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REVIEW

The effect of e-cigarette aerosol emissions on respiratory health: a narrative review

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ABSTRACT

Introduction: Due to the uptake in the use of e-cigarettes (ECs), evidence on their health effects is needed to inform health care and policy. Some regulators and health professionals have raised concerns that the respirable aerosols generated by ECs contain several constituents of potential toxicological and biological relevance to respiratory health.

Areas covered: We critically assess published research on the respiratory system investigating the effects of ECs in preclinical models, clinical studies of people who switched to ECs from tobacco cigarettes, and population surveys. We assess the studies for the quality of their methodology and accuracy of their interpretation. To adequately assess the impact of EC use on human health, addressing common mistakes and developing robust and realistic methodological recommendations is an urgent priority. The findings of this review indicate that ECs under normal conditions of use demonstrate far fewer respiratory risks than combustible tobacco cigarettes. EC users and smokers considering ECs have the right to be informed about the relative risks of EC use, and to be made aware that findings of studies published by the media are not always reliable.

Expert opinion: Growing evidence supports the relative safety of EC emission aerosols for the respiratory tract compared to tobacco smoke.

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1. Introduction

The use of electronic cigarettes (ECs) has significantly increased over the past decade. These consumer products have been rapidly gaining ground on conventional cigarettes due to their efficiency in reducing tobacco consumption, their competitive price, the consumer perception of EC as a less harmful alternative to smoking, and because EC provide 'smoking experience without smoking' [1–3]. Earlier designs have evolved over the past decade, and now the devices are available in multiple formats and models. Basically, ECs are battery powered electronic devices that operate by heating an element (most commonly, a metal coil) that vaporizes a solution (e-liquid) mainly consisting of glycerol, propylene glycol (PG), distilled water, and flavorings, and which may or may not contain nicotine. The user inhales the aerosol generated by vaporizing the e-liquid in a process commonly referred to as 'vaping.' ECs do not contain tobacco, do not create smoke, and do not rely on combustion to operate.

The composition of EC aerosol is by far less complex than that of cigarette smoke [4–10], which – by contrast – is known to contain thousands of harmful and potentially harmful constituents [11]. Some of main toxins identified in cigarette smoke are also present in EC aerosol emissions, but at much lower levels than in cigarettes smoke, and often at exposure levels no greater than present in the general environment.

EC use is regarded as having lower levels of risks than smoking as reported by the Royal College of Physicians [12], Public Health England [13] and others [14,15]. The RCP estimates that ECs are at least 95% less harmful than conventional cigarettes, although there is concern that long-term exposure to EC aerosol emissions could carry some health risks. Because the particle size in EC aerosols is well within the respiratory range [16,17], aerosol particles will penetrate into the lungs [18] making the airways the primary target of any potentially harmful effects.

Unfortunately, very little is known about the health effects of long-term vaping and this is of particular concern for those never smokers who have just started using ECs. Therefore, studies of the toxicological and biological effects of EC aerosol emissions, using in vitro human airway cell systems, animal models, and clinical studies, are needed to investigate the potential health risks of using these devices. Whether or not chronic exposure to EC will result in lung disease can only be evaluated by large scale, long-term studies of daily EC users who have never smoked in their life, a study that would be challenging to carry out at present, as most EC users have had prior or current cigarette smoke exposure. It goes without saying that nicotine consumption must be actively discouraged among youths, and – besides smoking – this includes vaping as well.

In this review article, we critically appraise published studies that have investigated the potential toxic effects of ECs using preclinical models, such as cell culture, animal models,

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and clinical studies (with the exclusion of case reports). Preclinical studies may not fully predict the response of the human body to the exposure, therefore animal studies are still required for regulatory toxicological testing. Significant technological advances with in vitro models are slowly being acknowledged as acceptable alternatives. With these methodological issues in mind, we present an overview of the emerging literature on respiratory health findings. The narrative review begins with a description of EC aerosols followed by discussions of the literature grouped by research type: cell testing, animal studies, and research on respiratory health.

2. Constituents of concern in EC aerosols

Upon activation, ECs generate respirable aerosols [16–18] containing several constituents of potential toxicological and biological relevance to respiratory health, but at much lower levels than in cigarette smoke [6–9]. One of the most comprehensive assessment of EC chemical emissions has shown that of the 150 constituents examined in the EC aerosol (including all tobacco smoke harmful and potentially harmful constituents, and additional toxic species reportedly present in EC emissions) 104 were not detected and 21 were present due to laboratory background [9]. Of the 25 remaining detected aerosol constituents, 9 were present at levels too low to be quantified. Only 16 were generated in whole or in part by the EC from: i) major e-liquid constituents (nicotine, PG, and VG); ii) recognized impurities in Pharmacopoeia-quality nicotine; and iii) 8 thermal decomposition products of PG or VG. By contrast, approximately 100 constituents were detected in mainstream cigarette smoke. The emissions of toxicants were from 82 to >99% lower on a per-puff basis from the EC compared with those from tobacco cigarettes. Although the aerosol from the EC is compositionally less complex than cigarette smoke and contains significantly lower levels of toxicants, a thorough characterization of EC chemical emissions will require non-targeted analytical assessments of a wide range of commercial products.

Among the commonly detected aerosol constituents, glycerol, PG and their thermal degradation products (i.e. carbonyl compounds), chemical flavorings, nicotine, and metals have attracted most attention. The US Food and Drug Administration (FDA) and the US Environmental Protection Agency (EPA) categorize vegetable glycerine (VG) and PG as Generally Recognized as Safe [19]. Although PG can be also found in cigarette smoke, high levels are normally present in EC aerosol emissions. Hence, it is necessary to have a better understanding of PG's safety by inhalation. All animal and human studies that analyzed the effect of the inhalation of PG have indicated that PG does not appear to pose a significant hazard via the inhalation route [20]. In fact, in several of these animal studies the concentrations of PG used were higher than the concentration used in EC and did not give rise to any toxic effects. However, human studies using PG concentrations similar to that in ECs are required to confirm the safety of inhalation of PG from vaping products.

Despite their good safety profile, exposure to glycerol and PG aerosols has been shown to elicit some irritant effects [21–23]. The possibility that chronic airway irritation may have long term consequences cannot be dismissed, and more research in this area is required. Additionally, PG, has been implicated as a potential cause of oral allergic contact dermatitis [24], and some users may report signs and symptoms compatible with contact dermatitis around the mouth or in the oral mucosa [25].

Thermal degradation of glycerol and PG during EC vaporization may generate toxic carbonyls including formaldehyde, acetaldehyde, and acrolein. Nevertheless, studies evaluating low-power cigalike and closed-system modular EC devices found formaldehyde, acetaldehyde, and acrolein at much lower levels than in cigarette smoke [8,9,26]. Aerosol generated from more-advanced high-power devices may produce levels of aldehydes approaching or even exceeding those of cigarette smoke [27]. However, it is now known that high aldehyde levels can be generated only when the EC is overheated, a condition generally seen in certain experimental protocols, and as a result these findings bear little relevance to normal use [28]. EC users find the taste delivered from overheated EC, known as 'dry puff,' to be so unpleasant that they cannot inhale the aerosol [29], thus avoiding potential exposure to high levels of aldehydes. Under normal vaping conditions, the aldehyde emission levels are far lower than in cigarette smoke and lower than the levels found in the environment [30,31]. Daily exposure from vaping (assuming a daily consumption of 5 g EC liquid) was 5 to 31-fold lower for formaldehyde, 191 to 528-fold lower for acetaldehyde and 25 to 193-fold lower for acrolein compared to daily smoking (assuming a daily consumption of 20 tobacco cigarettes). This represents a 79.0–96.8% reduction in formaldehyde, 99.5–99.8% reduction in acetaldehyde and 96.0–99.5% reduction in acrolein exposure from EC use (5 g/day liquid consumption) compared to smoking 20 tobacco cigarettes. Aldehydes such as formaldehyde are ubiquitous in the environment. According to the World Health Organization, indoor air of homes can have up to 250 $\mu g/m^3$ formaldehyde, but the average levels are under 50 $\mu g/m^3$. Therefore, considering a daily ventilation volume of 20 m^3, the daily formaldehyde exposure from breathing indoor air is approximately 1000 µg. This level is far higher than the total daily exposure from consuming 5 g of e-liquids. Moreover, newer devices are being fitted with better wicking designs (e.g. bottom coil vs top coil) and some have been fitted with automatic temperature control features to prevent overheating and excessive formation of carbonyls. Nonetheless, the possibility that temperature control features in some current devices may not perform accurately cannot be discounted [32].

