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ALLERGIES AND THE ENVIRONMENT (M HERNANDEZ, SECTION EDITOR)

Electronic Cigarettes: Their Constituents and Potential Links to Asthma

Phillip W. Clapp[1,2] · **Ilona Jaspers**[1,2,3]

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Abstract
Purpose of Review Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma.
Recent Findings Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms.
Summary Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers.

Keywords E-cigarette and asthma · E-liquid and asthma · Asthma · E-cigarette flavorings · E-cigarette and inflammation · Allergy

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✉ Ilona Jaspers
Ilona_jaspers@med.unc.edu

[1] Curriculum in Toxicology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

[2] Center for Environmental Medicine, Asthma, and Lung Biology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

[3] Department of Pediatrics, University of North Carolina, Chapel Hill, NC, USA

Introduction

Smoking has devastating effects on human health, and while nearly 18% of all adults in the USA currently smoke, tobacco

use among adults and high school students is declining [1–4] due to FDA-imposed regulations on the sale, distribution, advertising, and promotion of tobacco products [5–7]. In response to reduced cigarette consumption, tobacco companies are promoting new tobacco products, such as flavored electronic cigarettes (e-cigs), which are often represented as safer alternatives to traditional cigarettes. Inhalation of e-cig aerosols (commonly referred to as e-cig vapor) provides the sensation of smoking and desired nicotine effect without combusting tobacco. Since their US debut in 2007, e-cigs have gained popularity and usage has increased steadily, particularly among teenagers and young adults [8, 9] who often perceive these products as harmless [10]. However, recent findings suggest that e-cigs may cause respiratory harm in ways that are both similar to and different from traditional cigarettes.

E-cigs are battery-operated devices that deliver nicotine, flavorings, and other constituents to the user by heating flavored e-cig liquid (e-liquid) solutions to temperatures sufficient to form aerosols. Despite the different names for e-cigs, such as electronic nicotine delivery devices (ENDS), electronic vaporizers, cig-a-likes, vape pens, box mods, e-hookahs, or advanced personal vaporizers (APV), the basic physical components of these devices are the same: a battery, an atomizer, and a tank or cartridge to hold the e-liquid (Table 1). The e-liquid is composed of propylene glycol (PG) and vegetable glycerin (VG), which act as humectants, nicotine, and flavoring chemicals. The atomizers in more advanced e-cig devices (excluding first generation cig-a-likes and some e-hookahs) are powered by rechargeable lithium batteries. The construction and the materials that comprise the heating coils and wicking material of the atomizer vary significantly among the different device types. Common wicking materials include silica, organic cotton, rayon fibers, stainless steel mesh, and bamboo yarn. Heating coils are typically resistance wires made from various metal alloys, including Kanthal (iron, chromium, and aluminum), stainless steel (iron, carbon, and chromium), and Nichrome (chromium and nickel). However, pure metal coils of nickel and titanium are gaining popularity. Battery output, heating coil resistance, and wicking material determine the temperature range of the vaporizing device, which in turn significantly affects the thermal decomposition process of e-liquids and the resulting chemical mixture of the inhaled aerosol. Potentially harmful carbonyls have been detected in e-cig aerosols generated at high temperatures (> 200°C) [11, 12] and the emission of acrolein, a known inhalational toxicant, has been reported to increase 10-fold when the voltage of a device was increased from 3.3 to 4.8 V [13]. The composition of e-liquids also varies greatly due to the broad range of nicotine concentrations and flavoring chemicals used to make these products, thus resulting in an immensely large number of different chemical aerosol mixtures potentially inhaled by vaping e-cigs.

E-cig use has been touted as a much healthier alternative to cigarette smoking. Early advocates actively promoted e-cigs as cessation aids and claimed that vaping would significantly reduce smoking of conventional cigarettes [14, 15]. Recently, Public Health England (PHE) estimated that e-cigs are 95% less harmful than tobacco cigarettes, and when supported by a smoking cessation service, are effective at helping most people quit smoking [15]. Indeed, recent population level data suggest that increased e-cig use in the USA correlates with smoking cessation [16]. These trends have also been reflected among adolescents: cigarette smoking (defined as smoking a cigarette in the past 30 days) decreased in this population from 15.8% in 2011 to 8.0% in 2016, and e-cig use increased from 1.5 to 11.3% in the same time frame (https://www.cdc.gov/tobacco/data_statistics/fact_sheets/youth_data/tobacco_use/index.htm).

