A systematic review of health effects of electronic cigarettes
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Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>FeNO</td>
<td>fractional exhaled nitric oxide</td>
</tr>
<tr>
<td>NNK</td>
<td>nicotine-derived nitrosamine ketone</td>
</tr>
<tr>
<td>NNN</td>
<td>N-nitrosonornicotine</td>
</tr>
<tr>
<td>PAHs</td>
<td>polycyclic aromatic hydrocarbons/polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>PM</td>
<td>particulate matter</td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>particulate matter less than 2.5 micrometres in diameter</td>
</tr>
<tr>
<td>TSNAs</td>
<td>tobacco-specific nitrosamines</td>
</tr>
<tr>
<td>VOC</td>
<td>volatile organic compound</td>
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<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
1. **Methods**

1.1 **Search**
A search was carried out in PubMed, EMBASE and CINAHL (Annex 1).

Keywords were “electronic cigarette” or “e-cigarette” or “electrically heated cigarette” or “ENDS and cigarette” or “electronic nicotine delivery system” or “electronic nicotine delivery device” or “e-liquid”. The search was performed several times to update the evidence (Annex 1).

1.2 **Exclusion criteria**
Recommendations, expert statements, reviews, technical reports and other non-original papers were excluded, as were papers on smoking cessation, abuse liability, nicotine levels, withdrawal symptoms, poisonings (intentional and unintentional), prevalence, attitudes and beliefs.

1.3 **Eligibility criteria**
Original articles or abstracts on electronic cigarettes (or e-cigarettes) of any topic relevant to health, published before 26 November 2015, were considered eligible. Additionally, a few studies published after that date, found accidentally, have been included. We included studies in any language except a paper in Japanese by Ohta et al. (1) that we assumed to be the same paper as that by Uchiyama et al. (2). Almost all studies were peer-reviewed. A few risk modelling studies have been included as they are based on original findings and typically are presented for decision-makers or the media.

1.4 **Study selection**
The first part of the search was performed by two authors – Charlotta Pisinger (CP) and Dr Med. Martin Døssing – who both read and discussed the articles (3). The second updated search was performed by CP only.

First we screened the titles. After reading the abstract, papers that did not report a health-related topic were rejected. Agreement of the authors was necessary to exclude a paper (first review). Papers on adverse events were included even if the main focus of
the article was, for example, smoking cessation. Then, we excluded duplicates and papers describing the same study population or did not report original data. Full documents were obtained for the final inclusion. Additionally, we looked through the reference lists of the articles for missed papers and we investigated reports for overlooked papers. Finally, we included grey literature that we found accidentally or that others sent to us.

We investigated all papers for conflict of interest, funding and workplace of authors. If in doubt, we contacted the authors and asked about funding and conflict of interest or searched the Internet.
2. Overview of the studies

2.1 Topics
We identified 175 studies – 99 more than in Pisinger and Døssing (3) – the majority (n=105) of these investigating the content of e-cigarette fluid and vapour and/or performing experiments with cells, exposing them to e-cigarette fluid, vapour or extract of vapour. Thirty-one studies reported on adverse events, 32 were human experimental studies and 11 were animal experimental studies. Four papers investigated effects on both cells and animals (4–7). These papers are described in both sections but they only count as one paper.

Figure 1. Categorization of 175 studies identified

2.2 Conflicts of interest
In 34% of the studies the authors had stated a conflict of interest or described funding, or reviewers found a non-declared conflict of interest (for details, see footnotes in Tables 1 to 4 and Annexes 2 to 5). Most of the studies with conflict of interest were funded or otherwise supported by manufacturers of e-cigarettes, but many authors had also been consultants for manufacturers of medicinal smoking cessation therapy or received research grants from them. In several cases – for example, when an author had previously received lecture fees, research grants or travel expenses from a manufacturer (e.g. 8–10) no major influence on the actual study is expected. However, it is important
to note that in recent years the tobacco industry, a manufacturer of e-cigarettes, has published 17 out of the 60 studies with conflict of interest (28%), primarily studies investigating content of fluid. History has shown that we should be very careful in trusting results of studies influenced by the tobacco industry (11–13). Therefore, in-text citations for these studies are marked with an asterisk (*) to alert the reader. Studies funded by ecigarette manufacturers or performed in collaboration with the ecigarette industry are labelled with a chevron (^).
3. Presentation of results

3.1 Content of fluid and vapour
(See Table 1 for overview of studies; for details see Annex 2.)

General findings. Most studies have used conventional cigarettes as reference and investigated presence or concentrations of substances that are known to be harmful in conventional cigarettes. Some of the studies performed in vitro experiments with cells exposed to fluid or vapour, for example to test for cytotoxicity or viral defence. These studies are also mentioned in this section. Many studies found that the product labels did not show the ingredients (e.g. flavours, solvent, nicotine) or that the declaration did not correspond with the concentrations found (e.g. of nicotine).

Glycols. These are the major components in e-cigarettes. High amounts of propylene glycol (also called 1,2-propandiol) and glycerine were found in studies testing for these substances (8, 14–16, 17**, 18, 19, 20**, 21, 22).

Nicotine. Several studies found a large variability in nicotine concentrations across brands, labels, cartridges and refill fluids (14, 15, 22–32), while others found smaller variability (24, 33, 34, 35**, 36). “Nicotine-free” products were found to contain nicotine (14, 15, 25, 31, 37), sometimes in high concentrations, while others found that nicotine content corresponded to labels on the bottles (8, 16, 38^). There were also differences across countries (24). Two studies found the concentration of nicotine in e-cigarette vapour to be much lower than in tobacco smoke (20**, 39). A study found that in products labelled with strength of nicotine (“low”, “medium” or “high”), the actual nicotine concentration varied greatly across brands and could be 3 times higher in one product compared to another with the same strength (40).

Particles. There is no safe level of particulates. Smaller particulate matter less than 2.5 micrometres in diameter (PM_{2.5}) is particularly harmful (41). Particle pollution can

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1 Regarding potential health consequences, see section 3.7.
2 Regarding potential health consequences, see section 3.7.
increase the risk of heart disease, lung cancer and asthma attacks and can interfere with the growth and work of the lungs. One study found that e-cigarette liquids generate many nanoparticles, up to 3000 times more than found in ambient air (42). Some studies found that e-cigarettes and conventional cigarettes produce aerosols with comparable particle sizes (43, 44*, 45) with fine and ultrafine particles in vapour (18), but one study found particles from e-cigarettes much smaller (46*) and another much bigger (47) than in tobacco smoke.