Food flavorings are normally present in e-liquids. These chemicals have largely unknown effects when heated and inhaled. Chronic exposure to high levels of diacetyl, a butter flavoring processed in microwave popcorn factories, has been associated with cases of bronchiolitis obliterans ('popcorn lung') [33,34]. Although some e-liquids contain high concentrations of diacetyl [35,36], there have been no reports that this has caused bronchiolitis obliterans in EC users. Cigarette smoke also contains diacetyl, but at much higher levels (up to 750 times higher) than are found in EC aerosol [37]. Yet, cigarette smoke has not been linked conclusively with bronchiolitis obliterans as stated by the US Occupational Safety and Health Administration [38]. Nonetheless, it is reasonable to

assume that flavoring chemicals (or their thermal degradation products) in EC aerosol could have potential risks. Besides possible toxic effects upon the lung from chronic exposures, such as bronchiolitis obliterans, other respiratory effects to be studied should include respiratory irritation and potential allergic responses [39,40].

Most if not all of the lung damage observed in smokers is not caused by nicotine, but from the process of burning tobacco cigarette and the inhalation of thousands of toxic chemicals generated during combustion. The development of smoking-related diseases is currently attributed to oxidative stress, airway inflammation, and the direct toxic effects of thousands of chemicals and carcinogens present in tobacco smoke [41]. Nicotine is not classified as a carcinogen by the International Agency for Research on Cancer [42] and is relatively safe for human consumption at low concentrations [43]. The 2014 US Surgeon General's report examined the harm caused by nicotine and concluded that although nicotine may adversely affect fetal and adolescent brain development, it does not contribute to smoking-related diseases [44]. In terms of nicotine delivery, earlier ECs designs are generally less efficient than conventional cigarettes at delivering nicotine to the body [45–47], but the most recent and innovative EC devices have been reported to deliver nicotine at levels equivalent to those obtained from cigarettes [48].

Most likely class of compounds that may be found in some EC aerosols but not usually found in cigarette smoke (depending on materials used for the heating element in the vaping device – will be metals that may leach from metals and alloys sometimes used in ECs. Cigarette smoke also contains metals present in the tobacco leaves. When evaluating the hazardous potential of metals in EC aerosol, it must be noted that daily exposure levels from EC use are many order of magnitude lower compared to acceptable exposure from inhalational medications and by orders of magnitude lower than the regulatory limits for daily occupational exposure. Health risk assessment analyses show that levels of metals exposure from EC use were of minimal apparent health concern [49].

The key points about EC aerosols are that 1) EC aerosols contain constituents of concern, including formaldehyde, acetaldehyde, and acrolein, although, in almost every case, at substantially lower levels than cigarette smoke; 2) in the testing where higher levels were measured, the devices had been overheated, a condition very unlikely to occur in normal use; 3) the pyrolysis of flavouring components needs to be studied, as well as additional research on the base e-liquid components; and 4) prior research has already demonstrated that nicotine, which may or may not be a component in e-liquid, is not a carcinogen. With these facts in mind, we review the studies on EC research in cells cultures, animal models, and human subjects.

3. Effects of EC aerosols on airway cell cultures

The potential for EC toxicity has been investigated by exposing cell cultures to e-liquids or to aerosol generated by ECs. In general, these studies could help to gain insight into the biologic and toxicological effects of EC aerosols. These effects have been studied using a wide range of cell types, including neutrophils [50], macrophages [51], embryonic stem cells [52–54], murine fibroblasts [55], carcinoma cell lines [56,57], human endothelial cells [58], and lung fibroblasts [52,59,60]. The airway epithelium is primarily and extensively exposed to the aerosol emissions of ECs, so our focus will be on studies using human airway epithelial cells [61–64].

As there are many cell types available to researchers, it is important to be aware of their different sensitivity and responses across the test matrix. Differences in cellular metabolism, apoptotic rates and genetic characteristics can contribute to the observed variability in results between cell lines. For example, one study examined the response of several cell types to EC exposures to identify an appropriate test system [65]. Both A549 cells and the CL-1548 cell line showed reduced sensitivity (in term of cell viability) to e-liquid aerosol compared to primary NHBE cells, with increased sensitivity of CL-1548 compared with A549 cells. In another study [63], researchers investigated lung cell line, BEAS2B; the responses observed were similar to those in other cell lines. Strangely, in this study there was variability in the quantity of e-liquid consumed between identical EC exposure experiments.

The use of 3D tissue systems, whether 'home grown' or commercially available cultures, is becoming more routinely used for inhalation toxicology studies, due to improved tissue reliability and stability and the cost of production. A number of studies [64–66] have used 3D differentiated immortalized primary normal HBEC for EC assessment. Differentiated normal HBEC were exposed at the air–liquid interface (ALI) to EC aerosols (with or without nicotine), PG, VG, and reference 3R4F cigarette smoke, in a CULTEX® RFS compact module. Cigarette smoke led to eight times lower cell viability and five times higher oxidative stress than EC [64]. A shortcoming of this study is that it lacked a standard puffing regime and a standard protocol for aerosol generation.

In another study, Aufderheide et al. [66] repeatedly exposed differentiated immortalized primary HBEC (CL-1548) to cigarette smoke and EC aerosol to evaluate phenotypic changes associated with respiratory disease (e.g. COPD). Cultures exposed to mainstream cigarette smoke and e-cigarette vapor showed a clear reduction in mucus-secreting cells and their secretion activity as well as in cilia beating, with the effect less pronounced for the cells treated with the e-liquid aerosol. These observations suggest that EC aerosols may have a reduced risk for respiratory disease compared to cigarette smoke.

Shen et al. [67] conducted RNA sequencing analysis on differentiated normal HBEC exposed to 1R5F tobacco reference cigarettes and EC aerosols (with or without nicotine) at the ALI. Cigarette smoke elicited differential gene expression and cell cytotoxicity, but EC aerosol provoked less response. Neilson et al [68] reported on the use of the commercial 3D tissue culture EpiAirway™ (MatTek) to assess the irritancy of EC aerosols generated with the Vitrocell® VC1 system, and cigarette smoke exposure reduced cell viability to 12% while cells remained viable with EC exposure. The limitation of this study is the short exposure to EC aerosol that may minimize overall

toxicity. Other researchers used the 3D commercial tissue culture MucilAir™ (Epithelix) to evaluate EC toxicity. Matched nicotine doses of EC aerosol and cigarette smoke were tested with short repeated exposures [69] or a single acute exposure [70] to assess changes in gene-expression profiles using RNA sequencing. In both exposure studies, the more substantial changes in gene expression were observed with cigarette smoke than with EC aerosol. Even when the EC nicotine dose was doubled, the gene expression profiles remained significantly lower than those with cigarette smoke exposures. These studies were characterized by a short exposure to EC aerosol which minimized overall harmfulness.

The lack of high level of gene expression after EC aerosol exposure in the aforementioned studies contrasts with recent work in which nasal scrape biopsies, nasal lavage, urine, and serum from nonsmokers, cigarette smokers, and EC users were assessed for changes in immune gene expression profiles [71]. The authors found that vaping ECs resulted in decreased expression of immune-related genes similar to that from smoking cigarettes. However, little consideration was given to the fact that previous exposure to tobacco smoking in the vapers group (vapers are ex-smokers) would have induce irreversible epigenetic/gene expression changes [72]. Given that all EC users in the study were former smokers, it is impossible to decouple the effects of EC aerosol emission from those of previous tobacco smoke exposure. Also, the observed association between e-cigarette use and changes in gene expression does not imply causation given its cross-sectional design. Obviously, a longitudinal study of regular vapers who have never smoked in their life would have been more appropriate to establish potential causation of the e-cigarette exposure effect on gene expression and related clinical implications.