Many researchers and clinicians have strongly advocated for transitioning asthmatic smokers to e-cigs to reduce the healthcare burden of smoking-induced asthma exacerbations [17–19]. However, e-cig aerosols are poorly characterized complex mixtures of inert and reactive chemicals, and it is unclear whether long-term inhalation will improve or worsen asthma. The broad representation of e-cigs as safe alternatives to smoking has played a key role in the perception of e-cigs as harmless, particularly by adolescents [10]. A recent study found that e-cigs were perceived more positively, and usage was more prevalent among adolescents with asthma (12.4%) as compared to their non-asthmatic peers (10.2%) [20]. Similarly, data from the 2012 Florida Youth Tobacco Survey indicates that the rate of e-cig use in Florida high school students was greater in asthmatics than in non-asthmatics [21]. Outside of the USA, increased rates of e-cig use in asthmatic adolescents compared to non-asthmatics were found in Ontario, Canada [22], and South Korea [23]. Thus, while e-cigs may decrease the use of conventional cigarettes in adults with an existing smoking history, the prevalence of e-cig usage is greater in adolescent asthmatics relative to non-asthmatics, which is of concern. This review will focus on the specific components of e-cig aerosols and discuss how inhalation of these compounds could contribute to asthmatic phenotypes or promote exacerbation in asthmatic individuals.

E-liquid Components and Their Effects on the Lung

Propylene Glycol and Glycerin

PG and VG, the two compounds representing the greatest majority of e-liquid volume, keep nicotine and flavoring agents in suspension, enhance absorption of the wicking material, and generate plumes of aerosolized particles when heated to sufficient temperatures. Moreover, the humectant property of PG and VG (e.g., the ability to attract and retain

Table 1 Examples of common e-cig devices

1st Generation "Cig-a-like"	2nd Generation "Vape-pen"	3rd Generation "Box mod"	4th Generation "Temperature Control (TC) Box mod"
• Low price • Similar in size and shape to traditional cigarette • Disposable or rechargeable • Some new models have refillable cartridges • Available at most convenience stores • Power settings not adjustable • Often used by novice vapers or former smokers transitioning from cigarettes	• Low to moderate price • Refillable tank • Rechargeable lithium-ion battery • Some customizability with user-defined voltage (typically 3.0 to 6.0 V) • Most have heating coils with >1.0 Ω resistance • Often used by novice or intermediate vapers	• Moderate to high price • One or two rechargeable lithium-ion batteries • Highly customizable: user-determines settings for voltage or wattage • Can accommodate a wide variety of refillable tanks, rebuildable atomizers (RBAs), or tankless "dripping" atomizer • Heating coils with <1.0 Ω resistance are commonly used with these devices (sub-ohm vaping) • Heating coil temperatures may exceed 300° C with high wattage and low resistance • Generate significantly more aerosol than 1st or 2nd generation devices • Most often used by experienced vapers	• Among most highly priced devices due to more advanced electronics • User can program a maximal heating coil temperature to prevent overheating e-liquids and or burning the wicking material (i.e., a "dry hit") • Temperature control (TC) mode requires stainless steel, nickel, or titanium heating coils • One or two rechargeable lithium-ion or lithium polymer (LiPo) high amperage batteries • Other features and functions similar to 3rd generation box mods • Most often used by experienced vapers

moisture via absorption) provides the user with a "throat hit" similar to cigarette smoking. While the FDA has classified both PG and VG as generally recognized as safe (GRAS) for oral consumption, the effects of repeated inhalation of aerosolized PG and VG are unclear. To investigate the respiratory effects of PG and VG aerosol exposure, Boulay and colleagues at the Quebec Heart and Lung Institute conducted a randomized crossover, placebo-controlled study on the acute effect of nicotine- and flavor-free e-cig aerosol (70% PG, 30% VG) inhalation in healthy (n = 20) and asthmatic (n = 10) volunteers [24]. Volunteers inhaled aerosol three times per minute for 1 h and changes in respiratory mechanics and lung functions were evaluated. While some subjects in both groups experienced increased cough, chest tightness, and mucosal secretions with inhalation of PG/VG aerosol, the authors observed no consensual differences between the healthy or asthmatic volunteers. Furthermore, no significant decrements in lung function were observed in either group, and the fraction of exhaled nitric oxide (FeNO) and serum C-reactive protein (CRP) in asthmatic subjects was not significantly altered by aerosol exposure. The findings reported by Boulay et al. are consistent with earlier studies demonstrating that healthy non-smokers with acute (≤ 1 h) exposure to nicotine-free [25] or nicotine-containing [26] e-cig aerosols experience only minimal changes in lung function and FeNO. Although the acute exposures used in these studies did not cause significant functional pulmonary alterations, longer exposures may elicit changes in respiratory responses. Indeed, a previous study evaluating the effects of PG-based theatrical fog on respiratory symptoms and lung function found that entertainment industry workers exposed to PG aerosol over a 4-h shift experienced significant increases in acute cough and dry throat, but did not exhibit significant decreases in lung function as determined by pre- and post-shift comparisons of forced expiratory volume-one second (FEV1) and forced vital capacity (FVC). However, FEV1 and FVC values were both significantly lower in entertainment industry workers compared to a non-entertainment industry control group, and within the

group of entertainment industry workers, individuals that frequently worked within 10 ft or less of the fog source had significantly lower lung function than individuals working further away [27]. Thus, while acute exposure to PG and VG aerosols may not cause significant changes in pulmonary function, chronic exposures may result in impaired lung function.

