# Review

# A decade of e-cigarettes: Limited research & unresolved safety concerns

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ABSTRACT

It is well known that tobacco consumption is a leading cause of preventable deaths worldwide and has been linked to major diseases ranging from cancer to chronic obstructive pulmonary disease, atherosclerosis, stroke and a host of neurological/neurodegenerative disorders. In the past decade a number of alternative vaping products have hit the market, rapidly gaining consumers especially among the younger population. Electronic nicotine delivery systems or e-cigarettes have become the sought-after product due to the belief that they are much safer than traditional cigarettes. However, inadequate research and lack of regulatory guidelines for both the manufacturing process and the content of the vaping solution of the e-cigarette has become a major concern. Highly debated and unresolved questions such as whether e-cigarettes may help smokers quit and whether e-cigarettes will promote the use of nicotine among non-smokers add to the confusion of the safety of e-cigarettes. In this review article, we summarize the current understanding (and lack thereof) of the potential health impacts of e-cigarettes. We will also highlight the most recent studies (*in vivo/in vitro*) which seem to conflict with the broad safety claims put forward by the manufacturers. Finally, we provide potential solutions to overcome the research gap of the short and long-term health impact of e-cigarettes.

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## 1. Introduction

Electronic cigarettes (e-cigarettes), also known as electronic nicotine delivery systems (ENDS), are battery powered devices designed to vaporize (by heat) a solution of nicotine (e-liquid) and other additives (including propylene glycol, vegetable glycerin and ad hoc flavoring agents) into an aerosol which is then inhaled by the user (called vaping). These products are often made to look like cigarettes, cigars, pens or pipes in order to closely resemble traditional tobacco-based combustion products (TC) (Naik and Cucullo, 2015). Hon Lik, a Chinese pharmacist, invented the modern version of the e-cigarette in 2003, which was later patented internationally in 2007 (**Electronic Atomization Cigarette: US 20070267031 A1**) and was subsequently introduced into the global market including the U.S. (Rahman et al., 2014). The market size of this non-regulated product is booming because of the increase in popularity among young adults of the millennial generation (Grana et al., 2014). The accelerated adoption of e-cigarettes is supported by the common belief that they are indeed a safer alternative to traditional tobacco-based products (Goniewicz et al., 2014b) (Regan et al., 2013) or that they can be used to facilitate smoking cessation despite some opposing evidence (Kalkhoran and Glantz, 2016). A recent study on e-cigarette awareness and harm perception has shown that ≈ 95% of interviewed subjects (US adult population) believe e-cigarettes to be; 1) cleaner and healthier than conventional products, 2) cheaper (93%), 3) can be used to circumvent smoke-free policies (76–88%) and 4) are trendy (≈73%) (Pearson et al., 2012; Grana and Ling, 2014).

A recent report (Zhu et al., 2014) states that an astonishing 7764 unique flavors were available online in January 2014, with 242 new flavors being added per month, and sales occurring under 466 brands. A number of e-cigarette manufacturers minimized the health risk concerns on their products stating that the ingredients, including the flavoring agents, are not dangerous since they are all 'food grade' and '*generally recognized as safe*' (GRAS). However, GRAS designation by the Flavor Extracts Manufacturers Association (FEMA) refers to use of these substances up to specific concentrations for specific purposes in specific foods and does not pertain to the use of the same compounds for inhalation. In some instance e-liquids contain very high levels of flavoring agents, possibly exceeding concentrations sufficient to elicit irritant effects and inflammation in the respiratory and cardiovascular systems. This concern was recently brought up by a study from Farsalinos et al. (2015b) in which the authors tested 159 sweet e-cigarette flavors including chocolate, toffee, and caramel. The results clearly showed that ≈ 74% of the samples contained diacetyl and/or acetyl

propionyl which has been associated with bronchiolitis obliterans. Furthermore, certain flavored e-liquids demonstrated nicotine-independent *in vitro* cytotoxicity to various cells (Farsalinos et al., 2013a; Tierney et al., 2016). In addition to propylene glycol, glycerin and other flavoring agents, e-liquids contain a number of aldehydes (formaldehyde, benzaldehyde, acrolein, etc.) that form during the heating process. Small amounts of heavy metals and at least 20 known carcinogens and teratogenic agents have also been identified in the e-liquid as well as the vapors (Bahl et al., 2012; Grana et al., 2014; McAuley et al., 2012). Toxic aerosols released by e-cigarettes contain ultra-fine particles that can, in conjunction with air pollution, contribute to pulmonary and systemic inflammatory processes while decreasing macrophage and neutrophil antimicrobial activities (Hwang et al., 2016). Despite the proof that e-cigarettes are not as safe as popular belief, the long term health effects of e-cigarette vaping has only been marginally addressed (Orellana-Barrios et al., 2015). Hence, many unanswered questions remain about the overall toxicological effects, safety, efficacy of harm reduction, as well as the overall health impact of e-cigarettes.