The same methodological shortcoming occurs in another cross-sectional design study which investigated markers of innate lung responses in sputum samples from smokers, EC users and non-smokers [73]. The authors concluded that EC use and smoking alters the profile of innate defence proteins, but failed to consider the obvious confounder of previous andcurrent exposure to tobacco smoke among EC users who are ex-smokersor dual users). Proteomic analysis of sputum supernatants in former smokers has shown high levels of azurocidin 1, neutrophil elastase and CXCL8 [74]. Moreover, mucin concentrations are known to be elevated both in current and former COPD smokers with MUC5B and MUC5AC levels being approximately 3-fold (for current COPD smokers) and 10-fold (for former COPD smokers) higher than in controls who had never smoked [75]. It is important to consider the within subject instability of sputum endotypes due to the large variability observed with difference of the order of 1000 in the levels of analysed markers). The log-scales in the y-axis are typically used to make up for this variability.

Ghosh et al. [76] performed a proteomic investigation of bronchial brush biopsies and bronchoalveolar lavage obtained with a bronchoscopy of healthy non-smokers, cigarette smokers, and EC users, and found that ~300 proteins were differentially expressed in smokers and vapers airways. This paper is also subject to the same methodological flaws noted earlier, as vapers in the study were mostly dual users and the exclusive EC users were ex-smokers.

The least sophisticated studies expose two-dimensional (2D) submerged cellular systems to e-liquids, not aerosols. They employ continuous or immortalized cell lines or primary cells isolated from either human or animal tissues. These studies enable researchers to screen a large number of e-liquids using basic endpoints such as cell viability. Cytotoxic effects are measured using commonly used assays. One measurement is neutral red uptake where viable cells can take up neutral red via active transport whereas non-viable cells cannot so that the amount of released dye can be measured to determine the total number of viable cells. Thus estimating cytotoxicity. Another measurement is lactate dehydrogenase release (LDH) where a cytosolic enzyme is released into cell culture media to show plasma membrane damage and MTT (Abbreviation for the dye compound 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor; a marker of cell proliferation).

In one study [52], effects of 35 e-liquids were screened using human embryonic stem cells (hESC), mouse neural stem cells (mNSC), and human pulmonary fibroblasts (hPF). The MTT assay was used to determine NOAELs (no-observed-adverse-effect-level) and half maximal inhibitory concentration values, IC50s. It was found that hESC and mNSC were more sensitive to e-liquids than hPF. In other findings, the researchers attributed cytotoxicity to some flavors, but not to nicotine or PG/VG [52]. In an extension of this study, highly flavored e-liquids were examined and the researchers identified cinnamaldehyde, an ingredient in cinnamon-flavored e-liquids, as of particular toxicological concern [59]. In a later paper by the same group, cytotoxicity was also observed by aerosolized forms of these liquids, but in this case it was ascribed to VG/PG thermal degradation products (i.e. carbonyl compounds) rather than to flavourings [53].

Lerner et al. [60] also reported oxidative stress and cell morphological changes in human lung fibroblasts (hLF) in response to e-liquids, particularly cinnamon flavored e-liquids. that stimulated IL-8 secretion and caused loss of cell viability. They suggested that e-liquids have oxidative properties, and that sweet or fruit flavors made the e-liquids even stronger oxidizers. In the same study, exposure of human airway epithelial cells (H292) to EC aerosol at the ALI led to an increase in inflammatory cytokines. The authors explored the contribution of flavorings from e-liquids to lung barrier function and inflammation in human bronchial epithelial cells. In these studies, acetoin, diacetyl, pentanedione, maltol, ortho-vanillin, coumarin, and cinnamaldehyde, all flavors found in e-liquids, did not affect cell viability [77]. The conflicting results are due to lack of a standardized approach to in vitro toxicology assessments of vaping products (e.g. e-liquid vs EC aerosols, fibroblasts vs epithelial cells, etc).

Alveolar macrophages (AMs) are a unique lung cell population that eliminate airborne irritants and infectious agents, while also orchestrating resolution of lung inflammation, and impairments in AM function could therefore enhance susceptibility to airway infections and respiratory diseases [78]. Scott et al. [79] have shown that human AMs exposed to EC aerosol condensate increased cytotoxicity, ROS production, inflamma-

tory cytokines and inhibited their phagocytic activity, as would be expected given that EC aerosol contains oxidant and other pro-inflammatory constituents. Since EC are used almost exclusively by current or former smokers, the key question is how this adverse effect compares with that of exposure to cigarette smoke. Unfortunately, this study does not address that question.

High-content screening platforms provide useful tools for the initial hazard characterisation of e-liquids to help determine what toxic end points might need further investigation. Use of real-time cell analyzer (RTCA) technology (such as the xCELLigence platform) enables the screening of cytotoxic effects of e-liquid flavors on immortalized HBEC. Two studies have used these methods. First, Sherwood and Boitano [80] demonstrated that the different flavors tested varied in their cytotoxicity profiles, with vanillin and 2,5-dimethylpyrazine causing abnormalities in transepithelial resistance and ion conductance. This effect may have negative consequences for airway surface liquid homeostasis in individuals who use ECs regularly. Second, Iskandar et al. [81] discussed the utility of high-content screening of e-liquids using multiple basic endpoints, including cell viability, oxidative stress and cellular function on a Cellomics platform.

Omics tools have also been applied to investigate the global impact of e-liquids has in cellular systems. One study utilized untargeted metabolomics on primary normal human bronchial epithelial cells (HBEC) treated with e-liquids and cigarette smoke condensates demonstrated shifts in the HBEC metabolome following treatment. E-liquid exposure provoked a smaller response than cigarette smoke [82].

In addition to studies on toxicity, e-liquids have also been investigated for immune defense responses. Wu et al. [83] demonstrated that cultures of human airway epithelial cells infected with human rhinovirus increased viral load and production of anti-viral proteins with e-liquid treatment. Although cell viability was not altered, the data suggest that e-liquids could impair immune host defense.

These studies have shown a response to e-liquid exposure regardless of the cell systems and that some cell systems are more responsive than others. These studies have been criticized as not being representative of exposures under normal conditions of use, for example testing high doses and using continuous exposure protocols, in some cases as long as 48 hours. Also, some of these studies did not compare the effects of EC with conventional tobacco products. Tobacco products in most assays elicit significantly higher responses than ECs, as observed by Misra et al. [84]. A549 human lung epithelial carcinoma cells were exposed to e-liquids and aerosol extracts of e-liquids with or without nicotine and menthol flavoring, or to 3R4F total particulate matter (TPM). No cytotoxic, genotoxic, or inflammatory effects were observed for any of the EC treatments under investigation, whereas comparable exposures to cigarette smoke extracts resulted in markedly cytotoxic and genotoxic responses [84]. One obvious limitation of this study is the use of A549 cells that are known to be more resistant to cytotoxic stimuli. Moreover, the use of submerged cell monolayers does not reflect the real exposure of airway cells to EC aerosol.

In real life, exposures from ECs come from aerosols, not e-liquids. Therefore, exposing the cells and tissues to aerosol is a more appropriate way to investigate the biological responses to ECs. Extracts of aerosols can be generated by either collecting EC aerosol particulate matter onto a Cambridge filter pad [85] or generating aqueous extracts (AqEs) of aerosols by bubbling them through cell culture media [86] or by directly exposing cell cultures to EC aerosol emissions [54]. These exposures enable the delivery of more appropriate and realistic doses of EC.

