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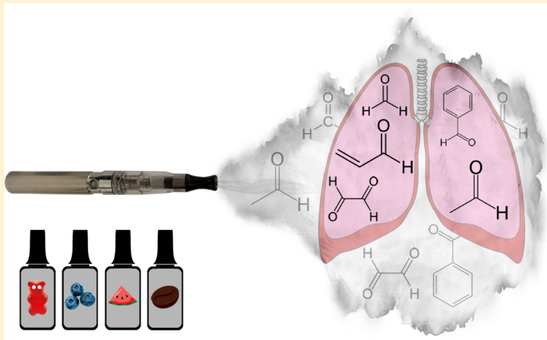
Flavoring Compounds Dominate Toxic Aldehyde Production during E-Cigarette Vaping

Andrey Khlystov* and Vera Samburova

Department of Atmospheric Sciences, Desert Research Institute, Reno, Nevada 89512, United States

Supporting Information

ABSTRACT: The growing popularity of electronic cigarettes (e-cigarettes) raises concerns about the possibility of adverse health effects to primary users and people exposed to e-cigarette vapors. E-Cigarettes offer a very wide variety of flavors, which is one of the main factors that attract new, especially young, users. How flavoring compounds in e-cigarette liquids affect the chemical composition and toxicity of e-cigarette vapors is practically unknown. Although e-cigarettes are marketed as safer alternatives to traditional cigarettes, several studies have demonstrated formation of toxic aldehydes in e-cigarette vapors during vaping. So far, aldehyde formation has been attributed to thermal decomposition of the main components of e-cigarette e-liquids (propylene glycol and glycerol), while the role of flavoring compounds has been ignored. In this study, we have measured several toxic aldehydes produced by three popular brands of e-cigarettes with flavored and unflavored e-liquids. We show that, within the tested e-cigarette brands, thermal decomposition of flavoring compounds dominates formation of aldehydes during vaping, producing levels that exceed occupational safety standards. Production of aldehydes was found to be exponentially dependent on concentration of flavoring compounds. These findings stress the need for a further, thorough investigation of the effect of flavoring compounds on the toxicity of e-cigarettes.



INTRODUCTION

Electronic cigarettes (or e-cigarettes) are battery-operated electronic devices that deliver nicotine or nicotine-free "vapors" to smokers in aerosol form. Since their introduction to the market in 2003, e-cigarettes have been increasing in popularity, especially among the younger population, including school-age children.[1] According to the 2015 report[2] of the National Center for Health Statistics (NCHS), approximately 3.7% adults in the United States use e-cigarettes on a regular basis while 12.6% of adults had tried an e-cigarette. The Adult Tobacco Survey (ATS), prepared by the Centers for Disease Control and Prevention (CDC), reported that the number of adult e-cigarette users doubled between 2010 and 2013,[3] while several studies showed that e-cigarette use is higher among 18−24-year-olds.[3,4] Bunnell et al.[5] reported the number of young e-cigarette users who never smoked before more than tripled (from 79000 to more than 263000) during the period of 2011−2013. According to Singh et al.,[1] in 2015, 25.3% of high school students have regularly used (one or more times per 30 days) any tobacco products (cigarettes, cigars, hookahs, pipes, etc.), with e-cigarettes being the most popular nicotine delivery device (16.0%). A similar pattern was observed among middle school smokers, where e-cigarette user group was dominant, 5.3%.[1] The popularity of e-cigarettes among young people raises serious concerns that e-cigarette usage could cause a future nicotine addiction and facilitate transition to regular cigarettes.

The growing popularity of e-cigarettes could be explained by marketing of these devices as a less harmful or even "healthy" alternative to traditional tobacco products. These claims are based on the assumption that "vapor" produced by "atomization" of e-cigarette liquid (or e-liquid) is harmless, because the e-liquid that is used for vaping is composed mostly of nontoxic components. However, with the exception of ultrasonic brands, e-cigarettes produce vapors using a heating element, which can lead to decomposition of e-liquid constituents. Thermal decomposition does indeed take place, resulting in the production of aldehydes[6−9] and other toxic compounds.[10] Toxic compounds produced by pyrolysis of e-liquid constituents could be the cause of immune and inflammatory response gene suppression in nasal epithelial cells observed in e-cigarette users.[11]

The studies hypothesized that the main source of carbonyl compounds is thermal decomposition of propylene glycol (PG) and/or vegetable glycerin (VG); each serves as a solvent for nicotine and flavoring compounds in e-liquids. Indeed, neat PG and VG were shown to produce aldehydes during vaping, with PG reportedly contributing more to aldehyde production.[6,7] The power and construction of e-cigarettes were also shown to

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have a strong effect on aldehyde emissions.[6,8,9,12] In addition to PG, VG, and nicotine, e-liquids often contain large quantities of flavoring compounds.[13] So far, only two studies have investigated the contribution of flavorings to toxic aldehyde emissions during vaping.[14,15] These studies have investigated direct emission due to evaporation of flavoring compounds, such as benzaldehyde and diacetyl. Thermal decomposition of flavoring compounds and its contribution to the production of aldehydes in e-cigarette vapor have been overlooked so far.