## 2. The device: basic design & operation

A variety of e-cigarette models have emerged over the last decade. Although the complexity in design and features has evolved, the key principle is the same. An e-cigarette has three basic parts: a rechargeable lithium battery, an atomizer and a reservoir (collectively known as a cartomizer; see Fig. 1) containing the e-liquid. Activation of the heating coil within the atomizer generates the nicotine aerosol (or mist).

Automatic models feature an airflow sensor which activates the heating coil when a user starts inhaling. Most models however, require the user to manually trigger the atomizer. By pressing and holding on an on/off switch a signal is sent to the battery to supply current to a heat coil which raises the temperature of the atomizer (up to 500 °F) vaporizing the e-liquid drawn from the cartridge/reservoir. This aerosol is then puffed by the users through a mouthpiece connected to the cartridge/reservoir. e-Cigarettes feature rechargeable batteriesavailable in different shapes and output power levels (expressed in milli-ampere-hours—**mAH**). Many e-cigarettes are equipped with a flame-looking light-emitting diode (LED) at the end of the battery to replicate the appearance of a regular burning cigarette (Orellana-Barrios et al., 2015; Rahman et al., 2014).

A user's vaping pattern and taste varies widely. Some consumers prefer to vape mild to moderate amounts of aerosol while others love to experience a smoke-like sensation or "hit" in
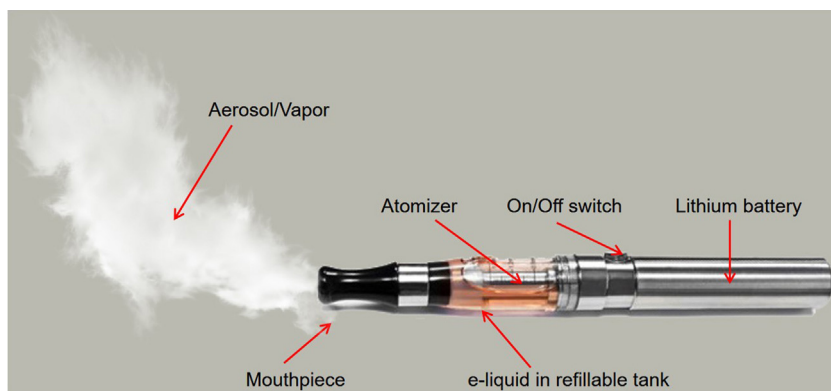


**Fig. 1.** e-Cigarette schematic illustration: This device is primarily a battery operated product designed to turn nicotine and other chemicals (including solvents and flavoring agents) into a vapor; an atomizer heats the e-liquid producing the vapor inhaled by the user. A rechargeable battery (w/wo LED indicator) of different sizes and output powers to the atomizer. Note that the battery output and the resistance of the heating coils in the atomizer determine the vaping capacity of the e-cigarette.

their throat by generating huge amount of vapors. To meet the consumer taste, e-cigarette manufacturers have developed customizable products which enable the user to adjust the atomizer's resistance (ohm, $\Omega$) and voltage (V) through replaceable parts and adjustable dials. These parameters work following the basic principle of Ohms law. Replaceable coils can vary in size to alter the resistance of the atomizer (1.2–1.8 ohm), inversely affecting the flow of the current and heat generated by the coil. This ultimately allows for the production of a denser vapor with intense taste and enormous feeling of throat "hit". Both variable voltage and variable wattage type manual devices are available in the marketplace. Variable voltage (usually 3.0–5.0 V) type devices allow the user to adjust the amount of current flow and hence the amount of aerosol depends on the resistance of the atomizer. Later models termed "temperature controlling vaping" enable the user to program a fixed temperature into the microchip. Like a thermostat, the current is maintained through digital adjustment to the voltage. These devices ensure fixed current output regardless of the resistance of the atomizer.

## 3. Chemical profile of e-liquid and nicotine intake

e-Cigarettes are either disposable (both the battery and the cartridge are to be discarded once the e-liquid has been consumed) or intended for multiple uses in which case, the reservoir (tank style) needs to be refilled with e-liquid or the prefilled cartridge needs to be replaced. E-liquid is the solution or "smoke juice" containing nicotine in an edible solvent approved by the FDA (usually propylene glycol or a mixture of propylene glycol and glycerin). Nicotine-free e-cigarettes are also available in the market. e-Cigarette manufacturers usually categorize the e-liquid strength as zero (0 mg/ml), low (6, 12 mg/ml), medium (18 mg/ml) and high (24 mg/ml) based on the nicotine concentration. e-Cigarette retail shops can purchase concentrated nicotine solutions (100 mg/ml) and dilute them according to the customer demand. E-liquids exceeding 24 mg/ml of nicotine (up to 36 mg/ml) are also available in the market (Bhatnagar et al., 2014). Wide ranges of flavoring agents can be added to the nicotine solution including chocolate, caramel, mint, menthol, coffee, cherry, apple and many more.