Taylor et al. [86] investigated oxidative stress responses in H292 cells exposed to AqEs from ECs and 3R4F reference cigarettes. As expected, the authors found that 3R4F induced significant oxidative stress, whereas no responses were observed with the AqEs from ECs. In this study, the quality control of AqE generation and nicotine quantification was integral to the understanding of the cellular responses and should be the best practice method for generating AqEs, but underestimation of the overall risk could have resulted from the use of a tumour lung cell line (H292) in submerged culture and from the fact that only soluble components of the EC aerosol are present in AqEs,

Yu et al. [56] used several cell types, including human head and neck squamous cell carcinoma cell lines HN30 and UMSCC10B, to assess the potential of EC to cause DNA damage and cell death compared to cigarette smoke. The authors generated AqEs by drawing EC aerosol (with or without nicotine) or cigarette smoke through media. EC-exposed cells were reported to show significantly reduced cell viability, clonogenic survival and DNA strand breaks, regardless of EC nicotine content. However, in this study the authors neglected to report how the extracts were made, how many puffs were taken, how much aerosol volume was collected, and how the extract was quantified and/or normalized to a constituent (e.g. nicotine). Without this information, it is difficult to reach meaningful conclusion.

Currently there are no standard protocols for the generation of AqEs for in vitro studies, and various methods are used. Taylor et al. [86] drew 10 puffs from ECs into 20 ml cell culture media under CORESTA CRM81 regime [87]. Other studies include bubbling 200 mg of vaporized e-liquid into 20 ml of cell culture media [55] or 50 ml EC aerosol bubbled through 10 ml cell culture media [88]. Irrespective of how the extract is made, it should be quality checked and assessed for nicotine content as a minimum to enable comparisons across studies and biological effects.

Over the years there have been advances to in vitro exposure systems. These systems have predominately been used to generate, dilute, and deliver aerosols, such as cigarette smoke, environmental particulates, and nanoparticles, to cellular cultures. Cell culture technologies to investigate aerosol properties have also evolved to enable air-liquid interface (ALI) 2D cell and three-dimensional (3D) tissue culture exposures. A number of reviews have outlined the value of commercially available systems, such as the Borgwaldt RM20S and the Vitrocell® VC1 and VC10 systems [89,90], and these tools have been adapted to be used for in vitro assessment of EC aerosol emissions. The problem is that these systems are expensive and as a consequence few entry-level scientists, academics, and new research groups have access to them. Instead, a plethora of poorly standardized, individually

made systems have been used to generate and deliver aerosols instead. The CRM81 regimen [87] has been suggested for EC aerosol generation, but is often overlooked or difficult to implement on a basic aerosol exposure system.

An example of an individually made system is Cervellati et al. [91]. The researchers used an exposure system that draws aerosols over cellular cultures using a vacuum pump, and the amount of aerosol and duration of exposure were not reported. Based on this unreliable procedure the authors conclude that unflavored EC aerosols produced less cytotoxicity than flavored EC aerosols and cigarette smoke. Similarly, Lerner et al [92], used a timer system to control a basic laboratory pump, and they too did not report the dosimetry and robustness of aerosol delivery. They observed an increase in inflammatory and mitochondrial stressors, but due to the basic generation system comparisons cannot be made with consumer usage or data from other reported studies.

Commercially available exposure systems have been used in studies to assess the cellular toxicity of e-cigarettes. When H292 were exposed for 1 hr to EC aerosol using the Borgwaldt RM20S, less cytotoxicity was observed compared to cigarette smoke [61]. The exposures were consistent to human exposures, and with the EC, the maximum concentration delivered was equivalent to a daily dose delivered within one exposure. A limitation of this study is that it focused on only one biological endpoint (cell viability). Other cytotoxicity endpoints could be effected differently.

Thorne et al. [93] reported that cigarette smoke induced DNA damage in BEAS2B at the ALI using a Vitrocell® VC10 system, in a dose dependent manner, whereas short-term exposure with two different ECs did not result in e any DNA damage, even at equivalent or greater doses than cigarette smoke aerosol. Using quartz crystal microbalance technology, nicotine delivery and deposited mass were both assessed at the exposure interface and demonstrated that EC exposures were 12–28 times greater than cigarette smoke. In another study, H292 cells were used to test aerosol from various types of EC devices, or a tank system with different e-liquid flavors, variable nicotine concentrations, and with modified battery output voltage, compared to cigarette smoke [62]. Exposure to EC aerosol resulted in decreased metabolic activity and cell viability and increased inflammatory cytokine levels, but all these factors were more adversely affected by exposure to cigarette smoke.

These studies indicate that product type, battery output voltage, and flavors significantly affected EC toxicity, with strawberry flavor being the most cytotoxic. However, the final flavour in an e-liquid is achieved by the combination of a variety of individual flavor ingredients. This can vary from just a few ingredients in a simple flavour to over a hundred ingredients and constituents in complex flavours. Without information on e-liquid compositions, no conclusions on the relative potency of individual flavour ingredients can be drawn. For the vast bulk of the in vitro papers published in this field, insufficient dose information and lack of information on e-liquid composition does not allow interpretation of the findings to consumer exposures.

Overall, a large number of in vitro studies have been published relating to the cytotoxic effects of vaping products and e-liquids, but we have identified many methodological limitations. The relevance of data obtained from direct e-liquid exposures, instead of aerosol exposures is questionable. Intuitively, aerosol exposure is more relevant to the real-life situation. However, it must be considered that there are no standard procedures for the generation of aerosol extracts (AqEs) or aerosols, and that custom-made exposure systems may not be able to generate consistent and reproducible aerosols. Yet another methodological shortcoming is that the effect of device liquid interactions is generally overlooked, and the device type and make, the e-liquids used, and the device settings are often not fully reported. Given the wide range of devices in the market place, the relevance of outcomes from any one single liquid-device combination cannot be extrapolated to vaping in general.

For robust findings that apply to real life vaping, in vitro exposures need to be contextualized with normal conditions (user exposures) and require the assessment of key dosimetry markers, such as nicotine or glycerol ratio, to 'normalize' exposures. Appropriate comparators must also be incorporated to be able to understand the effects attributed to ECs. Finally, the reliability and reproducibility of test systems need to be considered to ensure that the dynamic range captures EC and cigarette responses appropriately. Despite their limitations, studies on cellular systems and exposure systems offer a vast opportunity for researchers to compare responses to different e-liquids and aerosols and to understand the mechanistic pathways that are associated with biological effects and thus inform the hazard identification and characterization aspect of risk assessment.

4. Effects of EC aerosols on animal models

Despite the opportunities noted above, in vitro models have limitations, as they cannot fully predict the response of the human body to exposures. Although animal models have other shortcomings, they at least represent the complexity found in human bodies and are currently accepted as a reasonable alternative – their use is often required for regulatory toxicological testing. Animal models have been used traditionally to assess both the local and systemic effects of aerosols across a series of exposure durations ranging from hours to weeks. The practicalities, costs, species extrapolation to humans and ethical considerations of using animals makes extensive testing of ECs and their flavorings difficult.

For assessing inhalation toxicology, there are a number of regulatory accepted methods that include acute (OECD TG 403 and 436) [94,95], 28-day sub-acute (6 h per day, 5 days per week for 28 days) (OECD TG 412) [96], and 90-day sub-chronic (6 h per day, 5 days per week for 90 days) (OECD TG 413) [97] inhalation studies employing the controlled use of rodents for nose-only or whole-body exposures use various protocols. In addition to these methods, a variety of custom

methods have been commonly used in animal exposure models. Animal studies have recently investigated the toxicology of EC constituents or whole aerosols and have reported various effects, including minimal irritative responses, oxidative stress, inflammation, and impairment in the lung's immune defense against infection. These studies must be examined for their dosimetry and the appropriate exposures for the species, and, most importantly, how these extrapolate to realistic human doses and exposures.

For example, Phillips et al. [98] studied the toxicity of nicotine and nicotine and pyruvic acid aerosols in a 28-day rat inhalation study (OECD 412). Rats exposed to nicotine had decreased body weight and concentration-dependent increases in liver weight. The respiratory tract effects from nicotine exposures were localized in the larynx and limited to 'adaptive changes'. This study reported no toxicity with observed minimal changes to respiratory tract organs, suggesting minor biological effects on the lung related to nicotine aerosols. However, in this study the authors did not test e-liquids aerosols, which are more chemically complex than nicotine and nicotine and pyruvic acid aerosols on their own.