A study showed that the vapour size distribution alters in the human lung and leads to exhalation of smaller particles (19). Regarding particle concentration, two studies found extremely high doses deposited in a human lung model (48, 49); one found it to be double of the dose from tobacco smoke (49), two studies found it to be the same as in tobacco smoke (43, 44*), while three found the concentration to be lower, up to an order of magnitudes lower, than in tobacco smoke (18, 39, 50), and one study found that conventional cigarettes produce more particles initially, but particle counts converge to a level comparable to the condensed vapour (45). A simulation model found that e-cigarette droplets tend to grow larger in maximum size than conventional cigarette particles in the typically highly humid environment of the respiratory system (51*). Two “real-life” condition studies found that vaping e-cigarettes with nicotine showed only marginal particulate matter production in indoor air, while it was much higher after vaping e-cigarettes without nicotine (30, 52). The half-life of vapour was found to be very short – measured in seconds – due to rapid evaporation (47). A study also showed that deposited aerosol mass varied greatly from repeat experiments with all tested products (53*).

**Metals.** The heavy metals cadmium, mercury, lead and arsenic appear in the World Health Organization list of 10 chemicals of major public concern due to potential toxicity (54). A study found that concentrations of lead and chromium in vapour were within the range of conventional cigarettes, while nickel was up to 100 times higher than in conventional cigarettes (55), and one puff of e-cigarette vapour contained numerous metal particles, mainly tin, silver, nickel and aluminium (55). One study found more than 6 times higher content of copper in vapour than in conventional cigarette smoke (56), another found lead content in e-cigarette liquids to be in the same order as in conventional cigarettes (57), and a third found concentrations of cadmium, lead, nickel and arsenic considerably lower than in tobacco smoke but chromium concentrations comparable to smoke (22). Tin, chromium and nickel were found as nanoparticles. A “real-life” study showed a twofold increase of aluminium in indoor air after vaping (30). One study found cadmium, nickel and lead in almost all vapours of 12 brands but the amounts of toxic metals were low, comparable with amounts contained in a nicotine inhaler (nicotine replacement therapy) (9). Another study compared the levels of metals in these studies (9, 55) with regulatory standards and concluded that the levels of metals are unlikely to generate significant adverse health effects for smokers switching to ecigarette use (58). Finally, some studies found metals at lower limits than detection in fluid (38*) and vapour (20*), and trace quantities of mercury in vapour (46*) and of metals in indoor air (59*).
**Tobacco-specific nitrosamines (TSNAs).** These are probably the most important compounds associated with negative health effects in tobacco cigarettes, due to a combination of abundance and strong carcinogenicity (60, 61). N-nitrosonornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK) are classified as IARC group 1 carcinogens.³

Some studies found high maximum concentrations of total TSNAs in the vapour of most (9) or almost all fluids (62). One study found that the concentrations of carcinogenic TSNAs were up to 400 times lower in vapour than in smoke but that vapour concentrations of TSNAs are sufficiently high in some cases to give an elevated risk of tumour development (22). Other studies found that carcinogenic TSNAs were present in vapour at lower levels than tobacco smoke (50), and that TSNAs were present in all samples but the levels of TSNAs and nitrate in e-cigarette liquids were one to two orders of magnitude lower compared to tobacco products (35*). Other studies found trace levels of TSNAs (20*, 63, 64*, 65*, 66), or of TSNAs not present (16, 59*). Some studies detected TSNAs with no or weak carcinogenic effect or no TSNAs in the fluid (8, 14, 30, 32, 40).

Box 1 summarizes the findings on the identified content of fluids and vapour (glycols, nicotine, particles, metals, TSNAs).⁴

<table>
<thead>
<tr>
<th>Glycols are the major components:</th>
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<tbody>
<tr>
<td>• high amounts of propylene glycol and glycerine</td>
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**Glycols**

**Nicotine.** Several studies found a large variability in nicotine concentrations across brands, labels, cartridges, refill fluids – others found smaller variability

**Particles.** Many studies find particles in vapour:

- particle size: conflicting results:
  - fine and ultrafine particles
  - nanoparticles
  - comparable particle sizes as in tobacco smoke
  - much smaller particles than in tobacco smoke
  - much bigger particles than in tobacco smoke
  - alters in the human lung and leads to exhalation of smaller particles

- particle count: conflicting results:
  - up to 3000 times more nanoparticles than ambient air
  - double the dose from tobacco smoke
  - same as in tobacco smoke

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⁴ In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
This report was prepared at the request of WHO Prevention of Noncommunicable Diseases.
The findings, interpretations, and conclusions expressed in this work do not necessarily reflect the views of WHO.

- up to an order of magnitudes lower than in smoke
- tobacco smoke produce more particles initially, but particle counts converge to a level comparable to the condensed vapour
- marginal particulate matter production in indoor air after vaping of product with nicotine, while it was much higher after vaping without nicotine

**Metals.** Lead, chromium, tin, silver, nickel, copper, aluminium, cadmium and mercury identified in several studies:

- presence: conflicting results:
  - found in almost all vapours
  - found as nanoparticles
- concentrations: conflicting results:
  - up to 100 times higher than in conventional cigarettes
  - 6 times higher content in vapour than in smoke
  - within the range of conventional cigarettes/in smoke
  - comparable with amounts contained in a nicotine inhaler
  - trace quantity
  - considerably lower than in smoke
  - at lower limits than detection

**Tobacco-specific nitrosamines (TSNAs).** Total TSNAs, carcinogenic TSNAs and TSNAs with weak carcinogenic effect identified:

- presence: conflicting results:
  - all samples
  - most/almost all samples
  - not present
- concentrations: conflicting results:
  - high maximum concentrations
  - lower levels than tobacco smoke
  - trace level
  - one to two orders of magnitude lower compared to tobacco products
  - up to 400 times lower in vapour than in smoke

