Pomeranian J Life Sci 2024;70(2):75-81 doi: 10.21164/pomjlifesci.1065

The effects of e-cigarette aerosol components on the morphology and function of the conducting portion of the respiratory system: a narrative literature review

Paweł Szumilas[1, A], Kamila Szumilas[2, B], Aleksandra Wilk[3, C] ✉, Alicja Zawiślak[4, D], Beata Karakiewicz[1, 2, E]

[1] Pomeranian Medical University in Szczecin, Department of Social Medicine and Public Health, Żołnierska 48, 71-210 Szczecin, Poland
[2] Pomeranian Medical University in Szczecin, Department of Physiology, Powstańców Wlkp. 72, 70-111 Szczecin, Poland
[3] Pomeranian Medical University in Szczecin, Department of Histology and Embryology, Powstańców Wlkp. 72, 70-111 Szczecin, Poland
[4] Pomeranian Medical University in Szczecin, Department of Interdisciplinary Dentistry, Powstańców Wlkp. 72, 70-111 Szczecin, Szczecin, Poland

[A] ORCID: 0000-0002-3328-7904; [B] ORCID: 0000-0002-5635-1630; [C] ORCID: 0000-0002-1542-8371; [D] ORCID: 0000-0003-2036-2853; [E] ORCID: 0000-0001-6527-7287

✉ aleksandra.wilk@pum.edu.pl

ABSTRACT

As electronic cigarettes (e-cigarettes, vaping, etc.) are considered a safer alternative to traditional smoking, they are gaining popularity, especially among adolescents (10–14 and 14–18 years) and young adults (18–25 years). They are used by millions of users worldwide, and new generations of e-cigarette devices are being introduced. Some reports have suggested that e-cigarettes have harmful effects on human health, which is why it is important to introduce restrictions on the use of e-cigarettes by young people. The aerosols produced when the e-liquid is heated contain a complex of gases, toxic substances and various types of flavoring chemicals, which are then inhaled by users. In addition, when the products in the e-liquid are thermally degraded, more harmful reactive substances are produced. Exposure to e-cigarette aerosols appears to have harmful effects on human health, but it is critical that our understanding of these effects be expanded and that data be collected on the long-term effects of the substances in e-cigarette aerosols on the human body. Data on the various health risks associated with the use of e-cigarettes are mainly based on *in vitro* studies using established cell lines or cultured human cells, or on animal models. The substances contained in e-cigarette liquids and their aerosol – including the solvents propylene glycol and vegetable glycerin – are known to cause organ and tissue irritation in the upper respiratory tract when inhaled. The aim of this narrative review is to present our current knowledge on the effects of the chemical components of e-cigarette aerosols on the nasal cavity, trachea, bronchi, and organs of the conducting part of the respiratory system.

Keywords: e-cigarettes; e-aerosol; chemical components; ciliated columnar pseudostratified epithelium.

INTRODUCTION

There are many unknowns and controversies surrounding the use of electronic cigarettes (e-cigarettes; e-cigs), which are battery-operated electronic nicotine delivery systems (ENDS). Because they do not involve the burning of tobacco, ENDS are considered a less harmful alternative to tobacco and may therefore help smokers quit. E-cigarette users inhale and exhale ("vape") an aerosol – sometimes referred to as e-cigarette "vapor"– produced by heating e-liquid from a tank, cartridge, or pod to a temperature of 200–250°C, or sometimes over 300°C, depending on the type of ENDS, its power output, and the substances in the liquid [1, 2]. The e-liquids contain nicotine, water, propylene glycol (PG) and vegetable glycerin (VG) as solvents, as well as various toxic chemicals, many pharmacologically active substances, flavors and colorants [3, 4, 5].

During heating in the aerosolization process, the 2 main components of the e-liquid, PG and VG, as well as the flavoring chemicals, are thermally degraded; the resulting aerosols also contain a number of hazardous substances from the e-liquids, including formaldehyde, acetaldehyde, acrolein, propionaldehyde, 2-butanone, benzaldehyde, glyoxal, and hexaldehyde [6]. They also contain free radicals, nanoparticles, toxic metals and trace elements, and metals released from metal coils [7, 8]. The adverse effects of e-aerosol on human health have been reported for many years [9, 10]. The substances contained in the inhaled aerosol can be harmful to respiratory tissues and organs [2, 3, 4, 11].

Significantly fewer studies have been conducted on the composition of exhaled e-cigarette aerosol, but identified constituents include PG, glycerol, nicotine (Long), and aldehydes (Samburova et al.), as well as particulate matter smaller than 2.5 μm (PM 2.5) and ultrafine particles [12].

Four generations of e-cigarette devices are currently available [13, 14]. The first-generation non-refillable devices, called "cigalikes", are similar in shape and size to conventional cigarettes and contain a battery and cartomizer. Second-generation e-cigarette devices are larger than traditional cigarettes (the medium size is like a pen and the large size contains a tank) and have rechargeable batteries and a refillable tank of e-liquid. Third-generation devices, also called "mods", contain a battery and allow users to adjust the flow, thickness, and volume of the vapor. Fourth-generation e-cigarettes, known as "mod pods", are the most powerful and customizable e-cigarettes; they can be recharged via USB and are more economical than traditional cigarettes [13, 14, 15, 16]. Fourth-generation devices, such as those manufactured by JUUL, are popular with younger consumers, which distinguishes these devices from other e-cigarettes.