Waldum et al. [99] studied the longer-term in vivo effects of inhaled nicotine. In a 2-year study of chronic repeated exposure, rats exposed to inhaled nicotine at concentrations twice that found in the plasma of heavy smokers showed no resultant harmful effects. There were no increases in mortality, atherosclerosis, or increased frequency of tumors in treated rats compared with sham exposure controls, with only decreases in the body weight of nicotine-exposed rats reported. There were no attempts to measure markers of lung injury in this study.

Several studies have examined the systemic effects of EC excipients. The effect of inhaled PG was reported by Suber et al. [100] in a 90-day rat inhalation study. No significant differences in respiratory rates, minute volumes, or tidal volumes were observed between any of the groups. Body weights were generally not affected, with only females receiving the highest dose showing reduced body weights from day 50. Slight differences were observed between the treated and control groups in hematological and blood chemistries, but these did not show a dose-response relationship. However, there was an observed increase in nasal goblet cell numbers and mucin production with medium and higher doses of PG aerosol and nasal hemorrhage and ocular discharge with the highest dose. These findings are consistent with more recent data from mice exposed to PG aerosol for 20 min/day for 3 weeks [101] but inconsistent with in vitro results discussed earlier [74], further emphasizing caution in the interpretation of in vitro results. The study of Suber et al [100] suggests that sub-chronic exposures to PG resulted in dehydration and mucosal/tissue irritation, with no obvious signs of toxicity. In contrast, Glynos et al. [102] found that exposing C57BL/6 mice to EC aerosol increased bronchoalveolar lavage fluid (BALF) cellularity, MUC5AC levels, lung oxidative stress markers, airway hyperresponsiveness and pulmonary mechanics at least comparably if not more than tobacco cigarette smoke. However, an excessive 8 puff/min protocol – which equates to an unrealistic pattern of use of one puff every 7.5 seconds and the continuous running of their vaping machine throughout the whole session suggest that the experimental protocol did not reflect realistic exposure under normal conditions of use. Moreover, some study measurements in particular IL levels and respiratory mechanics, were adversely affected only after 3 days of exposure but not after 4 weeks of exposure, indicating temporary airway irritation that resolves over time.

Garcia-Arcos et al. [103] delivered aerosolized VG or PG, with or without nicotine, in A/J mice for 4 months. Exposure to inhaled nicotine-containing VG or PG stimulated the development of COPD-like effects, such as cytokine expression, airway hyper-reactivity, and emphysema-like tissue destruction. However, A/J mice are particularly susceptible to pulmonary emphysema (COPD-like effects) and lung tumors [104–106]. Increased mucin production and lung tissue destruction were seen with nicotine containing PG/VG but not with PG/VG alone, suggesting that nicotine itself causes lung toxicity. This is inconsistent with the rat inhalation study of Waldum et al. described earlier [99].

Another rat study by Salturk et al. [107] assessed the effects of EC aerosols on rats following 28-day exposures and compared outcomes to those in an untreated control group with eight rats per group. Two cases of hyperplasia and four of squamous metaplasia of the laryngeal epithelium were reported in the EC-exposed rats, but these changes were not statistically significant. Notably, there were no differences in epithelial distribution and inflammation in the laryngeal mucosa between the two groups. The study lacked a relevant comparator (i.e. tobacco smoke, which is known to cause consistent hyperplasia and squamous metaplasia in rats), used an unreasonable procedure for the generation of EC aerosol emissions, and overexposed animals to aerosols. In a longer-term exposure study, Werley et al. [108] reported only minor increase in BALF lactate dehydrogenase, total protein, alveolar macrophages, and neutrophils in treated rats following 42 days' recovery after 90 days of EC aerosol inhalation (with and without nicotine and flavors). However, Lerner et al. [60] reported an increase in inflammatory cytokine levels in BALF and reduced glutathione concentrations in the lungs of C57BL mice after three days of whole-body exposure to a similar low-power cigalike EC. Similarly, Sussan et al. [109], following 2 weeks of exposure to another popular cigalike model, reported increased lung lipid peroxidation and inflammation and increased susceptibility of mice to infection with influenza A and Streptococcus pneumoniae. However, the nicotine doses the animals were exposed to were at levels where acute toxicity in mice can be anticipated. Additionally, the control mice were not subjected to the same stress-inducing regime, namely, 1.5-hour, twice daily incarceration in a small box. The combined adverse effects of these factors were visible in the reduction in body weight in the test group versus the control sample. Stress is known to adversely affect the immune system in mice and is thus likely to be at least partially, if not fully, responsible for the increased susceptibility to infection.

Direct intratracheal instillation of e-liquids has also been employed in an asthma-like mouse model (e.g. ovalbumin sensitization) investigating respiratory allergic responses in pre-sensitized mice. Following 10-week intratracheal instilla-

tion of e-liquids, increases in pro-inflammatory cytokine levels in BALF and airway hyper-responsiveness to methacholine challenge were observed [110]. However, in this study as well, the nicotine dose used was higher than levels known to cause acute toxicity in mice. The effects described are consistent with the generic well-known increased sensitivity of 'asthmatic' lungs to inhaled respiratory irritants and do not indicate an e-liquid specific effect. This points to the importance of the inclusion of a reference group of animals exposed to tobacco smoke or instilled with tobacco smoke extracts resulting in equivalent nicotine doses, as a comparator. Such a reference group was missing in all of the animal studies discussed above.

Balancing the dosing of the comparison group is also an issue, Husari et al. [111] exposed mice to laboratory air, EC aerosols or cigarette smoke for 6 h per day for 3 days, and lung injury was assessed. EC aerosols, despite being delivered at higher doses than cigarette smoke (over eight times higher particulate and four times higher nicotine concentration), resulted in lower levels of lung inflammation. In comparison, cigarette smoke exposure resulted in significant increases in IL-1β, IL-6, TNF-α expression and oxidative stress in the lung and BALF, indicating that, despite higher exposure conditions, EC aerosols exhibited less toxic effects on the lungs of experimental animals when compared to cigarette smoke.

The aforementioned in vivo studies have reported effects in response to EC whole-aerosol and aerosol constituent exposures: adaptive responses to whole-aerosol exposure, dehydrating and irritative effects, localized inflammation and hyperplasia in the laryngeal and nasal epithelia, oxidative stress, and impairment in immune defense. However, these responses need to be put into context. The dosing and exposure of rodents to the various materials need to be considered, and have in some cases been higher than weight-adjusted daily doses in humans. It is questionable how reliable the specialist mouse models are; some strains of mice are predisposed to lung disease etiologies, such as emphysema and matrix remodeling, which must be considered when reporting data from these models. Finally, some of these studies have other limitations as they have not compared findings with conventional cigarette smoke exposure responses; when the latter is accounted for, the comparative degree of response from the EC aerosol or constituent may well be much reduced.

5. Effects of EC use on respiratory health

In vitro human cell systems and animal models are not robust indicators of the potential health risks of using ECs. The outcomes of clinical studies in most fields of medicine demonstrate how limited the value of these preclinical models are. When addressing the concern about health effects of ECs, human studies become extremely relevant, particularly when the test EC under normal conditions of use. Only prospective studies of large numbers of well-characterized EC users followed-up for several years can provide clear answers about the long-term health effects of ECs. Given the challenge of conducting multi-year studies, realistic alternatives can be the detection of early changes in subclinical injury in 'healthy' smokers switching to ECs with highly sensitive functional tests and biomarkers of lung inflammation and injury, and as well as modifications of more robust health effect indicators in EC users with pre-existing diseases. Additionally, epidemiologic survey data may provide useful information about the impact of EC use on respiratory health.

For acute reactions, some smokers switching from cigarettes to EC have self-reported transient throat irritation, dry cough, and other symptoms of respiratory irritation [22,23]. The acute changes detected with sensitive respiratory functional tests reported by some authors [112,113] indicates that the human respiratory tract enacts reflex defensive responses when exposed to non-specific stimuli such as hyperosmolar EC aerosols, with asthmatics exhibiting more intense (and efficient) reflex responses. Whether such acute irritation could translate into clinically meaningful lung disease remains unknown, but there is no evidence to suggest that such irritation may lead to clinically significant adverse lung effects. Another effect, the small increase in peripheral flow resistance immediately after EC use, is of questionable relevance to health outcomes [112,113], particularly given that in the same studies no significant changes could be detected by standard spirometry immediately after EC use [112,113]. Acute exposure of EC aerosols to 10 healthy individuals caused rapid changes in the biologic responses of small airway epithelium, alveolar macrophages, and lung capillary endothelium [114]. The relevance of such acute effects to clinical lung disease is however questionable, and can only be evaluated by future large scale, long-term studies of individuals who are not ex- or current cigarette smokers who have used only ECs.