**Carbonyls.** These are potential human carcinogens and toxicants (67). In one study, formaldehyde (carcinogenic, group 1), acetaldehyde (possibly carcinogenic, group 2B) and acrolein (toxic and a strong irritant to the skin, eyes and nasal passages) were detected in the vapours of almost all e-cigarettes (2, 9, 68); in another study, formaldehyde was detected in all the > 40 samples (66). A study found five carbonyl compounds in the refill solutions, including formaldehyde, acetaldehyde acetone, propionic aldehyde and butyraldehyde. Acetone was found in many samples at relatively high concentrations (40). Also, a study on flavoured e-liquids found that totals of flavour chemicals were high in general, and the concentrations of some flavour chemicals were sufficiently high to be of toxicological concern due to high aldehyde levels (69). A study found that some samples had extremely high concentrations of
carbonyls (2). High levels of carbonyls were found to be produced even in e-cigarettes without nicotine (68). A study found that the concentration of formaldehyde can be up to 3 times higher in e-cigarette vapour than in tobacco smoke (22). In this study, two apparently identical vaporizers made by the same manufacturer and filled with the same e-liquid yielded formaldehyde concentrations in vapour that differed by a factor of > 25, indicating that the concentration of formaldehyde in vapour depends on the vaporizer (22). Another study found exposure to formaldehyde comparable with smoking (9), as was also the case with vapour from high-voltage devices (10). A study also found high levels of “hidden formaldehyde” (formaldehyde-releasing agents) by use of high-voltage devices; formaldehyde hemiacetal was estimated to be 5 times as high as in conventional cigarette smoke (70). However, a paper concluded that even a low-voltage e-cigarette device can obtain the power of a high-voltage device with different ohmic values, with risk of dissemination of formaldehyde (71). The highest levels of carbonyls were observed in vapours generated from propylene glycol-based solutions (10) or in the second half of a vaping period, indicating overheating of wires (37). Direct dripping of e-liquid due to high temperatures attained in the atomizer may also expose users to increased volatile aldehyde levels relative to conventional e-cigarettes and even relative to conventional cigarettes, for a given nicotine yield (72). One study concluded that most carbonyls were detected at low concentrations in vapour, with the exception of acetone, formaldehyde and acetaldehyde (50). In a study, sucrose was found in all samples of e-liquids – this may be a source of aldehydes (73). Formaldehyde, acetaldehyde and acrolein were also found in vapour in other studies (22, 66), in comparison with conventional cigarettes at concentrations approximately 1/10 (65*) and 1/100 or less of those in smoke (20*, 28). One study found acrolein in vapour at a level comparable to mainstream cigar smoke (74), while other studies found acrolein in vapour at low levels (22, 38*), and acetaldehyde (38*) and formaldehyde at low levels (38*, 64*). The same author presented similar findings in another study, but in a newer version of the same abstract, acetaldehyde and acrolein were not mentioned (46*). Formaldehyde, acetaldehyde, acrolein and siloxanes were found in the aerosol profiles in another study; however, these compounds were never present in the liquids in this study (75). On the other hand, acetaldehyde and formaldehyde were detected in liquids in most samples in another study, at trace levels (35*). Formaldehyde was detected above the limit of quantification in indoor air, but was almost similar to background levels (76*). Finally, one study found that the release of formaldehyde was below the limit of detection (19). It is possible to reduce the levels of harmful substances: a study found that after a revised formulation the levels of acetaldehyde and acrolein decreased, or were not measurable (77).

**Volatile organic compounds (VOCs).** Long-term exposure to high levels of VOCs increases the risk of cancer and of damage to the liver, kidney and central nervous system (78). A study found 11 VOCs among the 15 VOCs analysed, among them benzene (carcinogenic, group 1), styrene and ethylbenzene (group 2B carcinogens), and toluene (40). Other studies also identified toluene (39) and p,m-xylene in almost all vapours (9). It is possible to reduce the levels of harmful substances: a study found that after a revised formulation the levels of acetaldehyde and acrolein decreased, or were not measurable (77).
formulation the levels of benzene decreased, or were not measurable (77). Benzene, toluene and 2,5-dimethylfuran were also found in vapers’ exhaled breath – but smokers had a much higher burden of VOCs than vapers (79). A study investigating fluid, vapour and aerosol found that all of the types of e-cigarette samples generally contained little or none of most of the target VOCs, except for acetic acid (80). In other studies, the concentrations were below the level of detection or quantification or existed at trace levels only in fluid (50) and vapour (20°).

**Hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).** Several PAH compounds, such as benzo(a)pyrene (carcinogenic, group 1), are classified as probable human carcinogens (81). A study found that PAHs in indoor air increased by 20% after vaping (30), and another study found high amounts of hydrocarbons in several products from one brand, in particular alpha-pinene and beta-pinene, probably present in the flavours (66). On the other hand, other studies found either no PAHs in fluid (14, 16), or that most PAHs were below detection level (50, 64°) or as traces only (40, 65°), in vapour (20°) and indoor air (59°).

**Phenols.** Phenol is highly irritating to the skin, eyes and mucous membranes after acute inhalation or dermal exposures, and is toxic via oral exposure (82). A study found five phenolic compounds in refill solutions, with total concentrations below 5 micrograms per gram (μg/g); levels differed dramatically among brands. No direct relationships were found between the levels of nicotine and the level of phenols, implying that phenolic compounds might originate from similar ingredients within the materials used by particular brands, such as flavours, rather than from the nicotine source per se (40). It is possible to reduce the levels of harmful substances: a study found that after a revised formulation the levels of cresols decreased, or were not measurable (77). In one study, total phenols were found to be present at levels 1200 times lower in all e-cigarette liquids than in conventional cigarette smoke (35°), and phenols were found at trace levels in vapour in another study (20°). An experimental study found that content of total phenols in exhaled e-cigarette aerosols was not distinguishable from content in exhaled breath blanks (17°).

**Other measures.** A recent toxicity assessment based on 42 samples (15 brands) concluded that none of the products were totally free from potentially toxic compounds and that a minority of liquids, especially those with flavourings, showed particularly high ranges of chemicals, causing concerns about their potential toxicity in case of chronic oral exposure (66). Other studies found that half of the liquids analysed contained up to 5 times the maximum amount of impurities specified in the European Pharmacopoeia (8), and that a number of the tested products contained tobacco alkaloids at concentrations that exceeded United States Pharmacopeia limits for impurities in nicotine used in pharmaceutical and food products (29).

A study tested for several of the above-mentioned harmful and potentially harmful substances but a further 150 substances were detected, many of them flavourants (22).
Diacetyl, a flavourant associated with respiratory disease (“popcorn lung”) when inhaled, and acetyl propionyl were found in a large proportion of sweet-flavoured e-cigarette liquids, with many of them exposing users to “higher-than-safety” levels (22, 83, 84).

The highly toxic diethylene glycol was found in trace amounts in two studies (22, 32) but not in other studies (8, 28). One study found potentially harmful additives, such as coumarin (37). Products advertised as containing tadalafil contained amino-tadalafil (25, 31). Products advertised as containing rimonabant contained rimonabant plus an oxidative impurity of rimonabant (25). One study found significant amounts of silicate beads in the aerosol (55). Most nicotine-containing e-cigarettes have a basic pH > 9, which seems to influence the doses of nicotine delivered (85). One study found solanesol, one of the major trisesquiterpenoid alcohols in tobacco, demonstrating that tobacco-related impurities are relevant when evaluating refill solutions (40).

Primary aromatic amines were found at trace levels only in vapour (20°). Tobacco industry studies with risk assessment models have been performed (86°, 87°).

**Problems regarding refilling process.** Fluids in cartridge reservoirs leak out of most brands and there are difficulties in assembling and disassembling e-cigarettes without coming into skin contact with the refill liquid (88).