Because the operating temperature of e-cigarettes is sufficient to decompose small molecules, such as PG and VG, it is possible that flavoring compounds could decompose, too. Many flavoring additives are aldehydes,[16] often containing unsaturated bonds. It was demonstrated that thermal decomposition of "chocolate" aldehyde (2-methylbutyraldehyde) leads to formation of formaldehyde, acrolein, and other aldehydes.[17] Another study has shown that unsaturated 2-alkenals and 2,4-alkadienals, while relatively stable in neat form, decompose at 200 °C in the presence of air and/or buffer, producing formaldehyde, acetaldehyde, and other small aldehydes.[18] Flavoring compounds, thus, could be an additional source of toxic aldehydes in e-cigarette emissions, which could explain the recent studies showing that flavorings significantly affect the inhalation toxicity of e-cigarette aerosols.[19,20]

In this study, we have investigated whether flavoring compounds could affect e-cigarette emissions of small, toxic aldehydes, such as formaldehyde, by measuring aldehyde concentrations in aerosols produced by vaping flavored and unflavored liquids.

■ MATERIALS AND METHODS

We have measured concentrations of 12 aldehydes in e-cigarette aerosols produced by flavored and unflavored liquids. To determine the role of flavoring compounds, in each experiment, we fixed all potentially important parameters that could affect aldehyde production (e-cigarette design, power output, and liquid PG/VG ratio)[6−9,12] and varied only the type and concentration of flavors. Under these conditions, any differences in aldehyde emissions could be due only to differences in the type and concentration of e-liquid flavor.

While comparison between e-cigarette brands was not the aim of this study, we have tested three popular brands of e-cigarettes to investigate whether results are not limited to one e-cigarette brand or construction type. The selected e-cigarette brands were chosen to represent the three most common types of e-cigarette "atomizers": bottom and top coil "clearomizers" and a "cartomizer". Two of the brands were single-coil types, while one was a double-coil type. General characteristics of the three types of e-cigarette devices that were tested in this study are listed in Table 1. The brands were chosen on the basis of ease of availability among the most popular brands to represent the three most common types of e-cigarette "atomizers".

Brand I was a double-bottom coil "clearomizer"; brand II was a single-coil "cartomizer", and brand III was a single-top coil "clearomizer". Though brand I offered a possibility to adjust output voltage (and thus power) between 3.2 and 4.8 V, it was operated at 4 V, the lowest common power setting according to the retailer. Brands II and III have a fixed, manufacturer-set power output. Thus, the possibility of overheating e-liquids during vaping that could lead to excessive aldehyde production (the so-called "dry puff") is excluded. Per the manufacturer's instructions, e-cigarettes were kept horizontal during sampling. Cartridges of brand I and III e-cigarettes were sampled with fresh coils, whose resistance was verified to be within the manufacturers' specifications, and filled up to two-thirds of their tank capacity. This was done to avoid wick starvation, which could also lead to "dry puff". Brand II was sampled with fresh manufacturer-prefilled cartridges.

Table 1. List of Tested E-Cigarette Devices

	brand I	brand II	brand III
brand	Kangertech eVod Glass[a]	V2 Standard	E-Cig CE4
type	bottom double coil clearomizer	single coil cartomizer	top single coil clearomizer
voltage (V)	4.0[b]	4.2[c]	3.9[d]
resistance (Ω)	1.5	3.4	3.1
power (W)	10.7	5.2	4.9
PG (%)/VG (%)	60/40	80/20	80/20
[nicotine] (mg mL^{-1})	12	18	12

[a]Used with a SmokTech Winder battery. [b]Voltage used for experiments. [c]Manufacturer-set voltage that cannot be modified by the user. [d]Used with a 1100 mAh eGo-T battery, a manufacturer-set voltage that cannot be modified by the user. Voltage and power are nominal values.

E-Cigarette vapor was produced by 4 s, 40 mL controlled "puffs" with a 30 s resting period between each puff. This protocol was adapted to simulate the most common vaping conditions.[14,21] E-Cigarettes were operated according to instructions from the manufacturer and retailer to mimic the most common vaping conditions. The schematic of the sampling setup is given in Figure 1. E-Cigarettes were operated manually to better represent real-life conditions. The operator manually depressed the e-cigarette power button, simultaneously switching a stainless steel three-way valve to sample position. The sample air was drawn by a pump through a mass flow controller (MassTrak 810C-DR-13-V1-S0, Sierra Instruments Inc., 0−50 sccm flow range, 810 ms response time constant) at a rate of 10 mL s^{-1}. The stability of the sample flow was monitored using the mass flow controller display and was checked before and after each experiment using a Gillibrator (Sensodyne, LP). After 4 s, on a signal from an electronic timer, the power button was released and the valve switched to the flush position, during which time the sampling

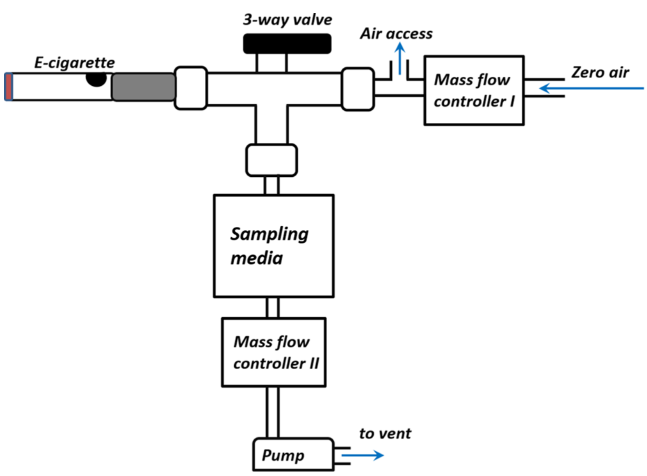

Figure 1. Schematics of the sampling system for e-cigarette emissions. The three-way valve was heated to 40 °C to prevent deposition and/or condensation of gaseous species.