Other researchers have confirmed the absence of airflow obstruction after single short-term EC use [115–117]. Furthermore, a 5 days confinement study of 105 healthy smokers reported no significant changes in pulmonary function (FVC, FEV1) in either the arm completely or partially switched from conventional cigarettes to EC or the arm completely discontinued using tobacco and nicotine products [118]. Likewise, in a high quality randomized control trial of 387 healthy smokers, Cravo and coll [119]. reported no significant changes in pulmonary function tests after 12 weeks between participants who switched to EC and those who were randomized to continue smoking. Although no serious acute respiratory symptoms were elicited after exposure to EC aerosols in any of the studies discussed here, the possibility that adverse events may occur in predisposed individuals responding to contaminants or by-products contained in EC aerosol cannot be excluded.

Improvements in pulmonary function tests may be observed after smoking cessation, but they may take months if not years to become clinically relevant and can be elicited only in smokers with preexisting airway obstruction. The impact of switching to ECs on long-term respiratory outcomes is less clear and it has been investigated only in a few studies. No change in pulmonary function tests was observed in a 1-year randomized controlled trial of smokers with normal spirometry at baseline switching to ECs, but improvements in respiratory symptoms (cough and shortness of breath) were reported [120]. Of note, progressive normalization of peripheral airways function (i.e. FEF 25–75%, a sensitive measure of obstruction in the more peripheral airways) among those who

completely gave up cigarette smoking was observed in this study [120].

For clinical trials, nitric oxide (NO) concentration in exhaled breath provides a practical measure of airway inflammation and high levels are generally found in the inflamed airways of asthmatics [121,122]. Low levels of NO [123,124] and high levels of carbon monoxide (CO) [125] are generally found in the exhaled breath of cigarette smokers and are known to normalize soon after quitting. The evidence about exhaled NO levels immediately after EC use is conflicting, with most of the studies showing either negligible or no change [112,113,115,117,126,127]. Switching from conventional cigarettes to combustion-free nicotine containing products (such as ECs) quickly and universally leads to normalization in exhaled CO levels [22,23].

Normalization of exhaled NO and CO levels have been observed among smokers who completely gave up cigarette smoking. In a 1-year randomized controlled trial [128], reversal to within normal non-smoking levels was already noted at 3 months with complete normalization at 6 and 12 months in quitters who stopped using ECs as well as those who were still using ECs. On the other hand, no significant changes were observed in individuals who failed to quit or reduce cigarette consumption. Complete abstention from smoking combustible cigarette is known to reduce toxic levels of exhaled CO to within normal limits; similar reductions in exhaled CO have been observed in acute [129,130] and long-term ECs studies [131,132]. Given that ECs are battery-operated devices that do not rely on combustion to operate, this was not surprising. Also, the reported improvements in exhaled NO and CO levels were associated with attenuations in composite symptom scores (cough, phlegm, shortness of breath, wheeze, tight chest, stuffy nose, sinus pain, and frontal headache), particularly in individuals who completely gave up smoking. These outcomes have been self-reported by a wide variety of vapers in the real world [2,25]. The reversal of inflammatory changes in the upper and lower airways after quitting smoking may be the mechanism for these improvements in symptom scores. When assessing respiratory health, it is however of outmost importance to disentangle health effects driven by chronic exposure to EC aerosol emissions from those related to previous smoking history. In a small cohort of daily EC users who have never smoked in their life, no deterioration in spirometric indices, development of respiratory symptoms, changes in markers of lung inflammation nor signs of early lung damage on HRCT were noted in any of the 9 subjects who completed the 3.5-year follow up [133]. The small sample size, the lack of a control smoking group, and the relatively short duration of the follow up were important limitations of this study.

The studies discussed above involved 'healthy' subjects, and only limited work has addressed health impact of EC use in users with pre-existing pulmonary diseases. The asthmatic smoker is a distinct disease phenotype with increased susceptibility to exacerbations and poor asthma-specific health status [134]. Quitting smoking can reverse the negative impact of tobacco smoke on asthma symptoms and lung function [135], and switching to EC use may produce significant respiratory benefits as well. A retrospective cohort study of regular EC users with mild to moderate asthma did not show any deterioration in respiratory physiology and subjective asthma outcomes [136,137]. On the contrary, smokers with asthma who quit or substantially decreased tobacco consumption by switching to ECs showed progressive significant improvement in the Juniper's Asthma Control Questionnaire (ACQ), FEV_1, FVC, and forced expired flow between 25% and 75% of the FVC (FEF25-75), as well as airway hyper-responsiveness (AHR) to inhaled methacholine [136]. A 2-year follow-up study confirmed that EC use ameliorated objective and subjective asthma outcomes and suggests that these beneficial effects may persist in the long term [137]. Remarkably, similar findings were found in the dual users of ECs and cigarettes. EC use was well tolerated, and exposure to e-liquid aerosol in this vulnerable population did not trigger any asthma attacks. These positive findings are consistent with results from a large internet survey of EC users with asthma [2]. Improvement in asthma symptoms after switching was reported in 65.4% of the respondents. Improved asthma symptoms were more often noted in exclusive EC users, while similar improvements were also described in dual users. Worsening of symptoms after switching was reported only in 1.1% of the asthmatics.

Another disease associated with tobacco smoking is COPD, a progressive disease characterized by a persistent inflammatory and remodeling response of the airways [138,139]. Smoking cessation is the only evidence-based strategy known to favorably modify the course of COPD and reduce mortality [140,141]. Reducing cigarette consumption by switching to EC use may yield considerable respiratory benefits in COPD. A retrospective-prospective study of patients with COPD found no deterioration in respiratory physiology (post-bronchodilator FEV_1, FVC, and %FEV1/FVC) in COPD patients who quit or substantially reduced their tobacco consumption by switching to EC use [142]. In smokers with COPD and irreversible airway obstruction, the lack of significant improvements in spirometric indices after smoking cessation is not unusual [143,144]. Nonetheless, participants in a three-year study experienced significant declines in yearly respiratory exacerbations, much improved overall health status (measured by the COPD Assessment Test [CAT]), and boosted physical activity (measured by the Six-Minute Walk Test) [142]. These improvement in health outcomes have also been reported in an internet survey of regular EC users with COPD [2]. Improvement in respiratory symptoms after switching was reported by 75.7% of the respondents, whereas worsening was reported by only 0.8%. Outcomes self-reported by general vapers also indicate improvement in respiratory symptoms [2,25]. A key finding is that respiratory exacerbations were halved in COPD patients who quit or reduced substantially their tobacco consumption after switching to ECs [142]. Smoking is known to increase susceptibility to respiratory infection to bacterial and viral pathogens and quitting smoking appears to lower the risk of respiratory infection [145–147]. Regular use of EC may reduce pathogens activity [148], probably due to the presence of propylene glycol in its aerosol form, which has antibacterial as well as antiviral activity [149,150]. Antibacterial activity has been recently shown in commercially available e-liquids [151].

