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QUANTITATIVE RESEARCH

Identification of flavouring chemicals and potential toxicants in e-cigarette products in Ontario, Canada

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Abstract

Objectives The current study examined constituents of e-cigarette products on the Canadian market, with a focus on the province of Ontario.

Methods E-cigarettes were systematically purchased at 80 retail outlets across 4 cities in Ontario, Canada, in January–February 2015. Product constituents were identified using gas chromatography and mass spectrometry. Additionally, tobacco-specific nitrosamines (TSNAs) were quantified in tested products using liquid chromatography with tandem mass spectrometry.

Results A total of 166 e-cigarette products were purchased, including disposable products (33%), refillable products (14%), and e-liquids (53%). Overall, e-cigarette products had an average of 6.2 (SD = 3.6) flavouring chemicals. E-cigarettes with sweet flavours (e.g., desserts, alcoholic drinks) had a significantly greater number of flavouring chemicals when compared with tobacco- and menthol-flavoured products ($p < 0.05$). Approximately one fifth (21%) of products contained flavouring chemicals with potential risk of inhalation toxicity (benzyl alcohol, benzaldehyde, vanillin). An additional 8 toxicants (e.g., acrolein, diacetyl) were detected in a total of 14 e-cigarette products. Measurable levels of TSNAs were detected in 70% of tested products.

Conclusion E-cigarettes purchased in Ontario, Canada, contained several constituents that may present excess risk, including some flavouring chemicals and carcinogenic nitrosamines. Further research is needed to determine whether the levels of these constituents have implications for the magnitude of risk to users. The findings reveal several policy gaps that may be addressed by developing regulatory product standards and labelling practices for e-cigarettes.

Résumé

Objectifs Examiner les ingrédients des produits de cigarette électronique en vente sur le marché canadien, en particulier dans la province de l'Ontario.

Méthode Des cigarettes électroniques ont été systématiquement achetées dans 80 points de vente au détail de 4 villes de l'Ontario, au Canada, en janvier-février 2015. Les ingrédients de ces produits ont été identifiés par chromatographie en phase gazeuse et par spectrométrie de masse. De plus, les nitrosamines spécifiques du tabac (NAST) ont été quantifiées par chromatographie en phase liquide avec spectrométrie de masse en tandem dans les produits testés.

Résultats En tout, 166 produits de cigarette électronique ont été achetés, dont des produits jetables (33 %), des produits rechargeables (14 %) et des e-liquides (53 %). Dans l'ensemble, les produits de cigarette électronique contenaient en moyenne 6,2 (écart-type = 3,6) arômes chimiques. Les cigarettes électroniques aux arômes sucrés (desserts, boissons alcoolisées) comptaient un nombre significativement plus important d'arômes chimiques que les produits aromatisés au tabac et au menthol ($p < 0,05$). Environ un cinquième (21 %) des produits contenaient des arômes chimiques comportant un risque potentiel de toxicité par inhalation (alcool benzylique, benzaldéhyde, vanilline). Huit autres substances toxiques (p. ex. acroléine, diacétyle) ont été détectées dans 14 produits de cigarette électronique. Des niveaux mesurables de NAST ont été détectés dans 70 % des produits testés.

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Conclusion Des cigarettes électroniques achetées en Ontario, au Canada, contenaient plusieurs ingrédients pouvant présenter un risque excédentaire, dont des arômes chimiques et des nitrosamines cancérogènes. Il faudrait pousser la recherche pour déterminer si les niveaux de ces ingrédients ont des conséquences sur l'ampleur du risque pour les utilisateurs. Ces constatations mettent au jour plusieurs lacunes dans les politiques, qui pourraient être comblées par l'élaboration de normes réglementaires sur les produits et de pratiques d'étiquetage pour les cigarettes électroniques.

