifiable levels of pyridine observed in the original experiments. The reagent blanks were also below the LOD. Overall, these observations are consistent with random and variable levels of air contamination as the source of these volatile organic compounds in our original e-cigarette emissions. Similarly, there was considerable consistency in toluene levels between air blanks and e-cigarette emissions; however, the measured emissions did not increase with increasing air volume, and the reagent blank showed the presence of toluene, albeit not at a quantifiable level, raising the possibility of combined air and reagent contamination. Propylene oxide was observed in two of the low volume experiments, at unquantifiable levels; the other four measurements conducted in this study showed below LOD levels, as were the corresponding air/method blank values. Therefore, there is a possibility of low-level propylene oxide emissions, but the lack of consistency in these observations precludes a firm conclusion at this time.

The aromatic amine measurements also showed evidence of both air and reagent contamination. Both 3- and 4-aminobiphenyl showed unquantifiable levels in most e-cigarette emission and air/method blank measurements. The most insightful observations were those of *o*-toluidine, which showed an increase in emissions from the e-cigarette with increasing puff volume, although the relationship was less than uniform. The air/method blank samples were not significantly different from the e-cigarette samples at any volume, and the reagent blank had detectable but not quantifiable levels.

The most complex behavior in these experiments was shown by carbonyls. We focus initially on the emissions of formaldehyde, acetaldehyde, and acrolein because these three compounds are known thermal decomposition products of glycerol,[80] and some commonality in drivers of emission levels from the e-cigarette might be expected. Although some contribution of air/method contamination was found for formaldehyde and acetaldehyde (but not acrolein) in our original experiments, the second experiments reflected a major contribution to the overall yields measured in the e-cigarette aerosol.

In these second experiments, both reagent blank and air/method blank levels of acrolein were below the LOD, confirming that observed acrolein levels are generated by the e-cigarette alone. In contrast, comparable levels of acetaldehyde were found in the reagent blank and air/method blank, and the air blank levels of acetaldehyde did not change with increasing air volume, suggesting that the acetaldehyde contamination in these e-cigarette emission measurements is reagent-based. Lastly, the reagent blank levels of formaldehyde were comparable to those of the lowest volume air/method blank, but the air/method blank formaldehyde emissions increased with increasing puff volume, showing a clear contribution of laboratory air to the measured formaldehyde levels.

The emissions of formaldehyde, acetaldehyde, and acrolein were all greater from the e-cigarette than from the air/method blanks, confirming a contribution from the device. Interestingly, the levels of these three species *decreased* with increasing puff volume. The magnitudes of the reductions were greater for acrolein (3-fold) than for the other two carbonyls. In the case of formaldehyde and acetaldehyde, at the highest puff volumes the contribution of the e-cigarette to the overall emissions was lower than the contributions of laboratory air and reagent blank. Subtraction of the air/method blank values from e-cigarette emissions for all three compounds showed that there was a 3−5-fold reduction in the e-cigarette generated emissions of these three carbonyls for a 4-fold increase in puff volume, implying an inverse quasi-linear correlation. Thermal decomposition of glycerol to form these three carbonyls has been attributed to both the temperature of the coil/wick and the wicking time of the e-liquid prior to aerosol formation.[41] Increasing air flow may serve to reduce coil and wick temperatures through convective cooling. Alternatively, there may be a reduction in aerosol residence time in the higher temperature zones of the e-cigarette. Both mechanisms could,

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in principle, reduce carbonyl generation; this is an area worthy of further investigation.

Of the remaining carbonyls, propionaldehyde showed detectable levels in the e-cigarette aerosol at low puff volumes only, whereas 2-butanone emissions from the e-cigarette, the air/method blank, and reagent blank were all not quantifiable, suggesting a reagent contaminant. One puff volume experiment gave unquantified levels of crotonaldehyde, whereas the other three measurements in the puff volume experiment and the original two measurements were below the LOD. Butyraldehyde, which was below the LOD in the original measurements, showed measurable levels from the e-cigarette at all puff volumes and was either not quantified or below the LOD for all air/method and reagent blank measurements. Similarly, acetone showed higher levels in the e-cigarette emissions than with the air/method blank and reagent blanks, all of which were not quantifiable. Both of these compounds therefore appear to be released from the e-cigarette.

In summary, these experiments signal the need to conduct background air, reagent, and method blank measurements when attempting to quantify e-cigarette emissions. Failure to do so raises the risk of both inaccuracies in the measured emissions and the reporting of false positives.