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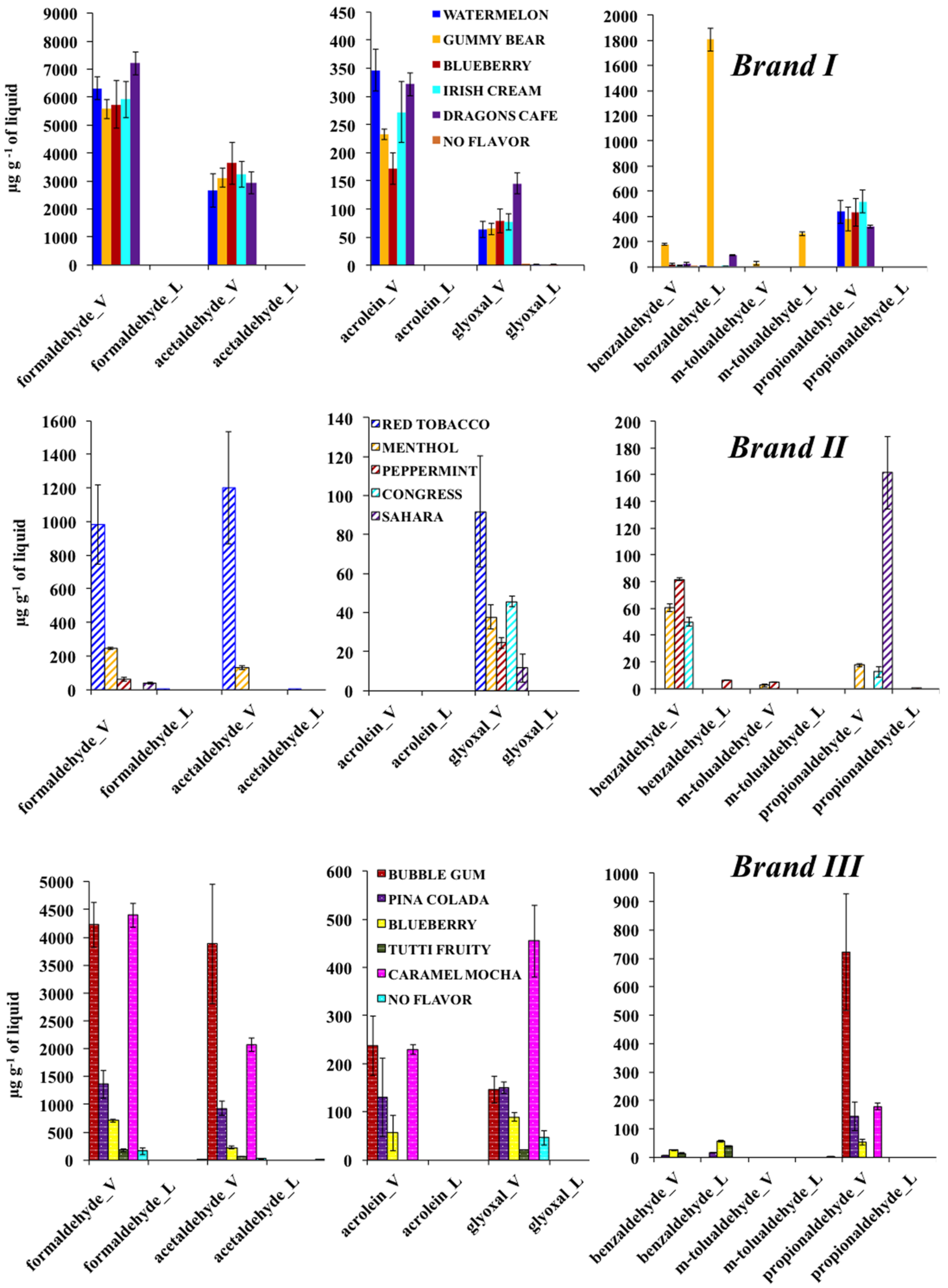

Figure 2. Amounts of aldehydes produced per gram of e-liquid. Error bars represent one standard deviation of triplicate measurements ($N = 3$). "V" designates "vapor" (aerosol), and "L" designates liquid.

line was flushed with zero air. All parts of the sampling system were made of stainless steel and were heated to 40 °C to minimize wall losses.

