

Chemistry Review of Vaping Products and Respiratory Injury

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ABSTRACT

Background: While the Public Health Agency of Canada notes 19 cases from May 2019 to February 2020 relating to e-cigarette or vaping product use-associated lung injury (EVALI) in Canada, there are likely many more unreported cases, including non-hospitalized and asymptomatic cases. E-cigarette use or vaping exposes users to numerous aerosolized chemical species, some of which have proven to be deleterious to health. These chemical species can include vitamin E acetate (VEA), flavourants, base / solvents (propylene glycol or vegetable glycerin), psychoactive substances, pesticides, endotoxins, metals, and pyrolysis by-products from e-cigarette heating coils.

Objectives: We aim to review current findings related to EVALI from the standpoint of known chemical species currently used in vaping products. We specifically examine the toxicological profiles of these chemical species and the mechanisms through which they cause lung injury.

Methods: A comprehensive literature search was performed with MEDLINE for EVALI-related human studies that were published between January 1, 2010, and May 15, 2020. This search strategy identified 832 case reports, case series, clinical trials, and in-vitro laboratory studies. From this group, 71 records were examined in greater detail.

Results and Conclusions: Although the chemical composition and toxicology of vaping products have largely been characterized, the physiological effects of the chemical interactions between various constituents of vaping products and the generation of new species remain inconclusive. Given the rapid increase in the popularity of vaping and e-cigarettes, there is a need for further research. Developing a comprehensive understanding of the chronic health effects of vaping through randomized controlled trials and physiological studies is prudent and necessary to reduce the long-term impacts on users and the health care system.

Introduction

Vaping devices, including electronic cigarettes (e-cigarettes), are often battery-operated devices that initiate the act of heating a liquid into a vapour, which then condenses into aerosols as it travels downstream from the device towards the lungs.¹ Most devices are composed of a battery, a heating element, a mouthpiece, and a chamber that contains the liquid solution. Vaping is the process of inhaling aerosols generated from e-cigarette devices. These devices have become highly popularized since their introduction into the North American market in 2007.^{2,3} Since then, they have assumed different names, including cig-a-likes, e-hookahs, electronic delivery systems (ENDS), mods, vapes, vape pens, sub-ohms, tank systems.^{1,4} Their success is largely due to originally being marketed to traditional cigarette smokers as a healthier alternative because they expose users to fewer chemical species.⁴ Traditional cigarette products contain over 600 chemical species, which increases to over 7000 species when heated due to the formation of combustion products.⁵ At least 69 of these resultant species are known to cause carcinogenic effects. In contrast, electronic liquids (e-liquids and e-juices) generally contain fewer chemical species overall.^{6,7} The compositions of different e-liquids vary; however, propylene glycol (PG) and/or vegetable glycerin (VG, glycerol) are typically used as the base (solvent) (Figure 1). The chemical structures of PG and VG are included because they are common structures and frequently found in vaping products. These simple structures

give further insight into the results of this study. Readers interested in the structure of other, more complex compounds mentioned in this paper can find them through a Google image search. E-liquids may contain flavouring compounds and nicotine.⁴ E-cigarettes may also be used to deliver many additives including tetrahydrocannabinol (THC), cannabidiol (CBD), and butane hash oils.^{4,8,9} However, unlike cigarettes where the chemical composition of different products has been clearly established (i.e. acetone, acetic acid, benzene, cadmium, carbon monoxide, formaldehyde, hexamine, naphthalene and toluene), the chemical interactions between the various constituents and the toxicological profiles of vaping fluids is not fully understood.^{5,10}

Rates of e-cigarette use are similar across age and sex demographics in Canada and the United States (US). Vaping is particularly popular amongst male youth and young adults, especially Americans who identify as Hispanic or non-Hispanic White Caucasian.¹¹⁻¹³ Overall, Black and Asian (South Asian and Southeast Asian) Americans were the least likely to use e-cigarette products. Prevalence within these ethnicities, however, did increase between 2017 and 2018.¹³ The 2017 Canadian Tobacco, Alcohol and Drugs Survey (CTADS) found that 15% (4.6 million) of Canadians aged 15 or older have tried vaping at least once in their life, which is an increase from 13% (3.9 million) in 2015.¹⁴ According to the 2019 Canadian Tobacco and Nicotine Survey, 4.8% of those surveyed reported the use of a vaping device in the prior 30-days, a growth from 3% in 2017.¹⁵ In 2018, 3.2% of US adults

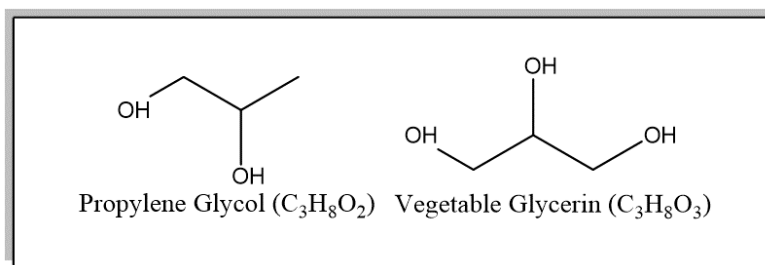


Figure 1 Structures of Common E-Liquid Bases.

reported current use of e-cigarettes.¹² Friedman and Horn found that the prevalence of e-cigarette usage did not discriminate between income or education levels,¹⁶ but a 2018 Swedish study found that lower levels of education correlate with use of vaping products.¹⁷ The variability of findings may be due to the different populations surveyed. A recommendation for further research into prevalence is warranted due to inconsistencies.

