Original investigation

Real-Time Measurement of Electronic Cigarette Aerosol Size Distribution and Metals Content Analysis

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Abstract

Introduction: Electronic cigarette (e-cigarette) use is increasing worldwide and is highest among both daily and nondaily smokers. E-cigarettes are perceived as a healthier alternative to combustible tobacco products, but their health risk factors have not yet been established, and one of them is lack of data on aerosol size generated by e-cigarettes.

Methods: We applied a real-time, high-resolution aerosol differential mobility spectrometer to monitor the evolution of aerosol size and concentration during puff development. Particles generated by e-cigarettes were immediately delivered for analysis with minimal dilution and therefore with minimal sample distortion, which is critically important given the highly dynamic aerosol/vapor mixture inherent to e-cigarette emissions.

Results: E-cigarette aerosols normally exhibit a bimodal particle size distribution: nanoparticles (11–25 nm count median diameter) and submicron particles (96–175 nm count median diameter). Each mode has comparable number concentrations (10^7 – 10^8 particles/cm³). “Dry puff” tests conducted with no e-cigarette liquid (e-liquid) present in the e-cigarette tank demonstrated that under these conditions only nanoparticles were generated. Analysis of the bulk aerosol collected on the filter showed that e-cigarette emissions contained a variety of metals.

Conclusions: E-cigarette aerosol size distribution is different from that of combustible tobacco smoke. E-cigarettes generate high concentrations of nanoparticles and their chemical content requires further investigation. Despite the small mass of nanoparticles, their toxicological impact could be significant. Toxic chemicals that are attached to the small nanoparticles may have greater adverse health effects than when attached to larger submicron particles.

Implications: The e-cigarette aerosol size distribution is different from that of combustible tobacco smoke and typically exhibits a bimodal behavior with comparable number concentrations of nanoparticles and submicron particles. While vaping the e-cigarette, along with submicron particles the user is also inhaling nano-aerosol that consists of nanoparticles with attached chemicals that has not been fully investigated. The presence of high concentrations of nanoparticles requires nano-toxicological consideration in order to assess the potential health impact of e-cigarettes. The toxicological impact of inhaled nanoparticles could be significant, though not necessarily similar to the biomarkers typical of combustible tobacco smoke.

Introduction

Electronic cigarettes (e-cigarettes) are perceived as a healthier alternative to combustible tobacco products, with the potential to aid users in quitting or reducing cigarette consumption.^{1–3} Lower biomarker levels from toxicants associated with tobacco-related disease were observed in e-cigarette users.⁴ From a technical perspective, the perception of the relative safety of e-cigarettes is based on their different mechanism of aerosol/vapor formation, compared with traditional combustible tobacco products. It is assumed that e-cigarettes do not generate many of the harmful and potentially harmful constituents (HPHCs⁵) inherent to combustible tobacco smoke because unlike the traditional cigarette that burns the tobacco leaf the e-cigarette heats the vegetable glycerine, propylene glycol, and other components of e-liquids at a lower temperature. This assumption seems reasonable, but published results show the presence of at least some HPHCs, including carbonyls and metals, in e-cigarette aerosol/vapor.^{6–10} The concentrations of carbonyls (formaldehyde, acetaldehyde, acetone, and acrolein) generated by e-cigarettes depend upon the conditions of usage, such as heating power and puff duration,^{6,11} and overall, the data reveal large variations in carbonyl levels.⁹ Analyses of e-liquids show the presence of high concentrations of tobacco alkaloids, a variety of flavoring agents, common plasticizers (diethyl phthalate and diethylhexyl phthalate), tri-, tetra-, and penta-ethylene glycol, and ethylene glycol.^{12–14} The flavoring additives used in e-liquids were not originally developed for inhalation or tested for toxicity via this exposure route, and the impact of heating on chemical composition is unknown. Additionally, the influence of high temperature and resulting chemical reactions of e-liquid components that are in direct contact with the heated wire have not been investigated.