Keywords Electronic nicotine delivery systems · Nitrosamines · Health policy

Mots-clés Dispositifs électroniques d'administration de nicotine · Nitrosamines · Politique de santé

Introduction

Electronic cigarettes (e-cigarettes) are battery-powered devices that deliver nicotine via an aerosol (Breland et al. 2017). E-cigarette solutions typically contain nicotine dissolved in propylene glycol and/or vegetable glycerin, and may contain various additives and flavours (Bertholon et al. 2013). E-cigarettes have been available on the Canadian market since 2007 (Standing Committee on Health 2015). At the time the current study was conducted, the Canadian market was comprised of e-cigarettes with and without nicotine (Hammond et al. 2015), despite a restriction on the sale of nicotine-containing e-cigarettes (Health Canada 2009). As of 2015, 13.2% of Canadian adults had ever tried an e-cigarette, while 3.2% reported use in the past 30 days, and 1.0% reported daily use (Reid et al. 2017). Use of e-cigarettes among Canadians is increasing and is most common among young people and among smokers (Reid et al. 2017).

Although e-cigarettes are likely to be substantially less harmful than combustible cigarettes (National Academies of Sciences 2018), they are not without harm. Nicotine is a pharmacologically active compound with a wide range of health effects. Although relatively benign among adult populations, nicotine has been linked with various adverse health outcomes for the developing fetus, including fetal growth restriction, risk of pre-term delivery, and stillbirth, and may have effects on brain development during adolescence (U.S. Department of Health and Human Services 2014). In addition, evidence suggests that nicotine poses risk of acute toxicity or poisoning from ingestion at high-enough doses; however, estimates for oral fatal doses among adults and youth have not been determined specifically (U.S. Department of Health and Human Services 2014).

Various other e-liquid constituents may pose health risks to e-cigarette users. Inhalation risks of two commonly used nicotine solvents, propylene glycol and vegetable glycerin, are currently not well characterized, despite their approved use for other purposes. For example, propylene glycol is commonly used as an additive in foods and cosmetics, a solvent in pharmaceuticals, an antifreeze, and as a key ingredient in theatrical mist or fog (Bertholon et al. 2013). Studies examining the health effects in theatrical staff exposed to such mist concluded that massive and prolonged exposure results in irritation of the airways (Bertholon et al. 2013). In addition, vegetable glycerin—although widely used in the food and chemical industry as a non-toxic additive—may pose risks as used in e-cigarettes due to the fact that it can generate toxic acrolein at high temperatures (Bertholon et al. 2013). Flavouring agents are also commonly added to e-liquids. Although most are commonly used in foods and indoor fragrances, data regarding the health effects related to their inhalation are limited (Breland et al. 2017; Bertholon et al. 2013). Finally, various contaminants, such as tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), metals, carbonyls, glycols, and aldehydes have been identified in some samples of e-liquids and their associated aerosols, in variable amounts, although typically at levels far below those found in cigarettes (Breland et al. 2017; Bertholon et al. 2013; Fernandez et al. 2015; Goniewicz et al. 2013).

To date, there is little empirical evidence examining constituents of e-cigarettes sold in Canada. While the nicotine content of e-cigarette products sold in Canada has been tested previously (Standing Committee on Health 2015; Czoli et al. 2018), to our knowledge, other constituents have not been examined. This evidence gap is critical to address for consumers, who may be largely unaware of e-cigarette constituents, as well as for regulators, who may use this evidence to inform Canada's new e-cigarette regulatory framework, introduced in May 2018 (Parliament of Canada 2016).

Given that e-cigarette products in other jurisdictions have been found to contain various toxicants (Goniewicz et al. 2013; Hutzler et al. 2014; Lisko et al. 2015; Kavvalakis et al. 2015; Varlet et al. 2015; Behar et al. 2016; Farsalinos et al. 2015a; Tierney et al. 2016; Farsalinos et al. 2015b; Hua et al. 2019), product testing and constituent analysis can provide information about the potential exposure of consumers to chemicals of public health concern, as well as whether exposure varies across markets. For example, it is unclear whether e-cigarette constituents differ in markets with current or past regulatory restrictions on nicotine-containing e-cigarettes, such as Canada (Health Canada 2009), relative to markets

where such restrictions have not applied. To this end, the current study examined constituents of e-cigarette products on the Canadian market, with a focus on the province of Ontario.