The 2018-2019 Canadian Student Tobacco, Alcohol and Drugs Survey found that students who vaped within the prior 30 days (with or without nicotine) doubled to 20% from 10% in 2016-17.¹⁸ In 2019, 27.5% of US high school students reported current use of e-cigarettes, a significant increase

from 1.5% in 2011.¹¹ A Canadian survey outlining the prevalence of vaping among youth between the ages of 16 to 19 from 2017 to 2019 showed similar findings (Figure 2a, Figure 2b).¹⁹ In 2018, this significant increase in e-cigarette use caused the Food and Drug Administration (FDA) Commissioner and the US Surgeon General to declare youth vaping an epidemic.²⁰ While some older users switch to vaping as an exit strategy for traditional cigarettes, the majority of youth users are using the product for recreation.¹⁵ Sixty-five percent of adolescents who tried an e-cigarette product reported that they had borrowed, purchased, or shared it with a friend or relative.¹⁸ This suggests the accessibility of vaping products to youth, despite some attempts to restrict sales and regulate the advertising of

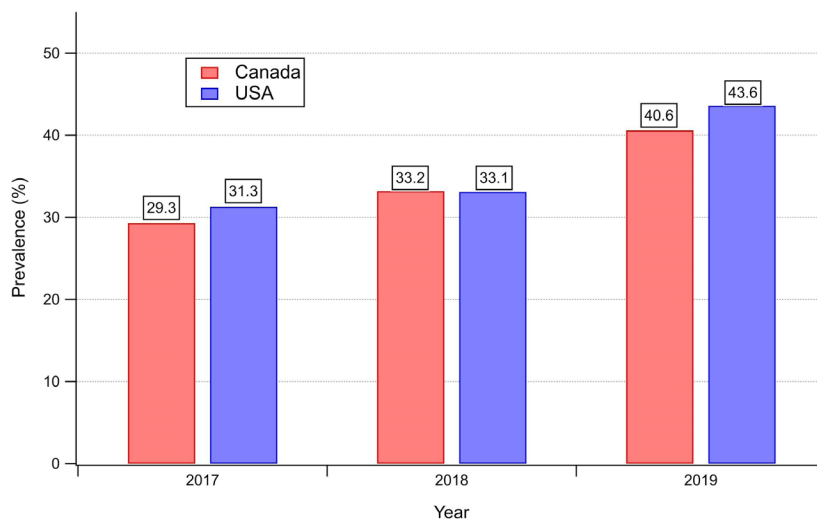


Figure 2a Prevalence of vaping in Canada and the United States from 2017 to 2019. The y-axis presents the proportion of adolescents (between 16-19 years old) who reported vaping at least once in their lifetime.*
*Adapted from Hammond et al. JAMA Pediatr. 2020.²¹

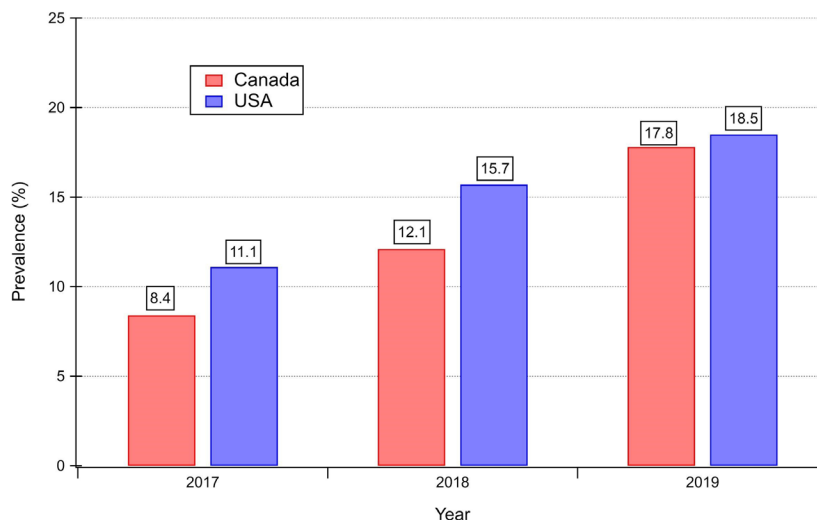


Figure 2b Prevalence of vaping in Canada and the United States from 2017 to 2019. The y-axis presents the proportion of adolescents (between 16-19 years old) who reported vaping within the prior 30 days.*
*Adapted from Hammond et al. JAMA Pediatr. 2020.²¹

such products in Canada and the US. The upward trend on e-cigarette usage among youth in Canada and the US is associated with the rising popularity of products that contain nicotine salts.¹⁹ Nicotine salts allow e-cigarette devices to deliver nicotine more efficiently than freebase nicotine solutions, adding to the addictive potential of e-cigarette products.⁴ In turn, this greater exposure can ultimately lead to detrimental health consequences.