4. DISCUSSION

4.1. Consistency of Measured Emission Levels with Literature Values. To understand the robustness of our data set, we compared our 3R4F emission measurements with those of Roemer et al.,[58] who provided 3R4F data measured under HCI and ISO smoking conditions. Our comparison of HCI data showed generally good agreement between our data set and that of Roemer et al. (within 30% for the majority of analytes). A small number of analytes showed more substantial differences, such as toluene, cadmium, and acrylonitrile (40−70% higher from those in Roemer et al.), whereas the present study provided higher values for emissions of formaldehyde (39%) and vinyl chloride (60%). There was no consistent bias between the data sets; our study tended to give higher carbonyl values and TSNAs, and the study of Roemer et al. measured higher levels of gaseous, volatile, semivolatiles, and PAH toxicants. We therefore concluded that the measurements provided by this study are a robust measure of the emissions from 3R4F.

We also sought to compare our e-cigarette emission data with literature values. This is a challenging undertaking, as there is little consistency in the experimental conditions used in historic studies. The wide range of puffing parameters used in previous studies is likely to have a particularly strong influence on the magnitude of published emission values.[68] A range of LOD/LOQ values apply in different studies, which prevents accurate comparison of low-level constituents. In addition, many studies do not disclose whether air/method blank values were measured as part of the experimental design. Hence, it is unlikely that quantitative comparisons of emission values across different studies is meaningful. However, some qualitative comparisons can be made with previous studies. The very low, unquantifiable, or undetectable emissions of TSNAs found from the e-cigarette in this study are consistent with the findings of Goniewicz et al.[30] Similarly, low levels of nicotine-related compounds in e-cigarette aerosols is consistent with the findings of Trehy et al.[76] Williams et al. also quantified chromium in e-cigarette aerosols.[35] Laugesen also presented data for chrysene emissions from an e-cigarette.[34] Hence, a number of the observations from this study are consistent with previous data. There are also some differences with previous studies; for example, the current study did not find toluene emissions to be generated by the e-cigarette, as reported previously.[32] Nevertheless, one of the main conclusions of this study, namely, that emission levels of many toxicants from e-cigarettes are several orders of magnitude lower than those from tobacco cigarettes, is entirely consistent with the collective e-cigarette literature on this topic.

4.2. Complexity of E-Cigarette Aerosol in Comparison to Cigarette Smoke. To compare the complexity of the e-cigarette aerosol to mainstream cigarette smoke, the emissions data in Supporting Information Tables S2−S11 were combined with the findings from the puff volume study (Table 4) to establish how many toxicants were undetectable, how many were present due to air/method blank factors, the number generated at unquantifiable levels, and the number of quantifiable toxicants.

One-hundred four chemical measurands were not detected in ePen emissions, and 21 were present due to laboratory background (Table 3). Among the remaining 25 compounds, 9 were present at levels too low to be quantified (Table 2) and therefore 16 compounds were generated by the e-cigarette at quantifiable levels. (Table 1 and Figure 2). Eight of these

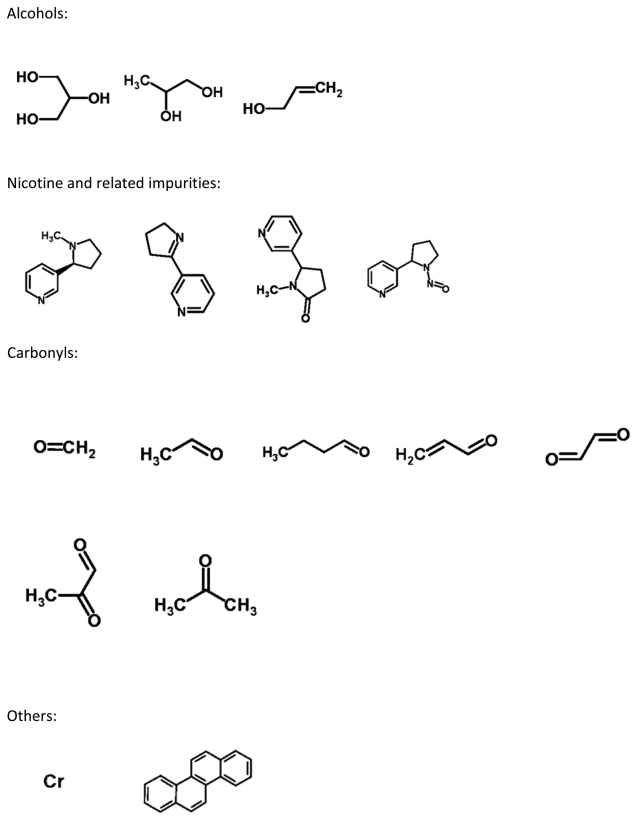

Figure 2. Emissions detected from the e-cigarette at quantifiable levels.

compounds are carbonyls or alcohols that have been linked to thermal decomposition of the aerosol carrier (i.e., PG or VG), three are major e-liquid ingredients, and three are impurities present in pharmaceutical-grade nicotine. One is a metallic element used in the e-cigarette coil, and one (chrysene) is of unknown origin. This is in clear contrast to Ky3R4F MSS, which contained approximately 100 compounds at quantifiable