Methods

Products

E-cigarette products were purchased using a systematic protocol at retail outlets across four Ontario cities (Toronto, Ottawa, Kitchener-Waterloo, Thunder Bay) in January and February 2015. A total of 80 retail outlets were visited, including five of each of the following types: vape shops, supermarkets, convenience stores, and gas station convenience stores. The study protocol has been described previously (Czoli et al. 2018). Products were classified according to the flavour that appeared on the product label into the following groups: tobacco (e.g., 'Tobacco Fusion', 'Marlboro Blend'); menthol (including mint flavours, e.g., 'Cool Menthol', 'Spearmint'); fruit (e.g., 'Cherry Crush', 'Honeydew'); non-fruit sweets (including desserts, e.g., 'Vanilla', 'Funnel Cake'); drinks (e.g., 'Rum Punch', 'Dark Coffee'); and other flavours (e.g., 'Hypnotic', 'Lumberjack').

Product testing

The purchased products were sent to the Nicotine and Tobacco Product Assessment Core (NicoTAR) at Roswell Park Comprehensive Cancer Center (Buffalo, USA) for testing. After arrival to the laboratory, each product was catalogued and assigned a unique sample number. All samples were stored in their original containers in a dark space at 4 °C prior to analysis, in order to minimize the risk of compound degradation. Aliquotes of 10 μL of each product were collected from each original container using the reverse pipetting technique and transferred to chromatography vials pre-filled with 1 mL of HPLC grade dichloromethane (Fisher Scientific). Analyses were performed using an Agilent 7890B GC with a 5977A MS. The DB-624, 30 m × 0.320 mm × 0.32 mm capillary column with flow rate of helium of 7 mL/min was used. Temperature of injector, mass transfer line, and ion source was 280 °C, column temperature increased from 110 to 250 °C (10 °C/min) with a hold for 1 min. The injection volume was 1 μL with a splitless injection. The full scan examined masses between 30 and 300 amu. Qualitative analyses of the flavoured liquids were carried out using the NIST 14 MS library as well as the FFNSC 3 flavouring library. All samples were run in triplicate.

Tobacco-specific nitrosamines, including N′-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), (S)-N-Nitrosoanatabine (NAT), and (R,S)-N-Nitrosoanabasine (NAB), were measured using the UPLC-MS/MS method. A 250 μL aliquot of each product was transferred using the reverse pipetting technique to 13 × 100-mm glass test tubes. Next, 2.5 mL of 100 mM ammonium acetate and 50 μL working internal standard (100 ng/mL, NNN-d_4, NNK-d_4, NAT-d_4, NAB-d_4) were added, and the samples were vortexed for 20 min. All samples were prepared in triplicate. Analyses were performed using the Waters XevoTM TQ-S with Acquity I-Class UPLC. The sample (2 μL) was injected onto a Waters Acquity UPLC BEH C18 2.1 mm × 100 mm, 1.7 μm column maintained at 40 °C, with a grandient mobile phase flow rate of 0.2 mL/min. The A mobile phase was 10 mM ammonium formate in water, and mobile phase B: methanol containing 0.1% acetic acid; the gradient started at 10% mobile B then up to 100% B at 5 min. The mass spectrometer (MS) was operated in a positive ESI mode, capillary voltage 3.2 kV, desolvation 500 L/Hr, cone 152 L/Hr, nebulizer 3.0 bar, desolvation 550 °C, and source temperature of 120 °C. The mass transitions were as follows: NNN m/z 178.1 → 148.08 and 178.1 → 120.06; NNN-d_4 m/z 182.13 → 152.12; NNK m/z 208.0 → 148.13 and 208.0 → 122.08; NNK-d_4 m/z 212.0 → 126.0; NAT m/z 190.1 → 160.1 and 190.1 → 160.0; NAT-d_4 m/z 194.1 → 164.1; NAB m/z 192.1 → 162.1 and 192.1; NAB-d_4 m/z 196.2 → 166.2. The method was validated as per the International Conference on Harmonization guideline Q2 (International Conference on Harmonization 2005). A calibration curve was generated to cover the range from 0 to 66 ng/mL for each analyte. To ensure accurate results for the samples, each calibration curves had linear coefficients of 0.99 ($R^2 \geq 0.99$) or above. The quantitation limits were 0.05 ng/mL for NNN, NAT, and NAB, and 0.5 ng/mL for NNK. The average analyte recovery rates were as follows: 101.8% (NNN), 101.0% (NNK), 102.9% (NAT), and 103.7% (NAB).

