On the toxicity of e-cigarettes consumption: Focus on pathological cellular mechanisms

Q1 On the toxicity of e-cigarettes consumption: ~~focus~~Focus on Q2 pathological cellular mechanisms

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Abstract

Q3 Tobacco smoking remains without a doubt one of the leading causes of premature death worldwide. In combination with conventional protocols for smoking cessation, e-cigarettes have been proposed as a useful tool to quit smoking. Advertised as almost free of toxic effects, e-cigarettes have rapidly increased their popularity, becoming a sought-after device, especially among young people. Recently some health concerns about e-cigarette consumption are being raised. It is well known that they can release several toxic compounds, some of which are carcinogenic to humans, and emerging results are now outlining the risks related to the onset of respiratory and cardiovascular diseases and even cancer. The present review shows the emerging evidence about the role of technical components of the devices, the e-liquid composition as well as customization by consumers. The primary topics we discuss are the main toxicological aspects associated with e-cigarette consumption, focusing on the molecular pathways involved. Here it will be shown how exposure to e-cigarette aerosol induces stress/mitochondrial toxicity, DNA breaks/fragmentation following the same pathological pathways triggered by tobacco smoke, including the deregulation of molecular signalling axis associated with cancer progression and cell migration. Risk to fertility and pregnancy, as well as cardiovascular risk associated with e-cigarette use, have also been reported.

Keywords:

E-cigarette, Genotoxicity, Oxidative stress, Cancer risk, Cardiovascular risk, Fertility

Abbreviations

No keyword abbreviations are available

1 Introduction

Since their introduction, the possible toxic effects associated with the consumption of electronic cigarettes (e-cigarettes; e-cigs) have been a matter of controversy among the scientific community and healthcare professionals [1]. Tobacco currently kills 8 million people per year [2] and it is little wonder that e-cigs have received great attention as a valid aid to stop smoking, due to their sudden sponsorship as almost risk-free even in the absence of proper scientific evidence-based data [1]. Thus, e-cigarettes have become increasingly popular, particularly among teenagers [3]. The key to their success can be mainly attributed to the possibility of vapers to inhale nicotine, maintaining the typical gestures associated with tobacco smoking without the exposure to typical toxic combustion by-products [4]. Since e-cigs become widely available worldwide, numerous studies have looked at usage patterns, safety, benefits, and risks. In the face of technological advances and the continuous placement on the market of input devices with unprecedented characteristics that can significantly influence the release of harmful compounds and, therefore, the associated toxicity, the scientific community is working hard to define a toxicological profile of these new electronic nicotine delivery systems (ENDS). It has long been known that not only the total number of smoked cigarettes but also the smoking methods can significantly affect the toxicological outcomes. The habit of reverse smoking, for example, is associated with higher risk of carcinoma of the palate [5]. Vaping introduces a huge number of variables such as the voltage, the resistance coil (one or more coils with different ohmic values), the e-liquid with variable portion of vegetal glycerin or propylene glycol, as well as different concentration of nicotine, further complicating the risk evaluation. The main purpose of this review is to focus on the emerging toxicological and molecular aspects related to the use of this new technology. In light of the growing interest on the topic and the resulting high number of studies, the present work will discuss a selection of the highest impact toxicological papers.

1.1 Electronic ~~Nicotine Delivery Systems~~nicotine delivery systems (ENDS) – basic e-cigarette configuration and main components

Conceived to help smokers to quit smoking, many tobacco-free nicotine delivery systems have been developed, including alternative products to nicotine [6]. The most common are summarized in Table 1. E-cigs have set a new standard that has never been achieved before. In contrast to nicotine vaporizers, e-cigs allow the consumers to mimic some gestures associated with smoking habits such as the hand-to-mouth act, the sensation of inhaling and exhaling something flavored, maintaining all social rituals that are common to smokers [7]. These features have strongly contributed to the rapid spread of this new device around the world. Although e-cigarette companies have been working hard to bring more and more innovative products to the market in recent years, e-cigs have common main components: battery, atomizer, and a tank or cartridge containing e-liquid (Fig. 1). Two main classes of e-cigs are identified: “closed” e-cigs, non-refillable with non-replaceable battery or atomizer, and “open” systems, designed to be refilled by users, allowing them to replace several components such as the atomizer (single or multiple coil) with a range of electrical resistances in terms of Ohm (Ω), voltage applied, and composition of the e-liquid. The e-liquid is composed of polypropylene glycol (PG) and/or vegetable glycerin (VG) at different rates; the consumer can customize it with a multitude of aromas and nicotine at different concentrations (usually from 0 to ~~36~~36 mg/mL) [8]. Contrary to what was initially thought, the nicotine concentration and the by-products of the thermal degradation of PG and VG are not the only toxic factors related to e-cigs to be considered. The technical characteristics of the device play a crucial role in the release of toxic compounds and hence on the risk associated with the use of e-cigs.

alt-text: Table 1

Table 1

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Medicinal and alternative nicotine delivery systems including ENDS.

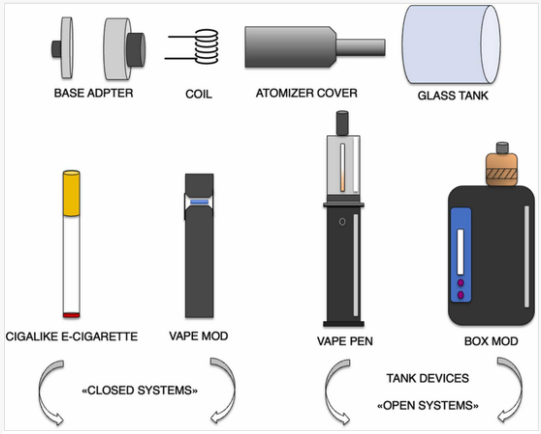
~~Non-tobacco nicotine delivery systems~~Non-tobacco nicotine delivery systems	
Medicinal nicotine delivery systems	**Alternative nicotine delivery systems**
Transdermal patch	ENDS (liquid e-cigarettes, heating tobacco systems)
Nicotine gum	Nicotine gel
Nasal spray	Nicotine candies
Inhalers	Nicotine lip balms
Sublingual tablets	Nicotine water

Modified from Rom et al. [6]. ENDS, electronic nicotine delivery systems.

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 1

Fig. 1



~~Main features of "closed" and "open" e-cigarette systems.~~Main features of "closed" and "open" e-cigarette systems. Common elements for different e-cigarette devices: coil, atomizer, tank (upper side). Schematic representation of different types of "closed" or "open" e-cigarette systems. "Closed systems" (left side) are non-refillable with non-replaceable battery or atomizer, "Open systems" (right side) allow the possibility of personalizing the vaping experience with adjustable voltage and wattage settings, as well as modifying nicotine concentration, polyethylene glycol/glycerol ratio and adding flavorings (bottom side).