After 15 warm-up puffs, which are necessary to bring e-cigarette output to steady state,[9] two puffs were sampled directly into 2,4-dinitrophenylhydrazine (DNPH) cartridges (Sep-Pak DNPH-Silica Short Body Cartridges, part WAT047205, Waters, Milford, MA) using the sampling setup presented in Figure 1. All samples were collected in triplicate; i.e., three DNPH cartridges were collected for each liquid. To verify the collection efficiency of DNPH cartridges, several tests were carried out with two cartridges in series. No aldehydes were detected in the second cartridge, indicating quantitative collection of aldehydes. Blank measurements were performed before and after experiments and showed no presence of aldehyde.

Because some aldehydes measured in this study, such as benzaldehyde, could be found as flavoring compounds in liquids and not produced during vaping, we have tested the aldehyde content of liquids. An aliquot (100 μL) of e-liquid was directly run through a DNPH cartridge, which was then extracted in a manner similar to that used for cartridges collected during vaping. Using DNPH cartridges to collect aldehydes from liquids has been reported elsewhere.[12]

Sampled cartridges were eluted with 2 mL of acetonitrile [high-performance liquid chromatography (HPLC) grade,

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EMD Millipore Corp., Billerica, MA] within a few hours of sampling and analyzed with a HPLC system (Waters 2690 Alliance System with a model 996 photodiode array detector) equipped with a Polaris column (C18-A, 3 μm, 100 mm × 2.0 mm HPLC column, Agilent). The following HPLC parameters were used: flow rate of 0.2 mL min^{-1}, injection volume of 2 μL, solvent A of ultrapure water, and solvent B of acetonitrile. The HPLC gradient was as follows: 50% A and 50% B for 10 min, 30% A and 70% B for 8 min, and 100% B for 1 min. The run time was 31 min. The photodiode array detector was operated in the range of 210−400 nm. The detection wavelength was set to 360 nm. Full spectrum readings were used to verify the identity of individual compounds by comparing spectra of individual peaks with the spectra of calibration compounds (DNPH−aldehyde adducts). The HPLC response is calibrated in micrograms per milliliter with a certified calibration mixture purchased from AccuStandard Inc. (New Haven, CT) that contains all 12 DNPH species listed in Table S1. Six-point external calibration was run prior to analysis, and one calibration check was run every 24 h. If the response of an individual compound is more than 10% off, the system is recalibrated, which did not occur during this study. Calibration curves for all aldehydes were linear with R^2 values of >0.99. Recovery rates for 12 standard aldehydes were 94.1−109%. The limit of detection for analyzed free (as opposed to DNPH adducts) aldehydes varied between 0.003 and 0.01 μg mL^{-1} (Table S1). Given the elution volume of 2 mL and the total of two puffs collected per cartridge, this translated into minimal detection limits of 0.003−0.01 μg/puff.

To investigate whether flavoring additives affect aldehyde production during vaping, five flavored e-liquids per each device were tested. In addition to flavored e-liquids, brands I and III were tested with unflavored e-liquids provided by the manufacturers. Brand II did not provide unflavored e-liquids. The relative amount of PG and VG in e-liquids was reported to have an effect on aldehyde production.[6,7,12] To control for this variable, e-liquids for each e-cigarette brand had the same PG/VG ratio. No information about the concentration or composition of flavoring compounds was provided by any of the manufacturers.

To determine whether the concentration of flavoring compounds affects aldehyde production, a series of experiments were performed with Brand III using “bubblegum” e-liquid diluted with the unflavored e-liquid of the same manufacturer and the same PG/VG content; 25, 50, and 75% dilutions were tested in addition to undiluted “bubblegum” and the unflavored e-liquids.

All measured aldehyde concentrations were normalized to the amount of e-liquid consumed. For this purpose, the amount of e-liquid per puff was determined by weighing cartridges before and after each experiment and dividing the weight change by the number of puffs made during each experiment. The liquid consumption per puff is reported in Table S2.

■ RESULTS

Figure 2 shows aldehyde concentrations detected in e-liquids and in aerosols (“vapors”) measured in this study. Among the tested brands, brand I produced the most aldehydes per liquid consumed (Figure 2) and per puff (Table S3) while brand II produced the least. There is anecdotal evidence that bottom coil construction is less prone to dry puffs, yet a bottom coil e-cigarette (brand I) produced the most aldehydes among the tested brands. This reflects the effect of power output on aldehyde production reported by other researchers, as brand I was the most powerful of the three tested brands (Table 1).

While a direct comparison with other studies is difficult because of the differences in e-cigarette construction, power setting, and e-liquid composition, amounts of aldehydes per puff observed in this study (Table S3) are in the range of or higher than those reported elsewhere.[8,9,12,15,22] For example, maximal formaldehyde emissions observed in this study are approximately 2−7 times lower than the steady-state emissions measured by Sleiman et al.,[9] who reported values ranging from 13000 to 48200 ng/mg. In terms of emissions per puff, our formaldehyde data [0.12−50 μg/puff (Table S3)] are comparable to values of 0.05−50 μg/puff reported by Gillman et al.[6] and 30−100 μg/puff reported by Sleiman et al.[9] Several earlier studies have reported significantly lower concentrations. Those studies, however, have used no warm-up puffs. As Sleiman et al. have shown,[9] the first few puffs significantly underestimate the actual emissions. This could be a reason for the low concentrations reported in those studies.