Moving from clinical data to surveys, these studies have investigated the impact of ECs on respiratory health by analyzing associations of their use with respiratory symptoms and respiratory illnesses. Although the evidence from clinical studies suggest that EC are unlikely to raise significant health concerns for the respiratory tract, most published surveys have suggested the opposite. Four studies examined respiratory symptoms in adolescents using or who have used EC [152–155] and all show an association between respiratory symptoms and EC use. All these surveys are cross-sectional, relying on inaccurate self-reporting of respiratory symptoms and respiratory illnesses, and failing to take into account relevant key confounders. These studies should be expanded in more appropriate longitudinal cohorts. In particular, the analysis conducted by McConnell and coll [152]. fails to confirm the association between asthma symptoms and EC use when controlling for tobacco smoking and second-hand smoke exposure. The association of EC use and self-reported chronic respiratory conditions (asthma as well as COPD) have been also reported in cross-sectional surveys of adults in the US [156,157], but these cross-sectional studies cannot demonstrate causation, and are not adjusted for baseline confounders such as smoking history. In a recent analysis from two observational cohorts, Bowler and coll [158]. concluded that EC use was associated with poorer respiratory health outcomes in adults at risk for or with COPD, but the study does not measure the frequency of EC use. Another potential confounder with the study is selection bias with the EC users having a more prolonged exposure to cigarettes (i.e. pack/years) which is associated with poorer COPD outcomes. Therefor the study's reported association of EC use and COPD may be in error from the misclassification of the level of EC use, or the differences in baseline cigarette use may have attenuated the negative association in the findings.

Human subject research on EC use and lung function has been conducted at the clinical and population levels. Clinical studies have observed some non-serious acute effects, but whether they result in lung disease is not known. Several studies and EC user surveys have reported beneficial effects after switching to EC use. To the contrary, cross-sectional surveys have indicated a negative association between EC use and lung disease, but these studies are limited because they do not adjust for confounders such as prior smoking history or frequency of EC use. There is a need for more long-term studies and population level epidemiological analysis of medical records.

6. Conclusions

ECs generate respirable aerosols [16–18] containing glycerol, PG and their thermal degradation products (i.e. carbonyl compounds), chemical flavorings, and metals, but at much lower levels than in cigarette smoke [6–9]. EC use (5 g/day) represents a 79.0–96.8% reduction in formaldehyde, 99.5–99.8% reduction in acetaldehyde and 96.0–99.5% reduction in acrolein exposure compared to smoking 20 tobacco cigarettes. Studies on the effect of the inhalation of PG in humans have indicated that it does not appear to pose a significant hazard [20], while noting that exposure to glycerol and PG aerosols has been shown to elicit some irritant effects [21–23]. Innovations in EC design and new technologies have been recently introduced to further minimize any residual harm and to improve user satisfaction. Newer devices with temperature controls prevent overheating and the dry-puff phenomenon that produce excessive formation of carbonyls.

The potential for EC toxicity has been investigated by exposing cell cultures to e-liquids or to aerosol generated by ECs, and our review focused on studies using human airway epithelial cells [61–64]. In real life, exposures from ECs come from aerosols, not e-liquids, therefore exposing the cells and tissues to aerosol is a more appropriate way to investigate the biological responses to ECs. This requires rigorous lab quality standard procedures for the generation of aerosol extracts (AqEs) or aerosols, and these are rarely applied. Another methodological issue with in vitro studies is that the effect of device liquid interactions is generally overlooked, and the device type and make, the e-liquids used, and the device settings are often not fully reported. Finally, with the large number of devices available, findings from any one single liquid-device combination cannot be extrapolated to vaping in general.

Animal studies have recently investigated the toxicology of EC constituents or whole aerosols and have reported various effects: adaptive responses to whole-aerosol exposure, dehydrating and irritative effects, localized inflammation and hyperplasia in the laryngeal and nasal epithelia, oxidative stress, and impairment in immune defense. These findings must be questioned because in some studies the dosing was substantially higher than for weight-adjusted daily doses in humans. Poisoning animals in their cages is not informative of what happens to consumer under normal condition of use. Studies with specialist mouse models are suspect because some strains of mice are predisposed to lung disease etiologies. Finally, some studies did not compare EC findings with conventional cigarette smoke exposure responses.

In vitro human cell systems and animal models are not robust indicators of the potential health risks of using ECs as preclinical studies have limited value. Human studies are the most relevant for addressing the health effects of EC, particularly when they have tested EC under normal conditions of use. For acute reactions, some smokers switching from cigarettes to EC have self-reported transient throat irritation, dry cough, and other symptoms of respiratory irritation [22,23], indicating that the human respiratory tract enacts reflexive defensive responses when exposed to non-specific stimuli [112,113]. There is no evidence to suggest that such irritation may lead to clinically significant adverse lung effects. Likewise, the small increase in peripheral flow resistance immediately after EC use is probably not indicative of negative health outcomes [112,113]. The relevance of acute effect findings is particularly questionable, given that no significant changes could be detected by standard spirometry immediately after EC use [112,113].

For short-term effects, a 5 days confinement study of 105 healthy smokers reported no significant changes in pulmonary function (FVC, FEV1) and a high quality randomized control trial of 387 healthy smokers [118] reported no significant changes in pulmonary function tests after 12 weeks.

Improvements in pulmonary function tests for smokers with preexisting airway obstruction may be observed after smoking cessation, but they may take months if not years to become clinically relevant.

The impact of switching to ECs on long-term respiratory outcomes is less clear. A 1-year randomized controlled trial of smokers with normal spirometry at baseline switching to ECs found no changes pulmonary function tests and reported improvements in respiratory symptoms (cough and shortness of breath) [119]. The study also observed progressive normalization of peripheral airways function (i.e. FEF 25–75%) among those who completely gave up cigarette smoking. Smokers switching from conventional cigarettes to combustion-free nicotine containing products (such as ECs) quickly and universally leads to normalization in exhaled CO levels [22,23]. When assessing respiratory health, it is of outmost importance to disentangle health effects driven by chronic exposure to EC aerosol emissions from those related to previous smoking history.

Only limited work has addressed health impact of EC use in users with pre-existing pulmonary diseases. A retrospective cohort study of regular EC users with mild to moderate asthma did not show any deterioration in respiratory physiology and subjective asthma outcomes [135,136]. Smokers with asthma who quit or substantially decreased tobacco consumption by switching to ECs showed progressive significant improvements [135], and a 2-year follow-up study confirmed that EC use ameliorated objective and subjective asthma outcomes [136]. EC use was well tolerated, and exposure to e-liquid aerosol in this vulnerable population did not trigger any asthma attacks. For smokers with COPD, a retrospective-prospective study determined that there was no deterioration in respiratory physiology in patients who quit or substantially reduced their tobacco consumption by switching to EC use [141].

Several surveys have contradicted these clinical findings, but their cross-sectional design cannot demonstrate causality. Surveys rely on self-report of respiratory symptoms and respiratory illnesses which can be inaccurate, and surveys fail to consider relevant key confounders, particularly smoking history, and other factors such as vaping frequency and duration. Longitudinal cohort studies could provide more robust data; for example, McConnell and coll [151]. failed to confirm the association between asthma symptoms and EC use when controlling for tobacco smoking and second-hand smoke exposure.

In summary, the human subject studies provide the most relevant data on the effects of EC aerosol on human lung function, and several studies demonstrate potential benefits for smokers switching to EC. No studies reported serious adverse events, although the potential for such reactions cannot be completely excluded. Minor acute reactions have been reported, but it is not known if they are indicators of potential future lung disease, and no significant changes in pulmonary function were observed in short term trials. Smokers who substituted EC use for smoking experienced improvements in symptoms (cough, phlegm, etc.) and exhibited lower levels of exhaled CO, particularly for those with complete EC substitution. For smokers with diseases such as asthma and COPD, EC use appears to have a beneficial effect on symptoms. Yet to completely test the effects of EC on lung function, specific data is needed for each of the hundreds of e-liquid flavor combinations and the many different types of devices. This data can be provided by cell studies and animal models, but the current research designs must be substantially improved to yield accurate findings for determining the respiratory health risks and benefits of EC use by smokers. Last but not least, only large long-range prospective studies of vapers who have never smoked can provide definitive data to demonstrate any potential impacts regular use of vaping products may have on long term health.