The presence of chromium, nickel, and lead (HPHCs⁵), as well as tin, silver, and aluminum, was reported in e-cigarette aerosols, including metal nanoparticles.¹⁰ Lead and chromium concentrations were within the range of conventional cigarettes, whereas nickel was about 2–100 times higher in e-cigarette aerosol than in Marlboro brand cigarette smoke.¹⁰ Inhaled lead causes adverse health effects and elemental chromium is a respiratory irritant, while the hexavalent chromium that could be formed during high-temperature oxidation is a known human carcinogen.^{15–17}

The chemical composition of e-cigarette emissions is an important, but not the only factor that contributes to the potential health impacts. Aerosol size is a critical parameter that defines the delivery of toxicants to the human respiratory system determining both delivery and deposition efficiency of each region of the respiratory tract.¹⁸ Analysis of literature shows that information on e-cigarette aerosol particle size is sparse and insufficient to draw definitive conclusions. Due to the presence of relatively volatile compounds (propylene glycol, glycerol, and water), the e-cigarette aerosol/vapor mixture is a highly dynamic system that, under different conditions, could result in either rapid particle growth or evaporation. Freshly formed combustible tobacco smoke requires high dilution before measuring aerosol particle size because the high concentration of particles leads to coagulation and changes in the size distribution.¹⁹ Particle concentration in e-cigarettes is smaller^{20,21} than in combustible cigarette smoke, thus e-cigarette aerosol coagulates at a slower rate. Also, unlike combustible tobacco smoke particles, which consist mostly of semi-volatile and low-volatile compounds, e-cigarette aerosol at high dilution evaporates quickly.²⁰ Therefore, in order to obtain an undistorted picture of aerosol size distribution, low

dilution is required, along with quick sample delivery and real-time measurement.

E-cigarette aerosol size was measured using different experimental methods.^{20–26} Real-time instruments were used including two-step dilution methods^{20,24–26} that likely change the aerosol size. Near real-time measurements that require long sample processing times, and could cause particle transformation while sampling and analysis, were also reported.^{22,23} Spectral extinction²⁰ and optical scattering²¹ methods that are applicable to log-normal particle size distributions, which are not proven for e-cigarette aerosol,²⁷ were used as well. The cascade impactor method (with the lowest cut-point 56 nm) was also recently applied.²⁸ These studies concluded that the particles in e-cigarette aerosols are similar in size to combustible tobacco smoke submicron particles; however, recent measurements that used an advanced real-time technique at low sample dilution showed a high number concentration of nanoparticles generated by e-cigarettes along with a comparable concentration of the submicron particles.²⁷

The presence of nanoparticles in e-cigarette aerosol was previously reported,¹⁰ although real-time analysis of the aerosol size distribution was not conducted. The greater surface area per unit mass of nanoparticles, compared with larger sized particles of the same chemistry, renders nanoparticles more active biologically.²⁹ Therefore, although the mass concentration of the nano-aerosol fraction could be significantly lower than the submicron particles, the toxicological effect of HPHCs and other chemicals attached to the nanoparticles could be high. High concentrations of metal nanoparticles can also be generated using the glowing wire technique,³⁰ which utilizes essentially the same resistively heated wire principle used in e-cigarettes. Although under normal operating conditions the presence of a wick impregnated with e-liquid provides a cooling effect, so-called “dry puffing” conditions,¹¹ the e-cigarette may function as a glowing wire nanoparticle generator.

The main objective of this study was to measure the particle size distribution of aerosols generated by different types of e-cigarettes (“cigalikes” and tank-style) using an advanced real-time technique, machine vaping, and direct sample delivery at low dilution. We also report the metal content of the e-cigarette aerosol and provide a preliminary assessment of nicotine’s influence on aerosol size and concentration.