Although the popularity of e-cigarettes is rapidly increasing, a comprehensive chemical characterization of the e-liquids (including sources and manufacturing processes, chemical composition of the solvents and flavoring agents, and the nicotine content of the aerosol) are seldom disclosed by the manufacturers. Chemical analyses of the e-liquids and popular e-cigarettes brands revealed high variability in the nicotine content (between refills) and nicotine delivery efficiency (between nominally identical e-cigarette models). Significant inconsistencies between the nominal (labeled) and the actual nicotine content of the e-liquids have been reported by a host of investigators (Etter et al., 2013; Famele et al., 2015; Goniewicz et al., 2015; Goniewicz et al., 2014a; Hahn et al., 2014; Lisko et al., 2015) These also include the presence of nicotine in "nicotine-free e-liquids" as well as variations (up to 12%) in nicotine concentration (Goniewicz et al., 2014a) and pH (Lisko et al., 2015) within the same batch of e-liquids. In addition to nicotine and flavoring agents the presence of other toxic compounds (especially carcinogens) have been recently reported. These include a cohort of toxic carbonyl compounds (acrolein, formaldehyde and acetaldehyde etc.), heavy metals (such as mercury—Hg, Cadmium—Cd, lead—Pb), volatile organic compounds (benzene, toluene), and nitrosamines (such as nitrosonornicotine, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone − NNK; see Table 1). Since many e-cigarette manufacturers use tobacco-derived nicotine, toxic chemicals are likely to be found in the vape as well (although to levels several hundred folds lower than those observed in TCs). A very recent but highly controversial report suggests that the reaction between the e-liquid vehicle (propylene glycol and glycerol) and formaldehyde (a known degradation byproduct of propylene glycol generated by heat) during vaping e-cigarette at high voltage (5.0 V) can lead to the formation of a highly carcinogenic hemiacetal in significant concentrations (Jensen et al., 2015). Hemiacetals release formaldehyde and are commonly used as biocides. The presence of

**Table 1**
Toxic components present in e-Cigarette aerosol and e-liquid.

| Components | Concentration | e-liquid/Aerosol | References |
|---|---|---|---|
| **Carbonyl compounds** | | | |
| Formaldehyde | 2.0-6.3 μg | 150 puff | Goniewicz et al. (2014) |
| | 1.9–120 μg | 10 puff | Uchiyama et al. (2016) |
| | 0.05–51 μg | 1 puff | Gillman et al. (2016) |
| | 0.1- 9.0 ug | 1 g of e-liq | Varlet et al. (2015) |
| Acetaldehyde | 1.1–13.6 μg | 150 puff | Goniewicz et al. (2014) |
| | 1.4 −73 μg | 10 puff | Uchiyama et al. (2016) |
| | 0.03-40.07 μg | 1 puff | Gillman et al. (2016) |
| | 0.05-10.2 μg | 1 g of e-liq | Varlet et al. (2015) |
| Acrolein | 0.7- 41.9 μg | 150 puff | Goniewicz et al. (2014) |
| | 2.1–16 μg | 10 puff | Uchiyama et al. (2016) |
| | < 0.02-5.5 μg | 1 puff | Gillman et al. (2016) |
| Propionaldehyde | 110 ng | 1 puff | Tayyarah and Long (2014) |
| | 40–1500 ng | 1 puff | Bekki et al. (2014) |
| **Volatile Organic Compounds** | | | |
| Toluene | 0.2–6.3 μg | 150 puff | Goniewicz et al. (2014) |
| p,m-xylene | | | |
| | 0.1-0.2 μg | 150 puff | |
| **Tobacco-Specific Nitrosamines** | | | |
| N-nitrosonornicotine (NNN) | 0.8-4.3 ng | 150 puff | Goniewicz et al. (2014) |
| | 0.5–16.7 ng | 1 ml of e-liq | Farsalinos et al. (2015b) |
| 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) | 1.1- 28.3 ng | 150 puff | |
| | 3.2 −10.8 ng | 1 ml of e-liq | |
| **Metals** | | | |
| Ni | 0.11-0.29 μg | 150 puff | Goniewicz et al. (2014) |
| Cd | 0.01-0.22 μg | 150 puff | |
| Pb | 0.03-0.57 μg | 150 puff | |

diacetyl, 2,3-pentanedione, and acetoin have also been reported in flavoring agents (Allen et al., 2015). Chronic inhalation of these chemicals may lead to respiratory complications including popcorn lung.