E-cigarettes are becoming increasingly popular among young adults, particularly teenagers, for a number of reasons: (i) many adolescents believe that e-cigarettes are safer than traditional smoking; (ii) e-cigarettes are cheaper than other tobacco products and can be used in places where other tobacco products, such as cigarettes, are not allowed; (iii) the e-liquid is often flavored with a variety of aromas, such as strawberry, chocolate, watermelon, blackcurrant, blueberry, cherry, grape and apple pie, which appeals to many users [17, 18, 19]. Many teens, both male and female, consider vaping to be safer than traditional smoking because they believe that e-cigarette aerosols are mostly water vapor. Use of e-cigarettes by adolescents and young adults is a risk factor for initiation of use of other tobacco products, such as conventional cigarettes. The use of e-cigarettes, especially if the liquid contains nicotine, is not safe and there is a large body of data showing that vaping actually affects younger smokers and their health. Vaping during pregnancy, commonly reported as a means of smoking cessation for expectant mothers, may be associated with health complications in the offspring, including impaired respiratory function [20, 21]. Inhaled and exhaled aerosols come into direct contact with tissues and organs of the respiratory system [22].

The purpose of the current narrative review is to collect and present data on the effects of toxic and irritating chemicals in e-cigarette aerosols on the morphology and function of the organs of the airway of the respiratory system. An electronic search of the English-language medical databases MEDLINE, Cochrane Collaboration and SCOPUS was performed using the key words "e-cigarettes", "vaping" and "vaping and its effects on the respiratory system".

NASAL CAVITY TISSUES AND EXPOSURE TO E-AEROSOLS

The nasal cavities, together with the paranasal sinuses, are part of the upper respiratory tract. Each nasal cavity chamber is divided into 3 regions: (i) a nasal vestibule, lined by skin and covered with stratified squamous epithelium that becomes pseudostratified posteriorly, characteristic of the next region; (ii) the respiratory region, with respiratory mucosa lined by a ciliated, pseudostratified columnar epithelium; (iii) the olfactory region, lined by olfactory mucosa and containing the olfactory epithelium and glands. The underlying lamina propria consists of connective tissue. These last 2 regions contain various types and densities of blood and lymphatic vessels, nerves, mucus with serous demilunes and serous (Bowman's or olfactory) glands, and cells including fibroblasts, mast cells, neutrophils, macrophages, and extracellular matrix. The airway mucosa also contains a variety of lymphocyte populations that reside in the airway epithelium (lymphoepithelium) and lamina propria and are involved in mucosal immunity [23]. The pseudostratified columnar ciliated epithelium, also called the respiratory or airway epithelium, acts as a physical barrier and first line of defense against invading pathogens, where it cooperates with immune cells to maintain homeostasis [24].

The surface epithelium of the nasal mucosa is often the first tissue to come into direct contact with various inhaled toxicants, including those present in e-cigarette aerosols. Users of e-cigarettes inhale these through the mouth and typically exhale through the nostrils. The inhaled and exhaled aerosol is likely to contain toxins and other harmful substances. The first case of a 26-year-old man with traumatic refractory bilateral epistaxis after vaping was described in 2017 [25]. The patient started regularly vaping different flavors of e-cigarettes after quitting smoking 1 month before. The authors reported that the incident was caused by a combination of the drying effect of the aerosol and the effect of the irritant chemicals it contained [25]. In addition, exhalation of the aerosol can dry out the nasal mucosa, sometimes causing nosebleeds. Other authors reported a nosebleed in one participant in a small (9 person) group of ENDS users recruited from users of supported temporary homeless accommodation in Ireland [26]. However, the association between vaping and nosebleeds has not been proven, suggesting that it may be an incidental adverse effect.

Both inhaled and exhaled e-cigarette aerosols contain many toxicants and irritants and may be harmful to nasal tissues and mucosal immunity. Changes in immune-related gene expression in epithelial cells of e-cigarette users, obtained from superficial scraping biopsies of the epithelium in the lower surface of the middle nasal turbinate of e-cigarette users, were evaluated. Total profile gene analysis revealed that exposure to e-cigarette aerosol decreased the expression of a large number (597) of immunology-related genes, which could affect the metabolism of epithelial cells and disrupt immune health in this upper part of the respiratory tract [27].

It is noteworthy that the impairment of nasal mucosal immune response in e-cigarette users may make users more susceptible to infections and alter the antiviral host defense response. In the study by Rebuli et al., volunteers of both sexes – all second- or third-generation e-cigarette users – were vaccinated with live attenuated influenza virus to assess their innate immune response to infection. Nasal epithelial lining fluid, nasal lavage fluid, and nasal scraping biopsy specimens were collected from the participants and analyzed for cytokine and chemokine levels, influenza-specific IgA, immune gene expression, and markers of viral load. The study showed that after inoculation, the nasal immune response was altered under the influence of e-cigarettes, and immune-regulated gene expression was downregulated; there were altered levels of cytokines (regulating antiviral host defense responses) and chemokine release (regulating monocyte recruitment and activation), while the level of IgA in e-cigarette users remained unchanged. These results show that e-cigarettes have a deleterious effect on nasal mucosal immunity and suggest that vaping may increase the risk of suppressed host defense functions during viral infections [28].

In addition, the inflammatory response of nasal and throat epithelial cells induced by exposure to e-cigarette vapor caused an influx of immune cells. Under physiological conditions, activated tissue macrophages participate in phagocytosis and release proinflammatory cytokines, leading to the differentiation of B cells and T cells that participate in downstream

signaling mechanisms. Exposure to e-vapor may also result in the release of elastase and matrix metallopeptidase-9, as well as various cytokines and chemokines by neutrophils. Cytokines and chemokines can activate signaling pathways, resulting in inflammation and apoptosis of epithelial cells by neutrophils. The cells release β-defensins with antimicrobial activity and participate in initiating an inflammatory response. In addition, exposure of nasal epithelial clone 1 to vapor results in decreased synthesis of short palate lung and nasal epithelial clone 1 (SPLUNC1) protein, a multifunctional innate defense protein that induces innate immunity [5, 29, 30].