Analysis

Product characteristics and e-cigarette constituents were examined using descriptive statistics. Analysis of variance and chi-square tests were used to examine differences in the number of flavouring chemicals and the presence of TSNAs among e-cigarette products across labelled flavour categories, respectively. Analyses were conducted using SPSS v.24.

Information regarding the health effects of various chemicals was drawn from 'The Good Scents Company Information System' (http://www.thegoodscentscompany.com/) and the US National Institute of Health TOXNET Toxicology Data Network (https://toxnet.nlm.nih.gov/).

Results

A total of 166 e-cigarette products were purchased, including disposable products (33.1%), refillable products (13.9%), and

e-liquids (53.0%) (see Table 1). The vast majority of products (95.2%) had glycerin as a solvent, while 4.8% had a mixture of glycerin and propylene glycol. E-cigarette products had an average of 6.2 (SD = 3.6) flavouring chemicals. The number of flavouring chemicals detected in e-cigarette products varied by labelled product flavour ($F = 2.804$, $p = 0.019$). Post hoc comparisons indicated that non-fruit sweet-flavoured e-cigarette products had a significantly greater number of flavouring chemicals when compared with e-cigarette products that were tobacco-flavoured (mean difference = 3.1, $p = 0.021$) and menthol-flavoured (mean difference = 2.7, $p = 0.048$). In addition, e-cigarettes with drinks flavours had a significantly greater number of flavouring chemicals when compared with e-cigarette products that were tobacco-flavoured (mean difference = 3.3, $p = 0.020$) and menthol-flavoured (mean difference = 2.9, $p = 0.044$). Other flavoured products also had a significantly greater number of flavouring chemicals when compared with tobacco-flavoured products (mean difference = 2.3, $p = 0.014$).

Overall, a total of 119 flavouring chemicals were detected among the sample of e-cigarette products. Flavouring chemicals detected in a minimum of 5% of products are presented in Table 2. Flavouring chemicals with potential risk of inhalation toxicity were detected among e-cigarettes, including benzyl alcohol (19.9%), benzaldehyde (21.7%), and vanillin (21.7%); the inhalation toxicity of the remaining flavouring chemicals has not been determined. Flavouring chemicals detected in a subsample of five cherry-flavoured products produced by different manufacturers are presented in Table 3. Each of these products had a distinct flavour profile, consisting of a different number of flavouring chemicals (ranging from 3 to 8), as well as different combinations of flavouring chemicals.

Additional chemicals with potential inhalation risks were detected among e-cigarettes, albeit at lower frequencies, including 2-acetyl pyrazine, acrolein, cinnamaldehyde, diacetyl, toluene, diacetin, acetone, and isopropyl alcohol (see Table 4). Other pharmacologically active chemicals detected included nicotine N-oxide, myosmine, and caffeine. TSNAs were detected in 70% of tested products ($n = 159$; 7 products were not tested for TSNAs due to insufficient e-liquid volume). Mean concentrations of TSNAs detected among e-cigarette products were as follows: NNN, 2.5 ng/mL (SD = 7.1; range BLOQ to 48.7; $n = 67$); NNK, 4.4 ng/mL (SD = 8.8; range BLOQ to 48.5; $n = 33$); NAT, 3.9 ng/mL (SD = 10.5; range BLOQ to 55.6; $n = 59$); and NAB, 1.3 ng/mL (SD = 3.6; range BLOQ to 18.9; $n = 64$). Products with very high concentrations of TSNAs were mostly e-liquids and commonly tobacco- or menthol-flavoured. Chi-square tests indicated a significant difference in the proportion of e-cigarette products with detectable levels of any TSNAs across labelled flavour groups ($\chi^2 = 12.040$, $p = 0.034$) (data not shown). Specifically, a significantly greater proportion of menthol-flavoured e-cigarettes had TSNAs present as compared with e-cigarettes with fruit flavours ($\chi^2 = 5.774$, $p = 0.016$) and non-fruit sweet flavours ($\chi^2 = 6.812$, $p = 0.009$). In addition, a significantly greater proportion of fruit-flavoured e-cigarettes had detectable levels of TSNAs as compared with e-cigarettes with other flavours

Table 1 Characteristics of tested products, overall and by labelled flavour ($n = 166$)