Between July and August 2019, the Centers for Disease Control and Prevention (CDC) detailed the first reported cases of electronic-cigarette or vaping product use-associated lung injury (EVALI), resulting in numerous media articles in both Canada and the US.⁸ Soon after the beginning of the outbreak, the CDC began to use the "EVALI" acronym as a way to report this public health crisis.^{21,22} As of April 7, 2020, there have been 19 cases of EVALI reported to the Public Health Agency of Canada, including 6 in Quebec, 5 in British Columbia, 4 in Ontario, 2 in New Brunswick, and 1 case each in Alberta and Newfoundland and Labrador.¹⁰ As of February 18, 2020, 2807 probable and confirmed EVALI cases were reported to the CDC in the US.⁶ Sixty-eight of these cases resulted in death.⁶ The demographics of US hospitalized patients were similar to those of e-cigarette users, mostly of the male sex (66%), and non-Hispanic White Caucasians (61%).^{6,23} Eighty-two percent of US patients reported the use of any THC-containing products and 33% reported exclusive use.⁶ Fifty-seven percent of patients reported any use of nicotine-containing products, and 14% exclusive use.⁶ The duration of symptoms before presentation to the emergency department (ED) and length of hospitalization varied from 0 to 155 days and 1 to 120 days, respectively.^{8,24-27} Individuals who actively vape can present with respiratory, gastrointestinal and constitutional symptoms; however, some patients may appear asymptomatic. Laboratory analyses in individuals who were actively vaping include bloodwork (complete blood counts

and non-specific inflammatory markers such as C-reactive protein), along with chest imaging (chest radiography or computerized tomography [CT]). If bronchoscopy was performed, a bronchoalveolar lavage (BAL) fluid sample was often collected for analysis. Aggregated case studies described 183 subjects of which just over half (53%) were admitted into the intensive care unit (ICU).^{8,24-27} A majority of the patients showed clinical improvement from treatment with glucocorticoids and antibiotics.^{8,24-27} The median age of EVALI-related death was higher than the median age of hospitalization (49.5 years versus 24 years, respectively).⁶ However, the age of death (15 to 75 years) was similar to hospitalized cases (13 to 85 years). Pre-existing medical conditions, obesity, and simultaneous use of combustible cigarettes while vaping appear to increase the risk of EVALI-related death.²³ Delayed treatment due to an incomplete exposure history may exacerbate the severity of the illness, leading to death.²³ The illicit nature of THC products can lead to incomplete exposure history. Consequently, it is critical for clinicians to nonjudgmentally and privately communicate with patients to ensure truthful disclosure.^{23,28} Despite the fall in the number of cases since the peak of the outbreak in September 2019, the number of cases has not fallen back down to pre-June 2019 levels.²⁹ Therefore, further research is necessary to establish causal relationships between EVALI and specific chemical products / compounds of interest.^{29,30}

While many questions regarding the EVALI outbreak remain, laboratory analysis completed by the FDA have established a link to vitamin E acetate (VEA or alpha-tocopherol acetate).^{9,31} VEA is commonly found in dietary supplements and dermatologic products. It is marketed as being safe for oral and topical intake, but it cannot be assumed that VEA has no adverse effects when inhaled.^{4,9} In two separate studies, Blount and colleagues detected VEA present in 100% and 94% of BAL fluid samples of EVALI patients, respectively.^{9,32} A 2019 study in Wisconsin, led by Pray et al., also detected VEA in all five THC cartridges belonging to two patients,

and in available BAL fluid in two patients.³¹ All eight patients interviewed admitted to the use of THC-containing e-cigarette products and to acquiring the products from the illicit market.³¹ Minnesota law enforcement seizures suggests VEA was introduced first into illicit products sometime in 2019 as a cost cutting measure, matching the timelines of the EVALI outbreak.^{9,33} Significantly, the findings directly link the outbreak to THC, VEA, and illicit e-cigarette products. No causal relationship, however, has been established to date. EVALI remains a diagnosis of exclusion following detailed clinical, radiologic and available pathologic and cytopathologic evaluations for individual cases.^{20,34} No clear single assay is available for confirmation of diagnosis at this time.^{8,35} We review below, from the perspective of chemical agents and toxicology, available literature pertaining to EVALI over the prior decade.

Methods

A literature search for EVALI-related articles was completed using MEDLINE, a frequently used database for medical and science publications. The search was limited to articles published between January 1, 2010 and May 15, 2020. Items pulled for a more detailed review include cases, case studies, clinical trials, and in-vitro laboratory studies. Items were excluded if they were a duplicate of another article, editorials, reviews, or animal studies. The search was further restricted to English-language articles only. Additional sources were identified through the reference section of relevant publications found from the literature search. Other primary references were obtained from official government websites (Health Canada and CDC).

Results

A total of 71 articles were retrieved from our search strategy and reviewed, many of which were case reports (n=20) and case series (n=7). Five small prospective clinical trials were also retrieved, 2 from Belgium, and 1 each from Canada, the US, and Sweden.

Case Reports and Studies:

Twenty-seven of the retrieved papers were case reports and case series (Table 1, Table 2).

Randomized Control Trials:

Five of the retrieved papers were randomized control trials (RCTs) (Table 3).

Overview of Potential Causes of EVALI and Establishing Toxicological Profiles:

E-cigarette products contain numerous potentially harmful chemical compounds, including psychoactive compounds (THC, CBD, nicotine), flavouring agents, diluents (VEA, medium-chain triglycerides [MCTs]), base (PG, VG), pesticides, and other contaminants.^{9,62-64} Detection of these chemicals constituents require the separation of e-liquid components by employing gas (GC) or liquid chromatography (LC). Mass spectrometry (MS) techniques, however, have become the primary method for detecting e-liquid constituents because their sensitivity and selectivity enables them to quantify compounds at the ng/mL scale.^{62,64,65} Coupling MS with GC or LC creates an additional dimension of separation, minimizing interferences. In addition to the use of analytical instruments, derivatization techniques may be required to further increase detection sensitivity for certain compounds. For instance, derivatization with 2,4-dinitrophenylhydrazine (DNPH) or O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) is required prior to instrumental detection of carbonyl-containing flavouring compounds.^{63,66-69} Nuclear magnetic resonance (NMR) can be used to confirm structural findings.⁶⁴ Multi-instrument analyses (with a combination of LC/GC-MS, GC/LC and/or NMR) are useful for comprehensive detection of e-liquid constituents. In the next sections, we discuss the chemistry and toxicology of various e-cigarette constituents.