To evaluate the effects of vaping on sinonasal symptom scores and mucociliary clearance (MMC), a prospective, randomized, single-blind clinical trial was conducted [31]. After 3 months of vaping, the Sino-Nasal Outcome Test-22 (SNOT-22) and saccharin transit time for MCC were assessed in e-cigarette users. The results indicated that e-cigarettes had adverse effects on both sinonasal symptoms and MMC [15, 31].

To evaluate the effects of e-cigarette aerosol exposure on the morphology and metabolism of epithelial cells and its potential adverse effects on nasal tissue structure, an experimental *in vitro* study was conducted using engineered human nasal mucosa. Epithelial cells were isolated from nasal biopsies and fibroblasts were isolated from the underlying tissue. Histologic and morphologic changes were observed in nasal epithelial cells after exposure to e-cigarette nicotine-free and nicotine-rich aerosol (eGo e-cigarette device), including reduced cell density, increased cell size, faint nuclei, and a significant reduction in the number of viable cells – as confirmed by increased lactate dehydrogenase (LDH) activity as a marker of cell death – compared to the control. Similar effects were observed in engineered human nasal mucosa. In the tissue model, significant disorganization of structure was observed as non-stratified layers and large epithelial cell size. The changes were worse in the tissue exposed to the nicotine-free aerosol. In addition, epithelial cells in the nasal tissue model were activated by e-aerosol to produce pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and mitochondrial pyruvate carrier 1 (MPC1) at higher levels than the control, which may indicate impaired function of the innate immune system [32].

The study by Song et al. showed that e-cigarette aerosols with higher nicotine concentrations induced mRNA expression and protein production of mucin-5AC (MUC5AC; gel-forming glycoprotein) but not mucin-5B (MUC5B; oligomeric mucus/ gel-forming) in cultured human nasal epithelial cells and NCI-H292 cells (airway epithelial cells). Both – MUC5AC and MUC5B – are glycoproteins that regulate the viscoelastic properties of airway mucus. These properties can be disrupted and alter mucin production [33].

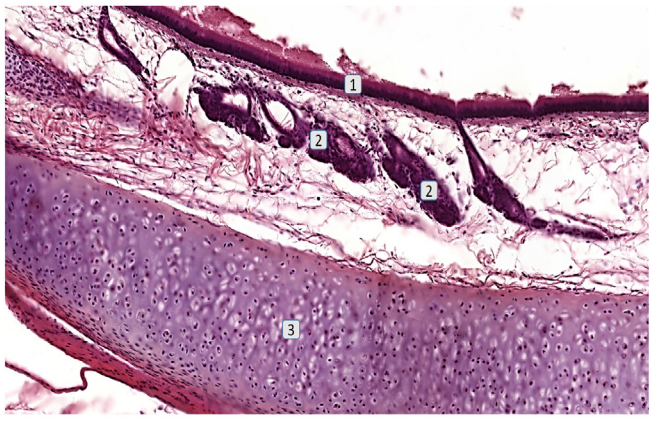
In a similar study by Kwak et al., the effects of exposure of human nasal epithelial cells to glyoxal and methyloglyoxal (the most toxic components of e-aerosols) on proinflammatory cytokines and MUC5AC/MUC5B expression and signaling pathways were determined. Both toxicants stimulated the synthesis of proinflammatory cytokines interleukin-1 beta (IL-1β) and IL-6 and increased MUC5AC/MUC5B expression via extracellular signal-regulated kinase 1/2 (ERK1/2), mitogenactivated protein kinase (p38 MAPK) and nuclear factor kappa beta (NF-κB) signaling pathways. Exposure did not affect the morphology or viability of cultured cells, but impaired mucin secretion [34].

The effects of cigarette smoking on odour in humans are well documented [35, 36]. It would be expected that long-term exposure to e-cigarette aerosols could cause olfactory dysfunction, including a reduced sense of smell. However, there are no documented data on the effects of e-cigarette use on olfactory epithelial function. In the study by Majchrzak et al., the odor perception of cigarette smokers and e-cigarette users compared to nonsmokers was estimated using the Sniffin' Sticks odor threshold (T), discrimination (D), and identification (I) tests. The composite score of Discrimination Threshold Identification (TDI) was then calculated. The results indicated that there was no negative effect of e-cigarette use on olfactory perception, in contrast to cigarette smokers [37].

There have also been studies in rats suggesting that exposure to e-cigarette aerosol for weeks can cause metaplasia and hyperplasia of the laryngeal mucosa, but the results were not statistically significant [15, 38]. However, this was a short-term experiment and the lesions or real consequences may not have had time to develop.

EXPOSURE OF THE TRACHEA AND BRONCHIAL TREE TO E-CIGARETTE VAPOR

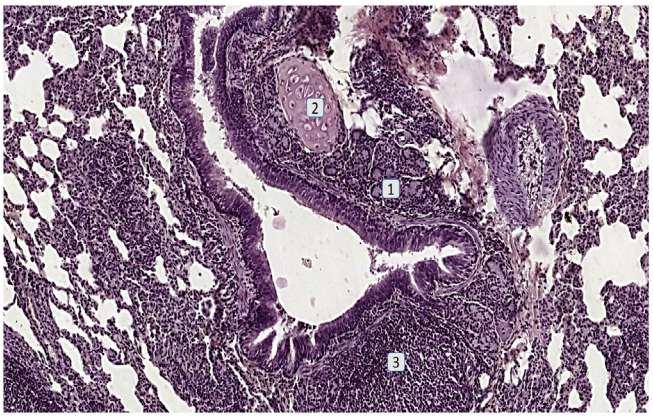
The trachea serves as a conduit for air, and its wall contains a mucosa lined (as in the upper part) by ciliated, pseudostratified, columnar respiratory epithelium with an underlying lamina propria rich in elastic fibers; the submucosa has connective tissue and a mucus-secreting submucosal layer with serous demilunes. Both layers are composed of diffuse lymphatic tissue. The submucosal layer connects to the perichondrium of the C-shaped tracheal hyaline cartilages, between whose free ends fibroelastic tissue and smooth muscle are found. The final layer, the adventitia, connects the trachea to adjacent structures along its course (Fig. 1).



