E-cigarettes and respiratory health: the latest evidence

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Abstract The E-cigarette market continues to expand at an alarming rate with thousands of flavours available for purchase and continuously evolving devices. Now that it is a multi-billion...
dollar industry and one without stringent regulation, there is rising concern over the safety of vaping products. Since June 2019, over 2800 cases of E-cigarette-associated acute lung toxicity have been reported in the USA, over 60 of which resulted in death. Many argue that E-cigarettes offer a safer alternative to smoking, but we are evidently far from fully understanding the potential hazards that they pose to respiratory health. Although the risk of an outbreak in the UK has been considered low due to tighter E-cigarette regulations, we cannot fully eliminate the possibility of similar events occurring in the future. With evidence frequently emerging of the harmful effects of E-cigarettes to pulmonary health, there is an urgent need to define the long-term implications of vaping. Studies show that E-cigarette exposure can disrupt pulmonary homeostasis, with reports of gas exchange disturbance, reduced lung function, increased airway inflammation and oxidative stress, downregulation of immunity, and increased risk of respiratory infection. In this review, the latest research on the effect of E-cigarette use on respiratory health will be presented.

**Introduction**

The first known E-cigarette (EC), described as a tobacco-free nicotine aerosol device, was invented in 1963 by a man named Herbert A. Gilbert. In 2003, a Chinese pharmacist named Hon Lik modernised the device, which later became the first commercially successful first generation EC. Now a rapidly growing multibillion-dollar industry, vaping is marketed as a safer alternative to smoking, due to the lack of tobacco combustion. EC use, however, raises a great deal of controversy. Although suggested as a useful tool in smoking cessation (Rahman et al. 2015), there is a great deal of convincing and conflicting evidence to dispute this claim and users are in fact switching from one addictive nicotine product to another (Ghosh & Drummond, 2017). EC use also appeals to those who have never smoked and is particularly popular with the younger generation (Bauld et al. 2017; Perikleous et al. 2018). Worryingly, there is considerable evidence to show that adolescent EC use can lead to tobacco smoking (Walley et al. 2019). At present, the long term and chronic health effects of EC exposure are yet to be determined. While studies may eventually show that vaping is less detrimental than smoking, new and emerging evidence of the potential adverse effects of vaping continue to be reported. An urgent need to fully define the health risks of EC or vaping-associated products therefore remains.

**Evolution of EC devices**

EC technology has changed significantly over the last decade. A range of devices have been manufactured with chronological advances in power capacity (Table 1) and resulting differences in health effects. First generation ECs, known as ‘cig-a-likes’ are fitted with fixed, low voltage batteries and were designed to resemble a regular cigarette. Second generation devices, referred to as ‘clearomisers’, are comparatively sleeker in design and fitted with larger volume fluid tanks. The third generation saw the introduction of modifiable battery devices, known as ‘mods’, fitted with adjustable wattage (Williams & Talbot, 2019). Fourth generation mods are advanced to contain highly customisable sub-ohm tanks and temperature control units, allowing the user to modulate voltage and intensity. Later generation devices allow inhalation of significantly larger volumes of E-liquid per puff, and therefore exhalation of large vapour clouds. The exhalation of large vape clouds has since become a popular phenomenon, with ‘cloud chasing’ events being held in numerous countries worldwide.

The chronological increase in power capacity has resulted in an associated rise in harmful emissions. Newer devices commonly use low-resistance heating coils, which render them sensitive to customisable wattage or voltage (Rankin et al. 2019). Increasing coil temperature is associated with elevated thermal degradation of E-liquid constituents, vegetable glycerine (VG) and propylene glycol (PG), and therefore, a resulting increase in the production of formaldehyde and other toxic carbonyl compounds (Kosmider et al. 2014; Sleiman et al. 2016; Rankin et al. 2019). Particulate matter (PM$_{1-10}$) emissions are also reported to increase progressively with each generation of EC (Protano et al. 2018). Studies specifically comparing the health consequences of evolving devices are limited and there is an urgent need for further research to define the potentially hazardous effects of newly emerging devices to human health. Lung inflammation and disturbances to gas exchange from the...
use of high power, fourth generation devices have been reported (Chaumont et al. 2019).

Now considered to be in the fifth generation, ECs have undergone a significant change in design to deliver nicotine more efficiently. In 2015, JUUL was released onto the market. With a sleek high-tech design that resembles a USB stick, JUUL uses pre-filled pods containing nicotine, PG, VG, benzoic acid and flavouring chemicals. Its significant popularity has encouraged ‘copycat’ devices to be manufactured and numerous pod devices are now available. Over the last couple of years a dramatic increase in demand has been reported, with Bloomberg estimating revenue of $3.4 billion dollars in 2019 compared to $1.3 billion in 2018. JUUL devices are fitted with a control circuit that limits coil temperature, presumably to reduce unwanted toxicants. Formaldehyde yields are indeed reported as reduced compared to that identified in previously studied ECs; however, evidence suggests that flavoured pods can generate a significant amount of cellular reactive oxygen species (ROS), contributing to inflammation and epithelial barrier dysfunction (Muthumalage et al. 2019). Furthermore, while the maximum nicotine concentration in JUUL pods is 18 mg/ml in the UK, in the USA, the maximum concentration available is 59 mg/ml. A study by Omaiye et al. (2019) showed that JUUL pod nicotine concentration can exceed this with the average pod at 60.9 mg/ml. The significantly high levels of nicotine have been suggested to increase the likelihood of addiction, especially in the adolescent population (Talih et al. 2019). JUUL and alike devices are relatively new in the vaping world and studies to assess health effects are therefore limited. Recently, a case study was reported of a 18-year-old male who presented with two episodes of pneumothorax, on two separate occasions within a fortnight. Prior to the onset of both episodes, the patient reported vaping JUUL mint-flavoured pods on the days leading to the event (Bonilla et al. 2019).