An emerging concern is that thermal decomposition of PG and VG results in the formation of carbonyl compounds with known inhalational toxicity and irritant properties [13]. Second, third, and fourth generation e-cig devices allow the user to manually increase the voltage applied to the atomizer with the push of a button, thereby increasing the heating coil temperature. Many e-cig users prefer to vape at high temperatures as more aerosol is generated per puff. However, applying a high voltage to a low-resistance heating coil can easily heat e-liquids to temperatures in excess of 300 °C; temperatures sufficient to pyrolyze e-liquid components. In a recent study, Wang and colleagues heated PG and VG under precisely controlled temperatures to determine whether common vaping temperatures are capable of pyrolyzing PG and VG into toxic volatile carbonyl compounds. They reported that significant amounts of formaldehyde and acetaldehyde were generated at temperatures ≥ 215 °C for both PG and VG, and heating VG to temperatures in excess of 270 °C resulted in the formation of acrolein [28]. Numerous studies have implicated exposure to reactive carbonyls, including formaldehyde, acetaldehyde, and acrolein, in the pathogenesis and exacerbation of asthma [29–32]. Thus, aerosolization of PG and VG at high temperatures often generated by users of more advanced third and fourth generation devices results in the formation and inhalation of reactive compounds known to exacerbate asthma.

Nicotine

Nicotine is a botanically derived parasympathomimetic alkaloid that is readily absorbed by the body through dermal, oral, and inhalational exposures, and easily crosses biological membranes including the blood brain barrier and placenta [33]. The system-wide physiologic effects of nicotine are mediated by its ability to affect the release and metabolism of neurotransmitters, and include increased blood pressure, increased pulse rate, increased free fatty acids in the plasma, mobilization of blood sugar, and increased concentrations of catecholamines in the blood [34–39]. In addition to stimulating the release of norepinephrine and dopamine, nicotine also directly activates nicotinic acetylcholine receptors (nAChRs) and stimulates cellular responses including increased c-fos and c-jun expression, increased expression of heat shock proteins, induction of chromosome aberration, reduced cell proliferation, and suppression of apoptosis [33, 40–44]. While muscarinic acetylcholine receptors (mAChRs) provide autonomic control of airway smooth muscle and activation of mAChRs causes muscle contraction and bronchoconstriction, whether or how nAChRs are involved in the pathogenesis or exacerbation of asthma is still unclear.

Recent work has also demonstrated that nicotine induces epigenetic alterations in rat germline cells and alters developmental signaling pathways necessary for normal fetal lung development [45]. In a landmark study by Rehan and colleagues, researchers injected pregnant Sprague-Dawley rats with nicotine to determine if repeated in utero nicotine exposures would transmit asthma to the second generation (F2) offspring. The authors report that acute, daily injections of nicotine throughout pregnancy (1 mg/kg; comparable to the dose of nicotine to which habitual smokers are exposed, that is, approximately 1 mg/kg/body weight) altered histone acetylation in the testes, ovaries, and lung tissues of F1 offspring and resulted in an asthmatic phenotype. Mating of the F1 rats produced similar epigenetic alterations and an asthmatic phenotype in both the male and female F2 offspring, although the F2 animals had never been directly exposed to nicotine. The nicotine-induced epigenetic alterations reduced expression of mesenchymal peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that plays a critical role in lung development, homeostasis, and repair. The authors further demonstrate that the observed asthma phenotype was a direct result of reduced PPARγ expression in the lung. Importantly, this study linked nicotine exposure during pregnancy to epigenetic modifications that cause multigenerational transmission of asthma. Follow-up studies by Rehan and colleagues solidify the link between nicotine-induced epigenetic alterations and transgenerational asthma by providing evidence of an asthmatic phenotype in F3 offspring [46, 47]. Most manufactured cigarettes contain 10–15 mg of nicotine per cigarette [48]. Unlike traditional cigarettes, e-cig refill liquids are available with varying concentrations of nicotine ranging from 0 to 36 mg/ml. Moreover, some e-cig users choose to purchase a concentrated nicotine solution and supplement their e-liquids with the amount of nicotine that provides the desired effect. The current literature on nicotine absorption from e-cig use varies greatly; thus, it is still unclear whether vaping will result in significantly greater nicotine exposures than smoking.