2 Impact of e-cigarette features in toxicological outcomes

In the following section we discuss the role of the main e-cigarette features that can alter the release of certain toxic compounds in the aerosol hence the toxicological outcomes. Recent evidence shows how the coil resistance as well as the nicotine content and the flavorings are particularly impactful variables. The present topic is of particular interest for the scientific community because, unlike traditional cigarettes, the toxicological effects associated with vaping depend largely on the type of device and how the consumer uses it.

2.1 Voltage and coil resistance

Unlike e-cig first generation, the latest models launched on the market allow the possibility to the consumers to regulate the voltage output of the battery *via* a wide selection of atomizers with electrical resistance values ranging from sub-Ohm (< 0,5 Ω) up to > 2.8 Ω coils [9].

Initially, low-voltage devices were commonly perceived as safer: the aerosol produced by e-cigs using higher-voltage devices caused a decrease in cell survival in H9c2 cardiomyoblast cells [10] and the influence of voltage on A549 cell line cytotoxicity was also observed; in fact, the increase of battery output voltage significantly reduces cell viability [11] . But from the very beginning it was hypothesized that the heating power of the device depended on a combination of the resistance value of the heating filament and the voltage applied to it by the Joule effect [12]. Thus, it is possible for a low-voltage e-cig to get the same power as a high-voltage e-cig as long as it reduces the resistance value. Growing evidence appears to support this hypothesis and some studies have reported increased carbonyl compound production when low resistance coils are used [13,14], because more e-liquid is consumed at higher wattage settings [15,16]. In addition, the temperature of the e-cig device may also be higher, and this can lead to an increase in carbonyl concentrations.

Interestingly, *in vitro* models have shown that switching from Ohm to sub-Ohm atomizers results in lower cell viability relating to a greater amount of ROS [13] and altered expression of key genes associated with oxidative stress [14]. Studies in rats confirm that sub-Ohm vaping causes marked airway remodeling with alveolar collapse and disorganization of the bronchial epithelium, together with the reduction of ciliated cells in the trachea and the presence of necrotic and apoptosis bodies [17]. It is noteworthy that these tissue alterations are reported by traditional smokers and COPD patients, and recently has been shown that exposure to e-cigarette aerosol is associated with inflammation along with loss of epithelial barrier function in lung cells [18].

The data on hematologic alteration are in line with the hypothesis that a low resistance vaporization can be considered a more intense exposure. The drop in the number of lymphocytes that occurs when naïve animals are exposed to sub-Ohm aerosol reflects what is observed when changing from light- to heavy-smokers [19] as well as in children with a history of exposure to tobacco smoke indoors [20]. The increase in neutrophils observed in the animal models is a key marker recorded in smokers and COPD patients who switch from light- to heavy-smokers [21–24]. Overall, the available data suggest that sub-Ohm atomizers trigger detrimental effects comparable to those induced by tobacco smoke that alter the gene expression of key genes associated with biotransformation, oxidative stress, and inflammation. However, it should be pointed out that in the above cited study [17], the authors did not perform a normalization considering the total aerosol released in “Ohm or sub-Ohm condition”. Since vapers dose themselves with aerosol, it cannot be ruled out that consumers may independently reduce the amount of inhaled vapor.

2.2 Nicotine

The concentration of nicotine in e-liquids and the ability of ENDS to deliver it in the mainstream of aerosol represents a key point for a satisfactory alternative to tobacco smoke or a valid aid for cessation [17]. However, the amount of nicotine delivered from e-cigarettes can be variable.

As discussed earlier, vaping patterns, including the power output setting and the type of coil, can greatly affect plasma nicotine levels [114]. Recent evidence shows that the lower temperature reached by e-cigs compared to those reached by tobacco combustion ~~(~690°C)~~(~690 °C) appears to promote the dehydrogenation of nicotine into nicotyrine, which could, in turn, increase the plasma level of nicotine by the inhibition of some cytochrome P450 isoforms, such as cytochrome P450 2A6, and cytochrome P450 2A13, both involved in nicotine metabolism.

Nicotine and nicotyrine concentrations in e-cig mainstream could be affected also by the PG and VG ratio. PG-based e-liquids have been found to deliver a higher nicotine concentration than VG-based e-liquids with the same output wattage[25] [26]. As PG is more volatile than VG, a higher percentage of PG will produce a higher vaporization rate at a given temperature and potency [27]. Additionally, liquids based on PG have been reported to generate much more toxic chemicals, such as formaldehyde and acetaldehyde, than liquids based on VG [28]. Recent evidence suggests that nicotine, by basifying the liquid, can catalyze the production of carbonyls, but new studies show how nicotine-free liquids, widely perceived as safer, can instead release a higher yield of carbonyls. [13,28].

These results are supported by the observation of higher levels of IL-6 and intracellular mucin in lung cells exposed to nicotine-free mainstream, while no significant differences were observed compared to controls in the case of nicotine e-

liquid [118]. Furthermore, exposure to nicotine e-cig has been shown to cause a response to lung inflammation/remodeling through the nAChRα7 receptor mediated signaling pathway [119], although marked tissue remodeling was also observed after acute or sub-chronic exposure to e-cig aerosol without nicotine. Interestingly, the dysregulation of MMPs, a marker of inflammation and extracellular matrix remodeling, was more evident with nicotine-free exposure [17,29], suggesting also nAChRα7 independent mechanisms. In conclusion, to date, data do not support the common belief that nicotine-free liquid are less harmful suggesting caution even for nicotine-free products use.

2.3 Flavors

Consumers can personalize their vaping experience by adding flavors to the liquid-base. Among the best sellers there are aldehydes (vanillin, vanilla; benzaldehyde, berry/fruit; cinnamaldehyde, cinnamon; damascenone, tobacco), benzyl alcohol, terpenes (linalool, flowery; farnesol, apple), pyrazines (coffee, chocolate), menthol, menthone, and other minty compounds, and sweet flavors, including ethyl maltol [30]. Although many flavors are safe as food additives, their airway toxicity after incomplete combustion is largely unknown. Data from cell-based and *in vivo* models suggest that the presence of vanillin and cinnamaldehyde correlates with cytotoxicity, ciliary impairment, and inflammation [17,31–33], while the presence of buttery flavor diacetyl is associated with pulmonary toxicity that can also occur through obliterate bronchiolitis [34]. To further complicate the matter, it should be borne in mind that aldehyde aromas can react with PG and VG to form irritant acetal compounds [35]. A study conducted on fetal, neonatal, and adult bovine pulmonary artery smooth muscle cells showed that exposure to the aromas of e-cig even without nicotine can damage immature lung [36], which is of special relevance for young consumers of e-cig, attracted by the variety of candy flavors. Moreover, aldehydes originated from flavoring agents influenced the activity of cytochrome P450-dependent monooxygenases [37,38], in particular by inhibiting cytochrome P450 2A6, involved in the oxidative metabolism of nicotine [39] leading to disruption of nicotine metabolism and a putative increase of plasmatic nicotine levels with a worsening of the addiction state as well as toxicological aspects related to nicotine intake. Thus, these results point to the need to investigate how flavors could have an impact on nicotine itself [40]. Moreover, these findings underline the concern that flavors may largely contain or potentially generate harmful species, according to the results of Gerloff and colleagues [41] who found an inflammatory response in human lung epithelial cells and fibroblasts and significant loss of epithelial barrier function after exposure to various components of e-cigarette aromas.