THC E-Liquids and VEA

Numerous articles hypothesized possible mechanisms by which VEA causes physiological dysfunction. One study examined the chemical

Table 1 Summary of 20 case reports identified in literature search

Author	Age/Sex	Summary
Bonilla et al. (2019) ³⁶	18/M	Within two weeks, the patient presented twice with pneumothoraces and reported vaping preceding to presentation. He was treated and was asymptomatic in his follow-up visit, reported that he stopped vaping.
Deliwala et al. (2020) ³⁷	41/M	Authors reported a patient case where symptoms occurred immediately after a switch from traditional cigarette to vaping products. CT scan showed presence of opacities.
Flower et al. (2017) ³⁸	33/M	While the patient used traditional cigarettes for 10 years, a 3 month vaping exposure resulted in respiratory symptoms and CT abnormalities. A lung biopsy was taken and microscopic findings were consistent to RB-ILD. A stoppage of vaping led to radiographic improvements.
Gay et al. (2020) ³⁹	46/M	Case of ELP due to use of cannabis-containing e-cigarettes. CT, X-ray, and lung biopsy showed many abnormalities. The analysis of BAL fluid found LLM.
He et al. (2017) ⁴⁰	54/M	The patient reported smoking cannabis oil for several years and reported respiratory symptoms. CT scan showed a nodular "tree in bloom" pattern, and BAL fluid analysis suggested DAH.
Hureaux et al. (2014) ⁴¹	43/F	Case of respiratory toxicity from vaping. The patient was a chronic cigarette smoker and switched to e-cigarettes, causing immediate illness. Ceasing e-cigarette use completely resolved all symptoms with no treatment.
Khan et al. (2018) ⁴²	40/F	Case of organizing pneumonia and pulmonary toxicity related to e-cigarette use. The symptoms, CT scans, and clinical course were similar to most EVALI cases.
Landman et al. (2020) ⁴³	17/M	Canadian patient case report that discussed symptoms, blood test results, chest scan imaging, biopsy findings, clinical course, and follow up visits at 1, 2, 3 and 4 months after discharge. The article included a literature review on relevant case reports and series.
Lu et al. (2020) ⁴⁴	17/M	The patient tested positive for cannabinoids. CT scan showed opacities, septal thickening and pneumomediastinum. Patient was admitted to ICU, provided supplemental oxygen, and treated with antibiotics and steroids.
Matta et al. (2020) ⁴⁵	16/M	Authors presented a case report of an individual with a 2 year history of daily vaping. They reported symptoms matching other EVALI cases, CT findings (abnormal imaging), clinical course and treatment (glucocorticoids and antibiotics).
Marasco et al. (2018) ⁴⁶	17/M	Authors presented a case of spontaneous pneumomediastinum. The patient reported taking a deep inhalation from an e-cigarette immediately prior to symptom onset. Patient was discharged after CT scans showed no further complications.
McCauley et al. (2012) ⁴⁷	42/F	Case report of a female with a 7 month history of vaping, which corresponded with onset of respiratory symptoms. Authors discussed laboratory findings (LLM in BAL fluid), provided patient's CT images (showing abnormalities), and discussed the ELP diagnosis.
Miskoff and Chaudhri (2020) ⁴⁸	41/F	Authors presented a female case report. The patient reported switching from combustible cigarettes to vaping products 4 to 8 weeks prior to ED presentation. Treated with antibiotics and steroids, the patient's condition stabilized and they were discharged.
Mittal et al. (2020) ⁴⁹	53/M	Case of chronic vaping that caused TM. This was the first case presented in literature related to innate immunity alterations due to chronic vaping. The patient was successfully treated with PAP therapy.
Puebla Neira et al. (2020) ⁵⁰	23/M	Authors report the first case of EVALI with AEP connected to vaping illicit THC oils. X-ray and CT both showed opacities.
Qarajeh and Kitchen (2019) ⁵¹	47/M	Authors present a patient with past medical conditions (diabetes and hypertension), vaping (with THC) and traditional cigarette use and presented to ED. Chest X-ray and CT showed opacities and pleural effusions. Treatment with corticosteroids led to clinical improvement.
Sechrist and Kanne (2019) ⁵²	25/M	25-year old man with worsening symptoms after vaping THC. CT imaging (with abnormal opacities) were shown. Uncommon EVALI imaging patterns (AEP and giant cell interstitial pneumonia) were discussed.
Thota and Latham (2014) ⁵³	20/M	AEP related to e-cigarette use. CT scans and symptoms were similar to most EVALI cases. The patient was treated with steroids and showed improvement upon follow-up.
Wilhite et al. (2019) ⁵⁴	22/M	Authors reported a patient case with DAH and pulmonary embolism. The patient admitted to vaping illicit THC products. He showed clinical improvement after steroid treatment.
Youmans and Harwood (2020) ⁵⁵	34/F	Reported the first EVALI-related death in the US. The patient with prior health conditions and concealed vaping an unknown cannabinoid oil to clinicians. She died 25 days after admission from deteriorating respiratory functions.