Numerous animal studies have reported beneficial effects of nicotine in the context of asthma, which are, at least partially, mediated by its anti-inflammatory properties and activation of the alpha7-nAChR [49–56]. Specifically, in a rodent study by Mishra et al., nicotine inhibited allergen-induced inflammation, eosinophilia, leukotriene release, allergen-specific IgE levels, and Th2 cytokine levels without significantly altering airway mucosa morphology or airway resistance [57]. In contrast, nicotine has been reported to increase airway mucus viscosity [58] and enhance mucus production in human bronchial epithelial cells, which is dependent on the

expression of alpha7-nAChR [59]. Nicotine-induced anti-inflammatory responses in the lung have also been associated with increased susceptibility to respiratory viral infections due to reduced migration of immune cells to sites of infection [60]. Thus, while nicotine may reduce inflammation in the lung, it also enhances mucus production and reduces the beneficial inflammatory responses in the context of viral infections, which are major triggers of asthma exacerbation.

Flavoring Agents

A major difference between the manufacture of e-cigs and traditional cigarettes is the broad use of flavoring agents to create thousands of uniquely flavored e-cig refill liquids. The 2009 Family Smoking Prevention and Tobacco Control Act banned the use of artificial or natural flavors (other than tobacco or menthol) in cigarettes due to concerns that tobacco with characterizing flavors promotes smoking among children and adolescents. However, e-cigs were not subject to this regulation and the manufacturer, marketing, and sale of flavored e-cig products surged in the USA. To better understand how the rapidly growing e-cig market was evolving, Zhu and colleagues investigated how the online market for e-cigs changed between August 2012 and January 2014 [61]. The authors report that by January 2014, there were 466 e-liquid brands (each with its own website) and 7764 unique flavors. In 17 months between their searches, there was a net increase of 10.5 brands and 242 new flavors per month. Furthermore, the products marketed in 2012 promoted their advantages over conventional cigarettes while the products marketed in 2014 emphasized flavor variety, consumer choice, and product versatility. The e-cig industry has continued to grow since 2014; however, exactly how many e-liquid brands and unique flavors are currently on the market is unclear.

Critics of the e-cig industry have denounced the use of flavoring agents citing concerns that some flavorings will promote e-cig use amongst youth [62, 63], increase nicotine dependence [64], and thwart the steady decline of smoking in this demographic [65, 66]. Indeed, current e-cig use in high school students (defined as use during at least 1 day in the past 30 days) reached 16% by 2015, surpassing the rate of conventional cigarette use for the first time [67]. However, the broad and unregulated use of flavoring agents may also present more immediate health concerns. Toxicological data on inhalational exposures of flavoring chemicals is extremely limited and recent studies have noted high concentrations of flavoring chemicals in e-liquids and e-cig aerosols [68, 69]. Furthermore, there is precedent for food-safe flavoring chemicals causing irreversible lung disease. Diacetyl (2,3-butanedione), a common food-safe flavoring agent used to provide a buttery or creamy flavor, has been shown to cause acute onset bronchiolitis obliterans, an irreversible obstructive lung disease, when inhaled by workers exposed to aerosolized flavoring agents containing diacetyl [70–72]. Diacetyl-induced bronchiolitis obliterans first occurred in workers at a microwave popcorn production facility, which resulted in the disease being described as "popcorn lung." A recent study investigating whether this known inhalational toxicant was present in sweet-flavored e-liquids revealed diacetyl in 110 (69.2%) of the 159 products tested [73]. Based on an estimated daily use of 3 ml of e-liquid per day, the investigators determined that use of 52 of the e-liquids tested would result in diacetyl exposures greater than the 5-ppb recommended 8-h time-weighted occupational exposure limit established by the National Institute for Occupational Safety and Health (NIOSH) and the Centers for Disease Control (CDC). Twenty-six of these e-liquids would result in diacetyl exposures more than five times greater than the 5-ppb limit and the e-liquid with the highest diacetyl content would result in an exposure 490 times greater than the NIOSH limit. However, there is little data available on the inhalation of flavoring agents and exacerbation or pathogenesis of asthma. Here, we will review relevant case reports, in vivo and in vitro studies, and molecular mechanisms that may provide insight into how the flavoring constituents of e-cigs interface with asthma.

Airway Irritants and Chemical Sensitizers

Workplace exposures to low-molecular-weight chemicals have been reported to cause sensitization and, subsequently, asthma. Low-molecular-weight chemicals with highly reactive side chains pose an even greater risk and, as such, are frequently identified as chemical sensitizers and airway irritants. While exposures to these types of chemicals have historically occurred in the workplace, e-cig users may also be exposed to similar levels of reactive, low-molecular-weight chemicals in the context of e-cig flavoring agents. The Flavor and Extract Manufacturers Association (FEMA) of the USA, a trade association of flavor ingredient manufacturers which evaluates the safety of food flavorings, has identified 1037 flavoring agents as potential respiratory hazards due to possible volatility and respiratory irritant properties [74]. Common e-cig flavoring agents on this list include, but are not limited to: diacetyl, acetoin, 2,3-pentanedione (buttery flavors), camphor and cyclohexanone (minty flavors), benzaldehyde (cherry or almond flavors), cinnamaldehyde (cinnamon flavor), cresol (leathery or medicinal flavor), butyraldehyde (chocolate flavor), and isoamyl acetate (banana flavor).