3 Evidence of toxic chemicals in e-cigarette emission

Since e-cigarettes heat the liquid to generate aerosol, initial studies focused mainly on the generation of toxic thermal breakdown products that could compromise users and bystanders' health. These include tobacco-specific nitrosamines (TSNAs), metals, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), carbonyl compounds, and aldehydes [42].

The first studies addressing this matter date back to the years ~~2008–2009~~2008–2009 [43,44]: eighteen brands of e-cigarette liquids available on the US market were studied, and then the US Food and Drug Administration (FDA) conducted a similar analysis. The results showed traces of aldehydes such as acrolein, formaldehyde, and acetaldehyde and some metals (mercury) [45]. In 2014, the study by Goniewicz and colleagues confirmed the presence of formaldehyde, acetaldehyde, and acrolein in e-cigarette emissions, as well as two VOCs: toluene and xylene [46] in a large number of e-cigarette brands.

Consistently with the literature, formaldehyde (group 1 carcinogens, International Agency for Research on Cancer, IARC, Lyon), acetaldehyde (group 2B), and acrolein, a strong irritant for the skin, eyes, and nasal passages [47], are the carbonyl compounds prevalent in e-cigarette emissions [46,48–51]. Fig. 2 shows the mechanism proposed by Klager and colleagues for the formation of aldehydes by pyrolysis and oxidation of glycerol and propylene glycol during the e-cig vaping process [51]. The release of aldehydes is closely associated with the vaping pattern, as effectively reported by Jensen et al. [52]. At low voltage, the formaldehyde amount was undetectable, whereas, moving toward higher values (*i.e.* 5.0 Volt), an average of 380 μg was recorded in the aerosol, allowing an e-cigarette user, vaping at a rate of 3 mL per day, to inhale close to 14 mg of formaldehyde per day [52]. These results are of particular concern, considering that the average formaldehyde output is about 3 mg per pack of 20 cigarettes [53]. Recently, the presence of dihydroxyacetone (DHA), the active ingredient of self-tanning products, has also been found in e-cig

aerosols [54]. Although the FDA approves the external use of DHA in cosmetics, the risk of inhalation at high temperatures is still unknown, while data on its genotoxicity are available [55].

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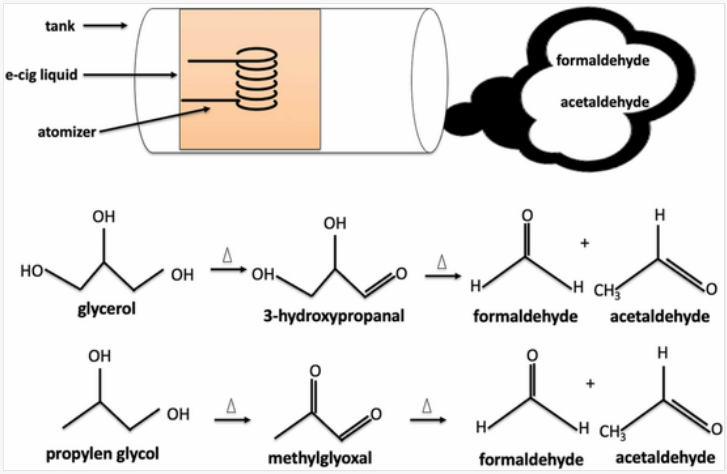
Fig. 2



~~Mechanism of formaldehyde and acetaldehyde formation from the heating of glycerol and propylene glycol during e-cigarette vaping.~~Mechanism of formaldehyde and acetaldehyde formation from the heating of glycerol and propylene glycol during e-cigarette vaping. Proposed mechanism for the thermal degradation of glycerol and propylene glycol and the generation of aldehydes from e-cigarette liquid independently of the presence of flavorings.~~[51]~~
The present figure has been modified from Klager et al. [51].

E-cigarette liquids consist of an unlimited combination of flavor additives, given that flavoring liquids is a more attractive experience, especially among young people [56]. While many of these flavors have been tested and recognized as safe for oral consumption by the FDA, thousands of flavor e-liquids are commercially available without a prior assessment of their inhalational toxicity [57]. Accordingly, several *in vitro* studies have highlighted toxic effects on human airway epithelial and immune cells [31,58,59]. These results have recently been confirmed in *in vivo* models that show alteration of asthma pathophysiology associated with flavor e-liquids even without nicotine [60], and they are of particular interest given that up to 66% of high school students who vape in US use flavor-only (nicotine-free) e-liquids [56]. Interestingly, a recent trial has reported a dose-response relationship between cinnamaldehyde levels from commercially available e-liquids and impaired respiratory immune cells [31].

Although cross-sectional and longitudinal studies have shown that e-cigarette use results in less overall systemic exposure to toxic substances than smoking [61,62], however, higher levels of metabolites from acrylamide, benzene, and possibly propylene oxide during e-cigarette using suggest a significant systemic exposure to these toxic/carcinogenic VOCs [62] that should be clarified in order to promote e-cig devices as a real healthier alternative to smoking [1]. However, as will be discussed below, the comparison in terms of chemical characterization, between traditional and electronic cigarettes is challenging because the aerosol chemical profile, including the concentration of toxic compounds, is a function of the device itself and its components. Even comparing first- and second-generation devices can be misleading.

3.1 Metals

The dangerous effects of heavy metal exposure are well known [63]. Although ENDS companies have stated the safety of e-cig compared to conventional cigarettes [64,65], several reports have shown the presence of different heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), arsenic (As), and chromium (Cr) in single devices and different brands of refill liquids [65–67].

It has been suggested that some e-cigs may also transfer Ni from the device to the aerosol, especially for those using NiChrome or Ni 200 resistance coils [68]. According to this, differences in the toxicological effects have been found

between devices with a stainless-steel atomizer or with NiChrome heating element or similar. In fact, animals exposed to the stainless-steel atomizer did not develop respiratory distress, whereas the ones exposed to NiChrome element did, suggesting that Ni in the device may transfer to the aerosol influencing the toxicological profile [69].

It is important to note that the amount of heavy metals in the aerosol of an e-cigarette is highly dependent on the refill solution [70]; some solutions result in lower levels of heavy metals than those found in the aerosols of conventional cigarettes [67,71], while others result in higher or equal concentrations as cigarette smoke [72].