Box 2 summarizes the findings on the identified content of fluids and vapour (glycols, nicotine, particles, metals, TSNAs).  

<table>
<thead>
<tr>
<th>Box 2. Identified content of fluids and vapour: carbonyls, VOCs, hydrocarbons and PAHs, other measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbonyls.</strong> Potential human carcinogens formaldehyde, acetaldehyde and acrolein detected in several studies:</td>
</tr>
<tr>
<td>• presence: conflicting results:</td>
</tr>
<tr>
<td>– all the &gt; 40 samples</td>
</tr>
<tr>
<td>– almost all samples</td>
</tr>
<tr>
<td>– not found</td>
</tr>
<tr>
<td>• concentration: conflicting results:</td>
</tr>
<tr>
<td>– extremely high concentrations</td>
</tr>
<tr>
<td>– high levels of carbonyls produced even in e-cigarettes without nicotine</td>
</tr>
<tr>
<td>– 3 times higher in vapour than in tobacco</td>
</tr>
<tr>
<td>– level comparable to mainstream cigar smoke</td>
</tr>
<tr>
<td>– approximately 1/10 of those in smoke</td>
</tr>
<tr>
<td>– 100/1 or less of those in smoke</td>
</tr>
<tr>
<td>– low/trace levels</td>
</tr>
</tbody>
</table>

5 In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
- almost similar to background level
- below the limit of detection

- special conditions with high concentrations:
  - e-cigarettes with flavours
  - vaporizer type
  - vapour from high-voltage devices
  - propylene glycol-based solution
  - second half of a vaping period (overheating)
  - direct dripping (overheating)

**Volatile organic compounds (VOCs).** Harmful substances as benzene (carcinogenic), toluene and 2,5-dimethylfuran (potentially neurotoxic) were identified:

- presence: conflicting results:
  - in almost all vapours
  - in little/none
  - Found in the aerosol but not in liquid
- concentrations:
  - smokers had much higher burden of VOCs
  - below the level of detection/quantification or trace level only

**Hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).** These include benzo(a)pyrene, a probable human carcinogen:

- presence: conflicting results:
  - no PAHs
  - in several products from one brand, in particular alpha-pinene and beta-pinene, probably present in the flavours
- concentration: conflicting results:
  - high amounts of hydrocarbons
  - most PAHs were below detection level or as traces only

**Other measures**

- none of the products were totally free of potentially toxic compounds
- half of the liquids analysed contained up to 5 times the maximum amount of impurities specified in the European Pharmacopoeia
- diacetyl and acetyl propionyl, chemicals associated with respiratory disease when inhaled, were found in a large proportion of sweet-flavoured liquids at “higher-than-safety” levels
- primary aromatic amines (suspected carcinogenic) were found at trace levels
- phenols present at trace levels
- potentially harmful additives such as coumarin identified
- significant amounts of silicate beads in the aerosol
3.2 Experiments with cells exposed to fluid, vapour or vapour extract: in vitro studies
(See Table 1 for overview of studies; for details see Annex 2.)

**Cytotoxicity.** Several studies have found e-cigarettes to be cytotoxic. An in vitro study demonstrated that menthol additives have a harmful effect on human periodontal ligament fibroblasts, causing a highly significant reduction of cell migration \(^{(89)}\). One study found that several samples were highly cytotoxic to human embryonic and mouse neural stem cells, and cytotoxicity was due to flavours. Cinnamon had a strong cytotoxic effect \(^{(90)}\), a finding that was supported by another study, though a less strong effect was found on cardiomyoblasts \(^{(91)}\). The latter study also found that that cytotoxicity was mainly observed in samples where tobacco leaves were used in production, and all vapour extracts were significantly less cytotoxic compared to conventional cigarette smoke extract \(^{(91)}\). Findings from another study indicated that e-cigarette fluids induced early and late apoptosis, with a major extent in nicotine-treated samples, but present anyway in the samples treated with nicotine-free fluids \(^{(92)}\). E-fluid containing tin particles was found to be cytotoxic on human pulmonary fibroblasts \(^{(55)}\). A study on human lung epithelial cells found toxicological effects of both ecigarette vapour and the pure carrier substances; cell viability was approximately 5 times higher than in cells exposed to conventional cigarette smoke \(^{(93)}\). Another study found that both e-cigarette and conventional cigarette smoke extracts reduced human alveolar cell proliferation, though conventional cigarette smoke exhibited effects at lower concentrations \(^{(4)}\). However, other studies found that vapour from only one out of 21 ecigarette fluids had cytotoxic effects on cultured murine fibroblasts \(^{(94^*)}\), that the tested ecigarette was not cytotoxic \(^{(95^*)}\), and that conventional cigarettes had significantly higher cytotoxicity \(^{(94^*, 95^*, 96, 97)}\). Finally, one study concluded that e-cigarette liquids and vapour do not produce any meaningful toxic effects in four widely applied in vitro test systems, in which the conventional cigarette smoke preparations are markedly cytotoxic and genotoxic \(^{(98)}\).

**Inflammation/oxidative stress.** Many studies have found stress and inflammation in cells exposed to e-cigarettes. A recent study has shown that e-cigarette vapour exposure leads to aggresome formation via proteostasis and autophagy impairment and serves as a mechanism to induce inflammatory oxidative stress, apoptosis, and senescence that can be ameliorated by an autophagy inducer. Thus, it suggests the mechanisms by which e-cigarette exposure can potentially induce chronic obstructive pulmonary disease \(^{(99)}\). Other studies found that vapours induce the release of cytokines and pro-inflammatory mediators \(^{(96)}\), and e-cigarette components exhibit oxidants and reactive oxygen species reactivity similar to used conventional cigarette filters, and oxidants and free radicals in e-cigarette aerosols were similar to oxidant reactivity in conventional cigarette smoke \(^{(56)}\). Findings from another study indicated that e-cigarette fluids induce oxidative stress, with a major extent in nicotine-treated samples, but present anyway in the samples treated with nicotine-free fluids \(^{(92)}\). This is in concordance with a study of Kupffer cells exposed to e-cigarette vapour.
extract showing inflammatory response, oxidative stress production and cytokine release, comparable to conventional cigarette exposure \((100)\), and a study using human, rat and mice bronchial and lung endothelial and lung-derived microvascular cells that concluded that soluble components of e-cigarettes, including nicotine, cause dose-dependent loss of lung endothelial barrier function, which is associated with oxidative stress and brisk inflammation \((7)\). A study using human innate immune cells found that e-cigarette exposure causes an inflammatory response from neutrophils and macrophages, and that the effects were similar to those caused by conventional cigarettes \((101)\). Other studies found that e-cigarette inhalation has an impact on cellular oxidative stress, redox imbalance and lung inflammation \((5)\). The latter study also showed that nicotine was probably not a sole contributing factor in increased oxidants and reactive oxygen species reactivity, and that that the state of the heating element after activation affects the generation of oxidants and reactive oxygen species \((5)\). "Dripping" e-liquids to produce e-cigarette vapour delivers a larger dose of oxidants and reactive oxygen species to consumers and there are at least two possible sources of oxidants and reactive oxygen species released from ecigarettes: from activation of the heating element, and from the process of vaporizing e-liquids \((5)\). A study using human lung epithelial cells found that oxidative stress was approximately 5 times lower than in cells exposed to conventional cigarette smoke \((93)\), and another study suggested that the intestinal epithelium inflammatory response is not altered by exposure to vapour from ecigarettes \((102)\). A study using young healthy human airway epithelial cells showed that e-cigarette fluid promotes pro-inflammatory cytokine IL-6 production and human rhinovirus infection \((103)\). Human lung fibroblasts exposed to e-cigarette liquid showed cell stress and other phenotypic abnormalities that were further exacerbated by nicotine \((5)\), and vacuolization and cell enlargement following treatment with 5% e-liquid containing nicotine was most similar to fibroblasts treated with 1% conventional cigarette smoke extract \((5)\).