Eugenol, a phenylpropene compound that is the major constituent of clove oil, and cinnamaldehyde, the α,β-unsaturated aldehyde that gives cinnamon its characteristic taste and odor, are two common e-cig flavorings that have both been identified as potent skin sensitizers in humans, and there is data linking these sensitizers to asthma. A 2015 case report describes a 34-year-old professional cleaner who developed cough, dyspnea, and maculopapular erythema after inhalation

and dermal exposures to an industrial cleaning agent containing eugenol [75]. Bronchial challenge with a 1:1000 dilution of eugenol produced a 17% decrease in FEV1 8 h after exposure, which was accompanied by cough and dyspnea. Based on these findings, the patient was subsequently diagnosed with eugenol-induced occupational asthma. Another report details the case of a 30-year-old hairdresser who developed nasal congestion, rhinorrhea, and eczema on his hands while working with haircare products [76]. His asthma symptoms progressively worsened leading to severe asthma attacks on three occasions. Skin prick testing with common aeroallergens, latex, and 2% eugenol were negative but specific inhalation challenge with eugenol (1:1000 dilution) produced rhinitis symptoms and an isolated late asthmatic response. Late asthmatic response and increased eosinophil and lymphocyte counts in sputum after eugenol challenge support the specificity of the airway reaction to eugenol.

Reports of contact dermatitis from cinnamon exposure date back to the late 1800s [77] and have been well-documented in recent years [78–83]. However, there is a paucity of reports directly linking cinnamon exposures to asthma. A study investigating the health effects of cinnamon dust exposures in 40 Sri Lankan cinnamon workers found that 20 workers (50%) experienced skin irritation, 15 workers (37.5%) had increased cough, and 9 workers (22.5%) were asthmatic [84]. The number of asthmatics in this study was reported to be disproportionately higher than other Sri Lankan industries with comparable working conditions and dust exposures. However, more evidence is needed to directly link inhalational exposures of cinnamaldehyde and the development or exacerbation of asthma.

Benzaldehyde is a highly reactive, low-molecular-weight aldehyde flavoring agent commonly used to create cherry- or fruit-flavored e-liquids. Benzaldehyde, in the context of occupational airborne exposures, has been reported to cause irritation to the eyes and mucous membranes of the respiratory passages [85]. Subacute inhalation exposures in rats at 500, 750, and 1000 ppm indicated hypothermia and other severe impairments of the central nervous system (CNS) at all doses, with abnormal gait, severe tremors, and convulsions occurring at the highest concentration [86]. Repeated inhalation of volatilized benzaldehyde produced eye and nasal mucosa irritation at 500 ppm and death in rabbits at 750 ppm [87]. While specific data on benzaldehyde exposures and asthma is lacking, inhalation of similar low-molecular-weight aldehydes is known to impair respiratory function [88, 89] and has been implicated in the development of asthma in children [90, 91]; thus, the use of this aldehyde flavoring agent in e-cigs is of concern. Kosmider and colleagues recently quantified benzaldehyde in aerosol generated from 145 flavored e-liquids [85]. One hundred eight of these e-liquids contained benzaldehyde, which ranged in concentrations from 5.129 μg/30 puffs to 141.2 μg/30 puffs. Based on their findings, the investigators estimated the median daily inhaled dose of benzaldehyde from cherry-flavored e-cigs to be 70.3 μg, an exposure more than 1000 times lower than the permissible exposure limit for benzaldehyde in the workplace. However, this study used a second generation vape pen (2.4 Ω resistance coil, 900 mAh battery, 3.4 V power setting) for 163 puffs (70 ml puff volumes) to estimate the median daily inhaled dose of 70.3 μg. A more current (3rd or 4th generation) device with significantly higher output power and sub-ohm resistance coils would likely expose users to much higher concentrations of benzaldehyde. In contrast to the irritant effects of benzaldehyde inhalation, a recent study reports that oral exposures to benzaldehyde suppress allergic inflammation in a murine model of allergic asthma and rhinitis [92]. Jang and colleagues dosed female mice with benzaldehyde (200 or 400 mg/kg; approximately 4–8 μg for a 20-g mouse) prior to allergen challenge and quantified markers of allergic inflammation. Mice treated with oral benzaldehyde had significantly less inflammatory cell infiltration in lung and nasal tissues, lower HIF-1α and VEGF expressions in lung tissue, fewer eosinophils and neutrophils in bronchial alveolar lavage (BAL), and lower Th2 cytokine titers in the BAL fluid. The seeming contradiction between the known CNS and irritant effects of benzaldehyde inhalation and the anti-inflammatory effects of oral benzaldehyde exposure reported by Jang suggest a significant difference in biological responses depending on the exposure dose and route of exposure. Further work is needed to elucidate whether and how benzaldehyde exposures from e-cig use will impact respiratory health, particularly in asthmatics with known sensitivity to aldehydes.