The E-liquid industry is also vastly expanding, with over 15,000 flavours currently available on the market. As flavours continue to be formulated and distributed at a rapid rate, there is growing concern over the toxicity of their chemical composition. Evidence of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons, ultrafine particles, ROS, cytotoxic metals and silicate particles have been reported (Pisinger & Døssing, 2014; Rubinstein et al. 2018; Hua et al. 2019). The vast variety of flavours allow numerous chemical combinations to exist, with some having previously raised concern. In 2016, the EU banned diacetyl as an E-liquid flavouring under the Tobacco Products Directive 2016 when it was linked to numerous reports of ‘popcorn lung’ or bronchiolitis obliterans, a condition that damages and exacerbates the bronchioles, causing severe obstruction to the lower airways. Worryingly, numerous E-liquids in Canada and the USA have been identified as containing diacetyl

| Table 1. E-cigarette device evolution |
|--------------------------------------|
| **First generation**                 |
| Known as ‘cig-a-likes’               |
| Fixed low voltage: ~3.7 V            |
| Led light automatically lights up upon inhalation |
| E-liquid is contained inside refill cartridges |
| **Second generation**                |
| Known as ‘clearomisers’ or ‘vape pens’ |
| Larger variable voltage: 3–6 V       |
| Manual ‘fire’ button to initiate inhalation |
| User refills E-liquid into the fluid reservoir |
| **Third generation**                 |
| Known as ‘mods’: Powerful and advanced sub-ohm battery ‘mod’ device |
| Modified batteries allow the voltage to be adjusted Up to 8 V |
| Mechanical mods: manual ‘fire’ button to initiate inhalation |
| User refills E-liquid into the fluid reservoir |
| **Fourth generation**                |
| Powerful and advanced sub-ohm battery ‘mod’ device |
| Regulated mods: user can control; voltage and/or wattage power output |
| Large puff volumes can be inhaled |
| Capable of producing ‘vape clouds’ upon exhalation |
| LED screen |
| User refills E-liquid into the fluid reservoir |
| **Fifth generation**                 |
| Known as ‘pods’                      |
| 3.7 V                               |
| No buttons or switches               |
| User must insert pre-filled E-liquid pods |
(Pierce et al. 2016; Czoli et al. 2019). Indeed, recently, a near-fatal case of acute bronchiolitis obliterans was reported when a 17-year-old male in Canada presented with severe respiratory symptoms after vaping flavoured E-liquids (Landman et al. 2019).

**Passive EC vaping**

With some devices capable of generating large clouds of exhaled vapour, there is concern as to whether passive vaping is harmful to respiratory health. Although the literature on potential risks for second hand vaping in non-EC users is slim, there is emerging evidence to suggest that EC aerosols exhaled by the user, referred to as second hand aerosols (SHAs), can contribute to environmental pollution. EC vapour exhaled from an individual differs from machine produced SHA, due to biological processes that occur within the respiratory tract. In a study that compared SHA from human breath with machine-generated SHA, particulate matter (PM$_{2.5}$) concentration was found to be 4.5 times higher in breath condensate (Czogala et al. 2014). This is an important distinction given the ability of PM$_{2.5}$ to penetrate the lower airways, and ultrafine PM to enter the circulation and reach distal organs (Terzano et al. 2010). Exhaled breath from vapers has also been identified as containing VOCs (Czogala et al. 2014).

Limited research has been conducted to investigate the acute effects of exhaled SHA on respiratory health; Tzortzi and colleagues showed that passive EC vapour exposure led to immediate alterations in respiratory mechanics and exhaled inflammatory biomarkers (Tzortzi et al. 2018), as well as a positive correlation between signs of respiratory irritation and increasing VOC exposure (Tzortzi et al. 2020). A study using 54 non-smoking volunteers to assess passive EC use in the home showed that participants absorbed comparable nicotine levels to those exposed to passive tobacco smoke, by measure of salivary and urine cotinine (Ballbè et al. 2014).

Of greater concern is the potential health effect of exhaled SHA on those with existing airway conditions, such as asthma and chronic obstructive pulmonary disease (COPD). While there does not seem to be any evidence on SHA and COPD, an association between SHA exposure and asthma exacerbations in the adolescent population has been identified (Kim et al. 2017; Bayly et al. 2019). A lack of research on SHA exposure in pregnant women is also of concern. The harmful effects of nicotine exposure on fetal development are well documented (England et al. 2015; Spindel & McEvoy, 2016). Given that nicotine concentration in EC SHAs are comparable to those from smoking, it is plausible that second hand exposure to vaping is potentially hazardous.

**Recent cases of EC, or vaping product use associated lung injury**

In 2019, an outbreak of EC-related conditions was reported in the United States, termed EC or vaping product use associated lung injury (EVALI) by the Centers for Disease Control (CDC). Incidents were first reported in the summer of 2019 after health department officials began to study an outbreak of cases across the country of severe and sometimes fatal EC-related lung injury that suddenly arose in otherwise healthy individuals. As of 18th February 2020 EVALI has resulted in the hospitalisation of 2807 cases, 68 of which have resulted in deaths across 29 states, the youngest of which was sadly a 13-year-old boy (Centers for Disease Control and Prevention, 2020). Regular updates are published on the CDC website, and although the number of new cases is thankfully declining, during its peak, numbers were reported at an alarming rate, with over 1000 new incidents reported between mid-October and December (Centers for Disease Control and Prevention, 2020). The most common symptoms described when presenting with suspected EVALI are chest pain, cough and dyspnoea, gastrointestinal symptoms such as diarrhoea, vomiting and abdominal pain, and flu-like symptoms such as fever, chills and headaches. Frequent hospitalisation was required, with the most severe cases needing mechanical ventilation (Siegel et al. 2019). Recently, vitamin E acetate, a thickening agent used in tetrahydrocannabinol (THC) containing vape products, was linked to EVALI cases when it was identified in all bronchoalveolar lavage samples from 29 patients, across 10 different states (Centers for Disease Control and Prevention, 2020). Similarly, a detailed case study of 53 affected EVALI patients in Wisconsin and Illinois revealed that 84% used THC-containing vape products (Layden et al. 2019). In an animal model of exposure, mice were exposed to vitamin E acetate aerosols over the course of 2 weeks. Following euthanisation, BAL fluid was extracted and lung sections were examined. Researchers observed an increase in markers associated with lung injury, in addition to increased levels of vitamin E acetate in BAL fluid (Bhat et al. 2020). These findings are similar to those observed in BAL fluids from EVALI patients (Blount et al. 2020). Nonetheless, while vitamin E acetate appears to be linked to reported cases, the evidence is not comprehensive enough to exclude the contribution of other chemicals (2019).