**Other findings.** Human bronchial cells that contained mutations found in smokers at risk of lung cancer were grown in a culture medium that had been exposed to vapour. The researchers found that cells exposed to high-nicotine vapour showed a similar pattern of gene expression to those exposed to tobacco smoke \((104)\). A study in human embryonic stem cells also showed dysregulation of gene expression indicating a negative effect of ecigarette use on heart development \((6)\). Another study found that at biologically relevant doses, vaporized e-liquids induced increased DNA strand breaks and cell death, and decreased clonogenic survival in both normal epithelial and head and neck squamous cell carcinoma cell lines independently of nicotine content \((105)\). Exposure to e-cigarette vapour also decreased the expression of cardiac transcription factors in cardiac progenitor cells, suggesting a persistent delay in differentiation \((6)\). Also, in definitive human cardiomyocytes there was a reduced expression of sarcomeric genes. E-cigarette fluid exposure had immediate and profound adverse effects on the metabolomic state of primary human bronchial epithelial cells similar to those seen with conventional cigarette smoke condensate \((106)\).
A study showed that platelet aggregation was enhanced when platelets were exposed to ecigarette vapour extract, and for the formulations with the highest concentration of nicotine, this enhancement mirrored the effects of mainstream and sidestream tobacco smoke extracts (107). Also, platelets were more likely to participate in coagulation-based reactions, suggesting an enhancement of the coagulation cascade, indicating increased risk of cardiovascular disease (107).

Box 3 summarizes the effects observed in experiments with cells: in vitro studies (cytotoxicity, inflammation/oxidative stress, other findings).

### Box 3. Effects observed in experiments with cells (in vitro studies)

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>Several studies have found e-cigarettes to be cytotoxic:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• compared with tobacco smoke:</td>
</tr>
<tr>
<td></td>
<td>▪ cell viability approximately 5 times higher than in cells exposed to smoke</td>
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<tr>
<td></td>
<td>▪ conventional cigarettes had significantly higher cytotoxicity</td>
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<tr>
<td></td>
<td>• cytotoxicity found to be due to flavours in several studies</td>
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<tr>
<td></td>
<td>• highly significant reduction of cell migration</td>
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<td></td>
<td>• no meaningful cytotoxic or genotoxic effects</td>
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</table>

<table>
<thead>
<tr>
<th>Oxidative stress and inflammation</th>
<th>Many studies have found oxidative stress and inflammation in cells:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• compared with tobacco smoke:</td>
</tr>
<tr>
<td></td>
<td>▪ most studies: comparable to conventional cigarette exposure</td>
</tr>
<tr>
<td></td>
<td>▪ one study: oxidative stress approximately 5 times lower than when exposed to smoke</td>
</tr>
<tr>
<td></td>
<td>▪ one study: intestinal epithelium inflammatory response not altered by exposure</td>
</tr>
<tr>
<td></td>
<td>• aggresome formation via proteostasis/autophagy impairment</td>
</tr>
<tr>
<td></td>
<td>• release of cytokines and pro-inflammatory mediators</td>
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<tr>
<td></td>
<td>• promotes pro-inflammatory cytokine IL-6 production</td>
</tr>
<tr>
<td></td>
<td>• the state of heating element affects generation of oxidants/reactive oxygen species</td>
</tr>
<tr>
<td></td>
<td>• more in nicotine-treated samples but also present in nicotine-free fluids</td>
</tr>
<tr>
<td></td>
<td>• &quot;dripping&quot; method delivers a larger dose of oxidants/reactive oxygen species</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Other findings</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>• a similar pattern of gene expression to cells exposed to tobacco smoke</td>
</tr>
<tr>
<td></td>
<td>• increased DNA strand breaks and cell death, and decreased clonogenic survival in both normal epithelial and head and neck squamous cell carcinoma cell lines</td>
</tr>
<tr>
<td></td>
<td>• dysregulation of gene expression indicating a negative effect on heart development</td>
</tr>
<tr>
<td></td>
<td>• immediate and profound adverse effects on the metabolomic state, similar to those seen with smoke condensate</td>
</tr>
<tr>
<td></td>
<td>• enhanced platelet aggregation, platelets more likely to participate in coagulation-based reactions</td>
</tr>
<tr>
<td></td>
<td>• promotes human rhinovirus infection</td>
</tr>
<tr>
<td></td>
<td>• dose-dependent loss of lung endothelial barrier function</td>
</tr>
</tbody>
</table>

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6 In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
3.3 Human experimental studies
(See Table 2 for overview of studies; for details see Annex 3.)

General findings. Most studies included smokers as volunteers and compared with a reference, mostly own-brand conventional cigarettes. All experimental studies report short-term exposure only, typically a few minutes of exposure to vapour.

Adverse events. These were very similar to those reported in studies reporting adverse events (Annex 3). There was low reporting of adverse events in regular users who were e-cigarette naive before study start, with the most frequent being light-headedness, throat irritation, dizziness and cough (108^, 109, 110^).