A drawback of these studies however, is that the quantitative and qualitative analytical methods were (in most cases) not properly validated. The use of dissimilar experimental conditions and tools with diverse detection limits, resolution or linearity ranges impacts data reproducibility and comparison, thus impairing their translational relevance (Goniewicz et al., 2014b). One such challenge is the pattern and strength of vape inhalation (vaping proficiency) and the vacuum needed to produce nicotine. Unfortunately, (for research purposes) the vaping proficiency of the users and the e-cigarette response to the user vaping (vacuum needed to activate the atomizer) vary widely. Usually smoke density remains unaltered throughout the entire burning of a conventional cigarette, while for e-cigarette aerosol density decreases with continued use (Norton et al., 2014; Trtchounian et al., 2010). Addressing this variance is of crucial importance for the preparation of experimental samples to be used for toxicological testing (Behar et al., 2015; Farsalinos et al., 2013b). Researchers have recently attempted to measure the amount and size of the particulate matter contained in the e-cigarette vapes vs TCs. Ruprecht et al. found lower levels of particulate matter in e-cigarettes (irrespective of the nicotine content) compared to TCs but these levels still exceed WHO guidelines. In other studies, the size of the particulate matter found in the vapor released by the e-cigarette was comparable to that of TCs' sidestream smoke until the aerosol reached the saturation point (first few puffs) but increased once the saturation point was crossed (Hua et al., 2013; Pellegrino et al., 2012; Ruprecht et al., 2014). The diameters of the e-cigarette aerosol particles were comparable to that found in sidestream smoke from conventional cigarettes with an average size of 250–450 nm (Ingebrethsen et al., 2012). In respect to nicotine delivery, a conventional cigarette releases approximately 1 mg of nicotine into the blood (Bhatnagar et al., 2014). When compared to that of e-cigarettes one-fourth to one-third of the nicotine content of the e-liquid will actually reach the blood stream (Farsalinos et al., 2014). Thus the total amount of nicotine effectively delivered into the blood stream depends on the effective strength of the e-liquid and the amount of e-liquid vaped. Considering a nominal nicotine concentration of 24 mg/ml then the total amount of nicotine potentially delivered into the blood stream of the vaper could be in the range of 6–8 mg. Nicotine delivery efficiency of the newest generation of e-cigarettes is even higher.

## 4. Toxicological studies and public health concern

The most common solvents used in the preparation of e-liquids are either propylene glycol and/or vegetable glycerin. Glycol mist (the same additive used in e-cigarettes) is also used in the show business and aviation industries to create "fog machine smoke". Varughese et al. studied the lung function of 101 employees at 19 theatres exposed to glycol fog during their work. The study revealed impaired lung function associated with dry throat and cough upon either acute or chronic exposure (Varughese et al., 2005). Wieslander et al. tested acute effects of glycol aerosol on personnel involved in aviation emergency training and demonstrated ocular and upper respiratory tract irritation in non-asthmatic people. In a few cases airway obstruction was also observed (Wieslander et al., 2001). Although a direct parallelism with e-cigarette exposure cannot be established, concerns remain regarding the use of these solvents in the preparation of e-liquids. Also troublesome is the fact that although glycerin is hygroscopic in nature and reduces the consistency of bronchial fluid it is still considered a safe additive (Callahan-Lyon, 2014).

Acute e-cigarette exposure may impair human lung function. As recently reported, e-cigarette exposure decreased the fraction of exhaled nitric oxide thus enhancing the total peripheral resistance (Vardavas et al., 2012). A 3% FEV1/FVC (forced expiratory volume in 1 s/forced vital capacity; the volume of air that can be maximally forcefully exhaled) reduction was also reported in response to e-cigarette exposure (Flouris et al., 2013). In vitro studies revealed direct cytotoxicity of e-liquid on human and mouse stem cells as although no effect was observed on human pulmonary fibroblast (hPF) (Bahl et al., 2012). Lerner et al., further demonstrated that e-cigarette aerosols induce oxidative stress in human lung epithelial cells and trigger an inflammatory response. They also observed similar results pre-clinically when exposing mice to e-cigarette aerosols (Lerner et al., 2015) (see Table 2).