Note: AEP = acute eosinophilic pneumonia, BAL = bronchoalveolar, CT = computed tomography, DAH = diffuse alveolar hemorrhage, ED= emergency department, ELP = exogenous lipoid pneumonia, LLM = lipid-laden macrophages, PAP = positive airway pressure, RB-ILD = respiratory bronchiolitis interstitial lung disease, THC = tetrahydrocannabinol, TM = tracheomalacia.

Table 2 Summary of 7 case series identified in literature search

Author	N	Summary
Billa et al. (2020) ⁵⁵	3	Detailed information of 3 adolescents (15M, 16M, 17M) was provided, including early symptoms, clinical course, radiographic imaging, and treatment. Also discussed pesticide components detected in patients' e-cigarette products that may explain symptoms.
Blagev et al. (2019) ²⁴	60	Patients presented with GI (90%), respiratory (98%) and constitutional (88%) symptoms. Majority were admitted to ICU (55%), administered antibiotics (90%) and steroids (95%). 10% of patients experienced symptom relapse after initial discharge, and most who followed up within two weeks still had abnormal CT and pulmonary function tests.
Carroll et al. (2020) ⁵⁶	15	Authors presented 15 patients with a mean age of 17.1 years. 13/15 completed bronchoscopy and all serum/urine findings were disclosed. Inpatient treatment course (glucocorticoids and antibiotics) and outpatient follow-up (pulmonary functions testing) was discussed.
Kalininskiy et al. (2019) ²⁵	12	Patients experienced a combination of GI, respiratory, and/or constitutional symptoms. 11/12 (92%) of patients reported using THC containing e-cigarette products. 67% of patients were admitted to ICU. Half of patients who followed up after discharge had resolved past CT abnormalities and normal spirometry.
Layden et al. (2020) ⁸	98	A total of 98 patients (48 probable and 50 confirmed cases) from Wisconsin and Illinois was used for the case series. Symptom onset was between April and August 2019. The authors provided extensive statistics on specific GI, respiratory and gastrointestinal symptoms.
Maddock et al. (2019) ²⁶	6	Symptoms and clinical findings were like other case series: abnormal chest radiograph scans, high CRP counts. The authors identified LLM found in BAL fluid in all patients. When EVALI is suspected and all possible infections are excluded, they suggest LLM may be a marker for EVALI.
Rao et al. (2020) ²⁷	13	Pediatric EVALI cases identified through chart review (92% had THC e-cigarette use). All patients had abnormal chest radiographs; glucocorticoid treatment led to clinical improvements.

Note: CRP = C-reactive protein, CT = computed tomography, GI = gastrointestinal, ICU = intensive care unit, LLM = lipid-laden macrophages, THC = tetrahydrocannabinol.

Table 3 Summary of 5 randomized control trials identified in literature search

Author	N	Control	Age	Summary
Antoniewicz et al. (2019) ⁵⁷	17	17	26 ± 3 years	Two visits - vaping with and without nicotine (49.4% PG/44.4% VG/5% ethanol, 19 mg/mL nicotine if added). Participants completed 30 puffs (3 s for each puff) over 30 minutes. Vaping with nicotine affects pulmonary (increased heart rate) and respiratory (airway obstruction) functions.
Boulay et al. (2017) ⁵⁸	30	30	Healthy, 20-37 years; Asthmatic, 21-40 years	Experimental (70% PG/30% VG) and placebo sessions involved 20 healthy and 10 asthmatic volunteers. Seated participants were to breathe into the e-cigarette 3 times per minute, for 1 hour. Respiratory tests (spirometry and forced oscillation techniques) showed that acute exposure does not affect pulmonary function
Chaumont et al. (2018) ⁵⁹	25	25	23 ± 0.4 years	Participants each took part in three sessions, sham-vaping, and vaping with and without nicotine (50% PG/50% VG, nicotine at 3 mg/mL). Every 30 s, participants inhaled from e-cigarette for 4 s, held the aerosol for 4s, and then exhaled for 25 times. Vaping with nicotine causes oxidative stress and changes to vascular function, and not due to high temperature PG and VG aerosolization.
Chaumont et al. (2019) ⁶⁰	Trial: 25; Trial 2: 20	Trial 1: 25; Trial 2: 10	Trial 1: 23 ± 0.4 years; Trial 2: not mentioned	Participants each took part in three sessions, sham-vaping, and vaping with and without nicotine (50% PG/50% VG, nicotine at 3 mg/mL). Serum club cell protein-16 increased; transcutaneous oxygen tension decreased after vaping. Vaping PG/VG with or without nicotine at high voltages (60 W) causes pulmonary epithelium injury.
Song et al. (2019) ⁶¹	30	15	21-30 years	Healthy, non-smokers were randomly assigned to the intervention (50% PG/50% VG) or control group. For 4 weeks, participants had to use the e-cigarette device 2 times per day, 20 puffs over a 60 minute time span. There was no significant difference in cell counts, cytokines, or gene expression after vaping. Urinary PG was correlated with changes in cell counts, lymphocyte, and macrophage count for the intervention group.