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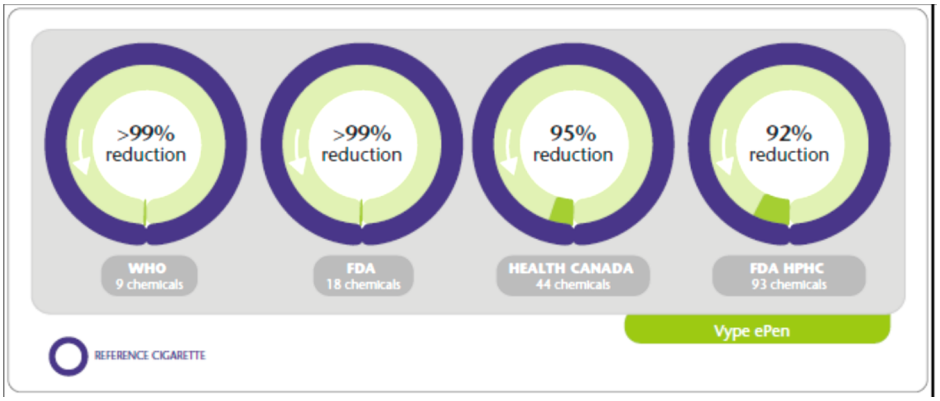

Figure 3. Comparison of percent reduction in e-cigarette emissions in comparison to those from Ky3R4F under HCI puffing conditions.

levels. These data demonstrate the comparative simplicity of the e-cigarette aerosol in comparison to tobacco cigarette smoke.

4.3. Comparison of E-Cigarette Emissions in Different Puff Blocks. Some authors have reported higher e-cigarette carbonyl yields from later puff blocks.[40] The current study design examined two separate puff blocks of 100 puffs each in an attempt to provide an overview of the emissions from the e-cigarette aerosol during the normal usage of a cartomizer. In terms of future investigations of this kind, it would be of interest to determine whether this is a necessary step or whether a single puff block can be used.

The e-cigarette emission measurements of puffs 1−100 and 101−200 in Table 1 were compared by *t*-test to establish whether any of the compounds showed a preferential bias for either puff block, focusing on the 16 compounds that were quantified in the e-cigarette aerosol at higher levels than in the corresponding air/method blank. None of the measurable constituents differed significantly between the puff blocks (*t*-test, significance criterion of $p < 0.05$). Consequently, we conclude that it is necessary to measure only one puff block when conducting e-cigarette emission measurements of this kind.

4.4. Comparative Emissions from ePen and Ky3R4F. This study has also shown substantial differences between the magnitudes of e-cigarette and Ky3R4F emissions. To summarize these differences, we grouped together the percentage differences between the e-cigarette and Ky3R4F per-puff emissions, organized by four toxicant lists: the nine WHO TobReg constituents proposed for mandated lowering in cigarette smoke,[17] the 18 constituents on the FDA abbreviated HPHC reporting list,[12] the Health Canada list of 44 tobacco smoke toxicants,[10] and the full FDA list of 96 HPHCs (other than the three species for which no analytical method was available). In conducting our comparison, ePen emissions were used "as-is", without subtraction of air/method blank values.

Figure 3 shows these comparisons between cigarette smoke and e-cigarette aerosol. In each case, the estimated exposure to the toxicant set was substantially lower for ePen than for Ky3R4F, with the reduction ranging between 92 and >99% depending on the toxicant list chosen.

As noted above, use of the HCI smoking regime provides higher emission levels than would occur if the ISO regime were used, and comparisons between Ky3R4F and e-cigarette yields would potentially differ under the different smoking regimes. The ISO regime is regarded by many as an underestimate of human exposure, and the HCI regime as more representative.[60] Nevertheless, to understand the impact on Ky3R4F emission levels, and our comparisons with ePen, of using less intense smoking parameters, we estimated the emissions that would be generated for the 150 measurands under ISO conditions. Our analysis showed that most estimated per-puff emission levels from Ky3R4F under ISO puffing were approximately one-half those measured under HCI. The percent reduction in emissions from the estimated Ky3R4F ISO values to those of the e-cigarette were slightly smaller (but similar overall) than those found using the measured Ky3R4F HCI data; for the WHO 9 and FDA 18 chemicals, e-Pen emissions were 99% lower than from the tobacco cigarette, 90% lower with the Health Canada list toxicants, and 82% lower for the FDA 93 HPHC.