EC related cases are not restricted to the United States. In the UK, respiratory failure caused by lipid pneumonia was reported in 2018 (Viswam et al. 2018). In September 2019, the death of a 57-year-old male, back in 2010, was found to be directly linked to EC use after he suffered lipid pneumonia, and in November 2019, a teenage boy suffered near fatal hypersensitivity pneumonitis from EC use. Similarly, in Belgium, the death of an 18-year-old male was recently reported as directly attributable to EVALI.
But, as of yet, an epidemic of EVALI has not been reported in countries outside the USA.

The effect of ECs on the respiratory system

Gas exchange

Pulmonary gas exchange is a tightly regulated mechanism, disruption of which can lead to low oxygen levels and/or the retention of carbon dioxide, having the potential to acutely or chronically impact lung function. One of the main factors involved in pulmonary gas exchange is surfactant. This is composed of a delicately balanced mixture of proteins and lipids, which if disrupted can lead to impaired gas exchange. EC vapour has been shown to cause fluctuations in surfactant composition, leading to gas exchange abnormalities. This was demonstrated in an animal model by Madison et al. (2019) in which PG, an organic solvent in E-liquid, caused disruption to the surfactant layer and gas exchange disturbance. In a randomised clinical trial, human participants displayed gas exchange disturbance by changes in transcutaneous oxygen tension and reduced lung function, after inhaling EC vapour from fourth generation ECs (Chaumont et al. 2018). These studies highlight the potential for ECs to cause long-term issues relating to hypoxia and hypercarbia.

Spirometry

Spirometry is the most commonly used method of assessing lung volume and function. Studies suggest that exposure to ECs can disrupt lung function. Larcombe et al. (2017) demonstrated reduced functional residual capacity in mice exposed to EC vapour versus healthy controls. Increased bronchial muscle reactivity was also reported, even when mice were exposed to non-nicotine containing EC vapour. With nicotine often cited to increase bronchial constriction, this study highlights the capacity for other components of E-liquid to impair lung function. This was further demonstrated by Khosravi et al. (2018), where anaesthetised guinea pigs were exposed to EC aerosol and measured for transpulmonary pressure and respiratory flow. Results showed that delivery of a single puff into the lung immediately activated transient bronchoconstriction. Similarly, in human studies of EC exposure, participants that vaped for 5–10 minutes displayed increased airway resistance after vaping (Gennimata et al. 2012; Vardavas et al. 2012).

Airway epithelium

The epithelial surface of the respiratory tract is continually exposed to inhaled toxins and provides a functional interface between external stimuli and the host immune response. It lines the respiratory tract from the nose to the alveoli, with a wide range of epithelial cell types. Numerous studies have cultured airway epithelial cells in vitro to investigate the physiological implications of EC use. Submerged cultures offer a relatively cheap and robust culture system for in vitro models but are unable to undergo mucociliary differentiation and thus lack the physiological features of airway mucosa. In order to create the pseudostratified mucociliary phenotype observed in vivo, cells require culturing at the air–liquid interface. Allowing the basal cell surface to be in contact with culture medium, while the apical surface is exposed to air, cells are able to undergo mucociliary differentiation and perform ion/fluid transport, thereby providing a more representative culture system of the respiratory tract in vivo. Mucociliary clearance is the initial host defence mechanism within the lung and is vital for the removal of foreign particles or pathogens from the airways. Disruption of mucociliary clearance, or normal secretions, impairs pulmonary function and increases the risk of infection. Exposure to cigarette smoke extract is well known to impair this key lung clearance mechanism (Stanley et al. 1986), with a recent study suggesting that acute EC exposure leads to comparable mucociliary dysfunction in vivo (Carson et al. 2017). This has been shown to occur throughout the airway with reports of impaired mucociliary action in the nasal mucosa (Kumral et al. 2016) and in bronchial epithelial cells in vitro (Chung et al. 2019). Recently, a study by Crotty Alexander et al. (2018) showed that primary human bronchial epithelial cells cultured to air–liquid interface and exposed to EC vapour for 15 minutes daily (3–5 days) displayed diminished airway barrier function.

The alveolar epithelium covers the vast majority of the surface area in the lungs. It is essential in gas exchange but also functions as an important protective barrier. Damage to the alveolar epithelium can have severe physiological consequences, such as acute respiratory distress syndrome (ARDS), a condition whereby increased capillary permeability leads to diffuse alveolar damage and the release of pro-inflammatory cytokines. To date, in vitro studies to investigate the physiological effects of EC use on alveolar epithelium have used the A549 type II alveolar epithelial cell line, derived from human adenocarcinoma. Cell cytotoxicity assays are often conducted using the lactate dehydrogenase (LDH) assay, in which the release of LDH is detected upon damage to the plasma membrane, indicative of cell death. The A549 cell line is relatively resistant to cell death and is highly proliferative (Lieber et al. 1976); however, alveolar epithelial cells exposed to EC extract in vitro displayed significantly elevated levels of LDH release compared to unexposed controls (Cervellati et al. 2014). In another study, the apical surface of A549 cells exposed to EC aerosol or EC extract showed a
dose-dependent increase in LDH release after just 2 hours of exposure (Hwang et al. 2016).