The principal component of the e-liquids delivered by the e-cigarettes is nicotine. Adverse effects associated with nicotine exposure include nausea, vomiting, dizziness and severe topical damage in case of accidental spill of highly concentrated nicotine solutions (Callahan-Lyon, 2014). Risk of accidental poisoning can arise from the use of undiluted nicotine concentrates if mistakenly used as "ready to vape" e-liquids. Few fatal cases from ingestion of e-liquid have been reported Chen et al. (2015) (2015; Ordonez et al., 2015; Thornton et al., 2014) and although the number of deaths is not highly significant this remain a concern to be addressed. The physiological impact of e-cigarette exposure has been investigated mainly on heart, lung and the vasculature suggesting sympathomimetic effects (Nelluri et al., 2016). Transient irritation of mouth and throat during the initial phase of e-cigarette inhalation has also been reported (Polosa et al., 2014). No change in complete blood count (CBC) has been observed in response to acute e-cigarette exposure (Flouris et al., 2012).

### 4.1. Availability of e-Cigarette to vulnerable population

The most recent nationwide survey conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC) estimated 12.6% US adults vaped e-cigarettes at

**Table 2**
Toxicological studies of e-Cigarette.

| | Study | Adverse effects | Reference |
|---|---|---|---|
| 1. | Human embryonic stem cells (hESC), mouse neural stem cells (mNSC), and human pulmonary fibroblasts (hPF) were exposed to e-Cigarette refill liquid. | Cytotoxicity was observed excluding hPF cells. | Bahl et al. (2012) |
| 2. | Human airway epithelial cells (H292) and wild type C57BL/6 J mice were exposed to e-Cigarette aerosol. | Oxidative stress and Inflammatory response. | Lerner et al. (2015) |
| 3. | Thirty healthy smoker inhaled e-Cigarette for 5 min. | Fraction of exhaled nitric oxide (FENO) was reduced while total peripheral airway resistance was enhanced. | Vardavas et al. (2012) |
| 4. | Fifteen smokers underwent acute e-Cigarette exposure. | 3% reduction in FEV1/FVC | Flouris et al. (2013) |
| 5. | Long term (6 months) use of e-Cigarette by forty participants. | Irritation of mouth and throat | Polosa et al. (2014) |

least once and 3.7% are current users (Schoenborn and Gindi, 2015). According to the CDC and the Center for Tobacco Products (CTP) of the Food & Drug Administration (FDA), the National Youth Tobacco Survey (2014) revealed an alarming rise of e-cigarette use among adolescents (see Fig. 2). It has been estimated that e-cigarette consumption has increased from 660,000 to 2 million and 120,000 to 450,000 among high and middle school students respectively (15 AD.). The report also states that e-cigarette vaping topped all other tobacco products consumed by students in the last three years (2011–2014) and their use has increased by ≈ 800% among high school students (2016b). The number of recurrent users has also increased from 0.3% to 6.8% (McMillen et al., 2014). The report also revealed that the number of young vapers (millennials) currently outpaces that of adults (14.2% vs. 8.6%). A 2014 study showed that over 8% of 8th graders, 16% of 10th graders and approximately 17% of 12th graders in US have vaped an e-cigarette during the one month survey period (Schraufnagel, 2015).

According to a recent public workshop by the FDA (Electronic Cigarettes and the Public Health; March 9–10 2015) (16 AD.), e-cigarette manufacturers have developed specific marketing strategies (including social networking services) to draw the attention and interest of young consumers. In contrast to TCs, the e-cigarettes market is not (yet) regulated, thus allowing anyone to purchase these products without the proof of age. e-Cigarette's manufacturers have also replaced the term "cigarette" (which has a negative inference among the general population) with "vaping device" or "hookah pen" to distance their products from TCs. In addition to traditional market advertising young consumers are further incentivized to purchase e-cigarettes with coupons and promotional (free) products (Schraufnagel, 2015). A recent study on more than 15000 American adolescents (from 6th through 12th grade) of different races and ethnic groups was conducted demonstrating lesser prevalence of e-cigarette smoking among female and young African-Americans. By contrast Hispanics, Caucasian, Asian, males and (in general) adolescents that already experienced smoking traditional cigarettes and/or have smoker friends are more prone to adopt e-cigarettes. The desire to quit smoking was not the driving force initiating e-cigarette vaping (Lippert, 2014).
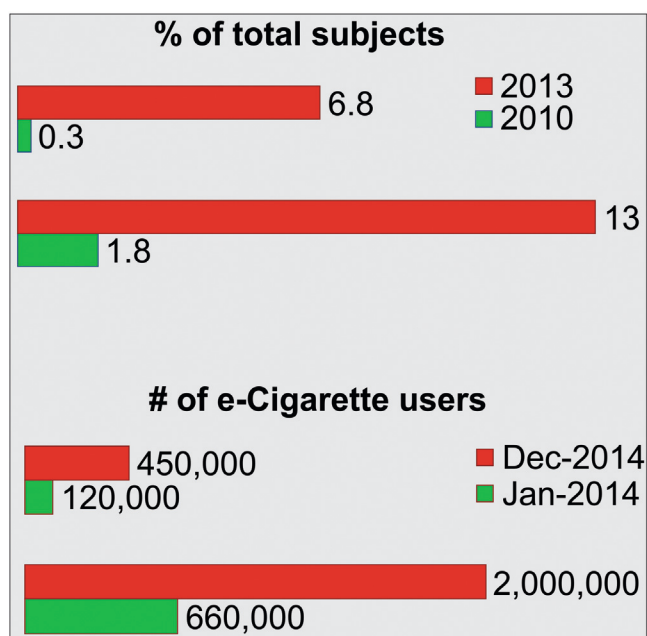