Note: PG = propylene glycol, VG = vegetable glycerin.

structure of VEA (a tocopherol) as an aliphatic molecule, containing a polar component and a nonpolar tail portion.⁹ The hydrophobic nature of the long tail portion in VEA has the ability to penetrate the surfactant layers in the lung, which are critical for respiration, and insert itself between surfactant phospholipids.⁷⁰ In doing so, phospholipids contained within the surfactant undergo a transformation from a gel to a crystalline state. Surfactant in the crystalline state causes an increase in the surface tension of the air-water interface of the lungs, increasing the difficulty of lung expansion during respiration. VEA is viscous in nature, allowing the compound to stay in and affect the lungs for long periods of time.³ Another study found the formation of a hydrogen-bonded THC/VEA complex as an avenue for potential lung injury.⁷¹ This heterodimer was found in unvaped e-liquids, as well as in aerosols of vaped e-liquid mixtures. Significantly, this finding demonstrates that VEA in the form of THC/VEA complex may be delivered to the site of lung damage, although the complex's significance remains under investigation. Lastly, the heating of VEA at sufficient temperatures may produce toxic ketenes, carcinogenic alkenes, and benzene.⁷² These findings were confirmed using analytical and theoretical chemistry approaches. Although many EVALI cases are associated with VEA in the US, no cases in Canada are believed to be linked to VEA. This suggests other compounds found in e-cigarette products may play a role in causing lung injury. While there is a strong association between VEA and EVALI, the true mechanisms by which VEA causes lung injury continue to remain under speculation.

Nicotine E-Liquids and Bases

PG and VG (Figure 1) are common nicotine e-liquid bases that may cause a toxicological effect or indirectly produce certain harmful compounds. Jensen et al. observed the formation of formaldehyde-containing hemiacetals from e-cigarette products at high voltages (5.0 V).⁷³ Under the right conditions, formaldehyde can

be a decomposition product of PG.⁷³ Assuming inhaling formaldehyde-releasing agents carries the same health risks as gaseous formaldehyde, Jensen et al. calculated the lifetime cancer risk to be between 5 to 15 times as high as the risk associated with long-term combustible cigarette use.⁷³ While long-term formaldehyde exposure is carcinogenic, acute exposure can irritate various mucous membranes, including the upper respiratory tract and eyes.⁷⁴ A similar study by the same authors found that pyrolysis of PG and VG created toxic organic compounds.⁷⁵ Corresponding compounds that may be generated from vaping pyrolysis of PG include acetaldehyde, acetic acid, acetone, acrolein, formaldehyde, formic acid, hydroxyacetone, lactaldehyde, prop-1-en-1-ol, and propanal. On the other hand, VG vaping pyrolysis by-products include acetaldehyde, acetic acid, acrolein, dihydroxyacetone, formaldehyde, formic acid, glyceraldehyde, glycidol, glycolaldehyde, and hydroxyacetone. Many of the mentioned compounds are considered to be volatile and present many inhalation health risks, such as irritation and difficulty breathing. The extent of pyrolysis by-product formation was dependent on the wattage and heat transfer efficiency of the e-cigarette device.⁷⁵

Flavouring Agents

Other constituents of concern include flavourants, especially diacetyl (2,3-butanedione), 2,3-pentanedione and acetoin, which are commonly found in e-liquids. Like VEA, flavouring compounds are safe for digestion, but not inhalation.⁶³ These compounds are used by e-liquid manufacturers to enhance a product's flavouring profile.²¹ The addition of flavourants and the use of attractive names attract both young adults and adolescents to use e-cigarette products. In May 2002, factory workers at a microwave popcorn-processing plant were diagnosed with bronchiolitis obliterans, also known as "popcorn lungs" (see Appendix A).⁶³ Later, federal investigations found a direct correlation between pulmonary dysfunctions, bronchiolitis obliterans, and butter-flavoured compounds used

in the facility, namely, diacetyl, 2,3-pentanedione, and acetoin. These compounds were found in 47 of 51 flavoured e-cigarette products tested by Allen et al.⁶³ Moreover, a chemical investigation showed acetoin slowly converting to diacetyl over time when stored in a sealed e-cigarette cartridge.⁶⁶ Conversion rates depend on numerous factors, including light exposure, ratio of PG/VG, pH, storage length, and amount of nicotine. Although occupational exposure limits are established for flavouring agents, there are no standards for general exposure. These occupational limits are particularly not applicable to youth, who are more vulnerable to exposures.²⁷ Diacetyl and 2,3-pentanedione downregulates the expression of genes crucial for cilia biogenesis (see Appendix A), and decreases the number of ciliated cells.⁷⁶ Overall, genetic material important for cytoskeletal and cilia processes are disturbed. Human airway epithelial cells (see Appendix A) exposed to e-cigarette vapours secrete proinflammatory cytokines (IL-6 and IL-8) (see Appendix A).⁷⁷ In a separate study on pulmonary epithelial cells, diacetyl exposure increased keratin 5 (Kr5) (see Appendix A) protein ubiquitination (see Appendix A), and decreased in Δ Np63 (see Appendix A) cell marker expression, which can potentially reduce airway basal cells' proliferative capacity.⁷⁸ At diacetyl concentrations below 50 mM over one hour, basal cells were able to recover and maintain cellular integrity without undergoing major damage or death.⁷⁸