**Direct lung parenchymal damage**

One of the most concerning effects of EC vapour is that of direct cell death in vivo. Epithelial membrane protein (EMP), can be detected in human serum and demonstrates a baseline level of cell turnover and homeostasis. A study by Staudt et al. (2018) showed that EC users demonstrated a dramatic rise in physiological EMP levels versus healthy controls, highlighting a potential link between cell death within the lung and EC vapour. Elevated secretion of fibroblast growth factor, which is indicative of diffuse alveolar damage (DAD), has also been reported (Cervellati et al. 2014). DAD is frequently seen in patients with ARDS and involves alveolar inflammation and haemorrhage. Indeed, DAD was reported in a case study earlier this year when a 47-year-old female chronic vaper presented with dyspnoea, nasal congestion, fevers and chills. Her lung biopsy showed thickening of alveolar septi from fibroblastic proliferation, as well as enlargement of the alveolar lining (Bakre et al. 2019).

**Effects on the immune system and host defence**

The respiratory system contains a rich microbiome, and must physiologically regulate a fine balance between the airway mucosa and the microbial environment to maintain pulmonary homeostasis. Although the specific factors that disrupt this equilibrium are not fully understood, inhaled irritants, such as pollution and cigarettes smoke can disturb homeostasis. This can lead to inflammation and a cascade of epithelial changes that can ultimately result in dissemination of potential pathogens, with resulting infection (Hakansson et al. 2018).

As previously mentioned, airway mucosa provides a physical barrier to invading microbes, and diminishing epithelial mucociliary function has been reported following exposure to EC vapour. However, other effects on respiratory immunity and the host defence system have been reported (Fig. 1). There is emerging evidence that EC use may increase susceptibility to respiratory infection by enhancing microbial capacity for cell invasion. In our study of 11 vapers, EC use increased the expression of a transmembrane receptor, platelet-activating factor receptor (PAFR), which during the process of internalisation is used as a Trojan horse by *Streptococcus pneumoniae* to adhere to and infect host cells. Furthermore, this study showed that bacterial adhesion to upper and lower airway cells was observed to increase following exposure to EC extract in vitro. A role for PAFR was confirmed, when pneumococcal adhesion was attenuated with a PAFR blocker (Miyashita et al. 2018). Supportive evidence is provided by the study of Shen et al. (2016) who showed that nicotine increases PAFR mRNA in lower airway cells in vitro, although nicotine is unlikely to be the sole driving factor since nicotine-free E-liquid significantly increased PAFR expression and pneumococcal adhesion in the same cell line (Miyashita et al. 2018). Increased susceptibility to viral infection has also been observed; Wu et al. (2014) reported elevated viral infection in primary human airway cells exposed to EC extract in vitro. In an animal model of EC exposure, Madison et al. (2019) showed that exposed mice infected with influenza virus displayed increased tissue damage, a profound increase in lung inflammation and a reduced cellular immune response. These studies suggest a capacity for vapourised E-liquids to enhance microbrial adherence and reduce pulmonary clearance, thereby disrupting pulmonary homeostasis between the airway mucosa and the microbial environment, and possibly predisposing vapers to respiratory infections.

There is also strong evidence that immune cell function itself is supressed by EC use. Alveolar macrophages (AMs) have long been known to provide one of the first lines of defence in the lower airways against invading pathogens and function to clear infectious or foreign particles by phagocytosis. It is becoming more apparent, however, that AMs are vital in maintaining pulmonary homeostasis by their role in surfactant and cell debris clearance, regulation of pulmonary inflammation and repair of tissue damage (Joshi et al. 2018). Disruption of AM function therefore leads to disequilibrium and enhances the capacity for respiratory infection. Sputum samples from healthy participants showed that bacterial phagocytosis was impaired following exposure to EC extract in vitro (Scott et al. 2018). Using a macrophage cell line, Ween et al. (2017) showed that, similar to cigarette smoke extract, macrophage phagocytosis of non-typeable *Haemophilus influenzae* was significantly reduced when cells were exposed to apple flavoured E-liquids and nicotine (18 mg/ml). Transcriptome analysis of AMs obtained from participants who inhaled 10 puffs from a well-known E-cigarette brand revealed changes in the expression of over 60 genes, including genes involved in inflammation and immunity (Staudt et al. 2018). For example, an increase in prostaglandin E3 receptor (PTGER3) expression was observed, deletion of which in a murine model has been shown to improve pulmonary host defence and protect mice from death following invasive pneumococcal disease (Aronoff et al. 2009). These findings are supported by a murine model of pneumococcal infection, in which EC vapour-exposed mice infected intranasally with *S. pneumoniae* displayed significantly reduced pulmonary clearance, partially due to impaired AM phagocytic function (Sussan et al. 2015). AM function may also be affected in patients diagnosed with lipoid pneumonia, a condition linked to EVALI associated respiratory failure, since both are
associated with the presence of lipid-laden AMs (Viswam et al. 2018).

Similarly, neutrophil function is impaired by exposure to EC vapour. Neutrophils play a vital role in controlling and eliminating lung infections, by releasing ROS and proteases at the site of infection. They also clear pathogens by phagocytosis and by forming web-like chromatin filaments coated in antimicrobial peptides, known as neutrophil extracellular traps (NETs) (Law & Grey, 2017). Excessive NET production, however, is cytotoxic to pulmonary epithelium and is identified in several chronic lung conditions and infections (Twaddell et al. 2019). Analysis of neutrophils obtained from regular vapers by induced sputum showed an increased tendency for NET formation compared to smokers and non-smokers (Reidel et al. 2018). Reduced antimicrobial activity has also been reported; using isolated human neutrophils, Corriden et al. (2020) showed that EC vapour exposure leads to a 4.2-fold reduction in chemotaxis towards the bacterial cell wall, while Hwang et al. (2016) showed that airway neutrophils acutely exposed to EC vapour displayed reduced antimicrobial function in vitro.