Pulmonary system. A single session of e-cigarette use in e-cigarette naive smokers, approximating nicotine exposure of one conventional cigarette, induced significant inhibition of cough reflex sensitivity, probably due to nicotine (111). Other studies in e-cigarette naive smokers found increased airway resistance (112–114) and a concomitant decrease in specific airway conductance (113), and an increase in impedance and overall peripheral airway resistance (114), effects that are reminiscent of those seen with tobacco smoking. Also, the same particle dose was received as with smoking and vaping (112). Two studies found immediate reductions in exhaled nitric oxide, similar to smoking (112, 114), and increased fractional exhaled nitric oxide (FeNO) (30), while another study found a decrease in FeNO (115). A study including both healthy volunteers and patients with asthma and chronic obstructive pulmonary disease also showed that 10 minutes of vaping caused immediate significant airway obstruction (116), which is in contrast to a retrospective review finding objective and subjective improvements in asthma outcomes (117). A study found that short-term vaping by e-cigarette naive users of flavoured e-cigarettes resulted in significant decrease in flow when 75% of forced vital capacity had been exhaled (118). Another study found that short-term usage was associated with increased flow resistance, even though spirometry-assessed lung function was deemed normal (119). Passive, but not active, vaping of one e-cigarette resulted in short-term lung obstruction, indicating insufficient inhalation by e-cigarette naive smokers (119). The last study found that short-term vaping of e-cigarettes generated non-significant decrease in lung function, approximately half of what was seen in smoking (120).

Cardiovascular system. Some studies in e-cigarette naive smokers found that short-term vaping resulted in increased heart rate (115, 121–125, 126^), an elevation in diastolic blood pressure (121–123, 127) comparable to the increase caused by smoking (126^), and a decrease in oxygen saturation (115). Other studies found no increase in heart rate (110^, 128, 129) or in blood pressure (110^), but an increase in oxygen saturation (110^). One study found no negative effect on elasticity and stiffness of ascending aorta (130). Active and passive vaping in e-cigarette naive smokers did not influence the complete blood count (131). One study using experienced e-cigarette users found no effect on cardiac function (127). One small study suggests
that nicotine, when inhaled via e-cigarette, does not impair the cerebral pressure–flow relationship (132).

**Cognitive function.** Two studies found improved time-based but not event-based prospective memory (133) and improved nicotine withdrawal impaired concentration/memory (134); these improvements were associated with cessation of conventional cigarette smoking.

**Toxicity.** Urinary toxicant and carcinogen metabolites were found to be significantly lower in current e-cigarette users than in conventional cigarette smokers, but a few e-cigarette users had higher-than-expected levels of total NNAL (metabolites of the tobacco-specific nitrosamine and lung carcinogen); lower than in smokers but higher than when exposed to second-hand smoking (135). Studies also found a metabolite of the pyrolysis product acrolein in urine, after vaping e-cigarettes with nicotine (30, 136). The latter found that in dual users e-cigarette use significantly reduced exposure to carbon monoxide and acrolein because of a significant reduction in conventional cigarette intake (136). Another study found benzene, toluene and 2,5-dimethylfuran in vapers’ exhaled breath, but smokers had a much higher burden of VOCs than vapers (79). An experimental study with experienced vapers found that e-cigarettes produce high levels of formaldehyde, acetaldehyde and acrolein only in dry puff conditions (the levels were increased by 30 to 250 times), in which the liquid overheats, causing a strong unpleasant taste; authors assume that vapers will avoid dry puff conditions (137).

**Other.** A marker of oxidative stress in exhaled breath was found to be significantly increased by vaping but less than by smoking (138).

Box 4 summarizes the effects observed in human experimental studies (adverse effects, toxicity, pulmonary system, cardiovascular system, other findings).

<table>
<thead>
<tr>
<th><strong>Box 4. Effects observed in human experimental studies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adverse events.</strong> Mild:</td>
</tr>
<tr>
<td>• most frequent: light-headedness, throat irritation, dizziness, cough</td>
</tr>
<tr>
<td><strong>Toxicity.</strong> Toxicants and carcinogen metabolites found in urine of vapers:</td>
</tr>
<tr>
<td>• concentrations:</td>
</tr>
<tr>
<td>• significantly lower than in smokers</td>
</tr>
<tr>
<td>• high concentration of NNAL (carcinogenic) found in some vapers</td>
</tr>
<tr>
<td>• high formaldehyde, acetaldehyde, acrolein only in dry puff conditions</td>
</tr>
<tr>
<td>• vapers’ exhaled breath: benzene, toluene and 2,5-dimethylfuran (harmful substances) identified</td>
</tr>
<tr>
<td>• smokers had much higher burden of VOCs than vapers</td>
</tr>
</tbody>
</table>

7 In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
Pulmonary system. Effects reminiscent of those seen with tobacco smoking:
- increased airway resistance, decrease in specific airway conductance, increase in impedance and overall peripheral airway resistance
- lung function:
  - non-significant decrease in lung function, approximately half of effect of smoking
  - normal but increased flow resistance
- both healthy volunteers and patients with asthma and chronic obstructive pulmonary disease: immediate significant airway obstruction
- same particle dose received in airways as with smoking
- significant inhibition of reflex sensitivity
- reduction in exhaled nitric oxide
- fractional exhaled nitric oxide:
  - increased
  - decreased

Cardiovascular system:
conflicting results on haemodynamic effect:
increased heart rate, elevation in diastolic blood pressure, decrease in oxygen saturation
no increase in heart rate or in blood pressure but an increase in oxygen saturation
no negative effect on elasticity and stiffness of ascending aorta
no effect on cardiac function

Other findings:
significantly increased marker of oxidative stress in exhaled breath
improved time-based but not event-based prospective memory
improved nicotine withdrawal impaired concentration/memory

3.4 Animal experimental studies
(See Table 3 for overview of studies; for details see Annex 4.)

General findings. The longest time of exposure in animal studies was four months (139). One study exposed animals for seven weeks (140), one during pregnancy and two weeks after (141), and another for four weeks (142) – otherwise it was short-term exposure only.

The long-term exposure study showed that exposure to e-cigarette vapour for five hours per day caused asthma and emphysema in mice (139). A study showed that mice treated intratracheally with e-cigarette fluid had increased infiltration of inflammatory cells, aggravated asthmatic airway inflammation and airway hyperresponsiveness, and stimulated the production of cytokines and ovalbumin-specific IgE production (143). This is in concordance with a study showing that exposure of mice to e-cigarette vapour increased pro-inflammatory cytokines and diminished lung glutathione levels, which are critical in maintaining cellular redox balance (5). Other murine studies also demonstrated that ecigarette exposure resulted in increased oxidative stress.
and moderate inflammation (7, 144) and impaired pulmonary antimicrobial defences, significantly impaired pulmonary bacterial clearance, and – in response to influenza A virus infection – increased lung viral titers and enhanced virus-induced illness and mortality (144). This is also in concordance with a study finding that e-cigarettes inhibit the expression of a host defence molecule against human rhinovirus infection in mice (103). Rats exposed to e-cigarette vapour developed hyperplasia and metaplasia in the larynx more frequently than non-exposed animals but the difference was non-significant, most probably due to very small study size (142). Another mice study found that second-hand exposure to e-cigarette vapour induced addiction-related neurochemical, physiological and behavioural alterations (140), and a mice study found increased levels of activity when exposed to vapour containing nicotine during late prenatal and early postnatal life – indicating that nicotine exposure from e-cigarette may cause persistent behavioural changes (140). Exposure to e-cigarette vapour – with or without nicotine – during the neonatal period resulted in a small negative impact on the weight of mice, and exposure to e-cigarette with nicotine caused diminished alveolar cell proliferation and a modest impairment in postnatal lung growth (145). In zebrafish, exposure to e-cigarette vapour extract resulted in broad, dose-dependent developmental defects coupled with severe heart malformation, pericardial oedema and reduced heart function (6). On the other hand, a mice study showed that despite higher exposure conditions, e-cigarettes exhibited less toxic effects on lungs of experimental animals after short-term exposure (4).