What appears to be evident is that the lack of regulation in the manufacturing process of e-cigarette devices could potentially be responsible for the presence of heavy metals in the e-cigarette aerosol [32].

4 Oxidative stress pro-inflammatory effect and lung tissue damage

Data from cell models have shown an increase of pro-inflammatory cytokine production in human bronchial epithelial cells (Beas2B) and human lung fibroblasts (HFL-1) after exposure to e-cig puffs (3.5 s at a rate of 33.8 mL/s airflow; 7.5 W with a coil resistance of 2.1 Ω) [41]. In addition, dysfunction of bronchial epithelial barrier function has also been observed, which could lead to easier infections by microorganisms. Furthermore, human lung fibroblasts exposed to e-cig aerosol showed stress signs and morphological changes, probably caused by increased reactive oxygen species (ROS) production and inflammatory response [73,74]. These findings are consistent with the significant increase in ROS levels along with the reduction of glutathione (GSH) levels and the increase of DNA damage rate found in human bronchial epithelial cells (HBECs) exposed to e-cig mainstream [33]. Furthermore, e-cigarette fluids and aerosols caused cytotoxicity of human pulmonary fibroblasts [75]; in addition the A549 cell line showed that an increase in cell death was observed regardless of the presence of nicotine [76–78]. These phenomena are associated with DNA strand breaks in short- and long- term exposure, reduction of GSH/GSSG ratio and increased levels of antioxidant response elements and interleukin (IL)─ 8 production [76,78]. The effect of e-cig aerosol on human monocyte-derived dendritic cells was also investigated; e-cigarette emission treatment significantly increased IL-6 production by lipopolysaccharide-matured dendritic cells. Furthermore, exposure to e-cig aerosol changed the expression of molecules associated with the RAK/TRAF6/TAB pathway, which also led to the activation of mitogen-activated protein kinase (MAPK), such as p38, extracellular regulated kinase (ERK1/2) and IL-1 receptor associated kinase [78]. Activation of MAPK is strongly related to tobacco smoke [79–81] and is associated with worsening of chronic airway inflammation and may contribute to cancer etiology [82]. In fact, c-Jun N-terminal kinase (JNK), p38 and ERK1/2 signaling pathways play a key role in regulating the initiation of the epithelium-mesenchymal transition (EMT) and redox balance in the respiratory tissues [83]. The pro-inflammatory effects of e-cigarette liquids have also been shown in *in vivo* systems. Murine models showed that intratracheal fluid treatment increases inflammatory cell infiltration, cytokine production, and airway hyperreactivity [84]. Glynos and colleagues have reported that e-cig ingredients (including PG and VG) can induce lung inflammation and cause changes in the respiratory parameters in mice [85]. A dose-dependent pulmonary inflammatory response was also found in the animals, with increased levels of IL-8 and oxidative stress [76]; this reaction, triggered by exposure to e-cig mainstream, involves the nuclear factor-κB signaling pathway [29].

Inflammatory response plays a role in the onset of asthma. In fact, cytokines induce the activation and proliferation of inflammatory cells that may lead to respiratory crisis or exacerbation of asthma in conventional cigarette smokers [86,87]. However, the role of e-cigarettes in asthma still remains unclear and proliferation of inflammatory cells in lung tissue has been observed in healthy and asthma mouse model [88].

Some studies have shown a greater susceptibility to respiratory pathogens in rodents exposed to e-cig volatile emission. It was observed that the exposed animals had altered antimicrobial lung defenses with lower phagocytosis activity by alveolar macrophages [89]. Other studies on e-cigs have confirmed the disruption of immunity homeostasis against pathogens in mice [90,91] along with a dysregulation of lipogenesis/myogenesis processes [29] and a higher rate of lipid deposition in the lung parenchyma, regardless of nicotine [91]. Interestingly, data from lipidomic analysis performed on the rat brain confirmed the impact of e-cig mainstream emission on lipid and cholesterol homeostasis, which could contribute to the onset of some neurodegenerative diseases [3]. More recently, it has been found that the redox imbalance leads to marked post-translational modification of proteins in lung tissue; images from transmission electron microscopy (TEM) and scanning electron microscopy (SEM) revealed that the lung parenchyma of e-cig exposed rats was markedly remodeled with alveolar collapse and disorganization of the bronchial epithelium; the

number of ciliated cells in the trachea was dramatically reduced and apoptotic and necrotic cells were observed [17]; these changes are consistent with those reported by smokers and chronic obstructive pulmonary diseases (COPD) patients.

Clinical data seem to not support the use of e-cigarette as a harm reduction strategy. E-cig consumption in adults with or at risk for COPD, was associated with worse pulmonary-related health outcomes, but not with quit conventional smoking [92]. Interestingly, both healthy subjects and mild asthma patients exhibited significant respiratory mechanical and inflammatory effects after a single session of e-cigarette smoking [93]. Even among young high school students, e-cig users showed an increased association with asthma [94].

4.1 E-cigarette, or vaping product, use associated lung injury

Since 2019, healthcare professionals have documented more than ~~1,800~~1800 cases of acute lung injury associated with e-cig use in US [95]. Given that e-cigarette, or vaping product, use associated lung injury (EVALI) is a new emerging pathology, clinical knowledge is still limited and mostly based on scanty reports of isolated cases showing mechanical injuries (spontaneous pneumothorax), pneumonias (organizing, eosinophilic, and lipoid), or hypersensitivity pneumonitis [96]. To date, a clear pathological mechanism has not yet been identified.

The emergence of EVALI has been attributed to the use of home-made tetrahydrocannabinol (THC)-containing e-liquids. One of the most feasible hypotheses is that e -liquid containing THC and poorly tested diluents can release some breakdown products (not yet fully characterized) when heated, triggering an inflammatory cascade resulting in EVALI [96]. Vitamin E acetate has recently been suggested to play a pivotal role in the occurrence of EVALI [97,98]. In particular, the esterified (semi-synthetic) form of vitamin E (VEA), normally used in the food industry as an antioxidant and preservative, seems to play an important role in lung injuries. Although to date little is known about lung injuries from VEA, the surfactant disruption or epithelial injury from ketene formation are some proposed mechanisms [99,100]. Although the use of VEA is recognized as safe for oral ingestion by the FDA, the use in inhalation products has not been approved [101]. However, scientific literature also reports cases of EVALI occurrence in patients who consumed only nicotine-containing products, and some animal models suggest that aerosolized PG and VG can trigger an inflammatory response that compromises lung function [70]; in addition, the volatile emission derived from e-cigarettes accumulating in the airway epithelium in a similar fashion as smoke from combustible cigarettes can be responsible for lipoid pneumonia, usually seen when breathing in oil mist [102].