With the exception of benzaldehyde and tolualdehyde, common flavoring compounds, aerosols contained significantly more aldehydes per gram of e-liquid consumed than the liquids used to produce these vapors did. None of the flavored liquids contained formaldehyde, acetaldehyde, or acrolein. Aerosols produced by flavored liquids, however, contained large amounts of these toxic aldehydes. This clearly demonstrates that these aldehydes are formed not by evaporation but by chemical breakdown of e-liquid components. This is consistent with several previous studies.[6,7,9]

Remarkably, there is a significant variation in the amount and relative abundance of individual aldehydes in vapors within each brand. It should be kept in mind that for each e-cigarette brand, the e-cigarette coil construction and power are the same; the e-liquid carrier composition (i.e., the PG/VG ratio) was also kept constant within each brand. These parameters could not explain the observed variations. Thus, the observed variations in emissions of individual aldehydes observed within each brand are not due to pyrolysis of carrier e-liquids (PG and VG). The only variable within one e-cigarette brand is the type of e-liquid flavor. This strongly suggests that flavoring compounds contribute to the production of aldehydes during vaping.

A comparison of aldehyde concentrations found in flavored and unflavored vapors shows that, in fact, decomposition of flavoring compounds dominates production of aldehydes during vaping. Unflavored brand I e-liquid produced detectable amounts of only glyoxal (2.53 ± 1.16 μg/g of e-liquid) and benzaldehyde (6.77 ± 1.05 μg/g of e-liquid); 11 other aldehydes were not detected (ND). In contrast, flavored brand I e-liquids produced large amounts of formaldehyde (5570 ± 330 to 7210 ± 410 μg/g of e-liquid), acetaldehyde (2670 ± 600 to 3640 ± 750 μg/g of e-liquid), acrolein (172 ± 27 to 347 ± 37 μg/g of e-liquid), glyoxal (64.2 ± 14.3 to 146 ± 18 μg/g of liquid), propionaldehyde (320 ± 10 to 518 ± 89 μg/g of e-liquid), and benzaldehyde (ND to 176 ± 7 μg/g of e-liquid). Brand III unflavored e-liquid produced formaldehyde (159 ± 54 μg/g of e-liquid), glyoxal (46.0 ± 14.5 μg/g of liquid), and acetaldehyde (26.9 ± 9.49 μg/g of e-liquid). Brand III flavored e-liquids produced formaldehyde (176 ± 18 to 4400 ± 200 μg/g of e-liquid), acetaldehyde (58.4 ± 1.1 to 3880 ± 1080 μg/g of e-liquid), acrolein (ND to 237 ± 61 μg/g of e-liquid), glyoxal (22.0 ± 3.4 to 455 ± 74 μg/g of e-liquid), propionaldehyde (ND to 722 ± 204 μg/g of e-liquid), and

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benzaldehyde (ND to 58.8 ± 3.2 μg/g of e-liquid). Because unflavored e-liquids produced relatively "clean" vapors, the large amounts of aldehydes found in flavored vapors must be due to pyrolysis of flavoring compounds.

It should be noted that our results do not suggest that PG or VG produces no aldehydes, but that flavoring compounds are responsible for the main part of the emitted toxic aldehydes. Nondetects for unflavored liquids reported in this study are likely due to the small number of puffs that we have used in our measurements. By collecting more puffs per measurement, we could have quantified emissions for unflavored liquids. This quantification, however, is of minor consequence, as the flavored liquids produce significantly more aldehydes than unflavored ones do.

To the best of our knowledge, only two studies have reported emissions from both flavored and unflavored liquids. Kosmider et al.[12] measured both flavored commercially available liquids and liquids containing only PG, VG, water, and nicotine. With the exception of butanal, detectable aldehyde concentrations were found only in flavored liquids. Gillman et al.[6] used 48% (w/w) PG and glycerin with 2% nicotine; it is not clear what the remaining 2% consisted of. For an atomizer that was identical to our brand III e-cigarette, but operated at a higher power setting (5.3 W), they reported formaldehyde emissions of 8.5 ± 8.9 μg/puff. Formaldehyde emissions from unflavored liquid measured in our study are 0.64 ± 0.22 μg/puff. Given the very large uncertainty in the data of Gillman et al. and the sample size (six) used in that study, the difference from our data is not statistically significant.

To provide further proof that flavoring compounds, not the carrier e-liquid (PG and/or VG), dominate production of aldehydes during vaping, we have performed a series of experiments in which a flavored brand III e-liquid ("bubblegum") was diluted with different amounts of the unflavored brand III e-liquid. Amounts per puff of formaldehyde, acetaldehyde, acrolein, and propionaldehyde as a function of the volume fraction of the flavored e-liquid are shown in Figure 3. Aldehyde concentrations increase exponentially with the concentration of flavoring compounds. While the reason for the superlinear relationship is not clear, it emphasizes the dominant effect of flavoring compounds on aldehyde concentration in e-cigarette vapors.