	Overall ($n = 166$)		Labelled flavour											
			Tobacco ($n = 55$)		Menthol ($n = 36$)		Fruit ($n = 41$)		Non-fruit sweets ($n = 5$)		Drinks ($n = 7$)		Other ($n = 19$)	
	% (n) or Mean (SD) [range]													
City*														
Toronto	40.0	(66)	34.5	(19)	42.9	(15)	41.5	(17)	12.5	(1)	42.9	(3)	57.9	(11)
Ottawa	18.8	(31)	21.8	(12)	14.3	(5)	17.1	(7)	37.5	(3)	28.6	(2)	10.5	(2)
Kitchener-Waterloo	21.8	(36)	21.8	(12)	28.6	(10)	17.1	(7)	25.0	(2)	–	–	26.3	(5)
Thunder Bay	19.4	(32)	21.8	(12)	14.3	(5)	24.4	(10)	25.0	(2)	28.6	(2)	5.3	(1)
Retail outlet type*														
Vape shop	53.3	(88)	56.4	(31)	34.3	(12)	53.7	(22)	62.5	(5)	71.4	(5)	68.4	(13)
Supermarket	13.3	(22)	18.2	(10)	22.9	(8)	7.3	(3)	12.5	(1)	–	–	–	–
Convenience store	27.3	(45)	20.0	(11)	28.6	(10)	36.6	(15)	25.0	(2)	14.3	(1)	31.6	(6)
Gas station convenience store	6.1	(10)	5.5	(3)	14.3	(5)	2.4	(1)	–	–	14.3	(1)	0.0	(0)
Product type														
Cartridge/cartomizer refill	13.9	(23)	16.4	(9)	25.0	(9)	7.3	(3)	12.5	(1)	–	–	5.3	(1)
Disposable	33.1	(55)	30.9	(17)	38.9	(14)	39.0	(16)	37.5	(3)	14.3	(1)	21.1	(4)
E-liquid	53.0	(88)	52.7	(29)	36.1	(13)	53.7	(22)	50.0	(4)	85.7	(6)	73.7	(14)
Solvents detected														
Glycerin (only)	95.2	(158)	98.2	(54)	100.0	(36)	87.8	(36)	87.5	(7)	100.0	(0)	94.7	(18)
Glycerin, propylene glycol	4.8	(8)	1.8	(1)	–	–	12.2	(5)	12.5	(1)	–	–	5.3	(1)
Average number of flavouring chemicals detected	6.2 (3.6) [1 to 21]		5.4 (3.5) [1 to 18]		5.8 (2.7) [1 to 14]		6.1 (2.9) [1 to 13]		8.5 (3.3) [4 to 13]		8.7 (4.7) [3 to 15]		7.7 (5.1) [1 to 21]	

* One (menthol-flavoured) product had missing information for city and retail outlet type of purchase

Table 2 Flavouring chemicals commonly detected in e-cigarette products ($n = 166$)

Chemical name	CAS no.	Odour type	Flavour and odour description	Potential inhalation toxicity (Y/ND)*	Frequency of detection % (*n*)	
1-Methyl naphthalene	90-12-0	Naphthyl	Naphthyl, chemical, medicinal, camphoreous	ND	69.3	(115)
2-Methyl naphthalene	91-57-6	Floral	Sweet, floral, woody, oily, aromatic	ND	62.7	(104)
Isoquinoline	119-65-3	Balsamic	Sweet, balsamic, herbal, almond, bitter almond, anise	ND	41.6	(69)
Menthol	2216-51-5	Mentholic	Peppermint, cooling, mentholic, minty, camphoreous, clean, spicy	ND	24.7	(41)
Ethyl vanillin	121-32-4	Vanilla	Sweet, creamy, vanilla, caramellic, smooth	ND	22.3	(37)
Benzyl alcohol	100-51-6	Floral	Floral, rose, phenolic, balsamic	Y	19.9	(33)
Benzaldehyde	100-52-7	Fruity	Strong, sharp, sweet, bitter, almond, cherry, oily, nutty, woody	Y	21.7	(36)
Vanillin	121-33-5	Vanilla	Sweet, vanilla, creamy, chocolate, creamy, spicy, phenolic, milky	Y	21.7	(36)
Ethyl maltol	4940-11-8	Caramellic	Sweet, caramel, jam, strawberry, cotton candy	ND	18.7	(31)
Terpineol	8000-41-7	Herbal	Fresh, clean, woody, pine, floral, lime	ND	15.7	(26)
Triacetin	102-76-1	Fruity	Clean, tropical fruit, creamy, oily	ND	7.8	(13)
Anisaldehyde	123-11-5	Anisic	Sweet, powdery, mimosa, floral, hawthorn, balsamic, creamy, vanilla, marshmallow	ND	6.6	(11)
Valeric anhydride	2082-59-9	N/A	N/A	ND	6.6	(11)
Gamma-decalactone	706-14-9	Fruity	Fresh, oily, waxy, peach, apricot, coconut, buttery, sweet, fruity, creamy	ND	6.0	(10)
Methyl, 3-hydroxy-hexanoate	21188-58-9	Fruity	Sweet, woody, ripe, fruity, pineapple, tropical, juicy, oily	ND	6.0	(10)
Cyclotene (3-methyl-1, 2-cyclopentanedione)	765-70-8	Caramellic	Sweet, caramel, maple, sugar, coffee, woody	ND	6.0	(10)
Creosol	93-51-6	Spicy	Spice, clove, vanilla, phenolic, medicinal, leathery	ND	6.0	(10)
Trimethylpyrazine	14667-55-1	Nutty	Nutty, musty, earthy, powdery, cocoa, roasted peanut	ND	5.4	(9)
Rheosmin	5471-51-2	Fruity	Sweet, berry, jam, raspberry, ripe, floral	ND	5.4	(9)