**Fig. 2.** Rise in e-Cigarette use among U.S. adults (2010–2014); Sample size >3000 each year (15 AD.; McMillen et al., 2014).

## 5. The success of e-Cigarette in smoking cessation program

The "Center for Drug Evaluation and Research" of the FDA assesses the potential therapeutic benefit over the risks of smoking cessation products and regulates their production, advertisement and sale. Accordingly, nicotine replacement therapies (NRTs)—e.g. nicotine patch/gum, bupropion SR, and varenicline are approved by the FDA for smoking cessation. This however, is not the case for e-cigarettes (despite the popular belief) since the rather scarce and quite contradictory scientific evidences do not presently justify their use as smoking cessation tools (Orr and Asal, 2014).

Bullen et al. conducted a randomized clinical trial on 657 people to observe the effect of e-cigarettes on reducing cravings and the relapse rate in smokers. Subjects were grouped into three different cohorts including nicotine patches, e-cigarettes and e-cigarette placebos. The study was inconclusive since the low success rate in quitting smoking among the participants did not yield sufficient statistical power to draw any conclusion on the effectiveness of e-cigarettes as smoking cessation tools when compared to nicotine patches. The investigators concluded that more extensive studies were needed (Bullen et al., 2013). Caponnetto et al. carried a recent randomized clinical study on 300 smokers with no desire to quit smoking. The difference in number of cigarettes smoked after vaping e-cigarettes was considered the main parameter to assess smoking cessation. Only 8.7% of smokers completely restrained themselves from smoking after 52 weeks. However, the investigators noticed a marked reduction of withdrawal syndrome and concluded that irrespective of the nicotine content e-cigarette vaping reduced the number of cigarettes smoked (Caponnetto et al., 2013). One of the most recent studies by Brose et al. suggests that the daily vaping of e-cigarettes bolsters the user effort to stop smoking or helps reduc the number of cigarettes smoked. By contrast, occasional vaping did not have any of these aforementioned effects (Brose et al., 2015).

The rate of smoking is considerably higher in people with mental disorders despite being more susceptible to tobacco related damage and injury. Studies aimed at evaluating the use of e-cigarettes as smoking cessation tools in healthy vs. mentally ill patients did not show statistically different results although the relapse rate in the latter category was higher (O'Brien et al., 2015). Further, no statistically significant differences were found when comparing the effectiveness of e-cigarettes vs. other nicotine delivery systems (e.g., nicotine patches, etc.) as a smoking cessation tool although the acceptability and consumer compliance was higher with e-cigarettes. The use of e-cigarettes as a replacement/alternative to TCs in cancer patients was also evaluated but results were not conclusive (Fillon, 2015).

## 6. e-Cigarette: regulatory issues

There is a rising demand by the regulatory authorities to bring the marketing, production and sale of e-cigarettes under legislative control and to limit their accessibility to vulnerable populations. Few countries have already developed regulatory guidelines for e-cigarettes such as Brazil, Uruguay, Singapore, Canada etc. In the US e-cigarettes will be brought under the FDA authority by August 8 2016 although the regulatory guidelines and the approaches to implement them are still a working in process far from being completed due to the scarce scientific data available to the regulators. Further, regulatory issues concerning the premises where e-cigarettes can or cannot be used is beyond the FDA's mandate; state and local governments have the authority to impose any restriction on e-cigarettes use in public places or any non-smoking zones (Orellana-Barrios et al., 2015).