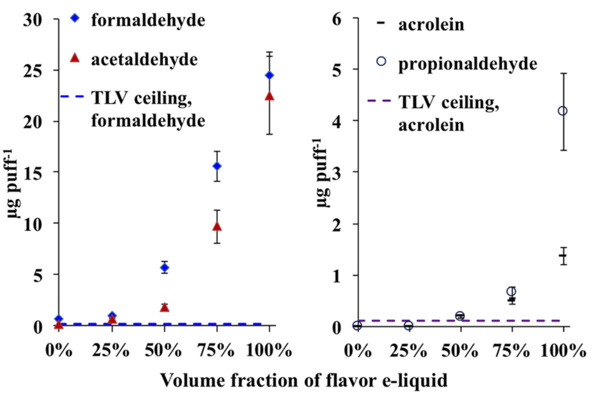

Figure 3. Amounts of formaldehyde, acetaldehyde, acrolein, and propionaldehyde as a function of flavored e-liquid volume fraction after dilution with unflavored e-liquid. Also shown are TLV ceiling levels for formaldehyde and acrolein, assuming each puff is diluted in 500 mL of air (a typical lung tidal volume). Error bars represent one standard deviation of triplicate measurements (N = 3).

It should be stressed that the amount of aldehydes produced by pyrolysis of flavoring compounds is dangerously large. The American Conference of Governmental Industrial Hygienists (ACGIH) establishes Threshold Limit Values (TLVs) for various hazardous chemicals. The ACGIH defines the threshold limit value-ceiling (TLV-C) as the concentration that should not be exceeded during any part of the working exposure,[23] thus representing a limit to instantaneous, not time-averaged, exposure. For formaldehyde, the TLV-C is 0.3 mg m^{-3}, and for acrolein, it is 0.23 mg m^{-3}. To compare exposure to these aldehydes from one puff, we have divided the amount per puff by 500 mL, the average tidal volume of a healthy adult.[24]

All flavored brand I vapors exceeded the ACGIH formaldehyde ceiling level by factors of 190−270 and the acrolein ceiling level by factors of 11−24, depending on the flavor used. Three of five liquids of brand II vapors exceeded the formaldehyde ceiling level by 2.0−13-fold, depending on the e-liquid flavor. No acrolein was detected in brand II vapors. All flavored brand III vapors exceeded the formaldehyde ceiling level by 2.9−66-fold. Four of brand III flavored vapors exceeded the acrolein ceiling by 1.5−6.0-fold, while no acrolein was detected in one of the liquids ("tutti fruity"). In other words, one puff of any of the tested flavored e-cigarette liquids exposes the smoker to unacceptably dangerous levels of these aldehydes, most of which originates from thermal decomposition of flavoring compounds.

In summary, our observations demonstrate that thermal decomposition of flavoring compounds is the main source of aldehydes in vapors produced by e-liquids tested in this study. These results demonstrate the need for a further thorough study of the contribution of flavoring additives to the formation of aldehydes and other toxic compounds in e-cigarette vapors. A study of the thermal behavior of individual flavoring compounds was beyond the scope of this paper and is part of a larger ongoing study, which also includes other decomposition products in addition to aldehydes. The dependence of toxic emissions on flavor concentration in e-liquids is another facet that needs attention. The results of our experiments indicate an exponential dependence of aldehyde emission strength on the concentration of flavoring compounds. For example, by diluting the flavored liquid by a factor of 4 in our experiments, we decreased the acrolein concentration below the TLV-C level (Figure 3). A better understanding of this dependence could offer a way to reduce the toxicity of vapors by controlling concentrations of flavoring compounds in e-liquids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05145.

Information about detection limits per compound, the average liquid consumption for each of the tested flavors, and a table with aldehyde concentrations per puff for each of the e-cigarette brands and e-liquids tested in this study (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: andrey.khlystov@dri.edu. Phone: +1-775-6747084.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Singh, T.; Arrazola, R. A.; Corey, C. G.; Husten, C. G.; Neff, L. J.; Homa, D. M.; King, B. A. Tobacco Use Among Middle and High School Students - United States, 2011−2015. *MMWR-Morbidity and Mortality Weekly Report* **2016**, *65*, 361−367.

(2) Schoenborn, C. A.; Gindi, R. M. Electronic Cigarette Use Among Adults: United States, 2014. Report NCHS Data Brief 217; National Center for Health Statistics: Hyattsville, MD, 2015.

(3) King, B. A.; Patel, R.; Nguyen, K. H.; Dube, S. R. Trends in Awareness and Use of Electronic Cigarettes Among US Adults, 2010−2013. *Nicotine Tob. Res.* **2015**, *17*, 219−227.

(4) Duke, J. C.; Lee, Y. O.; Kim, A. E.; Watson, K. A.; Arnold, K. Y.; Nonnemaker, J. M.; Porter, L. Exposure to Electronic Cigarette Television Advertisements Among Youth and Young Adults. *Pediatrics* **2014**, *134*, e29−e36.

(5) Bunnell, R. E.; Agaku, I. T.; Arrazola, R. A.; Apelberg, B. J.; Caraballo, R. S.; Corey, C. G.; Coleman, B. N.; Dube, S. R.; King, B. A. Intentions to Smoke Cigarettes Among Never-Smoking US Middle and High School Electronic Cigarette Users: National Youth Tobacco Survey, 2011−2013. *Nicotine Tob. Res.* **2015**, *17*, 228−235.

(6) Gillman, I. G.; Kistler, K. A.; Stewart, E. W.; Paolantonio, A. R. Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. *Regul. Toxicol. Pharmacol.* **2016**, *75*, 58−65.