Recently, the American Lung Association has underlined how vitamin E acetate seems to be the primary, but not only, cause of EVALI. A report by Centers for Disease Control and Prevention (CDC) analyzed bronchoalveolar lavage (BAL) fluid from several EVALI patients from 16 states and compared them to BAL fluid from healthy people. They identified vitamin E acetate, also found in product samples tested by the FDA and state laboratories, in BAL fluid from 48 of 51 EVALI patients; vitamin E acetate was not found in any of the BAL fluids of healthy people. In addition to vitamin E acetate, there are many other substances and product sources in vaping materials that are being examined as possible causes of EVALI [103].

As shown, the toxicological outcomes associated with the use of e-cigs depend largely on device settings, intensity of use, and type of e-liquid. This large collection of variables could be consistent with the wide range of clinical manifestations from mild dyspnea to acute respiratory distress syndrome and death [95]. Nevertheless, EVALI seems largely caused by improper use of e-cig by consumers and more studies are needed to better understand the pathological mechanisms that lead to the identification of presumed susceptible individuals, as well as which components of e-cig conduct to higher risk of EVALI.

5 Cancer risk and main oncogenic molecular mechanisms triggered by e-cigarettes

The relationship between e-cig use and cancer risk is one of the most controversial topics in the discussion around the health effects of ENDS. Due to the recent introduction of such devices on the market, knowledge of their long-term effects is, of course, limited, but recent evidence suggests that e-cigarette consumption could be far from benign [104]. As previously discussed, the e-cigarette emission contains many toxic chemical compounds also found in traditional cigarettes, such as acetaldehyde, formaldehyde, acetone, acrolein, chromium, N-nitrosamines, and others [102,105,106] , and, surprisingly, in particular settings, the concentration of some of these well-known carcinogens, such as

formaldehyde, can actually exceed the levels recorded in tobacco cigarettes [107]. The first data on the possible carcinogenic effects of e-cigarettes came from *in vitro* studies, in which human bronchial epithelial cells exposed to e-cig aerosol showed gene expression pathways changes (compared to baseline), similar to those seen in tobacco smoking models [108]. These findings were supported by the observation of malignant transformation features in airway epithelial cells exposed to e-cig emission [109].

To date, clinical trials are scanty: a cross-sectional study showed how the use of e-cigarettes seems to be safe for oral cells and should be suggested as an aid to smoking cessation [110], but no long-term studies on carcinogenicity were available.

However, there are growing evidence from basic studies on the carcinogenic potential of e-cigarette aerosol.

Below, the main hypothesized oncogenic mechanism are discussed.

5.1 DNA damage and epithelial-mesenchymal transition

The first *in vivo* toxicological study regarding the possible association between e-cigarette consumption and cancer risk, showed that e-cigs mainstream aerosol induces toxicological effects that can promote carcinogenesis by the up-regulation of the carcinogen-metabolizing enzymes, and inducing oxygen free radical production leading to oxidative damage to macromolecules including lipids, proteins, and DNA [4]. Furthermore, the results of mutagenesis tests such as the Ames reverse mutation assay and the Comet alkaline test [4] supported the hypothesis that the use of e-cig can promote genotoxic effects (Fig. 3). A significant breakthrough came from the study by Lee and colleagues that indicated that nicotine metabolites from e-cig emission such as nitrosamines, induced toxicological outcomes similar to those obtained with exposure to tobacco cigarette smoke in the lungs, blood, and heart mouse tissues [111]. Consistently, DNA oxidative damage together with lower activity of DNA repair machinery with down-regulation of the xeroderma pigmentosus group C protein complex (XPC) and the 8-oxoguanine DNA glycosylase (OGG1/2) repair proteins as well as an increased mutation susceptibility and carcinogenic transformation in lung and bladder tissues have been reported [111].

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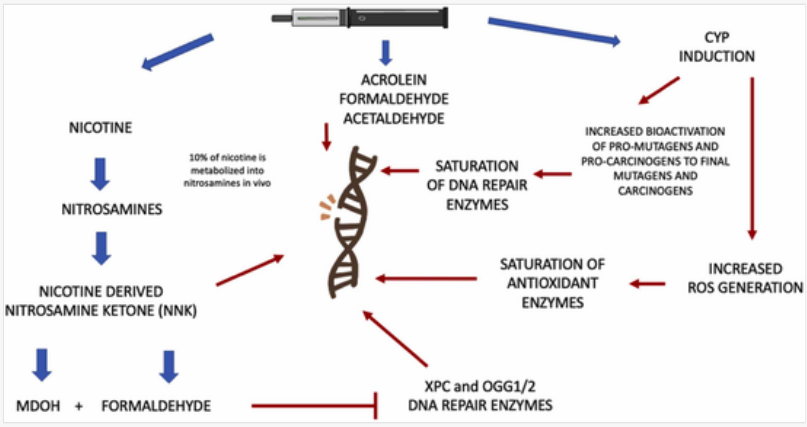
Fig. 3



~~Molecular mechanism proposed for e-cigarette DNA damage.~~Molecular mechanism proposed for e-cigarette DNA damage. Aldehydes released with e-cigarette aerosol, such as formaldehydes, can directly cause single-strand breaks in DNA and inhibit the expression of XPC and OGG1/2 DNA repair proteins in lung, bladder and heart tissue *in vivo*. Furthermore, a small portion of nicotine is metabolized into nitrosamines and nicotine-derived nitrosamine ketone which in turn is spontaneously degraded into methyldiazohydroxide and formaldehyde which also inhibits XPC and OGG1/2 DNA repair proteins. E-cig aerosol induces cytochrome (CYP) P450-dependent monooxygenase system which in turn leads to an increased bioactivation of pro-mutagens and pro-carcinogens to final mutagens and carcinogens. Again, the overproduction of reactive oxygen species (ROS) resulting from CYP induction is one of the well-documented ways in which CYP can play a key role in new cancer occurrence *via* a co-carcinogenesis mechanism [4,116,117].

To date, epithelial-mesenchymal transition ~~(EMT),~~(EMT), redox stress/mitochondrial toxicity, and DNA breaks/fragmentation are considered as the major molecular mechanisms suggested to be potentially involved in cancer risk associated with e-cig use [4,108,112]. Tobacco smoke is known to trigger EMT by altering the expression of various genes, including transcription factors, zinc-finger protein, and molecules involved in cellular adhesion (E-cadherin N-cadherin).

Recent evidence indicates that e-cig aerosol exposure leads to changes in the phenotype of A549 lung cancer cells with acquisition of fibroblast-like morphology, loss of cellular adhesion molecules, and increased motility [101]. These results are of enormous relevance, especially considering that EMT is often associated with an invasive feature.