Many studies have tested the physiological consequences from vaping exposure of other flavouring constituents. In a study from 2016-2017, there were over 15000 distinct flavours of e-liquids sold online, nearly double the number available in 2013-2014.⁷⁷ Cinnamon roll flavoured e-cigarette stimulated a far more significant increase in IL-8 than the other commercial e-liquids tested. In a study of cinnamon flavoured e-liquid products, cinnamaldehyde, 2-methoxycinnamaldehyde, dipropylene glycol, and vanillin were detected.⁷⁹ Inhalation of vanillin (or ethyl vanillin) may cause

respiratory irritation and inflammation.⁸⁰ This is supported by Gerloff and colleagues' findings, who found that vanillin exposure to respiratory epithelium caused IL-8 release in human bronchial epithelium (Beas2B).⁸¹ Moreover, it has been suggested that the pyrolysis of vanillin may lead to the formation of organic by-products, but this has to be assessed in vaping contexts. In the 2016 study, Behar and colleagues detected cytotoxic levels of cinnamaldehyde in 20 out of 39 different cinnamon-flavoured e-cigarette products.⁸² Cinnamaldehyde caused the depolymerization of microtubules within human pulmonary fibroblasts (see Appendix A). Additionally, lung cell exposure to cinnamaldehyde caused decreased growth, changed morphology and motility of cells, and increased breakage of DNA strands, leading to cell death. Kavvalakis et al.'s study of 263 e-liquid samples detected 2,5-dimethylpyrazine (chocolate flavour) and 3,4-dimethoxybenzaldehyde (cherry flavour) in 5 of 7 brands analyzed.⁸³ Respiratory epithelial cell exposure to 2,5-dimethylpyrazine activated the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, which in turn may compromise pulmonary epithelium salt and water homeostasis.⁸⁴ The toxicology of 3,4-dimethoxybenzaldehyde have not yet been assessed. Tierney et al.'s analysis found that maltol (cotton candy and caramel flavour) and ethyl maltol (caramel flavour) were detected in 8 and 9 out of 30 e-liquid products, respectively.⁸⁵ Out of all flavouring compounds tested by Hua and colleagues using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (which assesses cell metabolic activity), they found maltol to be the most cytotoxic.⁸⁶ Besides the detection of menthol and menthone in mint-flavoured e-cigarette products, pulegone was often detected in substantial concentrations.⁸⁷ Pulegone is carcinogenic and was banned by the FDA as a food additive in 2018. When inhaled, menthol itself may cause oxidative stress (see Appendix A), inflammation, and dysfunctional cell barriers.⁸⁰

E-liquid flavouring compounds may also react to form other compounds of varying toxicological profiles. Farsalinos and Voudris's study confirmed that the heating of flavoured e-liquids within an e-cigarette device contribute to aldehyde emissions (formaldehyde, acrolein, and acetaldehyde), to a minimal, but detectable extent.⁶⁸ Their findings were confirmed in a pilot study involving human participants, which found aldehyde emissions were higher in exhaled flavoured e-cigarette breaths than in pre-exposed, regular breaths.⁸⁸ Aldehyde emission is exponentially proportional to flavouring compound concentrations within e-liquids.⁸⁹ Moreover, lower nicotine concentrations and adjustable power settings directly increase acetaldehyde and formaldehyde production.⁹⁰ Muthumalage et al. and Gerloff et al. confirmed lung cell exposure to flavoured e-liquids lead to increased oxidative stress, reduced pulmonary epithelium barrier function and caused proinflammatory responses.^{81,91} Certain flavourants may interact with PG to form flavourant-PG acetals, which suggests the volatility of e-cigarette constituents immediately after mixing.⁹² These flavourant-PG acetals are highly stable, persisting in e-cigarette users after inhalation, and activating aldehyde-sensitive TRPA1 and aldehyde-insensitive TRPV1 irritant receptors (see Appendix A). These receptors, through increasing irritation sensation, contribute to chronic pulmonary conditions which include asthma.⁹² Lastly, flavouring compounds may influence the formation of free radicals in aerosolized e-liquids.⁹³ Reactive free radicals can induce oxidative stress, damaging crucial cell pathways. Other less common chemical compounds with the potential to cause injury include α or β damascenone (rose-scented), 3-methyl-1,2-cyclopentanedione (coffee flavour), acetamide, linalool, terpineol, citral, corylon (caramel flavour), anisaldehyde, trimethylpyrazine, eugenol, and limonene (citrus flavour).^{80,94} Limonene, in particular, was detectable in illicit products and was found in BAL fluid of an EVALI patient.³²

Other Constituents of Interest

In a comprehensive analysis of illicit, commercial, and medical-grade e-cigarette cartridges, Muthumalage et al. reported the detection of other potentially harmful compounds in e-liquids, including isoprene, acetaldehyde, ethylbenzene, toluene, acrolein, 1,3-butadiene, and benzene.⁶² The FDA lists these compounds as respiratory and cardiac toxicants. Many organic compounds detected in e-liquids may explain EVALI patient symptoms. For example, the inhalation of acetone and 1,2-dichloroethane can cause drowsiness. Inhalation of xylenes, and 1,3-pentadiene may cause dyspnea (shortness of breath) and chest tightness, while 1,3-butadiene can form peroxides when exposed to oxygen.⁶² Furthermore, exposure to methyl vinyl ketone and allyl chloride can lead to emphysema and edema. Muthumalage and colleagues' detection of pesticides in e-cigarette products confirm the findings of other studies.^{35,62,64} Tebuconazole, an antifungal agent, was one of many pesticides detected in THC-containing products that belonged to an EVALI patient.³⁵ While tebuconazole is not a pulmonary toxicant in itself, when heated above 150°C, it decomposes into hydrogen chloride (HCl) gas and nitrogen oxides.³⁵ HCl can further breakdown to chlorine gas (Cl₂) which can cause burning sensations in the lungs. Nitrogen oxides are pulmonary toxicants that can cause respiratory, gastrointestinal, and constitutional symptoms experienced by EVALI patients. Moreover, elements including silicon, copper, nickel, and lead were detected in illicit, counterfeit products.⁶² Although variable, metal concentration reached up to 600 ppm in e-liquids. Other metals can also be found in e-liquids, including chromium, manganese, aluminum, tin, iron and cadmium.²⁷ These detected metals may be due to the sloughing of the metal heating coil within e-cigarette devices due to use over time.⁹⁵ Significantly, among Rao and colleagues' cohort of 13 patients, the sickest patient's e-liquid cartridge tested positive for cadmium, while all other metals tested negative.²⁷ Cadmium potentially causes respiratory dysfunction.⁹⁵ Inhalation of other