These data demonstrate that, in addition to its simpler composition, the e-cigarette aerosol contains substantially lower levels of regulatory interest toxicants as compared with tobacco cigarette smoke. However, it is important to note that, although significantly lower than those from a tobacco cigarette, e-cigarette emissions may still represent a toxicologically significant dose. Quantitative risk assessment approaches and clinical and population studies may help to better understand this issue.

4.5. Sources of Background Toxicant Levels. Our study has demonstrated the importance of conducting air/method/reagent blank measurements when seeking to either establish the presence of a compound in an e-cigarette aerosol or to quantify it. We have presented evidence for low-level contamination from both laboratory air and analytical reagents. The presence of volatile organic species in occupational and residential room air is a well-recognized phenomenon, with indoor air quality standards established for a number of species. In addition, the presence of an analyte in the laboratory reagent used to analyze for it, such as carbonyls in 2.4-dinitrophenylhydrazine, is a well-recognized phenomenon.[54]

In our study, a number of steps were taken to minimize the potential sources of contamination. For example, separate laboratory rooms were used for the e-cigarettes and tobacco cigarettes when generating and collecting the aerosols. This arrangement was designed to minimize cross-contamination from tobacco cigarettes to e-cigarettes, particularly from the cigarette sidestream plume. The room used to generate e-cigarettes had historically been used for cigarette smoke generation, but it had been thoroughly cleaned and tested prior to use with e-cigarette analysis.

The puffing machines are also a likely source of contamination during metals analysis (data not shown). The

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puffing machines used in this study for e-cigarette aerosol generation were a mixture of machines that had never been used for cigarette smoking, and machines that had been repurposed from cigarette smoking to e-cigarette puffing. The latter puffing machines were thoroughly cleaned prior to use with e-cigarettes. Both e-cigarette analysis laboratories and puffing engines had been used exclusively for e-cigarettes for an extended period of time prior to this study.

One factor contributing to the measured contamination in e-cigarette aerosols is the number of puffs taken with the e-cigarettes in this study (200 puffs) in comparison to the number taken with tobacco cigarettes (10−11 puffs). With the e-cigarettes, approximately 20 times more laboratory air was drawn through the sampling system, corresponding to 11 L of air in comparison to 0.5 L with the tobacco cigarettes during the main study experiment. This represents a significant potential source of contamination in the e-cigarette aerosol measurement. A second factor is the low level of most e-cigarette emissions, with many compounds present at less than 1% of the levels in a cigarette; in comparison to these low levels, even trace level air contamination may appear significant.

In summary, despite efforts to minimize contamination in our study, we saw significant contributions to our data. We therefore recommend that laboratory background measurements are taken at the same time as e-cigarette aerosol emissions testing to contextualize and account for experimental artifacts.

5. CONCLUSIONS

We have reported an extensive study of toxicant emissions from an e-cigarette. Our data provide the broadest comparison available of e-cigarette and MSS smoke emissions. By covering all of the existing proposed and established tobacco cigarette toxicant lists identified by regulatory authorities and their advisory groups, as well as other compounds reported to be present in e-cigarette aerosols, we provide the most complete comparison of toxicant emissions possible with current scientific capability in this area. The study has demonstrated the relative chemical simplicity of the e-cigarette aerosol in comparison to that from a tobacco cigarette and also shown how levels of cigarette smoke HPHCs are, on average, between 82 and >99% lower per-puff from an e-cigarette than from tobacco cigarette smoke. These findings are an example of what can be achieved in the design of an e-cigarette product if extensive duty-of-care work is conducted to identify and use device parameters and ingredients that offer as little potential for toxicant generation as is possible. On a wider level, these measurements provide additional support to views that e-cigarettes may represent a less harmful alternative to tobacco cigarette smoking, although the presence of toxicants in e-cigarette aerosols means that their use is unlikely to be risk-free. Further studies—in particular, preclinical *in vitro* studies, clinical biomarker studies, and population studies—are necessary to test this hypothesis. However, the findings of the present study are an encouraging starting point for future research.

The study also provides key insights into how to conduct chemical measurements of this kind. In particular, we have demonstrated the necessity of both conducting air/method blank measurements and managing the chemical background of the testing environment in order to minimize the presence of contaminants and errors in the analytical data. This is a fundamental finding that must be considered by future researchers in this area.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.6b00188.

Description of Tables S1−S11 (PDF)
Methodology and experimental results (XLSX)

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■ ABBREVIATIONS

ISO, International Organization for Standardization; MSS, mainstream smoke; NAB, *N*-nitrosoanabasine; NAT, *N*-nitrosoanatabine; NDBA, *N*-nitrosodi-*n*-butylamine; NDELA, *N*-nitrosodiethanolamine; NDMA, *N*-nitrosodimethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NPYR, *N*-nitrosopyrrolidine; PAH, polycyclic aromatic hydrocarbon; VNA, volatile nitrosamine

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