Mint Flavoring Agents and Asthma

Mint-flavored e-cigs and e-liquids are among the most popular products used among vapers. However, there are reports of mint flavoring agents causing or exacerbating respiratory conditions. A 1990 New England Journal of Medicine Letter to the Editor describes a 21-year-old nonsmoking asthmatic female who developed wheezing within 10 min of using toothpaste containing mint or wintergreen flavoring [93]. The patient also disclosed that wheezing developed when chewing gum containing wintergreen or peppermint. Pulmonary-function tests demonstrated a significant decline in air flow after challenge with mint toothpaste as well as a moderate response to bronchodilators. A similar case report from Spain describes a 21-year-old female nonsmoker with asthma and aspirin intolerance who reported dyspnea after daily tooth brushing and bronchospasm after eating a menthol-containing candy [94]. Lung function monitoring revealed a 36% reduction in FEV1 immediately after the patient used mint-flavored toothpaste, but exposure to the same toothpaste without the flavoring agents did not significantly affect FEV1. A subsequent double-blind challenge with spearmint (*Mentha*

spicata), peppermint (*Mentha piperita*), and menthol, a terpenic alcohol obtained from the volatile oils of various species of *Mentha* (*labiateae*) caused an immediate and significant bronchial response. The patient did not show an immediate response to a skin prick test with these flavoring agents and no IgE activity against any of these flavors could be detected. More recently, a case was reported in which a 46-year-old nonatopic female with no significant medical history experienced several anaphylactic episodes after using mint-flavored toothpaste [95]. The change with the toothpaste caused immediate facial urticaria, abdominal colic, and bronchospasm requiring immediate treatment with epinephrine. Skin prick tests for reactivity to both the toothpaste and peppermint oil were strongly positive and the patient experienced symptoms of rhinitis and conjunctivitis following testing. Thus, in isolated cases, exposure to menthol compounds can produce strong allergic and/or asthmatic responses.

Menthol is widely used to flavor both e-cigs and traditional cigarettes. However, there is limited data on the direct effects of inhalational menthol exposures. Menthol is reported to have antitussive properties [96] and may mask early symptoms of respiratory disease [97]. Indeed, the potent antitussive action of this chemical has sustained the popularity of menthol-containing sore throat lozenges and topical cough-suppression ointments. Although there are few reported cases of severe mint allergy and fewer linking mint-flavoring exposures and asthma, the exposures associated with e-cig use present a unique concern. A study by Tierney and colleagues that identified and quantified the flavoring constituents in 30 popular e-cig products reported menthol concentrations ranging from 5.7 to 21.6 mg/ml (0.57 to 2.16% *w*/*v*) [98]. While the concentrations reported here are comparable to the menthol content of traditional menthol cigarettes (typically 3 to 20 mg per cigarette), the lack of regulation governing e-liquid formulations allows manufactures to add as much flavoring as they deem appropriate. The resulting variability in composition between products and even within batches of the same product makes determining an individual's exposure essentially impossible. It is currently unclear if menthol or other *Mentha*-based flavorings will have deleterious respiratory effects in the context of e-cig use. However, the growing popularity of these products, the expanding market, and the lack of regulation together with previously reported cases of allergic and respiratory effects caused by *Mentha*-based flavorings warrant caution when using or recommending these products.

Transient Receptor Potential Channel Activation by Flavoring Agents

Airways are highly sensitive to damage from inhaled pathogens, reactive chemicals, and foreign debris. Pulmonary reflex responses, such as sneezing and cough, protect the airways from the potentially harmful substances we inhale each day. Peripheral chemosensory and mechanosensory nerve endings are densely arrayed throughout the respiratory mucosa to provide respiratory feedback control and regulate pulmonary reflexes. Pulmonary unmyelinated afferent fibers (C-fibers) detect chemicals entering the lungs and, when stimulated by an airway irritant, induce sneezing, coughing, profuse mucus secretion, and pain as protective responses. Studies of the mechanisms governing pulmonary C-fiber activation have led to the identification of transient receptor potential (TRP) channels, which act as cellular sensors that respond to a wide variety of airway irritants, physical stimuli, and endogenous ligands. Broadly, TRP channels are transmembrane, cation-selective channels that play a critical role in various cellular processes including muscle contraction, cell proliferation, cell death, gene transcription, and neurotransmitter release, by altering intracellular calcium concentrations [99, 100]. Of the 28 sensory neuronal TRP channels, TRPV1, the capsaicin receptor, and TRPA1, the allyl isothiocyanate (mustard oil) receptor, play key roles in noxious chemical detection and initiation of pulmonary reflex responses.