mentioned metals may cause adverse neurologic, carcinogenic, respiratory, and cardiovascular effects.⁹⁵ All of these findings suggest EVALI is not singularly caused by VEA, but by a combination of VEA and other toxic substances in e-cigarette products. The CDC currently warns individuals against using informally acquired THC-containing e-cigarette products.³¹

Discussion and Conclusion

While e-cigarette products are marketed as being the “safer” alternative to traditional combustible cigarettes, it does not equate to being “safe” for use, especially after the large EVALI outbreak in the summer of 2019. The large outbreak suggests the heterogeneous nature of e-liquids and raises further questions regarding the safety of longstanding e-cigarette use. Many compounds found in e-liquids are potential pulmonary toxicants when inhaled. Products obtained by unlawful means often contain more compounds harmful to health, an outcome of cost-cutting to maximize profit, with a disregard to product safety. In doing so, certain premium compounds are replaced with cheaper ones, but still allow for a similar user experience. A lack of regulatory accountability during the e-liquid production process may also lead to the addition of endotoxins, bacteria, high metal content, and organic compounds uncommonly found in products for human consumption. Endotoxins can lead to the destabilization of respiratory immune-regulated disorders, such as asthma. Vaping chemistry, through pyrolysis and interactions between chemical species, further introduces new compounds of varying toxicological profiles to the user, many of which are harmful. Moreover, free radical reactions occurring amongst e-liquid constituents can give rise to reactive intermediates, which contribute to oxidative stress and adverse health effects. Chemical complexation and bonding interactions between e-liquid constituents may contribute to harm but have not been assessed physiologically. Therefore, e-cigarettes are a health risk to

consumers, of which adolescents and young adults are especially vulnerable, who are now the primary marketing targets of e-cigarette product manufacturers.

VEA remains a strong culprit for EVALI but more research is necessary to fully establish causal relationships, including the possibility of the injury being caused by multiple toxicants. As the lack of diagnostic tests further challenges clinicians when diagnosing patients with EVALI, there is a dire need for assessments of the toxicology and carcinogenicity of e-liquid components. In particular, from the list of EVALI-related case reports and case series summarized in this article (Table 1, Table 2), it is clear that the current clinical cases can only track down patients with acute symptoms that have obvious links to vaping practices. Clinical cases where chronic traditional cigarette smokers who fell ill immediately after transitioning to vaping products raise further concerns concerning vaping safety. The health impact of long-term and second-hand vaping is essentially unknown. More comprehensive chemical analyses of vaping products and their emissions would be helpful to reveal species that do not cause acute health outcomes but exhibit cumulative health impacts over time.

For research approaches, more RCTs and long-term studies on vaping should be conducted. Considering only hospitalized cases were reported, there are likely numerous cases that remain unreported to public health officials in Canada and the US. Therefore, despite a drop in the number of EVALI cases in the US and a low number of reported cases in Canada, we must remain vigilant and expect the possibility of another outbreak. A better understanding of the chemical composition of approved products available for purchase in Canada will allow better regulation of individual products, including taxation, minimizing long-term harm to the individual users and our health system.

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Declaration of Interests

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Appendix

Glossary of Terms

Cilia biogenesis: The synthesis of moving structures (cilia) in the lung that sweep debris and microbes from the airway.

Epithelial cells: Specific type of body cells that cover body surfaces, including the skin, and organs.

Fibroblast: Cells that synthesize collagen and the extracellular matrix (ECM), structures that provide support to surrounding cells.

Keratin 5 (Krt5): Forms the intermediate filaments in basal epithelial cells that make up the cytoskeleton.

Oxidative Stress: The imbalance between free radical generation and presence of antioxidants in the body.

Popcorn lungs: An informal term for bronchiolitis obliterans, the inflammation of the bronchioles.

Proinflammatory cytokines (including IL-6 and IL-8): Signaling proteins that promote inflammation. IL-6 and IL-8 are a specific type of proinflammatory cytokines. The increase in proinflammatory cytokines in the body increases inflammation.

TRPA1 / TRPV1: Specific types of ion channel receptors that senses and responds to irritants.

Ubiquitination: Refers to the ubiquitin-proteasome pathway (UPP), where protein complexes degrade damage or unused proteins by proteolysis, a cellular reaction that breaks peptide bonds.

Δ Np63: A cell marker found in airway basal cells. The reduction in Δ Np63 may result in suppression of cellular proliferative capacity.

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