Materials and Methods

Products Tested

Three brands of cigalike (fixed power, nonrefillable cartridge) e-cigarettes purchased in 2014–2015 were used at the preliminary stage of the study to obtain general understanding on aerosol size distribution measured using our approach: NJOY King (flavor: Bold; nicotine strength: 45 mg/mL or 4.5%, percentage only will be used to show nicotine content from now), V2 (flavors: Red, Menthol, Green Tea, Peppermint, and Sahara; nicotine strength: 0%–2.4%), and blu (flavors: Classic Tobacco, Magnificent Menthol, Cherry Crush, Java Jolt, Peach Schnapps, Pina Colada, and Vivid Vanilla; nicotine strength: 0%–1.6%). After preliminary tests that showed qualitatively similar results across all brands, flavors, and nicotine strengths, only few products were used for the next phase. A tank-style (adjustable power and refillable tank) Joyetech e-cigarette was also tested (flavors: Carolina Crush and Hypermint; nicotine strength: 0%–2.4%). A standard reference 3R4F cigarette was used to compare aerosol size distributions between e-cigarettes and combustible tobacco products.

Aerosol Particle Size Characterization

The Differential Mobility Spectrometer (DMS500; Cambustion Ltd, Cambridge, United Kingdom) is a powerful tool for measuring rapidly changing aerosols generated by e-cigarettes that may quickly evaporate or condense depending on conditions. It is a real-time (0.1 second response time), high-resolution (38 size classes) aerosol spectrometer that is able to measure wide particle size and concentration ranges (from 5 nm to 1 μm , and up to nine orders of magnitude, respectively) at high and low sample dilution.^{18,20,22} The DMS500 is combined with the Smoke Cycle Simulator (Cambustion Ltd), which controls puff profile and provides instantaneous direct sample delivery from the outlet of the product to the aerosol classifier chamber. Due to its rapid response time, the DMS500 can monitor the process of aerosol formation while the puff is developing, with software that defines count median diameter (CMD), particle number concentration (automatically corrected for dilution factor), and geometric standard deviation independently in both nano- and submicron size ranges.

During the preliminary effort, eight square-shaped, 5-second duration puffs were generated using a 1-minute inter-puff interval and three flow rates (15, 20, and 25 mL/s). These parameters are close to real human data topography measured for blu and V2 brands of e-cigarettes.³¹ Both high ($\sim 1/3000$) and low ($\sim 1/30$) sample dilutions were used. Minimum flow rates that demonstrated reliable lighting and stable aerosol generation were defined for blu (15 mL/s) and V2 (20 mL/s) e-cigarettes and used for further testing. The low flow rate allows for better wick saturation by the e-liquid, increases aerosol growth residence time, and therefore should provide more stable aerosol (higher flow rates were not investigated since the influence of the flow rate on e-cigarette aerosol generation was not the focus of this study). In the preliminary phase, only one or two replicates per cigalike brand, flavor, and nicotine strength were tested, and qualitatively similar results were obtained across all the tests.

In the next phase, two brands and few of the popular flavors were randomly selected for the tests described below. Three replicate sets of data per test were collected of two flavors of blu e-cigarettes (Classic Tobacco and Magnificent Menthol) at four nicotine strengths (0%, 0.6%–0.8%, 0.9%–1.2%, and 1.3%–1.6%) and one flavor of V2 (Red) at four nicotine strengths (0%, 0.6%, 1.2%, and 2.4%). A newly opened cartridge and freshly charged battery was used for each test. In the tank-style Joyetech tests, three replicate sets of data per test were collected of two flavors (Hypermint and Carolina Crush) of e-liquid loaded into the device at four nicotine strengths (0%, 0.6%, 1.2%, and 2.4%), using applied voltages of 3.2V for Hypermint and 4.5V for Carolina Crush, with a 1.8- Ω resistance heating element. The same puffing topography was used for the tank-style e-cigarette testing as was used for the blu e-cigarettes: eight 5-second puffs at 15 mL/s flow rate and 60-second inter-puff interval. All tests in this phase were conducted at low ($\sim 1/30$) sample dilution.