**Inflammation and oxidative stress**

Airway inflammation and oxidative stress are key events in chronic conditions such as asthma, COPD and infections such as pneumonia, and can be highly deleterious to the pulmonary system. These processes all have a similar degree of inflammation from the same pool of pro-inflammatory cytokines. This response has also been demonstrated in EC use, with some pro-inflammatory effects of vaping summarised in Fig. 2. Key studies include a murine model of chronic EC exposure, in which mice exposed to EC vapour for 1 hour daily over the course of 4 months subsequently displayed some features akin to human COPD, including airway hyper-reactivity, lung tissue destruction and increased expression of pro-inflammatory cytokines in lung homogenates, such as interleukin (IL)-8 (Garcia-Arcos et al. 2016). The cells driving inflammation after EC exposure include AMs, which are observed to secrete elevated levels of pro-inflammatory cytokines and chemokines (such as IL-6, tumour necrosis factor α and IL-8) following EC extract exposure in vitro (Scott et al. 2018). Similarly,
EC-exposed human airway epithelial cells have been reported to secrete increased cytokines such as IL-6 and IL-8 (Gerloff et al. 2017). These findings in human cells are mirrored in an in vivo murine model of EC exposure (Lerner et al. 2015; Gerloff et al. 2017). It should, however, be noted, that some studies have reported limited or no increase in the release of inflammatory cytokines in EC-exposed cells (Czekala et al. 2019). A possible explanation for differences between studies is variation in EC brands, E-liquids, nicotine strength and device, all of which widely impact the potential toxicity of EC emissions (Zhao et al. 2018). For example, in a single study of 17 E-liquids from four different brands, the effect that flavouring chemicals had on cell inflammation and cytotoxicity varied widely (Gerloff et al. 2017).

Another neutrophil-derived mediator that has a major role in pulmonary host defence, is neutrophil elastase, which if unchecked has the potential to cause considerable pulmonary damage and induce the release of pro-inflammatory cytokines. The importance of neutrophils in disease is illustrated by the association between the number of pulmonary neutrophils and disease severity in COPD (Pesci et al. 1998). It is therefore of interest, that EC-exposed neutrophils in vitro release increased elastase levels compared to unexposed controls, in addition to an elevation of activation markers CD11b and CD66b (Higham et al. 2016). Indeed, broncho-alveolar lavage samples obtained from regular vapers display increased neutrophil elastase levels compared to non-vapers (Ghosh et al. 2019).

ROS production is part of normal cellular metabolism, participating in maintenance of cellular redox homeostasis under physiological conditions. Excessive ROS production, however, elevates oxidative stress and airway inflammation. EC vapour is reported to contain up to $10 \times 10^3$ free radicals per puff (Goel et al. 2015), with high oxidative potential (Miyashita et al. 2018). More recently, research has expanded to determine the effect of varying operational voltage on emissions (Zhao et al. 2018). A recent study showed that adjustable fourth generation ECs are particularly harmful, when observing a positive correlation between increasing power and ROS emissions, with emissions being comparable to cigarettes at high power (Haddad et al. 2019). In addition to emissions from ECs, ROS production also occurs in EC-exposed alveolar macrophages, further elevating airway ROS exposure.

![Image of pro-inflammatory effects of E-cigarettes](Image created with BioRender.com)
EC-induced oxidative stress was shown to be a major factor in reducing AM phagocytic function, when antioxidant N-acetyl cysteine treatment of EC-exposed AMs was shown to restore function in vitro (Scott et al. 2018). EC vapour can also induce oxidative stress and inflammation in bronchial epithelial cells (Solleti et al. 2017) as well as pulmonary endothelial cells (Solleti et al. 2017; Munakata et al. 2018).

Future research

Evidence of the harmful respiratory effects of ECs from in vitro models, animal studies and human studies is constantly emerging; however, the vaping industry is evolving at an alarming rate. The constant expansion of flavours and evolution of EC devices pose a challenge in defining the key factors responsible for the reported deleterious respiratory effects. To keep up to date with this evolving field, there is a need for EC studies to develop aerosol systems that can be modulated in a similar fashion to fourth generation adjustable voltage systems.

A need to define chemical composition and chemical combinations is also evident. For example, although vitamin E acetate has been linked to the recent EVALI outbreak in the United States, there is not enough evidence to rule out the contribution of other chemicals, and with so many flavours on the market, a need to eliminate the risk of a similar outbreak in the UK and elsewhere is urgent.

Furthermore, the chronic effect of E-liquid chemicals needs to be assessed. ECs are relatively new, and as a result, no long-term studies exist to define the effects of ECs on human respiratory health. The lack of data obtained from animal studies to investigate the health effects of chronic vaping is also somewhat surprising. It is evident that vaping products contain numerous potentially dangerous chemicals; however, unlike pharmaceutical drugs, they do not require rigorous preclinical toxicology testing before marketing. Even the main ingredients in E-liquids (PG and VG) deemed safe for consumption have not been rigorously tested for inhalation risk and evidence of harm to respiratory health has been reported.

Finally, there is a need to define the effect of vaping on adolescent lung development, with reports of EC use rising in the younger generation, as well as to understand the implications of EC use in those with respiratory conditions such as asthma.

Conclusion

The respiratory system must maintain an intricate balance to maintain pulmonary homeostasis, which involves both physiological and immune-mediated processes. Evidence continues to emerge on the potential of EC use and vaping products to disrupt this equilibrium, leading to a cascade of events that result in lung inflammation, injury, reduced host defence and increased infection risk. As the popularity of ECs continues to rise and new cases of EVALI continue to be reported, it is clear, at least in the USA, that there is an urgent need to revisit the current EC regulatory environment (Grigg, 2019). ECs have a vast appeal to non-smoking adolescents and young adults, which is a cause of great concern. The huge variety of flavours and advances in EC technology have only increased the potential deleterious effects to respiratory health and it is vital that research into the long-term implications of vaping continues.