1 – epithelium; 2 – tracheal glands; 3 – hyaline cartilage

FIGURE 1. Histological structure of trachea (original photo: Department of Histology and Embryology, Pomeranian Medical University in Szczecin, Poland)

The trachea divides into a number of branches: primary bronchi, lobar secondary bronchi and segmental tertiary bronchi. The structure of their walls is similar to that of the wall of the trachea, but the cartilage forms cartilaginous plates of irregular shape. Along with the decrease in bronchial size, the cartilaginous plates are smaller and less numerous; neither cartilaginous plates nor glands are present in the wall of bronchioles [39]. The bronchial wall is rich in bronchial-associated lymphoid tissue (Fig. 2). The trachea and bronchi are in direct contact with the environment, and their exposure to e-cigarette vapor may have harmful effects.



1 – glands; 2 – plate of hyaline cartilage; 3 – BALT

FIGURE 2. Histological structure of bronchus: 1 – glands; 2 – plate of hyaline cartilage; 3 – bronchus-associated lymphoid tissue – BALT (original photo: Department of Histology and Embryology, Pomeranian Medical University in Szczecin, Poland)

Numerous *in vitro* tests have been developed to assess the effects of various inhaled environmental factors on cell viability, cytotoxicity, and genotoxicity with the goal of predicting potential respiratory tissue irritation *in vivo*; however, there are no standards for determining *in vitro* the effects of chemicals released during heating of e-fluid to e-aerosol. New *in vitro* techniques are needed to analyze different types of e-aerosols. Several tissue models are currently being used as effective tools for understanding complex physiological processes under *in vitro* conditions. The primary animal models used to assess the hazard and risk to humans of inhaling environmental chemicals and those released from e-cigarettes are rats and mice [40, 41, 42]. Recently, an alternative method using 3-dimensional (3D) *in vitro* tissue models has been used to assess inhalation toxicity.

An EpiAirway 3D tissue model, which provides a fully differentiated *in vitro* reconstruction of the primary human tracheobronchial epithelium, was used by Neilson et al. to study the direct toxicological response of the tissue to environmental stimuli. In the experiment, the reconstructed tissue was exposed to acute continuous cigarette smoke and e-aerosol. The results showed that exposure to e-aerosol did not result in a decrease in tissue viability, compared to the effects of conventional smoke [43]. However, a limitation of the study was that although acute continuous cigarette smoke exposure was used, exposure to e-aerosol was short-term; long-term exposure might be expected to yield different results.

An *in vitro* model of human bronchial epithelium (MucilAir) was used to study the effect of e-cigarette aerosols on tracheal epithelial integrity, mainly in terms of barrier resistance. The cells were derived from primary human cells isolated from human bronchial tissue obtained from 3 donors. The researchers described a new system of their own development useful for studying the tissue response of human airway epithelium to inhaled smoke, e-aerosols, and various environmental pollutants. The experiment used an organic electrochemical transistor to measure the barrier resistance at the air-liquid interface (ALI). Cultured epithelial cells were briefly exposed to an elevated concentration of e-aerosol generated by heating an e-liquid containing PG, VG, and nicotine. Exposure of MucilAir cells to e-aerosol had negative effects on ALI and was capable of producing cytotoxic effects [41].

The same ALI system was used by Noël et al. to study the effects of exposing human bronchial epithelial (HBE) cells (H292) to either butter-flavored or cinnamon-flavored e-cigarette aerosols (third-generation devices) for 2 h per day for 1 or 3 consecutive days. The e-cigarette aerosols were generated under "sub-ohm" (<0.5 Ω) or regular vaping conditions (resistance >1 Ω and voltage >4.5 V), and the cells were exposed to the aerosol at the ALI. The data showed that aerosols produced with the butter flavored liquid under sub-ohm conditions were characterized by high levels of carbonyls such as formaldehyde, acetaldehyde, and acrolein. Both types of aerosols affected the morphology and function of H292 cells. Exposure exerted significant cytotoxic effects and decreased integrity of tight junctions between epithelial cells (apoptosis and cell necrosis) was observed. The production of reactive oxygen species was also increased. At the molecular level, exposure led to dysregulation of gene expression in HBE cells, such as downregulation of genes associated with biotransformation, inflammation, and oxidative stress. It has also been documented that oxidative stress is involved in toxicity processes in cells exposed to cinnamon-flavored e-cigarette aerosol [44].

The devices with the sub-ohm coil have lower resistance and are able to deliver more power than other types of e-cigarettes, producing a larger cloud and stronger flavors, making them especially attractive to the younger user population. Sub-ohm devices are designed to work with lower levels of nicotine in e-liquids, but with a higher ratio of VG to PG. Volatile aldehyde emissions and e-liquid consumption in sub-ohm vaping products with 3 different configurations of 0.15 Ω coils (dual, quadruple, and octuple) were measured in the study by Cancelada et al. There were no significant differences between carbonyl emission levels and coil resistance or configuration. In addition, the results were compared to the carbonyl emission levels of e-cigarettes with lower tank systems and the lower emission levels found in sub-ohm devices [45]. The e-cigarette aerosols produced by heating the liquid in different types of devices contain many harmful toxins and chemicals, the effects of which on human health from short- and long-term exposure have not

yet been fully established. It should also be noted that many chemicals, including aldehydes, persist in indoor air.