The findings of experimental studies and clinical trials consistently showed how the use of e-cig results in the generation of free radicals associated with oncogenesis [4,89,112,113]. Moreover, exposure to e-cigarette aerosol increases mitochondrial ROS generation with disruption of the electron transport chain *in vitro* [96], which in turn is involved in mediating the signaling of inflammation and DNA instability. As discussed, basic studies have shown that the mainstream of the e-cigarette (containing or free of nicotine) includes compounds capable of inducing DNA damage such as single- or double-stranded breaks, DNA fragmentation and mutation [4,75,111]. The generation of double-strand-breaks is of particular concern considering that this form of DNA damage is largely repaired by the "non-homologous end joining" pathway, which is known to be error-prone, and is correlated with the acquisition of mutations and deletions [104].

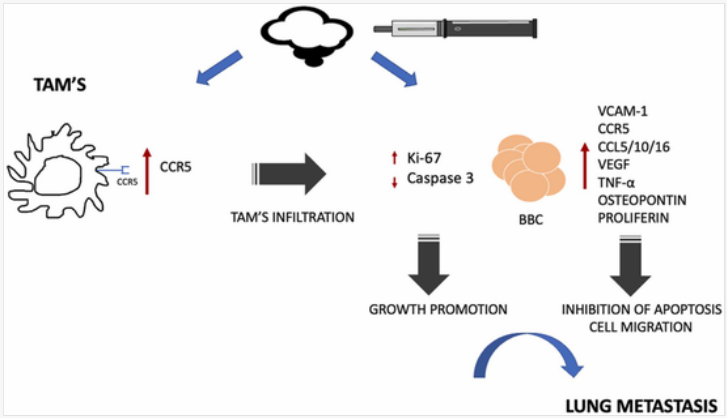
XPC and OGG1/2 are both crucial for DNA nucleotide excision repair and base excision repair, respectively. Data from mouse models of exposure to e-cigarettes aerosol have shown a significant decrease in the expression of XPC and OGG1/2 in lung tissue [111]. This observation is in line with the presence in e-cig mainstream aerosol of aldehydes, such as acrolein, acetaldehyde, crotonaldehyde, and 4-hydroxy-2-nonenal, which are known to affect the DNA repair proteins [114,115], causing the enzyme degradation that alters DNA repair capability. Furthermore, nicotine can be nitrosated to nicotine-derived nitrosamine ketone and then transformed into methyldiazohydroxide, pyridyl-butyl derivatives and formaldehyde, *in vivo* in lung, bladder and heart tissue [111]. The presence of these metabolites in the tissues above mentioned induces the formation of O6-methyldeoxyguanosines, and γ-hydroxy-1,N2-propano-deoxyguanosines, markers of DNA oxidative damage, coupled with reduced activity of XPC and OGG1/2, thereby significantly increasing the risk of new cancer occurrence in naïve models (Fig. 3).

5.2 5.3. E-cig exposure boosts breast cancer progression and pulmonary metastasis promotion increasing macrophage-tumor cell crosstalk *via* CCL5 and VCAM-1 pathways

Recent findings suggest how exposure to e-cigs enhances breast cancer progression and pulmonary metastasis in both cellular and *in vivo* models [118]. Exposure to e-cigarettes increases the expression of the antigen Ki-67, a tumor proliferator factor, and reduces the level of pro-apoptotic caspase-3, leading to significant tumor growth. Furthermore, exposure to e-cigs leads to an increase in circulating monocytes and drives the infiltration of monocytes in both primary tumor and in the lung colonized mass *via* the C-C motif chemokine ligand 5 (CCL5) secreted by breast cancer cells (BCC), and its chemokine receptors C-C chemokine receptor type (CCR1/CCR5), upregulated in monocytes [118]. This chemokine crosstalk axis increases the migration of BCC when exposed to e-cig aerosol. Up-regulation of vascular cell adhesion molecule 1 (VCAM-1) promotes the binding between BCC and tumor-associated macrophages and it provides a protective effect against apoptosis, allowing metastatic seeding in the new microenvironment [118]. Moreover, the e-cigarette mainstream aerosol drives the BCC to release higher levels of several cytokines such as tumor necrosis factor-α, CCCL5/10/16, matrix metalloproteinases (MMPs), osteopontin, proliferin, vascular endothelial growth factor, which increase tumor progression and metastasis (Fig. 4).

alt-text: Fig. 4

Fig. 4



~~Electronic cigarettes promote the growth and migration of breast cancer cells through the CCL5/CCR1/CCR5 axis and VCAM-1.~~Electronic cigarettes promote the growth and migration of breast cancer cells through the CCL5/CCR1/CCR5 axis and VCAM-1. E-cigarette aerosol leads to an upregulation of Ki-67 tumor proliferation factor coupled with an inhibition of tumor cell apoptosis caspase-3. Furthermore, the increase of CCL release from breast cancer cells and the up-regulation of CCR5 receptor by tumor-associated macrophages (TAM's) promote cell migration. In addition, the direct binding of macrophages to cancer cells, *via* E-cig-regulated VCAM-1 expression by breast cancer cells (BCC) provides a protective effect against apoptosis, allowing metastatic seeding in the new microenvironment.

5.3 E-cigarettes modulate the nAChR-E2F1-Sox2 axis and sympathoadrenal system

E-cigarette condensate can promote the self-renewal and maintenance of stem cell properties in non-small cell lung adenocarcinoma inducing the expression of embryonic stem cell factor Sox2. The proposed molecular mechanism involves the downstream signaling axis of the nicotinic acetylcholine receptor (nAChR)-transcription factor E2F1 (E2F1). Once nicotine binds the nAChR and β-arrestin-1 scaffolding protein is recruited it activates the proto-oncogene tyrosine-protein kinase (Src kinase), which subsequently activates the rapidly accelerated fibrosarcoma protein kinase (Raf-1). Activated Raf-1 then phosphorylates the retinoblastoma (Rb) tumor suppressor protein that in cellular quiescence is normally bound to transcription factor E2F1. The phosphorylation of the Rb factor promotes the dissociation from E2F1, resulting in the induction of Sox2, which plays a key role in self-renewal and maintenance of stem cell properties in non-small cell lung adenocarcinoma cells [119].

These findings suggest how e-cigarette consumption can fuel the self-renewal of stem-like cells as well as tumor growth.

Furthermore, it has been hypothesized that e-cigarette use can increase cancer risk by means of the sympathoadrenal system stimulation; the e-cig mainstream aerosol leads to a sympathomimetic effect attributable to nicotine content which in turn increases plasmatic norepinephrine and epinephrine levels [120]. Preclinical and clinical studies suggest that norepinephrine and epinephrine directly stimulate the proliferation of tumor cells by binding to β-adrenergic receptors and β-blockers are reported to counteract the course of cancer progression, as documented by many preclinical and clinical studies [121]. Interestingly, recent evidence indicates that catecholamine-induced increase of systemic vascular resistance might redirect blood flow to cancer tissue [122].