Box 5 summarizes the effects observed in animal experimental studies.

### Box 5. Effects observed in animal experimental studies

Effects observed in animal experimental studies are summarized as follows:

- increased infiltration of inflammatory cells and pro-inflammatory cytokines
- increased oxidative stress and moderate inflammation
- asthmatic airway inflammation and airway hyperresponsiveness
- impaired pulmonary antimicrobial defences
- enhanced virus-induced illness and mortality
- asthma and emphysema
- hyperplasia and metaplasia in the larynx
- developmental defects coupled with severe heart malformation
- neonatal exposure: diminished alveolar cell proliferation and a modest impairment in postnatal lung growth
- increased levels of activity by late prenatal and early postnatal exposure

### 3.5 Adverse events

(See Table 4 for overview of studies; for details see Annex 5.)

**General findings.** There are no studies with long-term follow-up. The longest follow-up period is two years. As most smokers have no or few and mild symptoms, for example
a mild cough for decades, potential serious adverse effects of e-cigarette use should not be expected in short-term studies.

**Population-based survey.** One large population-based survey with high representability has been performed in Chinese adolescents. The study included more than 45,000 students, aged approximately 12 to 18 years. E-cigarette use was significantly associated with respiratory symptoms in analyses adjusted for sex, age, perceived family affluence, second-hand smoke exposure, and school clustering effect (146).

**Surveys and interviews with e-cigarette users.** Most adverse events have been from the mouth/throat and the respiratory system, but symptoms from many organ systems have been reported. On the other hand, many regular e-cigarette users reported a decrease in respiratory symptoms and improvements in general health. Regular users of e-cigarettes typically reported few negative symptoms, such as mouth and throat irritation, cough, vertigo, headache, gastrointestinal discomfort, epigastric burning or nausea, and many positive health effects, such as improved breathing, reduced cough and expectoration, improved health and physical fitness, improved quality of life, improved sleep, and improved smell and sense of taste (147^, 148–150, 151^, 152, 153). Often, a majority or all of the regular users included in studies had quit smoking, and the positive side-effects are identical with health improvements after smoking cessation. On the other hand, vapers in a chat forum mostly reported negative symptoms, from many organ systems. In particular, symptoms for respiratory, mouth and throat, neurological, and sensory organ systems were reported, and users with negative symptoms often reported more than one symptom. Interactions were often seen between organ systems. Positive effects most frequently affected the respiratory system (154). A summary of adverse events reported to the United States Food and Drug Administration (155) categorized eight out of almost 50 reports as serious adverse events: hospitalization for illnesses such as pneumonia, congestive heart failure, disorientation, seizure, hypotension, possible aspiration pneumonia, second-degree burns to the face, chest pain and rapid heartbeat, possible infant death secondary to choking on an e-cigarette cartridge, and loss of vision requiring surgery. In most cases (except burns, choking and loss of vision) there was no information on causality. Other adverse events reported were headache/migraine, chest pain, cough/sputum, nausea/vomiting, dizziness, feeling sick, confusion/stupor, sore throat, shortness of breath, abdominal pain, pleurisy, blurry vision, and sleepy/tired.

**Prospective studies and randomized trials.** One possible serious adverse event (myocardial infarction) was recorded in a study (156). A randomized controlled trial on smoking cessation (13 weeks) found a higher number and proportion of adverse events occurred in the nicotine–e-cigarette group than in the nicotine–patches group; however, there was no evidence of an association with e-cigarettes, and the event rate was not significantly different (157). A substudy of this trial found that mentally ill persons tolerated e-cigarette well (158). Two other randomized trials reported that adverse events such as cough, dry mouth, shortness of breath and headache declined
over 12 months of follow-up (159), whereas a short-term dual use group reported both positive and negative symptoms (160). In some studies the time association between e-cigarettes and adverse events was registered by a health professional; participants primarily experienced mouth/throat and respiratory symptoms, headache, palpitations and nausea, but there were no serious adverse events (159, 161–164). Causality seems probable. In three studies, symptoms waned spontaneously over weeks or months (159, 162, 163). In one study, however, users experienced a slight increase in mouth/throat irritation and dry cough over time. This study had the longest follow-up period, amounting to two years (164). One study included schizophrenic patients (162). This study showed that positive and negative symptoms of schizophrenia did not increase after smoking reduction or cessation in patients using e-cigarettes. No safety concerns were raised during another prospective study, although the limitations in recording of adverse events prevented the authors from drawing any conclusions (156).

**Case reports.** A case of contact dermatitis was most probably caused by use of a nickel-containing e-cigarette device (165). Other case reports on different lung diseases (166–168), reversible cerebral vasoconstriction syndrome (169), atrial fibrillation (170), lichen planus (171), lingua villosa nigra (172), colonic necrotizing enterocolitis in a newborn child (his mother was vaping an e-cigarette during pregnancy) (173), relapse of colitis ulcerosa symptoms (174), and remission in a colitis ulcerosa patient and beneficial effects on idiopathic chronic neutrophilia (175) have been reported, as they found time association or reversibility, but causality can only be hypothesized. One of the case reports is in a dual user (169).