(7) Jensen, R. P.; Luo, W.; Pankow, J. F.; Strongin, R. M.; Peyton, D. H. Hidden Formaldehyde in E-Cigarette Aerosols. *N. Engl. J. Med.* **2015**, *372*, 392−394.

(8) Geiss, O.; Bianchi, I.; Barrero-Moreno, J. Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapours. *Int. J. Hyg. Environ. Health* **2016**, *219*, 268−277.

(9) Sleiman, M.; Logue, J. M.; Montesinos, V. N.; Russell, M. L.; Litter, M. I.; Gundel, L. A.; Destaillats, H. Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. *Environ. Sci. Technol.* **2016**, *50*, 9644−9651.

(10) Fromme, H.; Schober, W. Waterpipes and e-cigarettes: Impact of alternative smoking techniques on indoor air quality and health. *Atmos. Environ.* **2015**, *106*, 429−441.

(11) Martin, E.; Clapp, P. W.; Rebuli, M. E.; Pawlak, E. A.; Glista-Baker, E. E.; Benowitz, N. L.; Fry, R. C.; Jaspers, I. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. *American Journal of Physiology - Lung Cellular and Molecular Physiology* **2016**, *311*, L135−L144.

(12) Kosmider, L.; Sobczak, A.; Fik, M.; Knysak, J.; Zaciera, M.; Kurek, J.; Goniewicz, M. L. Carbonyl Compounds in Electronic Cigarette Vapors: Effects of Nicotine Solvent and Battery Output Voltage. *Nicotine Tob. Res.* **2014**, *16*, 1319−1326.

(13) Tierney, P. A.; Karpinski, C. D.; Brown, J. E.; Luo, W.; Pankow, J. F. Flavour chemicals in electronic cigarette fluids. *Tobacco Control* **2016**, *25*, e10−e15.

(14) Allen, J. G.; Flanigan, S. S.; LeBlanc, M.; Vallarino, J.; MacNaughton, P.; Stewart, J. H.; Christiani, D. C. Flavoring Chemicals in E-Cigarettes: Diacetyl, 2,3-Pentanedione, and Acetoin in a Sample of 51 Products, Including Fruit-, Candy-, and Cocktail-Flavored E-Cigarettes. *Environ. Health Perspect.* **2016**, *124*, 733−739.

(15) Kosmider, L.; Sobczak, A.; Prokopowicz, A.; Kurek, J.; Zaciera, M.; Knysak, J.; Smith, D.; Goniewicz, M. L. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. *Thorax* **2016**, *71*, 376−377.

(16) Fisher, C.; Scott, T. R. *Food Flavours: Biology and Chemistry*; Information Services, Royal Society of Chemistry: London, 1997.

(17) Rosado-Reyes, C. M.; Tsang, W. Thermal Stability of Larger Carbonyl Compounds: 2-Methylbutyraldehyde. *Int. J. Chem. Kinet.* **2014**, *46*, 285−293.

(18) Zamora, R.; Navarro, J.; Aguilar, I.; Hidalgo, F. Lipid-derived aldehyde degradation under thermal conditions. *Food Chem.* **2015**, *174*, 89−96.

(19) Lerner, C.; Sundar, I.; Yao, H.; Gerloff, J.; Ossip, D.; McIntosh, S.; Robinson, R.; Rahman, I. Vapors Produced by Electronic Cigarettes and E-Juices with Flavorings Induce Toxicity, Oxidative Stress, and Inflammatory Response in Lung Epithelial Cells and in Mouse Lung. *PLoS One* **2015**, *10*, e0116732.

(20) Leigh, N.; Lawton, R.; Hershberger, P.; Goniewicz, M. Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). *Tobacco Control* (September 15, 2016).

(21) Dautzenberg, B. Real-Time Characterization of E-Cigarettes Use: The 1 Million Puffs Study. *J. Addict. Res. Ther.* **2015**, *6*, 229.

(22) Goniewicz, M. L.; Knysak, J.; Gawron, M.; Kosmider, L.; Sobczak, A.; Kurek, J.; Prokopowicz, A.; Jablonska-Czapla, M.; Rosik-Dulewska, C.; Havel, C.; Jacob, P.; Benowitz, N. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco Control* **2014**, *23*, 133−139.

(23) ACGIH, TLV Chemical Substances Introduction (http://www.acgih.org/tlv-bei-guidelines/tlv-chemical-substances-introduction), 2016 (accessed August 1, 2016).

(24) Barrett, K. E.; Barman, S. M.; Boitano, S.; Brooks, H. *Ganong's Review of Medical Physiology, Section VII, Pulmonary Function*, 24th ed.; McGraw-Hill: New York, 2012.

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SUPPORTING INFORMATION

Flavoring Compounds Dominate Toxic Aldehyde Production During E-cigarette Vaping

Andrey Khlystov* and Vera Samburova

Desert Research Institute, Department of Atmospheric Sciences,
Desert Research Institute, Reno, NV 89512

*Corresponding author's e-mail: andrey.khlystov@dri.edu
and phone: +1-775-6747084.

3 pages, 3 tables

Supplemental information

Tables

Table S1. Minimum detection limits for HPLC determination of free aldehydes.