Analysis of Metals in E-cigarette Aerosol

The blu e-cigarettes were machine vaped to collect samples for analysis of metal content in the e-cigarette aerosol. A 5-port linear smoking machine (Hawktch, FP2000; Tri-City Machine Works, Chester, VA) applied puffing topography conditions similar to those that were used for aerosol size and concentration measurements: square-shaped puff profile at 17.5 mL/s flow rate, 4.3-second puff duration, and 60-second inter-puff interval. The aerosol from 75 puffs was collected on a quartz fiber filter. Triplicate samples of all the

flavors (Classic Tobacco, Magnificent Menthol, Cherry Crush, Java Jolt, Peach Schnapps, Pina Colada, and Vivid Vanilla) were individually collected at high (1.2%–1.6%) and zero nicotine levels. Total particulate matter (TPM) yield was determined gravimetrically in a temperature- and humidity-controlled balance room. Each filter was recovered and microwave digested in 1:1 nitric acid. Samples were analyzed using an inductively coupled plasma mass spectrometer (Elan DRC-e ICP-MS, Perkin Elmer). Filter method blank samples were handled and digested identically to vaped filter samples.

Results

Aerosol Particle Size and Concentration

In most cases, all the e-cigarettes tested at a low sample dilution exhibited a bimodal particle size distribution. As shown in [Figure 1](#), at the beginning of the puff (during the first 0.3–0.5 seconds), the nanoparticle mode appears with peak number concentration at particle size approximately 10–15 nm, then the submicron size mode starts to grow; both modes then coexist through the rest of the 5-second puff. At high dilution ([Supplementary Figure S-1](#) and [Supplementary Table S-1](#)), nanoparticles fraction increased, but the submicron fraction decreased. This behavior is different from combustible 3R4F cigarette ([Supplementary Figures S-2 and S-3](#) and [Supplementary Table S-2](#)), where at both low and high dilution, most of the particles remain in the submicron range.

[Table 1](#) shows results of measurements with the cigalike and tank-style Joyetech e-cigarettes taken at low dilution. For all e-cigarettes tested (all flavors and nicotine strengths), the particle number concentrations for the 5–50 nm size range were at 10^7 – 10^8 particles/cm³, CMD varied from approximately 11 to 25 nm, and geometric standard deviation was within 1.9. Results for the 50–1000 nm size range particle number concentrations were also at 10^7 – 10^8 particles/cm³, CMD varied from 96 to 175 nm, geometric standard deviation was within 1.76.

“Dry Puffing” Tests

Additional tests were conducted using a Joyetech tank-style e-cigarette (at 4.5V applied voltage and 1.8- Ω heating element resistance) with an empty tank to determine if aerosol was generated by an e-cigarette while operated with no e-liquid. For these tests puffing parameters were the same as previously used (15 mL/s flow rate, 5-second puff duration). Under these conditions only nanoparticles

Figure 1. Aerosol size distribution, blu ClassicTobacco, 5-second puff, 15 mL/s flow rate at low dilution ($\sim 1/30$).