References

Aronoff DM, Lewis C, Seregni CH, Eaton KA, Goel D, Phipps JC, Peters-Golden M & Mancuso P (2009). E-prostanoid 3 receptor deletion improves pulmonary host defense and protects mice from death in severe Streptococcus pneumoniae infection. J Immunol 183, 2642–2649.

Bakre SA, Al-Farra TS & Al-Farra S (2019). Diffuse alveolar damage and e-cigarettes: Case report and review of literature. Respir Med Case Rep 28, 100935.

Ballbé M, Martinez-Sánchez JM, Sureda X, Fu M, Pérez-Ortuño R, Pascual JA, Saltó E & Fernández E (2014). Cigarettes vs. e-cigarettes: Passive exposure at home measured by means of airborne marker and biomarkers. Environ Res 135, 76–80.

Bauld L, MacKintosh AM, Eastwood B, Ford A, Moore G, Dockrell M, Arnott D, Cheeseman H & McNeill A (2017). Young people’s use of e-cigarettes across the United Kingdom: Findings from five surveys 2015–2017. Int J Environ Res Public Health 14, 973.

Bayly JE, Bernat D, Porter L & Choi K (2019). Secondhand exposure to aerosols from electronic nicotine delivery systems and asthma exacerbations among youth with asthma. Chest 155, 88–93.

Bhat TA, Kalathil SG, Bogner PN, Blount BC, Goniewicz ML & Thanavala YM (2020). An animal model of inhaled vitamin E acetate and EVALI-like lung injury. N Engl J Med 382, 1175–1177.

Blount BC, Karwowski MP, Shields PG, Morel-Espinosa M, Valentín-Blasini L, Gardner M, Brasilton M, Brosius CR, Caron KT, Chambers D, Corstvet J, Cowan E, De Jesús VR, Espinosa P, Fernandez C, Holder C, Kuklenyik Z, Kusovschi JD, Newman C, Reis GB, Rees J, Reed C, Silva L, Seyler T, Song MA, Sosnoff C, Spitzer CR, Tevis D, Wang L, Watson C, Wewers MD, Xia B, Heitkemper DT, Ghinai I, Layden J, Briss P, King BA, Delaney LJ, Jones CM, Baldwin GT, Patel A, Meaney-Delman D, Rose D, Krishnasamy V, Barr JR, Thomas J, Pirkle JL & Group LIRIW (2020). Vitamin E acetate in bronchoalveolar-lavage fluid associated with EVALI. N Engl J Med 382, 697–705.

Bonilla A, Blair AJ, Alamro SM, Ward RA, Feldman MB, Dutko RA, Karagounis TK, Johnson AL, Folch EE & Vyas JM (2019). Recurrent spontaneous pneumothoraces and vaping in an 18-year-old man: a case report and review of the literature. J Med Case Rep 13, 283.

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Carson JL, Zhou L, Brighton I, Mills KH, Zhou H, Jaspers I & Hazucha M (2017). Temporal structure/function variation in cultured differentiated human nasal epithelium associated with acute single exposure to tobacco smoke or E-cigarette vapor. *Inhal Toxicol* **29**, 137–144.

Centers for Disease Control and Prevention (2020). Outbreak of Lung Injury Associated with E-cigarette use, or Vaping. https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html.

Cervellati F, Muresan XM, Sticozzi C, Gambari R, Montagner G, Forman HJ, Torricelli C, Maioli E & Valacchi G (2014). Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells. *Toxicol In Vitro* **28**, 999–1005.

Chaumont M, van de Borne P, Bernard A, Van Muylem A, Deprez G, Ullmo J, Starczewska E, Brik R, de Hemptinne Q, Zaher W & Debas N (2019). Fourth generation e-cigarette vaping induces transient lung inflammation and gas exchange disturbances: results from two randomized clinical trials. *Am J Physiol Lung Cell Mol Physiol* **316**, L705–L719.

Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, Sabater J, Abraham WM, Kim MD & Salathe M (2019). Electronic cigarette vapor with nicotine causes airway mucociliary dysfunction preferentially via TRPA1 receptors. *Am J Respir Crit Care Med* **200**, 1134–1145.

Corriden R, Moshensky A, Bojanowski CM, Meier A, Chien J, Nelson RK & Crotty Alexander LE (2020). E-cigarette use increases susceptibility to bacterial infection by impairment of human neutrophil chemotaxis, phagocytosis, and NET formation. *Am J Physiol Cell Physiol* **318**, C205–C214.

Crotty Alexander LE, Drummond CA, Hepokoski M, Mathew D, Moshensky A, Willeford A, Das S, Singh P, Yong Z, Lee JH, Vega K, Du A, Shin J, Javier C, Tian J, Brown JH & Breen EC (2018). Chronic inhalation of e-cigarette vapor containing nicotine disrupts airway barrier function and induces systemic inflammation and multiorgan fibrosis in mice. *Am J Physiol Regul Integr Comp Physiol* **314**, R834–R847.

Czekala I, Simms L, Stevenson M, Tschierske E, Maione AG & Walete T (2019). Toxicological comparison of cigarette smoke and e-cigarette aerosol using a 3D in vitro human respiratory model. *Regul Toxicol Pharmacol* **103**, 314–324.

Czogala J, Goniewicz ML, Fidelus B, Zielenkiewicz A, Travers MJ & Sobczak A (2014). Secondhand exposure to vapors from electronic cigarettes. *Nicotine Tob Res* **16**, 655–662.

Czóli CD, Goniewicz ML, Palumbo M, Leigh N, White CM & Hammond D (2019). Identification of flavouring chemicals and potential toxicants in e-cigarette products in Ontario, Canada. *Can J Public Health* **110**, 542–550.