CAS, Chemical Abstract Service; *Y*, yes; *ND*, not determined

Chemicals presented are those that were detected in a minimum of 5% of all tested products

* Inhalation toxicity determined using a flavourings database, available at: http://www.thegoodscentscompany.com/search2.html

($\chi^2 = 4.221, p = 0.040$). Finally, a significantly greater proportion of e-cigarettes with other flavours had detectable levels of TSNAs as compared with non-fruit sweet-flavoured products ($\chi^2 = 5.855, p = 0.016$).

Discussion

Our results confirm findings from previous studies showing that e-cigarette liquids consist of a wide variety of flavouring chemicals (Hutzler et al. 2014; Kavvalakis et al. 2015; Tierney et al. 2016; Hua et al. 2019; Bitzer et al. 2018). In particular, commonly detected flavouring chemicals included cyclotene (methyl cyclopentenolone), ethyl maltol, and ethyl vanillin (Hutzler et al. 2014; Kavvalakis et al. 2015; Hua et al. 2019; Bitzer et al. 2018), as well as vanillin, trimethylpyrazine, terpineol, benzaldehyde, anisaldehyde, and benzyl alcohol (Hutzler et al. 2014). The frequency with which benzyl alcohol, benzaldehyde, and vanillin were detected among e-cigarette products warrants attention, given the risk of inhalation toxicity posed by these chemicals.

The flavouring chemicals most commonly detected in the current study included 1-methyl naphthalene, 2-methyl naphthalene, and isoquinoline, differing from previously published studies (Hutzler et al. 2014; Kavvalakis et al. 2015; Tierney et al. 2016; Hua et al. 2019; Bitzer et al. 2018). This difference may stem from the varying flavour profiles of tested e-cigarette products. Alternatively, it may reflect differences in product availability across markets, given that at the time of the analysis, the Canadian e-cigarette market was distinguished from those where previous testing has taken place by the absence of major international e-cigarette brands, such as blu and NJOY in the US (Hammond et al. 2015).

Table 3 Flavour profiles of cherry-flavoured e-cigarette products ($n = 5$)

Chemical name	CAS no.	Presence of flavouring chemical				
		Product A	Product B	Product C	Product D	Product E
1-Methyl naphthalene	90-12-0		✓	✓	✓	✓
2-Methyl naphthalene	91-57-6		✓	✓	✓	✓
Isoquinoline	119-65-3				✓	✓
Menthol	2216-51-5	✓		✓		✓
Ethyl vanillin	121-32-4					✓
Benzaldehyde	100-52-7		✓		✓	
Vanillin	121-33-5				✓	
Ethyl maltol	4940-11-8					✓
Terpineol	8000-41-7	✓				
Triacetin	102-76-1					✓
Anisaldehyde	123-11-5			✓		
Total number of flavouring chemicals		2	3	4	5	8