Table 1. Results of Measurements of Electronic Cigarettes Taken at Low Dilution

		5–50 nm			50–1000 nm			Nicotine (%)
		Total N/cc	CMD (nm)	GSD	Total N/cc	CMD (nm)	GSD	
blu Classic Tobacco	Average	6.68E+07	11.72	1.79	4.17E+07	132.33	1.73	Zero
	SD	1.34E+07	0.14	0.09	1.58E+07	12.76	0.02	
	RSD	20.12%	1.21%	5.26%	38.01%	9.64%	1.06%	
	Average	8.68E+07	12.20	1.77	5.59E+07	131.69	1.73	0.6–0.8
	SD	1.24E+07	0.43	0.05	1.40E+07	10.54	0.01	
	RSD	14.26%	3.52%	2.58%	24.99%	8.01%	0.69%	
	Average	7.03E+07	12.96	1.69	3.75E+07	130.43	1.74	0.9–1.2
	SD	2.63E+07	0.35	0.03	1.36E+07	3.98	0.01	
	RSD	37.33%	2.71%	1.96%	36.37%	3.05%	0.37%	
blu Magnificent Menthol	Average	6.72E+07	12.07	1.76	3.97E+07	133.86	1.76	1.3–1.6
	SD	2.62E+07	0.71	0.05	9.90E+06	8.91	0.01	
	RSD	38.94%	5.92%	2.57%	24.95%	6.66%	0.71%	
	Average	5.11E+07	10.98	1.81	2.90E+07	132.94	1.75	Zero
	SD	1.08E+07	0.46	0.06	1.33E+07	4.51	0.03	
	RSD	21.19%	4.23%	3.30%	45.90%	3.39%	1.66%	
	Average	5.93E+07	14.57	1.68	3.82E+07	126.34	1.73	0.6–0.8
	SD	5.00E+06	1.14	0.12	3.08E+07	13.78	0.05	
	RSD	8.43%	7.84%	7.31%	80.67%	10.91%	2.79%	
V2 RED	Average	7.75E+07	16.84	1.74	8.80E+07	124.65	1.67	0.9–1.2
	SD	1.50E+07	3.57	0.10	1.97E+07	13.61	0.02	
	RSD	19.35%	21.18%	5.51%	22.34%	10.92%	1.44%	
	Average	6.75E+07	18.34	1.75	9.51E+07	124.88	1.65	1.3–1.6
	SD	1.02E+07	3.54	0.05	9.20E+06	8.82	0.02	
	RSD	15.16%	19.31%	3.10%	9.68%	7.07%	0.95%	
	Average	1.06E+08	14.58	1.62	5.83E+07	119.03	1.62	Zero
	SD	2.43E+07	1.12	0.07	2.17E+07	4.80	0.02	
	RSD	23.04%	7.65%	4.45%	37.21%	4.03%	1.18%	
Joyetech Carolina Crush, (4.5 V, 1.8 Ω)	Average	1.30E+08	12.57	1.70	5.86E+07	111.10	1.61	0.6
	SD	1.23E+07	0.26	0.01	3.59E+06	2.24	0.01	
	RSD	9.43%	2.06%	0.54%	6.12%	2.02%	0.87%	
	Average	1.34E+08	13.93	1.62	5.82E+07	110.10	1.62	1.2
	SD	2.81E+07	0.22	0.04	1.92E+07	1.94	0.02	
	RSD	20.90%	1.61%	2.39%	32.93%	1.76%	0.96%	
	Average	2.07E+08	21.61	1.50	9.66E+07	96.10	1.58	2.4
	SD	9.98E+06	1.12	0.01	1.65E+07	2.93	0.01	
	RSD	4.82%	5.20%	0.56%	17.03%	3.05%	0.77%	
Joyetech Hypermint (3.2 V, 1.8 Ω)	Average	3.46E+07	16.96	1.69	4.23E+07	143.02	1.70	Zero
	SD	4.52E+06	0.50	0.06	1.47E+07	7.57	0.02	
	RSD	13.05%	2.94%	3.72%	34.67%	5.29%	0.89%	
	Average	2.12E+07	20.43	1.74	4.29E+07	172.00	1.73	0.6
	SD	1.42E+07	6.37	0.20	1.32E+07	40.57	0.02	
	RSD	67.32%	31.16%	11.57%	30.75%	23.59%	0.96%	
	Average	1.68E+07	25.39	1.49	3.74E+07	163.73	1.75	1.2
	SD	2.82E+06	0.88	0.01	7.61E+06	1.56	0.00	
	RSD	16.83%	3.46%	0.74%	20.37%	0.95%	0.27%	
Joyetech Hypermint (3.2 V, 1.8 Ω)	Average	1.61E+07	22.07	1.65	6.33E+07	175.36	1.66	2.4
	SD	5.35E+06	0.75	0.02	1.21E+07	9.45	0.03	
	RSD	33.35%	3.40%	1.42%	19.06%	5.39%	1.53%	
	Average	3.94E+07	15.11	1.82	7.09E+07	156.56	1.68	Zero
	SD	3.33E+06	0.40	0.00	1.27E+07	3.23	0.02	
	RSD	8.45%	2.66%	0.24%	17.86%	2.06%	1.31%	
	Average	4.93E+07	15.23	1.85	7.84E+07	147.72	1.68	0.6
	SD	7.29E+06	0.99	0.04	1.08E+07	1.96	0.02	
	RSD	14.78%	6.49%	2.11%	13.76%	1.33%	1.49%	
Joyetech Hypermint (3.2 V, 1.8 Ω)	Average	4.97E+07	12.88	1.90	6.09E+07	146.02	1.69	1.2
	SD	1.20E+07	1.72	0.03	1.66E+07	11.07	0.03	
	RSD	24.11%	13.37%	1.62%	27.31%	7.58%	1.51%	
	Average	3.99E+07	15.10	1.78	7.17E+07	160.47	1.69	2.4
	SD	5.67E+06	0.15	0.04	1.14E+07	5.32	0.02	
	RSD	14.24%	0.99%	2.31%	15.83%	3.32%	1.32%	