The respiratory epithelial model, EpiAirway, established using primary cells from a disease-free, non-smoking donor, was used in an experiment by Czekala et al. in which the tissue was exposed to conventional cigarette smoke and e-aerosol with and without blueberry flavoring. It was shown that exposure to e-aerosols with and without flavoring did not affect tissue morphology or function compared to air-exposed tissues [46]. Similar effects were observed in another study using a 3D organotypic model of human airway epithelium (MucilAir, Epithelix) in which tissues were repeatedly exposed to smoke from a 3R4F reference cigarette and its nicotine-rich e-aerosol at the ALI for 4 weeks. There were no significant changes in morphology or function, such as levels of proinflammatory mediators, cilia beating, barrier integrity, or cytotoxicity, compared to air controls [47].

Conventional cigarette smoking has a well-documented association, including with chronic bronchitis and other diseases, and acquired ion transport abnormalities have been considered as potential mechanisms for mucus obstruction in respiratory tissues [48, 49]. Although e-cigarettes are considered a healthier alternative to traditional smoking, it is now known that they may have adverse effects on the respiratory system [11].

The study by Lin et al. sought to determine whether e-cigarette vapor could disrupt ion transport in the airway epithelium, which is associated with chronic bronchitis. Primary HBE cells, isolated from tissue from donors with no disease affecting ion transport, or Calu-3 (American Type Culture Collection) cells were used in the experiment. Both cell types – Calu-3 and HBE passages 1-2 – were cultured at the ALI until full differentiation. The e-aerosol was prepared from Red Oak Domestic e-cigarette liquid containing nicotine. Exposure of Calu-3 cells to e-aerosol reduced chloride transport, but this effect was not observed after exposure of the cells to e-liquid. It was shown for the first time that e-cigarette vapor could exert a dose-dependent inhibitory effect on chloride anion transport through the cystic fibrosis transmembrane conductance regulator (CFTR). The effect was not associated with alteration of monolayer integrity or cell viability, although such effects were observed when cells were exposed to the e-aerosol for 60 min. Exposure of primary HBE cells to e-aerosol also inhibited the epithelial sodium channel (ENaC) in a dose-dependent manner. After 5 min, the activity of the ENaC was reduced by 95.8% in primary HBE cells. These effects were observed after exposure to the e-aerosol, indicating that vaporization of the e-liquid is required to induce ion transport dysfunction. During heating of the e-aerosol, acrolein, a simple unsaturated highly reactive aldehyde, is released among a variety of thermal degradation products [50]. Thus, ion channel dysfunction in the airway epithelium may be partly controlled by acrolein, which can modify specific amino acid residues on CFTR and inhibit channel gating [50, 51]. Thus, some toxic chemicals, such as acrolein, released from the e-liquid as thermal degradation products, may lead to bronchial and lung disease with chronic use of e-cigarettes.

The study by Chung et al. used human cultured cells and a novel *in vivo* animal model to determine the effects of nicotine-containing e-aerosols on airway mucociliary function. For the *in vitro* experiment, differentiated HBE cells (primary HBE cells) were isolated from donor lungs of never-smokers; for the *in vivo* part, the airways of an ovine large animal model were exposed to e-aerosol. Exposure of cultured and differentiated HBE cells to e-aerosol at the ALI for 4 weeks reduced surface liquid hydration and increased mucus viscosity in a nicotine dose-dependent manner. Acute exposure of cells also increased intracellular calcium levels. This effect is primarily dependent ontransient receptor potential ankyrin 1 (TRPA1), a nicotine receptor, and is controlled by inhibiting the receptor with the selective antagonist A967079. The results of the *in vitro* study were confirmed in the *in vivo* animal model. Exposure of ewes to e-cigarette vapor containing nicotine also produced effects similar to the *in vitro* study and dose-dependently reduced tracheal mucus velocity and increased plasma cotinine (the major metabolite of nicotine) [52]. The study showed that e-cigarette liquid containing nicotine had detrimental effects on airway mucociliary function under *in vitro* and *in vivo* conditions. E-cigarette liquid and aerosol contain several other toxic and irritating substances that may have similar adverse effects.

Like conventional smoking, e-cigarette use may affect gene expression. In the study by Corbett et al., bronchial airway epithelial cells obtained during fiberoptic bronchoscopy with an endoscopic cytobrush from: (i) current smokers, (ii) current e-cigarette users who are former smokers, and (iii) former smokers were included in the experiment to assess the effect of e-cigarette aerosol and smoke on the gene expression profile. For all genes examined in bronchial epithelial cells, e-cigarette users were found to have altered gene expression profiles more comparable to former traditional smokers. However, there was a group of genes in e-cigarette users whose expression was altered specifically in the subjects. The data may indicate that e-cigarette use by former cigarette smokers may lead to a change in the expression of new genes in the airway epithelium and that this may also affect epithelial function [53].