CAS, Chemical Abstract Service

Chemicals presented are those that were detected in a minimum of 5% of all tested products

Additional flavouring chemicals with potentially adverse health effects previously detected in e-cigarettes include various aldehydes (Hutzler et al. 2014; Varlet et al. 2015; Behar et al. 2016; Farsalinos et al. 2015a; Tierney et al. 2016), and in particular, benzaldehyde in the aerosol from cherry-flavoured products (Kosmider et al. 2016) and cinnamaldehyde in a variety of flavoured e-liquids (Behar et al. 2016). Furthermore, it has been shown that e-cigarette flavouring chemicals can directly impact the formation of free radicals, which may induce oxidative stress at the cellular level (Bitzer et al. 2018). Although these flavouring chemicals, as well as many others found in e-cigarettes, are used as additives in foods and are considered 'safe' for

Table 4 Potentially harmful and pharmacologically active chemicals detected in e-cigarette products ($n = 166$)

Chemical name	CAS no.	Harmful effect	Frequency of detection % (n)	
Potential inhalation chemicals				
2-Acetyl pyrazine	22047-25-2	Potential respiratory irritant, skin irritant, eye irritant	1.8	(3)
Acrolein	107-02-8	Respiratory toxicant, skin toxicant, eye irritant	1.2	(2)
Cinnamaldehyde	14371-10-9	Respiratory irritant, skin irritant, eye irritant	1.2	(2)
Diacetyl	431-03-8	Respiratory irritant, skin irritant; associated with bronchiolitis obliterans, spirometry abnormalities, and respiratory symptoms	1.2	(2)
Toluene	108-88-3	Respiratory irritant, skin irritant, eye irritant	0.6	(1)
Diacetin	25395-31-7	Potential respiratory irritant, skin irritant, eye irritant	0.6	(1)
Acetone	67-64-1	Respiratory irritant, skin irritant	0.6	(1)
Isopropyl alcohol	67-63-0	Respiratory irritant, skin irritant	–	–
Tobacco-specific nitrosamines (TSNAs)				
Any TSNA			69.8	(111)
NNN	16543-55-8	Potential carcinogen	42.1	(67)
NNK	64091-91-4	Carcinogen	20.8	(33)
NAT	71267-22-6	N/A	37.1	(59)
NAB	37620-20-5	N/A	40.3	(64)
Other chemicals				
Nicotine N-oxide	63551-14-4	Tobacco minor alkaloid, oxidation product of nicotine	3.0	(5)
Myosmine	532-12-7	Tobacco minor alkaloid, oxidation product of nicotine	1.2	(2)
Caffeine	58-08-2	N/A	1.2	(2)

CAS, Chemical Abstract Service; *N/A*, not available

ingestion, this does not necessarily mean they are safe for inhalation (Farsalinos et al. 2015a; Barrington-Trimis et al. 2014). Indeed, the potential inhalation toxicity of many flavourings and the long-term health effects of e-cigarette use remain largely unknown (Breland et al. 2017; Bertholon et al. 2013).

Several additional potentially harmful chemicals were detected in our sample of e-cigarette products. For instance, acrolein is a respiratory toxicant, skin toxicant, and eye irritant that has previously been detected in e-cigarette liquids and aerosols (Parliament of Canada 2016; Kavvalakis et al. 2015; Khlystov and Samburova 2016; Farsalinos et al. 2015c). In addition, concerns have been raised regarding the presence of diacetyl in e-cigarettes, given its role in skin and respiratory system irritation, as well as its association with the lung disease bronchiolitis obliterans (Farsalinos et al. 2015a; Allen et al. 2016). Our product testing also demonstrated the presence of the active chemicals myosmine and caffeine, which have been previously detected in e-cigarettes (Lisko et al. 2015; Lisko et al. 2017). Last, product testing revealed the widespread presence of TSNAs in e-liquids, at least one of which was detected in 70% of tested products. However, quantitative analyses indicated that TSNAs were, on average, present only in trace amounts, consistent with previous research (Goniewicz et al. 2013; Farsalinos et al. 2015b). Notably, such levels of TSNAs translate into aerosol emissions and exposures that are many times lower than those of conventional cigarette smoke (Goniewicz et al. 2013; Farsalinos et al. 2015b, c). Further research is needed to delineate the extent to which the presence of TSNAs and minor tobacco alkaloids, such as nicotine N-oxide and myosmine, is the result of contamination from nicotine extracted from tobacco, or the products of nicotine oxidation or other chemical processes associated with the use of e-cigarette devices.