From a regulatory perspective, if any product contains synthetic compounds and claims any therapeutic effect it should be treated

as a drug and must be approved by the appropriate controlling agency. The initial approach of the FDA was to treat e-cigarettes as drug delivery devices. This argument was met with significant resistance by the manufacturers which counter-argued that no therapeutic claims were linked to e-cigarettes. In the US, products containing synthetic nicotine are regulated and need to meet specific standards; if nicotine is obtained from a plant source it is treated as a tobacco product. Since the e-liquids manufacturers seldom disclose the constituents and the nicotine source(s) used in the preparation of their products, these issues remain unresolved (Rahman et al., 2014). Grana et al. in a comprehensive review on e-cigarette use advocated for the following guidelines to be included in the corresponding legislative policy: the use of e-cigarettes needs to be put under levels of restriction similar to conventional cigarettes; these include e-cigarette use in public places (smoke free zone), age requirement to purchase the product, premise for sale and commercialization methods (television, social media, etc.). Further, the use of flavoring agents should also be strongly regulated requiring detailed characterization (toxicological studies) for all the ingredients used in the preparation of e-liquids. Any unproven (deceiving) marketing claim should be forbidden until verified by the scientific community (e.g., e-cigarette use as smoking cessations tools). Finally, any marketing policy promoting the dual use of both e-cigarettes and TCs should be prohibited or at least strongly discouraged by the legislators (Orellana-Barrios et al., 2015).

## 7. Secondary exposure and non-user risk of e-cigarette:

e-Cigarettes have been reported to cause secondary exposure to nicotine but to a much lesser extent compared to TCs. The exhaled e-cigarette aerosol is also free from combustible toxic ingredients present in traditional cigarettes (Czogala et al., 2014). Schober et al. conducted a study on indoor air quality after six vaping sessions of two-hour duration by nine e-cigarette smokers. The results demonstrated an elevated number of suspended particles containing nicotine, glycerin and 1,2-propanediol (Schober et al., 2014). After evaporation of the solvents from the exhaled e-cigarette aerosols the residual nicotine deposited on solid surfaces was shown to react with atmospheric nitrous acid yielding tobacco specific nitrosamines which are carcinogenic in nature (Kuschner et al., 2011; Riker et al., 2012). Flavoring agents used in the preparation of e-liquids could also be appealing to children. The intrinsic risk is that children could be tempted into ingesting the e-liquid which also contains a high concentration of nicotine. The effect could be potentially fatal (Callahan-Lyon, 2014). As for the use of e-cigarettes during pregnancy (also as a "safer" alternative to TCs), it is important to note that there are no data currently available to assess the potential risks to the fetus.

## 8. Promoting e-Cigarette research

To establish a rationale and sustainable policy to regulate e-cigarette manufacturing, marketing and usage, the long term biological and physiological impact on these products need to be dissected out and evaluated accordingly. The physical characteristics and mode of operation of e-cigarettes are rapidly evolving and their modulatory effects on aerosol generation (time & force required while inhaling), composition (presence of any unwanted degradation products), physical characteristics (diameter of aerosol particles) and user exposure (total amount and density) need to be investigated.

Traditional cigarettes are usually classified as full flavor, light and ultra-light in order of decreasing nicotine and tobacco residue (TAR) concentrations whereas consumers might not be fully aware of the exact nicotine content truly delivered by e-

cigarettes as discussed above. Although nominal nicotine concentrations (0–24+ mg/ml) are clearly labeled on every e-liquid refill or cartridge, the effective nicotine yield delivered in the vapor is highly variable, thus extensive pharmacokinetic studies are needed to assess the bioavailability and relevant physiological parameters (absorption, distribution, metabolism and excretion) of nicotine from various e-liquid refills (nicotine strengths) and e-cigarette designs (Etter et al., 2013). Establishing e-cigarettes as a controlled and dose dependent nicotine delivery system might open a new avenue for the efficient delivery of chemicals other than nicotine. Chemical profiling of both inhaled and exhaled aerosols of e-cigarettes needs to be done with no discrepancy in sample collection and experimental conditions. About half of the chemical profiling studies that have been performed so far are on products sold outside US. Thorough analysis of products available in US market is, as of now, scarce. The discrepancies (Jensen et al., 2015) regarding heat-generated formaldehyde during vaping and the presence of di-acetal in e-liquid vehicles should be further investigated and resolved. Further, stringent regulatory parameters relative to the design, and technical characteristics of e-cigarettes (e.g., max battery output and temperature of the heat coil) should be developed and enforced.

### 8.1. Health impact of e-Cigarette: the little we know

One of the major obstacles in regulating e-cigarettes is that the possible long term adverse effects are still unknown. Although a few studies are available with mixed results, the data are insufficient to draw any conclusion. The effects of e-cigarettes in altered medical conditions such as asthma, chronic obstructive pulmonary disease (COPD) or even pregnancy have not been investigated. Both COPD and asthma can be triggered/aggravated by chronic exposure to traditional combustible cigarettes (Moerloose et al., 2006). Once these patients are diagnosed the best possible way to prevent worsening of these conditions is to quit smoking. However, the craving for nicotine and/or the act of smoking itself becomes a strong deterrent to quit and a catalyst for switching to e-cigarettes. According to the American Lung Association, approximately 80% of COPD deaths are caused by smoking (2014) and a most recent CDC survey revealed that about 21% of asthmatic patients in the US currently smoke (2016a). Further, recent *in vivo* studies have shown that e-cigarette exposure can induce proteostasis/autophagy impairment leading to oxidative stress, apoptosis, and senescence, thus suggesting a potential role in COPD-emphysema pathogenesis (Shivalingappa et al., 2015).