Bronchial epithelial cells (Lonza Human Bronchial Epithelial Cells) were used to study the effects of exposing the cells to 3 e-liquid apple flavors, nicotine, PG and VG. Exposure to e-cigarette aerosol had a cytotoxic effect and induced increased necrosis and apoptosis of cultured bronchial epithelial cells. In addition, macrophage function was impaired, with decreased efferocytosis via a reduction in the expression of the efferocytic receptor – CD36 (a macrophage apoptotic cell receptor). E-cigarette vapor also impaired the secretory function of bronchial epithelial cells, as reflected by the inhibition of the release of inflammatory cytokines such as TNF-α, interferon-gamma-inducible protein 10 (IP-10; CXCL10), macrophage inflammatory protein-1 alpha (MIP-1α), and macrophage inflammatory protein-1 beta (MIP-1ß). However, different components of the e-cigarette extract, cigarette smoke extract, and three tested flavors were able to reduce the release of different types of cytokines, resulting in changes in cell function of bronchial epithelial cells [54]. The same researchers conducted similar

studies in which human bronchial epidermal cells (16HBE cells), normal human bronchial epithelial (NHBE) cells, and alveolar macrophages from healthy donors were exposed to an e-aerosol of ten different flavored e-liquids. Bronchial epithelial cell viability and apoptosis, as well as phagocytosis of bacteria and apoptotic cells, were assessed. A flavor-dependent increase in apoptosis and necrosis of 16HBE cells was observed; NHBE cells also showed high sensitivity to different flavors. The phagocytic activity of alveolar macrophages was also altered in a flavor-dependent manner, and the secretory activity in the release of cytokines by NHBE cells and macrophages was also altered [55]. This study suggests that flavors in e-liquids may play a key role in altering bronchial epithelial morphology and alveolar macrophage function and phagocytic activity.

CONCLUSIONS

Limited long-term human data are available to assess the physiological and pathophysiological consequences of e-cigarette use and exposure to inhaled and exhaled e-cigarette aerosol and to determine the actual impact of the use of such devices on human health. Toxicological analyses indicate that the aerosols produced by e-cigarette devices contain harmful chemicals at levels that could cause short-term health effects, but the potential long-term effects have not been adequately studied. In addition, chemicals in e-cigarette aerosol have been found to persist indoors, making second-hand exposure a health concern. Respiratory organs and tissues are the first to be exposed to e-aerosols, which can cause biochemical, morphological, and functional adverse effects in airway epithelial cells, as documented in *in vitro* and *in vivo* studies. Diseases of the airways may be associated with mucus hypersecretion, such as the major pathophysiological features of chronic bronchitis and chronic obstructive pulmonary disease. Thus, the mucus on the surface of the airway epithelium plays a very important and even key role in the proper functioning of the epithelium. As the use of electronic cigarettes and their rapid increase in popularity has public health implications, e-cigarette users should be studied holistically, especially younger groups of addicted and experimental users.

REFERENCES

1. Bhatt JM, Ramphul M, Bush A. An update on controversies in e-cigarettes. Paediatr Respir Rev 2020;36:75-86. doi: 10.1016/j.prrv.2020.09.003.
2. Perez MF, Crotty Alexander LE. Why Is Vaping Going Up in Flames? Ann Am Thorac Soc 2020;17(5):545-9. doi: 10.1513/AnnalsATS.201910-802PS.
3. Bonner E, Chang Y, Christie E, Colvin V, Cunningham B, Elson D, et al. The chemistry and toxicology of vaping. Pharmacol Ther 2021;225:107837. doi: 10.1016/j.pharmthera.2021.107837.
4. Eshraghian EA, Al-Delaimy WK. A review of constituents identified in e-cigarette liquids and aerosols. Tob Prev Cessat 2021;7:10. doi: 10.18332/tpc/131111.
5. Kaur G, Pinkston R, McLemore B, Dorsey WC, Batra S. Immunological and toxicological risk assessment of e-cigarettes. Eur Respir Rev 2018;27(147):170119. doi: 10.1183/16000617.0119-2017.