7. Expert opinion

There is growing evidence to support the relative safety of EC emission aerosols for the respiratory tract compared to tobacco smoke [4,14,159]. Public Health England estimated, on the basis of a review of 185 studies, that vaping an e-cigarette is likely to be at least 95% less harmful than smoking a regular cigarette [13]. In 2016, the Royal College of Physicians reaffirmed this figure, estimating the risk of long-term inhalation of e-cigarette aerosol to be unlikely to exceed 5% of the risk associated with long-term cigarette smoking [12]. This review article shows that although some potential effects on respiratory cell types can be shown in vitro, and low levels of chronic irritation of the respiratory tract can be anticipated at certain levels of vaping, these effects are much less than those of smoking. The clinical evidence confirms that ECs are unlikely to raise significant health concerns for the respiratory tract under normal conditions of use. Former smokers using and smokers intending to use ECs as a substitute for smoking should receive correct information about residual risks and potential benefits of these products. Promoting further access to ECs may offer an opportunity to reduce or prevent some of the otherwise inevitable burden of respiratory morbidity and mortality caused by tobacco smoking [160].

To this end, Public Health Institutions and the Ministry of Health in the UK support ECs use as an integration to the already existing Tobacco Control policy. The National Centre for Smoking Cessation and Training (NCSCT) and the National Health Service (NHS) are now actively supporting EC-based intervention along to their standard tobacco control programs and smoking cessation interventions to local stop smoking services [161,162]. The results of such UK policy have been encouraging, with an accelerated decline of smoking prevalence in the adult population from 19.8% (7.7 millions) in 2011 to 14.9% (6.1 millions) in 2017 [163]. Nevertheless, in most countries there is resistance in accepting the UK model of introducing ECs in smoking cessation clinics.

This review article also draws attention to the potential for misinformation from poorly designed and largely misinterpreted experimental studies. As for the majority of existing observational and epidemiological studies [164], preclinical (i.e. in vitro systems and animal models) and clinical models can be also uninformative or even misleading due to problem with methodology and interpretation of these studies. It is urgent to address common mistakes and to develop robust

and realistic methodological recommendations in order to adequately assess the impact of EC use on human health under normal condition of use.

The adoption of standardised methods will also enable a better understanding and a reliable comparison and extrapolation of results obtained across various studies and research groups. There are a number of initiatives in existence, driven by industry groups and non-governmental agencies. For example, CORESTA, (Cooperation Centre for Scientific Research Relative to Tobacco) has recently recommended a method and puffing regime for the generation and collection of EC aerosols [165]. The Institute of In Vitro Sciences (IIVS) has recently hosted a series of workshops, 'Assessment of In Vitro Chronic Obstructive Pulmonary Disease (COPD) Models for Tobacco Regulatory Science' and 'In Vitro Exposure Systems and Dosimetry Assessment Tools for Inhaled Tobacco Products', bringing together a community of industry, academic and regulatory scientists to support the development, harmonization and standardization of in vitro applications for tobacco product and EC testing [166,167]. The workshops have explored methods and standards supporting these topic areas and are driving in vitro standardization with the support of technical working groups, sharing of data and publication of key findings.

More prospective clinical trials are needed to provide meaningful insights on the effects of EC aerosol on lung health and to generate findings that are most relevant to researchers, policy makers and users. Clinical research has described no serious adverse events, although the potential for such reactions cannot be completely excluded. Minor acute reactions have been reported, but it is not known if they are indicators of potential future lung disease, and no significant changes in pulmonary function were observed in short term trials. Smokers who substituted EC use experienced improvements in smoking symptoms (cough, phlegm, etc.) and exhibited lower levels of exhaled CO, particularly for those with complete EC substitution. For smokers with diseases such as asthma and COPD, EC use may have a beneficial effect on symptoms. Yet to completely test the effects of EC on lung function, specific data is needed for each of the hundreds of e-liquid flavor combinations and the many different types of devices. This data can be provided by cell studies and animal models, but the current research designs must be substantially improved to yield accurate findings for understanding the respiratory health risks and benefits of EC use by smokers.

In an *Expert Review in Respiratory Medicine* article published about 7 years ago [168], we discussed several important research developments and future avenues for e-cigarette science. In the authors' view, those expert opinions have been substantiated by the growing body of evidence. We therefore reiterate our prediction that EC use is the most effective method of substituting tobacco cigarette for those smokers who are unable or unwilling to quit and we are now confident that current vaping products are much less harmful than conventional cigarettes as well as earlier EC designs.

This narrative review has identified many gaps in EC science and identified specific research needs important for advancing current knowledge about health effects from e-cigarette use. In particular it is paramount to improve research methods, data quality and interpretation of study findings. In relation to experimental in vitro and animal models, exposure studies must be representative of human inhalation exposure to e-cigarette aerosols under normal condition of use and include relevant controls. In relation to human behavioral/market research, it is important to develop and standardize new questionnaires for improved assessments of dependence on e-cigarettes, patterns and frequency of use, as well as device characteristics. In relation to clinical and epidemiological studies, it is mandatory to include as comparison groups individuals who continue to smoke, those who try to quit with other evidence-based tobacco cessation treatments, and those who are not users of tobacco products, including e-cigarettes.

Looking into the future, it is likely that the interest among medical community and patients' associations about risk reduction with ECs among COPD patients will grow because poor quality of life in patients with COPD remains an unmet need and medical management is quite unsatisfactory. Anything that can improve quality of life of COPD patients should not be dismissed lightheartedly. Given that many COPD patients continue smoking despite their symptoms, it will be important to substantiate the role of the EC as a viable, much less harmful alternative.

In the next 5 years there will be more evidence supporting the possible trade-off between vaping products as an 'off-ramp' for adult smokers and an 'on-ramp' to nicotine use for youth. Millions of deaths from cigarette smoking are an immediate, stark, and preventable tragedy that should be fully factored in to a rational risk-benefit analysis.

Independent research will become increasingly important. Tolerability, safety, efficiency, and harm reduction potential of these new technologies will have to be endorsed through independent research. Such an approach is strongly needed to provide rigorous feedback to the industry and informed answers to the regulators.

Potential concern on the absolute risk of existing ECs will be resolved by technological innovation in EC design with the creation of superior and much safer new generation products. A clear understanding of the residual risk of these new products will resolve current concerns about long term health effects. Nonetheless, we should not lose sight of the potential benefits of ECs compared to cigarettes as a lot of people still smoke conventional cigarettes and this will be a public health issue for a number of years to come.

More disruption will occur. The critical distinction in public health and consumer policy is that of a fast-moving tech innovation that is obsoleting combustible tobacco products. This is likely to bring more disruption among the enemies of innovation and lovers of status quo in tobacco control. This disruption has been already set in motion; more countries will follow the positive developments in Japan, Korea, England, New Zealand, Canada and Iceland that by promoting a widespread and complete adoption of new technologies it is possible to substantially accelerate declines in smoking prevalence.

Author contributions

All authors revised the article critically for important intellectual content and approved its final version.

Declaration of interest

In relation to his work in the area of tobacco control, R Polosa has received lecture fees and research funding from Pfizer and GlaxoSmithKline, manufacturers of stop smoking medications. He has also received support from The Consumer Advocates for Smoke-free Alternatives (CASAA) for publication and open access costs of one paper. He has also served as a consultant for Pfizer, Global Health Alliance for treatment of tobacco dependence, ECITA (Electronic Cigarette Industry Trade Association, in the UK), Arbi Group Srl., and Health Diplomats (consulting company that delivers solutions to global health problems with special emphasis on harm minimization). Lectures fees from a number of European electronic cigarette industry and trade associations (including FIVAPE in France and FIESEL in Italy) were directly donated to vapers advocacy no-profit organizations. Publication costs for previous work have been met by independent e-cigarette industry including Happy Liquid, Ritchy Europe, Cuts Ice e-Liquid Laboratories and VDLV e-Liquids. R Polosa is the Director of the Center of Excellence for the acceleration of Harm Reduction at the University of Catania (CoEHAR), which has received a grant from Foundation for a Smoke Free World to develop and carry out 8 research projects. R Polosa is also currently involved in the following pro bono activities: scientific advisor for LIAF, Lega Italiana Anti Fumo (Italian acronym for Italian Anti-Smoking League), the Consumer Advocates for Smoke-free Alternatives (CASAA) and the International Network of Nicotine Consumers Organizations (INNCO); Chair of the European Technical Committee for standardization on "Requirements and test methods for emissions of electronic cigarettes" (CEN/TC 437; WG4). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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