CMD = count median diameter; GSD = geometric standard deviation; RSD = relative standard deviation.

(Figure 2) were generated, with average CMD approximately 7.5 nm and concentration approximately 10^7 particles/cm³.

Metals Analysis

The results of the analysis of the metal content of the aerosol generated by e-cigarettes were blank subtracted, normalized with respect to the mass of TPM collected, and are shown graphically in Figure 3. The SD bars and outlier points in Figure 3 show that arsenic, chromium, nickel, copper, antimony, tin, and zinc levels vary widely across the nicotine- and non-nicotine-containing flavors, with relative standard deviations ranging from 35.2% to 57.0%. The levels of those same metals in the filter method blank samples had an average relative standard deviation of 25% and were mostly at or less than four times the instrument detection limit. The filters themselves (taken from a freshly opened package) showed copper, chromium, and lead contamination at roughly four times the instrument detection limit, and solvent blank levels were less than the instrument detection limit for all metals. Other HPHCs (beryllium, cadmium, cobalt, lead, and selenium) were not detected in any of the e-cigarette aerosol samples.

Discussion

Aerosol Size Distribution and Chemical Composition of Nanoparticles

We applied a real-time, high-resolution technique with a rapid sample delivery (similar to what was used by other researchers,²⁰ but at

Figure 2. Aerosol size distribution, Joyetech (Tank-style), “Dry Puffing” Test, 5-second puff, 15 mL/s flow rate at low dilution (~1/30).

Figure 3. Metal levels in electronic cigarette aerosol.