6. Son Y, Khlystov A. An Automated Aerosol Collection and Extraction System to Characterize Electronic Cigarette Aerosols. Front Chem 2021;9:764730. doi: 10.3389/fchem.2021.764730.
7. Li Y, Burns AE, Tran LN, Abellar KA, Poindexter M, Li X, et al. Impact of e-Liquid Composition, Coil Temperature, and Puff Topography on the Aerosol Chemistry of Electronic Cigarettes. Chem Res Toxicol 2021;34(6):1640-54. doi: 10.1021/acs.chemrestox.1c00070.
8. Uchiyama S, Noguchi M, Sato A, Ishitsuka M, Inaba Y, Kunugita N. Determination of Thermal Decomposition Products Generated from E-Cigarettes. Chem Res Toxicol 2020;33(2):576-83. doi: 10.1021/acs.chemrestox.9b00410.
9. Behar RZ, Davis B, Wang Y, Bahl V, Lin S, Talbot P. Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids. Toxicol *In Vitro* 2014;28(2):198-208. doi: 10.1016/j.tiv.2013.10.006.
10. Salam S, Saliba NA, Shihadeh A, Eissenberg T, El-Hellani A. Flavor-Toxicant Correlation in E-cigarettes: A Meta-Analysis. Chem Res Toxicol 2020;33(12):2932-8. doi: 10.1021/acs.chemrestox.0c00247.
11. Marques P, Piqueras L, Sanz MJ. An updated overview of e-cigarette impact on human health. Respir Res 2021;22(1):151. doi: 10.1186/s12931-021--01737-5.
12. Li L, Lin Y, Xia T, Zhu Y. Effects of Electronic Cigarettes on Indoor Air Quality and Health. Annu Rev Public Health 2020;41:363-80. doi: 10.1146/annurev-publhealth-040119-094043.
13. Farsalinos KE, Polosa R. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. Ther Adv Drug Saf 2014;5(2):67-86. doi: 10.1177/2042098614524430.
14. Hussain S, Shahid Z, Foroozesh MB, Sofi UF. E-cigarettes: A novel therapy or a looming catastrophe. Ann Thorac Med 2021;16(1):73-80. doi: 10.4103/atm.ATM_190_20.
15. Kar M, Emre IE, Bayar Muluk N, Cingi C. Effect of Electronic Cigarettes on the Inner Mucosa of the Craniofacial Region. J Craniofac Surg 2019;30(3):e235-38. doi: 10.1097/scs.0000000000005190.
16. Trucco EM. A review of psychosocial factors linked to adolescent substance use. Pharmacol Biochem Behav 2020;196:172969. doi: 10.1016/j.pbb.2020.172969.
17. Jones K, Salzman GA. The Vaping Epidemic in Adolescents. Mo Med 2020;117(1):56-8.
18. Khlystov A, Samburova V. Flavoring Compounds Dominate Toxic Aldehyde Production during E-Cigarette Vaping. Environ Sci Technol 2016;50(23):13080-5. doi: 10.1021/acs.est.6b05145.
19. Sapru S, Vardhan M, Li Q, Guo Y, Li X, Saxena D. E-cigarettes use in the United States: reasons for use, perceptions, and effects on health. BMC Public Health 2020;20(1):1518. doi: 10.1186/s12889-020-09572-x.
20. Szumilas K, Szumilas P, Grzywacz A, Wilk A. The Effects of E-Cigarette Vapor Components on the Morphology and Function of the Male and Female Reproductive Systems: A Systematic Review. Int J Environ Res Public Health 2020;17(17):6152. doi: 10.3390/ijerph17176152.
21. Nagpal TS, Green CR, Cook JL. Vaping During Pregnancy: What Are the Potential Health Outcomes and Perceptions Pregnant Women Have? J Obstet Gynaecol Can 2021;43(2):219-26. doi: 10.1016/j.jogc.2020.05.014.
22. Delles C, Olfert IM. Electronic cigarettes: how bad are they for your health? Cardiovasc Res 2020;116(6):e64-6. doi: 10.1093/cvr/cvaa041.
23. Harkema JR, Carey SA, Wagner JG. The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol 2006;34(3):252-69. doi: 10.1080/01926230600713475.
24. Rha MS, Kim CH. Are Electronic Cigarettes Harmful? Mucin May Be the Key. Clin Exp Otorhinolaryngol 2021;14(3):249250.
25. Aziz A, Md Shukri N, Ramli RR. Vape Till Your Nose Bleed. J Med 2017;18(1):47-51. doi: 10.3329/jom.v18i1.31178.
26. Scheibein F, McGirr K, Morrison A, Roche W, Wells JSG. An exploratory non-randomized study of a 3-month electronic nicotine delivery system (ENDS) intervention with people accessing a homeless supported temporary accommodation service (STA) in Ireland. Harm Reduct J 2020;17(1):73. doi: 10.1186/s12954-020-00406-y.
27. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, et al. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. Am J Physiol Lung Cell Mol Physiol 2016;311(1):L135-44. doi: 10.1152/ajplung.00170.2016.