The study findings have several implications for policy. Currently, flavouring chemicals and additives are often undeclared on e-cigarette product packages, meaning consumers are likely unaware that they may be exposed to these substances. The mandatory labelling of e-liquid constituents would address this information gap, providing greater transparency and information to consumers. The current study also identified the presence of several harmful and potentially harmful constituents in e-cigarette products. Although the study findings demonstrate their presence in e-cigarettes available on the Canadian market, their potential health impacts will be determined by their dose. While the concentrations of constituents other than TSNAs were not directly examined in the current study, several quantitative analyses have demonstrated that the level of toxicants detected in e-cigarette aerosols is substantially less than that in conventional cigarette smoke (National Academies of Sciences 2018; Goniewicz et al. 2013). The presence of these chemicals and contaminants represents an avoidable risk, as evidence shows many e-cigarette products do not have detectable levels of these substances, including acrolein (Khlystov and Samburova 2016), diacetyl (Farsalinos et al. 2015a), and TSNAs (Kavvalakis et al. 2015). Regulators may consider establishing product standards to ensure e-cigarettes meet specified quality criteria and are free of toxicants and contaminants. Passage of the Tobacco and Vaping Products Act in May 2018 paves the way for development of regulations pertaining to the testing, reporting, and labelling of e-liquids by manufacturers, as well as the potential for regulators to impose regulatory limits on certain constituents (Parliament of Canada 2016). Last, the study findings reflect the wide range of flavouring chemicals used in e-cigarette products. E-cigarette flavours have been identified as a policy-relevant product characteristic, given their potential role in encouraging cessation among smokers, as well as in encouraging uptake, particularly among youth (e.g., Farsalinos et al. 2013; Pepper et al. 2016). Although the labelled product flavour implies a collection or profile of flavouring constituents, these profiles are not consistent across similarly flavoured products, meaning multiple chemical combinations can yield a given flavour. These findings imply that regulatory restrictions applied to specific flavours, such as cherry, will be difficult to implement and are unlikely to be successful, given the many flavouring chemicals manufacturers have at their disposal.

Limitations

The current study examined constituents in e-liquids, not in e-cigarette aerosol, which is ultimately inhaled by users; therefore, the findings reflect the potential exposure of users to several chemicals of public health concern. Despite the fact that previously published analyses have shown that some compounds, such as diacetyl, are readily transferred from e-cigarette liquid to aerosol (Farsalinos et al. 2015a), direct inhalation exposures were not examined in the current study. In addition, the findings are limited due to their largely qualitative nature; quantification of e-liquid and aerosol constituents is needed to assess potential toxicological risks, given that these are dose-dependent. Last, although the sample of products tested in the current study was not necessarily representative of the Ontario or Canadian market, it is nonetheless geographically diverse and large, particularly in comparison with other published studies (Hutzler et al. 2014; Lisko et al. 2015; Varlet et al. 2015; Behar et al. 2016; Tierney et al. 2016; Farsalinos et al. 2015b; Hua et al. 2019; Bitzer et al. 2018; Khlystov and Samburova 2016; Lisko et al. 2017).

Conclusion

Findings from the current study demonstrate that e-cigarettes purchased in Ontario, Canada, consisted of a wide variety of flavouring chemicals and contained several constituents of

public health concern, including some flavourings and tobacco-specific nitrosamines. Although the total number and level of toxicants detected in e-cigarettes are substantially less than toxicants in conventional cigarette smoke (National Academies of Sciences 2018), reductions may help minimize any excess risk due to variability in how e-cigarette liquids are manufactured. Such policy gaps may be addressed by developing regulatory product standards and labelling practices for e-cigarettes.

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Compliance with ethical standards

Conflict of interest DH has provided paid testimony in tobacco litigation on behalf of governments and class-action plaintiffs on issues related to tobacco product science and regulation. MLG reports grants from and served as an advisory board member to pharmaceutical companies that manufacture smoking cessation drugs. The other authors have no competing interests to declare.

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