TRPV1 is activated by capsaicin, heat, pH, and vanilloid compounds, and has been demonstrated to be involved in neurogenic inflammation and various symptoms associated with airway diseases [101–105]. Activation of TRPV1 elicits reflex responses, which include cough, bronchoconstriction, and mucus hypersecretion [104]. Loss of function mutations in the *TRPV1* gene have been associated with reduced susceptibility to asthma [106]. Accordingly, peripheral blood TRPV1 expression was increased in children with asthma [107], and its expression is increased in murine models of allergic airway inflammation [108]. However, other studies have not detected a role for TRPV1 in allergic airway inflammation or exacerbation of asthma [109, 110].

There is an emerging link between TRPA1-mediated neurogenic inflammation and asthma [111–113]. TRPA1 is believed to be the major reactive irritant receptor in the airways as TRPV1-null mice display normal respiratory sensitivity to airway irritants, responding with profound respiratory depression and normal pulmonary reflex responses [114, 115]. Once inhaled, reactive electrophiles, such as flavorings commonly used in e-liquids, activate TRPA1 by covalent modification of cysteine residues, altering protein complex conformation and facilitating inward flow of cations [116–118]. In turn, TRP-mediated cation influx stimulates C-fiber responses including thermal and mechanical hyperalgesia, acute pain, respiratory depression, and neurogenic inflammation [119, 120]. Accordingly, it is plausible that highly reactive electrophilic flavoring agents in e-cigs, through direct activation of TRPA1 and subsequent neurogenic inflammation, may also contribute to the pathogenesis or exacerbation of asthma. Activation of TRPA1 on C-fibers stimulates the release of neurokinin A (NKA), substance P (Sub P), and calcitonin gene-related

peptide (CGRP). These neuropeptides bind to tachykinin (NK1, NK2, and NK3) and CGRP receptors on effector neurons to promote and modulate early inflammatory responses including vasodilation, extravasation of plasma protein, leukocyte recruitment, mucus hypersecretion, and airway constriction [113]. Furthermore, these neuropeptides act on nonneuronal cells to stimulate the release of additional proinflammatory mediators, including ATP, adenosine, bradykinin, prostaglandins, leukotrienes, histamine, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), proteases, and glutamate [121]. Transient stimulation of respiratory neurons and induction of pulmonary reflexes are believed to protect the airways by eliminating harmful substances and promoting tissue repair and healing. However, sustained activation of TRPA1 and persistent inflammatory neuronal stimulation may exacerbate chronic respiratory conditions including asthma [114]. Recently, Caceras and colleagues demonstrated that genetic ablation of TRPA1 in mice inhibits allergen-induced leukocyte infiltration in the airways, reduces proinflammatory cytokine release and mucus production, and almost completely abolishes airway hyperreactivity to contractile stimuli [122]. Furthermore, pharmacological inhibition of TRPA1 recapitulated the response in wild-type mice. Common e-cig flavoring agents, including cinnamaldehyde (cinnamon), cannabidiol (cannabis oil), linalool (floral/spicy flavor), menthol (mint), eugenol (clove), limonene (citrus), gingerol (ginger), and ethyl vanillin (vanilla) are potent TRPA1 agonists [123–128]; These data further illustrate a potential role for TRPA1 in the pathogenesis of asthma and bring into question the safety of repeated inhalation of e-cig flavoring agents that are known TRPA1 agonists.

Conclusions

A recent study of cigarette smoking among adults with asthma exacerbations reports that one in three emergency department patients with asthma are current smokers [129] and asthmatic smokers often have more severe symptoms, accelerated decrements in lung function, and impaired short-term therapeutic