5.4 E-cigarette exposure accelerates brain tumor growth through EGFR-ERK activation

Patient-derived glioblastoma stem-like cells exposed to e-liquid showed a significant increase of phospho- epidermal growth factor receptor (EGFR) in a dose-dependent manner, which in turn led to an upregulation of phospho-ERK as a downstream effector of the EGFR signaling pathway [123]. These results are of particular interest since it has long been known how EGFR is implicated in the carcinogenesis of various types of cancer [124,125]. These results were supported by studies showing that nude mice injected with patient-derived brain tumor cells experienced accelerated tumor growth as measured by MRI after being exposed to e-liquid compared to controls. Furthermore, brain tumor lesions occurred within 20 days in the e-liquid treated group, compared to controls, and the increase in phospho-EGFR expression was associated with a poor survival rate in mice [124,125].

These results, once again, suggest the role of the e-cigarette in cancer progression, and they are of particular concern considering that the EGFR-ERK pathway could be induced in other types of tissues as well.

6 Fertility and pregnancy

Robust evidence supports a large number of severe adverse effects of cigarette smoking during pregnancy, including premature birth, premature rupture of membranes, placental abruption, and placenta premature. In addition, infants have higher incidence of teratogenic effects such as orofacial clefts, heart defects, limb reduction, clubfoot, craniosynostosis, gastroschisis, anal atresia, hernia, and cryptorchidism, as well as neurological disorders [126]. Tobacco consumption strongly affects female fertility; smokers require almost twice the number of *in vitro* fertilization attempts to get pregnant compared to non-smokers [127], as it has been established that smoking negatively affects semen parameters by reducing the male fertility factor [128].

On the other hand, data from *in vivo* models indicate that even when nicotine-free liquid is used, e-cig mainstream emission also contains many harmful substances including endocrine disruptors that can impair male and female fertility [129]. Male rats treated with e-cig refill with or without nicotine showed histopathological changes in testicular morphology, including premature sloughing of germ cells [84]. Furthermore, circulating testosterone levels are lower in exposed animals due to a decrease in 17β-hydroxysteroid dehydrogenases gene expression (involved in testosterone synthesis) and sperm collected from the epididymis cauda revealed a significant decrease in sperm count and viability [84]. Similar results were obtained when rats were exposed to the aerosol generated by a low-voltage device loaded with a nicotine-free liquid. High levels of ROS were also detected in testicular tissue of exposed animals along with a significant increase in several markers of oxidative stress status [130] revealing its negative role, also for male fertility. Although, to date, there are no studies evaluating the effects of e-cig consumption by women during pregnancy, data from animal models indicate that e-cig exposure adversely affects conception and can cause harmful effects on the embryo and fetus [131,132]. These observations are particularly alarming considering that a recent survey has reported that almost 65% of respondents perceive the e-cig as safer than conventional cigarettes, and approximately 75% of smoker pregnant women are willing to switch to e-cigs, suggesting a lack of awareness of the dangers [128]. Overall, data from basic studies on the toxicological effects of e-cig consumption on fertility and pregnancy indicate that e-cigs use cannot be considered a safer alternative to tobacco smoking, even when they are nicotine-free.

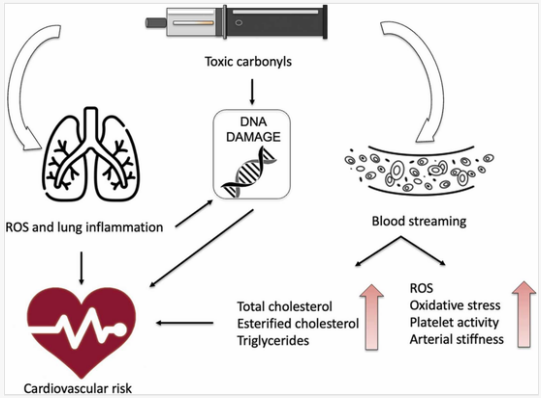
7 Cardiovascular risk

Tobacco smoking is the leading preventable cause of morbidity and premature death for cardiovascular diseases. The main mechanisms of smoking-induced cardiovascular diseases, proposed also for e-cigs, include oxidative injuries, chronic inflammation, endothelial damage, and disruption of lipid homeostasis [133].

Animals exposed to e-cigarette aerosol for 3–6 months developed an increase in systolic blood pressure, a decrease in heart rate, and cardiac fibrosis [134]. Cardiac fibrosis was also detected in 8–9 week-old Wistar rats that were exposed to e-cig aerosol [135]. Increased systemic inflammation, increased levels of cardiac fibrosis, along with increased formation of DNA mutagenic adducts in various tissues, including the heart, all of which contribute to higher cardiovascular risk, have also been reported [111,134]. E-cig condensate, especially when the liquid used contains nicotine, has caused an increase in endothelial permeability as well as an alteration of cell morphology [136,137], resulting in loss of endothelial barrier function, associated with oxidative stress and inflammation, effects which are also associated with higher cardiovascular risk [138]. These observations are in line with those of some clinical trials that have shown significant increased levels of endothelial progenitor cells in blood samples, a marker of vascular injury after e-cigarette exposure [139]. Data from a lipidomic study in the brain of rats exposed to e-cig mainstream emission for 4 or 8 weeks showed changes in lipid peroxidation of polyunsaturated fatty acids in line with those previously reported for tobacco smoke. Furthermore, analysis of the total fatty acid profile indicates that both the atherogenic index and the thrombogenic index increased dramatically after 8 weeks of exposure [3]. In general, studies available in the literature to date [133] indicate that e-cigarette use may be associated with adverse cardiovascular effects, through various mechanisms including oxidative stress, inflammation, DNA damage, and impaired hemodynamics and platelet activity (Fig. 5).

alt-text: Fig. 5

Fig. 5



~~Mechanisms of e-cigarette induced cardiovascular dysfunction.~~Mechanisms of e-cigarette induced cardiovascular dysfunction. Recent evidence shows how e-cigarette consumption could increase the risk of cardiovascular diseases through various mechanisms such as oxidative stress, inflammation, DNA damage, arterial stiffness, altered lipid homeostasis, and platelet activity.~~[133]~~
The present figure has been modified from Buchanan et al. [133].

A significant breakthrough on the molecular mechanisms underlying the endothelial dysfunction and oxidative stress associated to e-cigarette exposure comes from the study by Kuntic and colleagues [140]. The results show how e-cigarette aerosol increases oxidative stress by means of the up-regulation of NOX-2 activity leading to endothelial dysfunction by increased oxidative breakdown of NO and peroxynitrite formation, as well as limiting the availability of tetrahydrobiopterin, an essential eNOS cofactor. Interestingly, by the use of NOX-2 knock-down animals, the authors got the evidence of the association between aldehydes release and e-cigarette mediated endothelial dysfunction and inflammation.