England LJ, Bunnell RE, Pechacek TF, Tong VT & McAfee TA (2015). Nicotine and the developing human: a neglected element in the electronic cigarette debate. *Am J Prev Med* **49**, 286–293.

Garcia-Arcos I, Geraghty P, Baumlin N, Campos M, Dabo AJ, Jundi B, Cummins N, Eden E, Grosche A, Salathe M & Foronjy R (2016). Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. *Thorax* **71**, 1119–1129.

Gennimata S-A, Palamidas A, Kaltsakas G, Tsikrika S, Vakali S, & Gratziou C, Koulouri N (2012). Acute effect of e-cigarette on pulmonary function in healthy subjects and smokers. *Eur Respir J* **40**, 1053.

Gerloff J, Sundar IK, Freter R, Sekera ER, Friedman AE, Robinson R, Pagano T & Rahman I (2017). Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography-mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts. *Appl In Vitro Toxicol* **3**, 28–40.

Ghosh A, Cookey RD, Ghio AJ, Muhlebarg MS, Esther CR, Alexis NE & Tarran R (2019). Chronic e-cigarette use increases neutrophil elastase and matrix metalloprotease levels in the lung. *Am J Respir Crit Care Med* **200**, 1392–1401.

Ghosh S & Drummond MB (2017). Electronic cigarettes as smoking cessation tool: are we there? *Curr Opin Pulm Med* **23**, 111–116.

Goel R, Durand E, Trushin N, Prokopczyk B, Foulds J, Elias RJ & Richie JP (2015). Highly reactive free radicals in electronic cigarette aerosols. *Chem Res Toxicol* **28**, 1675–1677.

Grigg J (2019). E-cigarette regulation: getting it wrong costs lives. *Lancet Respir Med* **7**, 994–995.

Haddad C, Salman R, El-Hellani A, Talih S, Shihadeh A & Saliba NA (2019). Reactive oxygen species emissions from supra- and sub-ohm electronic cigarettes. *J Anal Toxicol* **43**, 45–50.

Hakansson AP, Orihuela CJ & Bogaert D (2018). Bacterial-host interactions: physiology and pathophysiology of respiratory infection. *Physiol Rev* **98**, 781–811.

Higham A, Rattray NJ, Dewhurst JA, Trivedi DK, Fowler SJ, Goodacre R & Singh D (2016). Electronic cigarette exposure triggers neutrophil inflammatory responses. *Respir Res* **17**, 56.

Hua M, Omaiye EE, Luo W, McWhirter KJ, Pankow JF & Talbot P (2019). Identification of cytotoxic flavor chemicals in top-selling electronic cigarette refill fluids. *Sci Rep* **9**, 2782.

Hwang JH, Lyes M, Sladewski K, Enany S, McEachern E, Mathew DP, Das S, Moshensky A, Bapat S, Pride DT, Ongkoke WM & Crotty Alexander LE (2016). Electronic cigarette inhalation alters innate immunity and airway cytokines while increasing the virulence of colonizing bacteria. *J Mol Med* **94**, 667–679.

Joshi N, Walter JM & Misharin AV (2018). Alveolar macrophages. *Cell Immunol* **330**, 86–90.

Khosravi M, Lin RL & Lee LY (2018). Inhalation of electronic cigarette aerosol induces reflex bronchoconstriction by activation of vagal bronchopulmonary C-fibers. *Am J Physiol Lung Cell Mol Physiol* **315**, L467–L475.

Kim SY, Sim S & Choi HG (2017). Active, passive, and secondhand smoking cessation tool: are we there? *Pulm Med* **2017**, 17789.

Kosmider L, Sobczak A, Fik M, Knysak J, Zaciera M, Kurek J & Goniewicz ML (2014). Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. *Nicotine Tob Res* **16**, 1319–1326.

Kumral TL, Saltürk Z, Yildirim G, Uyar Y, Berkilen G, Atar Y & Inan M (2016). How does electronic cigarette smoking affect sinonasal symptoms and nasal mucociliary clearance? *B-ENT* **12**, 17–21.
E-cigarettes and respiratory health

Landman ST, Dhillon I, Mackenzie CA, Martinu T, Steele A & Bosma KJ (2019). Life-threatening bronchiolitis related to electronic cigarette use in a Canadian youth. *CMAJ* **191**, E1321–E1331.

Larcombe AN, Janka MA, Mullins BJ, Berry LJ, Bredin A & Franklin PJ (2017). The effects of electronic cigarette aerosol exposure on inflammation and lung function in mice. *Am J Physiol Lung Cell Mol Physiol* **313**, L67–L79.

Law SM & Gray RD (2017). Neutrophil extracellular traps and the dysfunctional innate immune response of cystic fibrosis lung disease: a review. *J Inflamm* **14**, 29.

Layden JE, Ghinai I, Pray I, Kimball A, Layer M, Tenforde M, Navon L, Hoots B, Salvatore PP, Elderbrook M, Haupt T, Kanne J, Patel MT, Saathoff-Huber L, King BA, Schier JG, Mikosz CA & Meiman J (2019). Pulmonary illness related to e-cigarette use in illinois and wisconsin—preliminary report. *N Engl J Med* **382**, 903–916.

Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, Larcombe AN, Janka MA, Mullins BJ, Berry LJ, Bredin A & Landman ST, Dhaliwal I, Mackenzie CA, Martinu T, Steele A & Weinberg C (2019). Neutrophil extracellular traps and the dysfunctional innate immune response of cystic fibrosis lung disease: a review. *J Inflamm* **14**, 29.

Lieber M, Smith B, Szakal A, Nelson-Rees W & Todaro G (1976). A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int J Cancer* **17**, 62–70.

Madison MC, Landers CT, Gu BH, Chang CY, Tung HY, You R, Hong MJ, Baghaei N, Song LZ, Porter P, Putluri N, Salas R, Gilbert BE, Levental I, Campen MJ, Corry DB & Kheradmand F (2019). Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. *J Clin Invest* **129**, 4290–4304.