Several preclinical studies have been conducted on the effects of nicotine on the brain and neurological disorders such as stroke. Adverse effects (Bradford et al., 2011) have been reported where nicotine has been administered subcutaneously via osmotic pump or direct injection but did not expose the animals to nicotine aerosols to simulate chronic and frequent use of e-cigarettes. This is a serious concern with respect to adolescents because of the negative impact of nicotine on cognitive function and brain development (Yuan et al., 2015). As smoking has been reported to cause neurobiological dysfunction with altered blood flow and morphology in different parts of the brain (Durazzo et al., 2015; Toda and Toda, 2010) similar effects arising from the chronic use of e-cigarettes are likely. Vaporized e-cigarette liquids have been shown to induce increased DNA strand breaks and cell death independently from their nicotine content (Holliday et al., 2016; Yu et al., 2016). However, nicotine enhances the leakiness of the blood-brain barrier (BBB) by modulating expression and translocation of tight junction protein (Hawkins et al., 2004) that may originate or aggravate neurodegenerative disease conditions.

A recent pilot study has clearly shown that e-cigarette vapors can trigger oxidative stress and inflammation of lung endothelial cells with concurrent loss of lung endothelial barrier functions (Schweitzer et al., 2015). Hence, the lack of studies focusing on the effect of chronic e-cigarette exposure on BBB permeability is quite concerning and should be addressed. Alternatively, nicotine has been shown to have beneficial effects on Parkinson's (Jurado-Coronel et al., 2016; Li et al., 2015; Liu et al., 2015 Lombardo and Maskos, 2015; Quik et al., 2015) and Alzheimer's disease (Gao et al., 2014; Kem, 2000; Kihara et al., 1997; Xue et al., 2015). Further, nicotine's major non-addictive metabolite cotinine has been proven to reduce the burden of post-traumatic stress disorder (PTSD) and schizophrenia (SCHZ) (Barreto et al., 2015; Echeverria et al., 2011, 2016; Echeverria and Zeitlin, 2012). Perhaps aside from the recreational purpose, e-cigarettes might be reconfigured as efficient nicotine delivery systems (devoid of all the other chemicals and flavoring agents) to treat these disease conditions but extensive preclinical and clinical studies need to be performed to prove substantial therapeutic efficacy.

## 9. Conclusion and future directions

The common belief that "e-cigarettes release merely water-based vapors" is incorrect. In addition to nicotine, e-cigarette vapors contain potentially toxic substances which are solvent byproducts (generated by heat) released in the vapor and/or trace constituents of the flavoring additives. Many of the toxic substances present in TC's sidestream and mainstream smoke are absent or negligible although e-cigarette vapors have been shown to contain traces of heavy metals as well as carcinogenic and teratogenic agents derived from flavoring additives.

The rapid adoption of e-cigarettes among consumers of all ages and especially millennials is a major concern. The primary reasons are; 1) the lack of studies addressing the long term health impact of these products (Cheng, 2014), 2) the lack of standardized manufacturing processes and 3) the lack of regulatory guidelines concerning the use of e-cigarettes. Claims concerning the possible use of e-cigarettes not only as recreational devices but also as smoking cessation tools are unverified and need to be scientifically proven since the scant data available today are rather contradictory or lack sufficient power analysis to be relevant. If these claims are not confirmed, then e-cigarettes should be treated on par with TC products and regulated likewise.

As more studies addressing the potential biological and pathogenic impact associated with e-cigarette vaping becomes available there is the significant possibility that many of the safety claims put forward to the public by the manufacturers will fade. To further these investigations, organ specific long term functional and toxicological studies will be necessary. These should include side by side comparative studies against TC products, thus providing a sound scientific basis to help the FDA regulate the manufacturing processes of e-cigarettes (including functional vaping parameters and technical standards of the hardware components) and the allowable contents (qualitative and quantitative parameters) of the e-liquids (Ribisl et al., 2016).

## Conflict of interest

None.

## Authors **contribution**

Mohammad A. Kaisar drafted the review; Shikha Prasad and Taylor Liles also contributed to the drafting of the manuscript and proof reading. Luca Cucullo supervised the work and provided guidance during manuscript preparation and revisions. All authors have read and approved the final version of the manuscript.

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