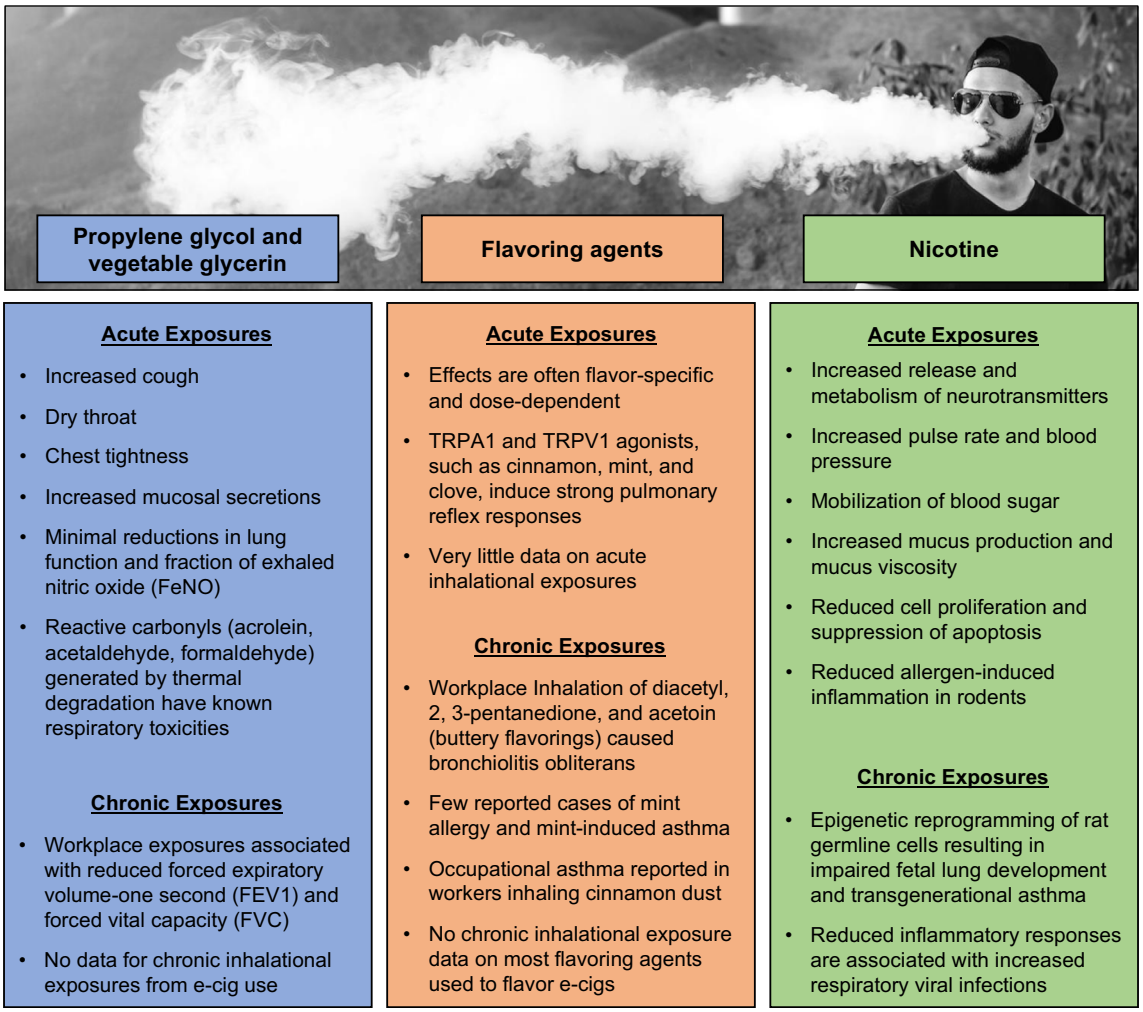

Fig. 1 Biological effects of exposure to chemicals in e-cig aerosols (published with permission from Shutterstock)

response to corticosteroids [130]. While cessation should be strongly encouraged in asthmatics who smoke, it is unclear whether substituting e-cigs for cigarettes is a universally safer option. There is a justifiable concern that any broad statement promoting e-cig safety may be unfounded considering the lack of inhalational toxicity data on the vast majority of the constituents in e-cigs. This is particularly true for individuals with existing lung disease such as asthma. Based on the limited data for the health effects of e-cig use in the context of asthma, we identify several key points of concern (summarized in Fig. 1):

- Many adolescents with asthma hold a positive view of e-cigs, and the prevalence of e-cig usage is greater in adolescent asthmatics as compared to non-asthmatics.
- Current e-cig devices are capable of pyrolyzing e-liquids resulting in thermal degradation on constituents and the generation of known respiratory toxicants such as acrolein, formaldehyde, and acetaldehyde.
- Nicotine exposure during pregnancy may induce epigenetic alterations, disrupt developmental signaling pathways necessary for normal fetal lung development, and play a role in multigenerational transmission of asthma.
- Common flavoring agents, which are often present at high concentrations in e-liquids and e-cig aerosols, are chemically similar to known airway irritants and sensitizers, and have been reported to cause occupational asthma. Moreover, e-cig exposures of some of these chemicals may exceed workplace exposure standards.
- There is no data on the potential long-term effects of e-cig use and incidence or exacerbation of asthma.

While e-cig use may help some asthmatics quit smoking, it is imperative that we continue addressing knowledge gaps to fully assess whether and how vaping e-cigs can significantly modify asthma and allergic airway disease.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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