low sample dilution) and found that the aerosol size distribution generated by e-cigarettes is uniquely different from that of combustible tobacco smoke. E-cigarette aerosols normally exhibit a bimodal size distribution: a high number concentration of nanoparticles and a comparable number concentration of particles in the submicron range.²⁷ Although size-segregated chemical analysis data are not available (these experiments are in progress), a few hypotheses about the chemical content of the particles can be made based on the data presented here. At high dilution (Supplementary Table S-1), the concentration of nanoparticles increased and CMD increased slightly (while the concentration of submicron particles and CMD decreased), and this particle size distribution remained unchanged across the wide range of sample dilution (from 1/3180 to 1/26 500). These results suggest that the nanoparticles consist of less volatile compounds that do not readily evaporate, whereas the submicron particles contain both volatile and less volatile compounds, and can lose their volatile compounds via evaporation at higher sample dilution. The highest concentration of nanoparticles appears at the beginning of the puff, during the first 0.3–0.5 seconds, when evaporation of the e-liquid is not yet fully developed. It is plausible that nanoparticles could form from: (1) direct emissions from the surface of the metal wire, such as compounds that the wire itself contains, (2) compounds that are in direct contact with or near the wire surface, such as wick material and the so-called “gunk,” or dark oily liquid that typically accumulates around the wire after some period of use, and (3) the primary less volatile components of the e-liquid, that is, vegetable glycerine, nicotine, and perhaps flavorings. Elemental analysis (Figure 3) has shown that the sum of the elements detected was approximately 10 ng/mg of TPM, whereas the mass calculation of the nanofraction (derived from the DMS500) assuming density 8 g/cm³ (average between Ni and Cr) yielded approximately 100 ng/mg TPM (average mass of TPM/puff was ~2 mg). From this rough assessment, metals may represent roughly only approximately 10% of the total nanoparticle mass and thus, other low volatile compounds may contribute as well. After the first 0.3–0.5 seconds of puff development, more e-liquid is drawn towards the heating element, evaporates, and forms a plume of submicron particles that should mainly consist of the primary components of the e-liquid. Formation of the submicron particles may occur on the nanoparticles that would serve as the centers of vapor condensation if present in the aerosol formation (nucleation) zone. The nucleation zone is a place where the critical vapor supersaturation is achieved and therefore phase transition (vapor to aerosol) happens.³² In a flow type system (like e-cigarette), nucleation occurs in a very narrow zone.^{32,33} Because the aerosol formation rate is an exponential function of vapor concentration and temperature³², it is very difficult to perform valid calculations, accurately predict conditions of aerosol formation in the e-cigarette, and define the location of the nucleation zone. It is possible that part of the nanoparticles do not pass through the nucleation zone, or there may be not enough vapor to condense on all of the nanoparticles in which case some of the nanoparticles would not grow to submicron size. As a result, the aerosol generated by e-cigarettes has a complicated, typically bimodal particle size distribution that contains both nanoparticles and submicron particles, and this distribution changes during puff development. After the puff is fully developed and a significant fraction of the nanoparticles become encapsulated within the submicron particles, the concentration of nano-aerosol measured by DMS500 decreases. At high dilution e-cigarette aerosol quickly evaporates enhancing concentration of nanoparticles in the air and decreasing submicron fraction.

Metals Analysis Data

Metals were detected in e-cigarette emissions, and the amounts in most cases varied by several orders of magnitude for the same flavor and nicotine content. The large variations in metal levels could be caused by e-cigarette manufacturing inconsistencies, such as varied wire resistance and the material and precision of the application of the solder.¹⁰ Variations in wire resistance and battery voltage could result in different heating temperature. The duration of e-liquid exposure to the high temperature may also vary, because the e-liquid delivery rate to the heated wire may not be well controlled in commercial e-cigarettes.

Influence of Nicotine Content on Aerosol Size Distribution

From the data obtained, it is difficult to draw firm conclusions as to how nicotine concentration influences particle size and concentration. It does not seem that the nicotine level affected aerosol parameters for blu Classic Tobacco (Table 1 and Supplementary Figures S-4 and S-5). At the same time, an increase in nicotine level caused a moderate increase in particle number concentration for blu Magnificent Menthol and for V2 Red (Table 1 and Supplementary Figures S-6 and S-7). Also for these two cigalike e-cigarettes, an increase in nicotine concentration caused a slight increase in the CMD of nanoparticles (Table 1 and Supplementary Figures S-8 and S-9). A similar trend was observed for the tank-style Joyetech e-cigarette data obtained for Carolina Crush (Supplementary Figures S-10 and S-11). Joyetech Hypermint data did not show effect of nicotine on aerosol concentration and size (Table 1 and Supplementary Figures S-12 and S-13).

Potential Influence of Instrumental Artifacts on Accuracy of Measurements

As noted earlier, in order to avoid particle evaporation, tests were conducted at a low sample dilution of approximately 1/30. Additional measurements were taken using the blu e-cigarette (Classic Tobacco, mid-nicotine level) varying the dilution factor from 1/13 to 1/35. Data shows that varying dilution in this range did not change the bimodality of the e-cigarette aerosol (Supplementary Figures S-14 and S-15). Also in the DMS500, a sheath flow is supplied to the sample before it enters the particle classifier column, which results in an additional small dilution factor of approximately 1/2.7. Likely playing a larger role, is the reduced pressure in the column (~250 mbar), which may enhance evaporation of the particles while they are passing through the classifier that contains the electrometers that measure the particle size distribution. Because nanoparticles are measured at the entrance to the classifier column, the reduced pressure mostly affects evaporation of the larger submicron particles, which have a longer residence time while passing through the column. Theoretical calculations of CMD of the submicron particles based on TPM data (~2 mg/puff), using as example parameters measured for blu Classic Tobacco (0.6%–0.8% nicotine), have shown that CMD should be approximately 365 nm rather than approximately 132 nm measured by DMS500. Therefore, the submicron particles size measured by the DMS500 is underestimated, as previously observed by other researchers²¹.