Data from clinical trials seem to support the observation raised from basic studies. The use of e-cigs in ex-smokers was associated with a higher risk of stroke compared with never smokers although unexpected reduction in systolic blood pressure by ~~9–10~~9–10 mmHg, and diastolic blood pressure by ~~6~~6 mmHg were observed [141]. The daily e-cigarette consumption, adjusted for smoking conventional cigarettes as well as other risk factors, is associated with increased risk of myocardial infarction [142], as well as significantly higher odds of cardiovascular disease among dual users of e-cigarettes and tobacco cigarettes compared with smoking alone [143]. Results from a recent cross-sectional study indicate that switching from combustible cigarettes to e-cigarettes does not confer stroke benefits. However, sole e-cigarette use is not associated with greater odds of stroke in young adults. Nevertheless, the e-cigarette consumption in former or current combustible cigarette users, increases the odds of stroke significantly even compared with current sole combustible cigarette use. Overall, the switch from tobacco smoke to e-cigarettes cannot be considered beneficial [144].

8 Discussion and final remarks

Due to their recent release on the market, epidemiological data on the toxicological risk associated with the long-term use of e-cigs are not yet available, in particular with regard to the new onset of cancer or cardiovascular events.

The delay between exposure to carcinogens and the manifestation of the disease, at least in most cases, does not yet allow us to observe a presumed correlation; and, in addition, e-cig consumers are often dual-users or ex-smokers, which makes it more difficult to make a good estimate on the discrete health impact of ENDS.

Some short-term observational studies have reported promising results on the null effect of the use of e-cigarette on some health outcomes such as blood pressure, heart rate, alteration of lung tissue or changes in the rate of COPD exacerbation [145], however, these typical harms from tobacco cigarettes often take decades to manifest and only occur in a fraction of smokers, making long-term conclusions impossible [146].

Technical features of e-cig, such as the battery output, the temperature, the atomizer setting, or the liquid composition, allow consumers to personalize their vaping experience, leading to a “personalized toxicological profile” with unpredictable health consequences. In addition, there are many confounding factors that depend not only on patient cohorts, but rather on the settings and technical aspects of the devices, making it difficult to draw clear conclusions about the studies. Basic studies could partially fill these gaps.

Several animal studies have been conducted to address the question of whether ENDS could be less harmful than burnt tobacco products. The problem is not whether or not e-cigarettes are as harmful as classic blondes; the key question is whether or not these new devices are harmful by themselves. The presence of several carcinogenic chemical compounds found in the main emission of electronic cigarette, as well as the toxicological results reported in the basic studies, modify indeed the initial scenario of perception of a typical safe aerosol emitted by the electronic cigarettes. Not only, emerging data seem to indicate that ENDS can represent a "gateway" to nicotine addiction especially for young people who are often attracted by the technological appeal of these new devices [145–149], with an estimated threefold increase in the risk of subsequent initiation to smoke with e-cig use [150,151]. In addition, even if e-cigs could help smokers to quit or reduce tobacco consumption, as some studies would suggest [6,152,153], comparative trials are also needed to evaluate the efficacy and risk/benefit ratio of e-cigs over other commonly used smoking cessation therapies.

Since we are far from definitively understanding the health impact of ENDS, its promotion as a part of the health organization's effort to quit smoking is at least premature and potentially harmful [4]. Public health campaigns should refrain from presenting ENDS as "healthier than tobacco smoke". As recently reiterated by the World Health Organization, not only there is no evidence that e-cigarette helps in quitting smoking, but governments need to expand evidence-based interventions to fight tobacco smoke [154].

Funding

~~Conceptualization: D.C., F.V., M.P., I.C.-C., A.C.-V., and C.F.; Project~~This work was supported by a grant from the Italian Ministry of Education~~administration:~~, University and Research~~F.V., D.C., M.P., P.R., S.C., I.C.-C., A.C.-V., and C.F.; Investigation and data curation: F.V., D.C., L.R., and S.G.; Visualization: F.V., D.C., L.R., and S.G.; Writing original draft preparation: F.V., D.C., L.R., M.P.; and S.G.; Review and editing: M.P., S.G., M.M., F.V, D.C., I.C.-C., A.C.-V., C.F., and~~. S.G., PhD fellowship grants were awarded from the Italian Ministry of Education, University and Research. L.R. and F.V. postdoctoral fellowship grant was cofounded by D.C., M.P., S.C., and P.R.; ~~Funding acquisition: D.C., C.F., M.P., F.V., P.R., S.C. All authors have read and agreed to~~ I.C-C. was supported by a postdoctoral fellowship from the Andalusian Government Ministry of Economy, Knowledge, Business, and University ~~version~~ (DOC_00587/2020~~of the manuscript.~~).

Uncited reference

[25].

CRediT authorship contribution statement

Fabio Vivarelli: Conceptualization, Project administration, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition. **Silvia Granata:** Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Laura Rullo:** Investigation, Data curation, Visualization, Writing – review & editing. **Matilde Mussoni:** Writing – review & editing. **Sanzio Candeletti:** Project administration, Funding acquisition. **Patrizia Romualdi:** Project administration, Writing – review & editing, Funding acquisition. **Carmela Fimognari:** Conceptualization, Project administration, Writing – review & editing, Funding acquisition. **Ivan Cruz-Chamorro:** Project administration, Conceptualization, Writing – review & editing. **Antonio Carrillo-Vico:** Project administration, Writing – review & editing, Conceptualization. **Moreno Paolini:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing, Funding acquisition. **Donatella Canistro:** Conceptualization, Project administration, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to Dr. Lorna Rettig, Medical Writer, for the support received during the revision of the manuscript.

Conflicts of ~~Interest~~interest

The authors declare no competing interests.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Graphical ~~abstract~~Abstract

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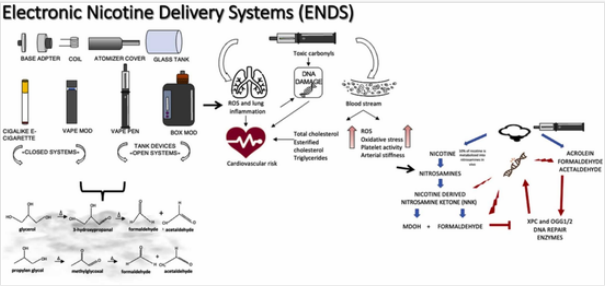

Highlights

- E-cigarettes release toxic aldehydes derived from e-liquid thermal ~~degradation;~~degradation.
- Exposure to e-cigarette aerosol triggers oxidative stress and ~~inflammation;~~inflammation.
- E-cigarette use is associated with DNA damage and it could increase cancer ~~risk;~~risk.
- E-cigarette consumption could impair male ~~fertility;~~fertility.
- E-cigarette use can raise risk of pulmonary and cardiovascular chronic diseases.

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