Miyashita L, Suri R, Dearing E, Mudway I, Dove RE, Neill DR, Robinson R & Rahman I (2015). Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One* **10**, e0116732.

Miyashita L, Suri R, Dearing E, Mudway I, Dove RE, Neill DR, Robinson R, Hong MJ, Baghaei N, Song LZ, Porter P, Putluri N, Salas R, Gilbert BE, Levental I, Campen MJ, Corry DB & Kheradmand F (2019). Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. *J Clin Invest* **129**, 4290–4304.

Pesci A, Majori M, Cuomo A, Borciani N, Bertacco S, Cacciani G & Gabrielli M (1998). Neutrophils infiltrating bronchial epithelium in chronic obstructive pulmonary disease. *Respir Med* **92**, 863–870.

Pierce JS, Abelman A & Finley BL (2016). Comment on “Flavoring chemicals in e-cigarettes: diacetyl, 2,3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes”. *Environ Health Perspect* **124**, A100–A101.

Pisinger C & Dossing M (2014). A systematic review of health effects of electronic cigarettes. *Prev Med* **69**, 248–260.

Protano C, Avino P, Manigrasso M, Vivaldi V, Perna F, Valeriani F & Vitali M (2018). Environmental electronic vapor exposure from four different generations of electronic cigarettes: airborne particulate matter levels. *Int J Environ Res Public Health* **15**, 2172.

Rubinstein ML, Delucchi K, Benowitz NL & Ramo DE (2018). Adverse health outcomes following e-cigarette use: a review of the literature. *Front Sci Rep* **6**, 23984.

Rubinstein ML, Delucchi K, Benowitz NL & Ramo DE (2018). Adverse health outcomes following e-cigarette use: a review of the literature. *Front Sci Rep* **6**, 23984.

Sleiman M, Logue JM, Montesinos VN, Russell ML, Litter MI, Gundel LA & Destaillats H (2016). Emissions from electronic cigarettes: key parameters affecting the release of harmful chemicals. *Environ Sci Technol* **50**, 9644–9651.

Solleti SK, Bhattacharya S, Ahmad A, Wang Q, Mereness J, Rangasamy T & Mariani TJ (2017). MicroRNA expression profiling defines the impact of electronic cigarettes on human airway epithelial cells. *Sci Rep* **7**, 1081.
Spindel ER & McEvoy CT (2016). The role of nicotine in the effects of maternal smoking during pregnancy on lung development and childhood respiratory disease. Implications for dangers of e-cigarettes. *Am J Respir Crit Care Med* **193**, 486–494.

Stanley PJ, Wilson R, Greenstone MA, MacWilliam L & Cole PJ (1986). Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. *Thorax* **41**, 519–523.

Staudt MR, Salit J, Kaner RJ, Hollmann C & Crystal RG (2018). Altered lung biology of healthy never smokers following acute inhalation of E-cigarettes. *Respir Res* **19**, 78.

Sussan TE, Gaighate S, Thimmulappa RK, Ma J, Kim JH, Sudini K, Consolini N, Cormier SA, Lommicki S, Hasan F, Pekosz A & Biswal S (2015). Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model. *PLoS One* **10**, e0116861.

Talih S, Salman R, El-Hage R, Karam E, Karraghlanian N, El-Hellani A, Saliba N & Shihaedeh A (2019). Characteristics and toxicant emissions of JUUL electronic cigarettes. *Tob Control* **28**, 678–680.

Terzano C, Di Stefano F, Conti V, Graziani E & Petroiani A (2010). Air pollution ultrafine particles: toxicity beyond the lung. *Eur Rev Med Pharmacol Sci* **14**, 809–821.

Twaddell SH, Baines KJ, Grainge C & Gibson PG (2019). The emerging role of neutrophil extracellular traps in respiratory disease. *Chest* **156**, 774–782.

Tzortzi A, Teloniatis S, Matiampa G, Bakelas G, Tzavara C, Vyzikidou VK, Vardavas C, Behrakis P, Fernandez E & Investigators TP (2020). Passive exposure of non-smokers to E-Cigarette aerosols: Sensory irritation, timing and association with volatile organic compounds. *Environ Res* **182**, 108963.

Tzortzi A, Teloniatis S, Matiampa G, Bakelas G, Vyzikidou, V, Vardavas, CI, Behrakis P, Fernandez E (2018). Passive exposure to e-cigarette emissions: Immediate respiratory effects. *Tob Prev Cessation* **4**, 18.

Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulos V, Connolly GN & Behrakis PK (2012). Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest* **141**, 1400–1406.

Viswam D, Trotter S, Burge PS & Walters GI (2018). Respiratory failure caused by lipoid pneumonia from vaping e-cigarettes. *BMJ Case Rep* **2018**, 224350.

Walley SC, Wilson KM, Winickoff JP & Groner J (2019). A public health crisis: electronic cigarettes, vape, and JUUL. *Pediatrics* **143**, e20182741.

Ween MP, Whittall JJ, Hamon R, Reynolds PN & Hodge SJ (2017). Phagocytosis and inflammation: Exploring the effects of the components of E-cigarette vapor on macrophages. *Physiol Rep* **5**, e13370.

Williams M & Talbot P (2019). Design features in multiple generations of electronic cigarette atomizers. *Int J Environ Res Public Health* **16**, 2904.

Wu Q, Jiang D, Minor M & Chu HW (2014). Electronic cigarette liquid increases inflammation and virus infection in primary human airway epithelial cells. *PLoS One* **9**, e108342.

Zhao J, Nelson J, Dada O, Pyrgiotakis G, Kavouras IG & Demokritou P (2018). Assessing electronic cigarette emissions: linking physico-chemical properties to product brand, e-liquid flavoring additives, operational voltage and user puffing patterns. *Inhal Toxicol* **30**, 78–88.

Additional information

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None declared.

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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