Nanotoxicological Implications

The presence of high concentrations of nanoparticles in e-cigarette aerosol substantially differentiates it from combustible tobacco

smoke. While vaping the e-cigarette, along with submicron size particles that mostly should consist of the main components of e-liquid, the user is also inhaling nano-aerosol that consists of nanoparticles with attached chemicals that has not been fully investigated. Based on the results of this study and limited information available from the literature,¹⁰ we hypothesize that nanoparticles contain metals (and/or metal oxides) and other unknown low volatile chemicals. Our data indicate that at the beginning of each puff, the user inhales nanoparticles only. The same situation may apply if the e-liquid is exhausted from the cartridge or from the tank. If there is not enough liquid in the immediate vicinity of the heated wire, then only nanoparticles can be generated. The toxicological impact of inhaled nanoparticles could be significant, though not necessarily similar to the biomarkers typical of combustible tobacco smoke. Inhaled nanoparticles may deposit efficiently by diffusion in all regions of the respiratory tract.³⁴ Their small size facilitates uptake into cells and transcytosis across epithelial cells into the blood and lymph circulation systems to reach potentially sensitive target sites such as bone marrow, lymph nodes, spleen, and heart.³⁵ Access to the central nervous system and ganglia via translocation along axons and dendrites of neurons has also been observed.³⁶

Conclusions

The particle size distribution of e-cigarette aerosol was measured using advanced real-time instrumentation with high resolution at conditions that provided minimal distortion during the particle sampling and delivery. The typical e-cigarette aerosol size distribution is different from that of combustible tobacco smoke and exhibits a bimodal behavior with comparable number concentrations of nanoparticles and submicron particles. The highest concentration of nanoparticles is observed at the beginning of the puff. Bulk aerosol analysis suggests that metals contribute to nanoparticle formation, but comprehensive particle size segregated chemical analysis is required to obtain full information about the chemical composition of nanoparticles. The presence of high concentrations of nanoparticles requires nanotoxicological consideration in order to assess the potential health impact of e-cigarettes.

Limitations

We used three brands of commercially available cigalike e-cigarettes (NJOY, blu, and V2) and one tank-style e-cigarette (Joyetech). Although most of the cigalike e-cigarettes are designed similarly, some brands may have differences that could result in a different particle size distribution. Tank-style e-cigarettes have more variety in the design of the heating element and physical construction, plus a wider range of voltages, and varied resistance of the heating elements. Therefore, although all of the e-cigarettes we tested typically showed bimodal particle size distribution, to obtain a full picture will require measurements of different brands at different flow rates, puff durations, applied power, and e-liquid content.

Although the DMS500 is a powerful instrument to measure nanoparticles in real time, it underestimates the particle size of the submicron fraction (due to partial evaporation). The upper size limit of the DMS500 could be extended up to 2.5 microns, thus other methods, such as the cascade impactor,²⁸ should be applied for the larger size particles analysis to complement DMS500 measurements.

This study was focused on measurement of particle size and concentration of the aerosol generated by e-cigarettes but not on how the aerosol can be transformed once it enters the human body.

Because environmental conditions (such as humidity and temperature) along with dilution may strongly affect aerosol parameters, this important investigation should be pursued, perhaps in coordination with modelers, to predict how the aerosol will deposit in the human respiratory tract.

Supplementary Material

Supplementary Figures S-1 to S-15 and Tables S-1 and S-2 can be found online at <http://www.ntr.oxfordjournals.org>

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Declaration of Interests

None declared.

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