Aldehyde	***MDL, μg/mL***
formaldehyde	0.009
acetaldehyde	0.007
acrolein	0.01
propionaldehyde	0.013
crotonaldehyde	0.008
methacrolein	0.008
n-butyraldehyde	0.009
benzaldehyde	0.009
valeraldehyde	0.011
glyoxal	0.003
m-tolualdehyde	0.008
hexaldehyde	0.007

Table S2. Average liquid consumption per puff for e-cigarette brands and e-liquids tested in this study. Liquid consumption within triplicate measurements did not vary by more than 20%.

Brand	***Flavor***	***Liquid consumption, mg/puff***
Brand I	Watermelon	7.85
	Gummy Bear	6.25
	Blueberry	7.627
	Irish Cream	7.027
	Dragon's Cafe	6.38
	No flavor	13.85
Brand II	Peppermint	6.19
	Menthol	4.6
	Congress	5.138
	Sahara	3.06
	Red Tobacco	2.456
Brand III	Bubble Gum	5.785
	Pina Colada	6.115
	Blueberry	5.988
	Tutti Frutti	6.117
	Caramel Mocha	3.319
	No flavor	4.04

Table S3. Concentration of aldehydes (units: µg puff^{-1}) in e-cigarette emissions from three e-cigarette devices, n/a – e-liquid was not available; ND – not detected (below detection limit); each sample was collected and analyzed in triplicates (N=3)

Aldehyde	***Flavors (concentration level in vapor emission)***					
Brand I	***No flavor***	***Watermelon***	***Gummy Bear***	***Blueberry Pomegranate***	***Kahlua & Irish Cream***	***Dragon Café***
formaldehyde	ND	49.5±3.2	34.8±2.1	43.8±6.6	41.57±4.5	46.0±2.6
acetaldehyde	ND	20.9±4.7	19.5±2.1	27.7±5.7	22.79±3.3	18.63±2.5
acrolein	ND	2.72±0.29	1.45±0.06	1.31±0.21	1.91±0.38	2.05±0.13
propionaldehyde	ND	3.44±0.72	2.38±0.59	3.28±0.83	3.64±0.62	2.04±0.08
crotonaldehyde	ND	ND	ND	ND	ND	ND
methacrolein	ND	ND	ND	ND	ND	ND
butyraldehyde	ND	ND	ND	ND	ND	ND
benzaldehyde	0.09±0.01	ND	1.10±0.05	0.15±0.05	0.06±0.02	0.13±0.10
glyoxal	0.04±0.02	0.50±0.11	0.40±0.06	0.60±0.16	0.54±0.10	0.93±0.79
valeraldehyde	ND	ND	ND	ND	ND	ND
m-tolualdehyde	ND	ND	0.15±0.10	ND	ND	ND
hexanaldehyde	ND	ND	ND	ND	ND	ND
Brand II	***No flavor***	***Sahara***	***Red Tobacco***	***Peppermint***	***Menthol***	***Congress***
formaldehyde	n/a	0.12±0.01	2.41±0.58	0.37±0.07	1.14±0.03	ND
acetaldehyde	n/a	ND	2.95±0.82	ND	0.60±0.06	ND
acrolein	n/a	ND	ND	ND	ND	ND
propionaldehyde	n/a	0.038±0.012	0.40±0.07	ND	0.080±0.003	ND
crotonaldehyde	n/a	ND	ND	ND	ND	ND
methacrolein	n/a	ND	ND	ND	ND	ND
butyraldehyde	n/a	ND	ND	ND	ND	ND
benzaldehyde	n/a	ND	ND	0.51±0.01	0.28±0.01	0.26±0.02
glyoxal	n/a	0.035±0.021	0.22±0.07	0.15±0.02	0.17±0.03	0.23±0.04
valeraldehyde	n/a	ND	ND	ND	ND	ND
m-tolualdehyde	n/a	ND	ND	0.030±0.001	0.012±0.005	ND
hexanaldehyde	n/a	ND	ND	ND	ND	ND
Brand III	***No flavor***	***Bubble Gum***	***Pina Colada***	***Blueberry***	***Tutti Fruity***	***Caramel Mocha***
formaldehyde	0.64±0.22	24.4±2.3	8.34±1.54	4.27±0.16	1.08±0.11	14.6±0.7
acetaldehyde	0.11±0.04	22.5±6.2	5.67±0.75	1.35±0.13	0.36±0.01	6.88±0.38
acrolein	ND	1.37±0.35	0.80±0.49	0.34±0.22	ND	0.76±0.03
propionaldehyde	ND	4.18±1.18	0.88±0.31	0.32±0.06	ND	0.59±0.04
crotonaldehyde	ND	ND	ND	ND	ND	ND
methacrolein	ND	ND	ND	ND	ND	ND
butyraldehyde	ND	ND	ND	ND	ND	ND
benzaldehyde	ND	ND	0.036±0.002	0.15±0.01	0.091±0.008	ND
glyoxal	0.19±0.06	0.85±0.16	0.92±0.07	0.54±0.05	0.14±0.02	1.51±0.25
valeraldehyde	ND	ND	ND	ND	ND	ND
m-tolualdehyde	ND	ND	ND	ND	ND	ND
hexanaldehyde	ND	ND	ND	ND	ND	ND