28. Rebuli ME, Glista-Baker E, Hoffman JR, Duffney PF, Robinette C, Speen AM, et al. Electronic-Cigarette Use Alters Nasal Mucosal Immune Response to Live-attenuated Influenza Virus. A Clinical Trial. Am J Respir Cell Mol Biol 2021;64(1):126-37. doi: 10.1165/rcmb.2020-0164OC.
29. Higham A, Rattray NJ, Dewhurst JA, Trivedi DK, Fowler SJ, Goodacre R, et al. Electronic cigarette exposure triggers neutrophil inflammatory responses. Respir Res 2016;17(1):56. doi: 10.1186/s12931-016-0368-x.
30. Wu Q, Jiang D, Minor M, Chu HW. Electronic cigarette liquid increases inflammation and virus infection in primary human airway epithelial cells. PLoS One 2014;9(9):e108342. doi: 10.1371/journal.pone.0108342.
31. Kumral TL, Saltürk Z, Yildirim G, Uyar Y, Berkiten G, Atar Y, et al. How does electronic cigarette smoking affect sinonasal symptoms and nasal mucociliary clearance? B-ENT 2016;12(1):17-21.
32. Rouabhia M, Piché M, Corriveau MN, Chakir J. Effect of e-cigarettes on nasal epithelial cell growth, Ki67 expression, and pro-inflammatory cytokine secretion. Am J Otolaryngol 2020;41(6):102686. doi: 10.1016/j.amjoto.2020.102686.
33. Song SY, Na HG, Kwak SY, Choi YS, Bae CH, Kim YD. Changes in Mucin Production in Human Airway Epithelial Cells After Exposure to Electronic Cigarette Vapor With or Without Nicotine. Clin Exp Otorhinolaryngol 2021;14(3):303-11. doi: 10.21053/ceo.2020.01907.
34. Kwak S, Choi YS, Na HG, Bae CH, Song SY, Kim YD. Glyoxal and Methylglyoxal as E-cigarette Vapor Ingredients-Induced Pro-Inflammatory Cytokine and Mucins Expression in Human Nasal Epithelial Cells. Am J Rhinol Allergy 2021;35(2):213-20. doi: 10.1177/1945892420946968.
35. Ajmani GS, Suh HH, Wroblewski KE, Pinto JM. Smoking and olfactory dysfunction: A systematic literature review and meta-analysis. Laryngoscope 2017;127(8):1753-61. doi: 10.1002/lary.26558.
36. Siegel JK, Wroblewski KE, McClintock MK, Pinto JM. Olfactory dysfunction persists after smoking cessation and signals increased cardiovascular risk. Int Forum Allergy Rhinol 2019;9(9):977-85. doi: 10.1002/alr.22357.
37. Majchrzak D, Ezzo M-C, Kiumarsi M. The effect of tobacco- and electronic cigarettes use on the olfactory function in humans. Food Quality Preference 2020;86:103995. doi: 10.1016/j.foodqual.2020.103995.
38. Salturk Z, Çakır Ç, Sünnetçi G, Atar Y, Kumral TL, Yıldırım G, et al. Effects of Electronic Nicotine Delivery System on Larynx: Experimental Study. J Voice 2015;29(5):560-3. doi: 10.1016/j.jvoice.2014.10.013.
39. Ball M, Hossain M, Padalia D. Anatomy, Airway. In: StatPearls. Treasure Island (FL): StatPearls Publishing LLC; 2022.
40. Cao X, Coyle JP, Xiong R, Wang Y, Heflich RH, Ren B, et al. Invited review: human air-liquid-interface organotypic airway tissue models derived from primary tracheobronchial epithelial cells-overview and perspectives. *In Vitro* Cell Dev Biol Anim 2021;57(2):104-32. doi: 10.1007/s11626--020-00517-7.
41. Ferro MP, Leclerc L, Sleiman M, Marchiori B, Pourchez J, Owens RM, et al. Effect of E Cigarette Emissions on Tracheal Cells Monitored at the Air-Liquid Interface Using an Organic Electrochemical Transistor. Adv Biosyst 2019;3(3):e1800249. doi: 10.1002/adbi.201800249.
42. Movia D, Bruni-Favier S, Prina-Mello A. *In vitro* Alternatives to Acute Inhalation Toxicity Studies in Animal Models – A Perspective. Front Bioeng Biotechnol 2020;8:549. doi: 10.3389/fbioe.2020.00549.
43. Neilson L, Mankus C, Thorne D, Jackson G, DeBay J, Meredith C. Development of an *in vitro* cytotoxicity model for aerosol exposure using 3D reconstructed human airway tissue; application for assessment of e-cigarette aerosol. Toxicol *In Vitro* 2015;29(7):1952-62. doi: 10.1016/j.tiv.2015.05.018.
44. Noël A, Hossain E, Perveen Z, Zaman H, Penn AL. Sub-ohm vaping increases the levels of carbonyls, is cytotoxic, and alters gene expression in human bronchial epithelial cells exposed at the air-liquid interface. Respir Res 2020;21(1):305. doi: 10.1186/s12931-020-01571-1.
45. Cancelada L, Tang X, Russell ML, Maddalena RL, Litter MI, Gundel LA, et al. Volatile aldehyde emissions from "sub-ohm" vaping devices. Environ Res 2021;197:111188. doi: 10.1016/j.envres.2021.111188.
46. Czekala L, Simms L, Stevenson M, Tschierske N, Maione AG, Walele T. Toxicological comparison of cigarette smoke and e-cigarette aerosol using a 3D *in vitro* human respiratory model. Regul Toxicol Pharmacol 2019;103:314-24. doi: 10.1016/j.yrtph.2019.01.036.
47. Czekala L, Wieczorek R, Simms L, Yu F, Budde J, Trelles Sticken E, et al. Multi-endpoint analysis of human 3D airway epithelium following repeated exposure to whole electronic vapor product aerosol or cigarette smoke. Curr Res Toxicol 2021;2:99-115. doi: 10.1016/j.crtox.2021.02.004.
48. Kreindler JL, Jackson AD, Kemp PA, Bridges RJ, Danahay H. Inhibition of chloride secretion in human bronchial epithelial cells by cigarette smoke extract. Am J Physiol Lung Cell Mol Physiol 2005;288(5):L894-902. doi: 10.1152/ajplung.00376.2004.
49. Raju SV, Lin VY, Liu L, McNicholas CM, Karki S, Sloane PA, et al. The Cystic Fibrosis Transmembrane Conductance Regulator Potentiator Ivacaftor Augments Mucociliary Clearance Abrogating Cystic Fibrosis Transmembrane Conductance Regulator Inhibition by Cigarette Smoke. Am J Respir Cell Mol Biol 2017;56(1):99-108. doi: 10.1165/rcmb.2016-0226OC.
50. Lin VY, Fain MD, Jackson PL, Berryhill TF, Wilson LS, Mazur M, et al. Vaporized E-Cigarette Liquids Induce Ion Transport Dysfunction in Airway Epithelia. Am J Respir Cell Mol Biol 2019;61(2):162-73. doi: 10.1165/rcmb.2017-0432OC.
51. Gaurav R. Vaping Away Epithelial Integrity. Am J Respir Cell Mol Biol 2019;61(2):127-9. doi: 10.1165/rcmb.2019-0016ED.
52. Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, et al. Electronic Cigarette Vapor with Nicotine Causes Airway Mucociliary Dysfunction Preferentially via TRPA1 Receptors. Am J Respir Crit Care Med 2019;200(9):1134-45. doi: 10.1164/rccm.201811-2087OC.
53. Corbett SE, Nitzberg M, Moses E, Kleerup E, Wang T, Perdomo C, et al. Gene Expression Alterations in the Bronchial Epithelium of e-Cigarette Users. Chest 2019;156(4):764-73. doi: 10.1016/j.chest.2019.05.022.
54. Ween MP, Hamon R, Macowan MG, Thredgold L, Reynolds PN, Hodge SJ. Effects of E-cigarette E-liquid components on bronchial epithelial cells: Demonstration of dysfunctional efferocytosis. Respirology 2020;25(6):620-8. doi: 10.1111/resp.13696.
55. Ween MP, Moshensky A, Thredgold L, Bastian NA, Hamon R, Badiei A, et al. E-cigarettes and health risks: more to the flavor than just the name. Am J Physiol Lung Cell Mol Physiol 2021;320(4):L600-14. doi: 10.1152/ajplung.00370.2020.