Box 6 summarizes the effects of reported adverse events.8

<table>
<thead>
<tr>
<th>Box 6. Reported adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported adverse events are summarized as follows:</td>
</tr>
<tr>
<td>• no long-term use effects reported</td>
</tr>
<tr>
<td>• large population-based survey: e-cigarette use significantly associated with respiratory symptoms</td>
</tr>
<tr>
<td>• a higher proportion of adverse events seen in e-cigarette group in a randomized trial, but difference not significant</td>
</tr>
<tr>
<td>• possible serious adverse events reported, but causality is not known</td>
</tr>
<tr>
<td>• most common adverse events: mild, such as mouth and throat irritation, cough, headache, nausea</td>
</tr>
<tr>
<td>• conflicting results on symptoms:</td>
</tr>
<tr>
<td>- new users often report several negative symptoms from more organ systems</td>
</tr>
<tr>
<td>- regular users often report improvement in cough and breath and general well-being – some of these attributed to smoking cessation</td>
</tr>
<tr>
<td>- conflicting results on increase/decrease in reported adverse events over time</td>
</tr>
<tr>
<td>• many case reports from all organ systems – but causality is unknown</td>
</tr>
</tbody>
</table>

8 In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
3.6 Passive exposure to vapour
(For details of studies see Annexes 2–4; relevant studies are marked with Θ)

Human experimental studies have shown that passive vaping resulted in short-term lung obstruction and increased cotinine (119, 120), but passive vaping did not influence complete blood count indices in smokers and never smokers (131). A “real-life” study found that non-smokers passively exposed to e-cigarette vapour absorb approximately as much nicotine as when exposed to smoke from conventional cigarettes (176). Relatively high concentrations of propylene glycol and glycerol could be quantified in the air of chamber tests, indicating risk of passive vaping (177). Two studies have investigated third-hand exposure to nicotine: an experiment showed significant increases in the amount of nicotine on all surfaces (178), whereas a study in households showed significantly less nicotine on surfaces compared to smoking conventional cigarettes (179). A study found that emission rates of organic compounds (including alkanes and organic acids), as well as total emission of inorganic elements and metals, were also significantly reduced in vaping compared to smoking. However, analysis of elemental emissions indicated the presence of toxic metals in e-cigarette aerosol, with nickel and silver having higher indoor emission rates compared to conventional cigarettes (180). Analyses of indoor air quality showed that there were high concentrations of ultrafine particles (PM$_{2.5}$), that the concentration of putative carcinogenic PAHs in indoor air increased by 20%, and that aluminium increased 2.4-fold after vaping sessions (30). A real-life vaping study showed that e-cigarettes emit PM$_{2.5}$, although the concentration was notably lower than from smoking (181). Benzene, toluene and 2,5-dimethylfuran were also found in the exhaled breath of e-cigarette users (79).

One study investigated the interaction between radon (significant risk for lung cancer) and e-cigarette sidestream vapour and found that the increase in the attached potential alpha energy concentration was higher for the e-cigarette than for the traditional conventional cigarette. Therefore, the aerosol from e-cigarettes operates as a carrier of the radon progeny and, as a consequence, it decreases the plate-out of radon daughters (182).

On the other hand, one study found that vaping does not produce detectable amounts of toxic and carcinogenic substances in the air of an enclosed space (183$^*$). Formaldehyde was detected above the limit of quantification in indoor air in one study; however, these levels were overlapping the range of the background levels (76$^*$). A study investigating vapour and aerosol found that all of the types of e-cigarette samples generally contained little or none of most of the target VOCs, except for acetic acid (80), and a real-life study showed trace quantities of metals and low levels of carbonyls in indoor air, below the WHO Indoor Air Quality Guidelines (59$^*$). Other studies performed by the tobacco industry concluded that exhaled e-cigarette aerosol did not increase bystander exposure for phenolics and carbonyls above the levels observed in exhaled breaths of air (17$^*$).

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Note: Study not sponsored by e-cigarette industry but first author has performed other studies sponsored by the industry.
and that exposure of bystanders to the chemicals in the exhaled e-cigarette aerosol was below current regulatory standards that are used for workplaces or general indoor air quality \(^{(59^*)}\), and a mathematical modelling model concluded that the exposure of bystanders to nicotine in the exhaled aerosol is not at levels that would be expected to cause health concerns \(^{(184^*)}\).

Box 7 summarizes the findings from studies on passive vaping (human experiments; indoor air, particles and emissions).\(^{10}\)

**Box 7. Findings from studies on passive vaping**

**Human experiments:**
- short-term lung obstruction but no influence on complete blood count found in acute exposure studies
- non-smokers passively exposed to vapour absorb approximately as much nicotine as when exposed to smoke
- total phenols and carbonyls in exhaled aerosols not distinguishable from content in exhaled breaths blanks

**Indoor air, particles and emissions:**
- significant increases in the amount of nicotine on all surfaces
- high concentrations of ultrafine particles (PM\(_{2.5}\)), concentration of putative carcinogenic PAHs in indoor air increased by 20%, and aluminium increased 2.4-fold after vaping sessions
- benzene, toluene and 2,5-dimethylfuran found in exhaled breath
- vaping does not produce detectable amounts of toxic and carcinogenic substances in the air of an enclosed space
- formaldehyde above limit of detection but not higher than background levels
- phenols and carbonyls in exhaled aerosol as in exhaled breath blanks
- compared to smoking:
  - presence of toxic metals in aerosol, with nickel and silver having higher indoor emission rates compared to tobacco smoke
  - emission rates of organic compounds and inorganic elements and metals reduced compared to smoking
  - significantly less nicotine on surfaces compared to smoking
  - PM\(_{2.5}\) notably lower than in smoke

### 3.7 The major ingredients: glycols, nicotine and flavours

**Glycols.** Of special concern is the fluid carrier or vehicle and major ingredient of e-cigarettes that create the visible fume: the glycols, propylene glycol and glycerine.

Even though these are recognized as safe for oral intake \(^{(185)}\), and concentrations found in ecigarettes typically have been below occupational safety standards \(^{(186)}\), it

\(^{10}\) In general, studies with severe conflicts of interest have findings indicating little or no harm to health.
must be noted that occupational safety standards are not intended to establish “safe” exposure concentrations for a general population but to diminish harm in exposed workers during working time (187), and that eating and inhaling are not the same. The lungs have a very large surface and completely different values may apply when a vaper is exposed for several hundred daily direct inhalations in decades. An internal technical report commissioned by vapers and vendors of e-cigarettes concluded that estimated levels of exposure to propylene glycol and glycerine are close enough to threshold limit values to warrant concern, and that the threshold limit values are based on uncertainty rather than knowledge (188, 189). Glycols are used as theatrical smokes and fogs and a study of more than 100 employees showed that chronic work-related wheezing and chest tightness were significantly associated with increased cumulative exposure to theatre fogs (mineral oil and glycols) over the previous two years. Acute cough and dry throat were associated with acute exposure to glycol-based fogs; increased acute upper airway symptoms were associated with increased fog aerosol overall. Lung function was significantly lower among those working closest to the theatre fog source (190).

**Propylene glycol** is a solvent used in pharmaceutical products, in cosmetics, as a food additive, as theatrical fog and as industrial antifreeze. An old experimental study showed that continuous residence of monkeys and rats for a year or more in an atmosphere supersaturated with the vapour of propylene glycol was without deleterious effect on the lungs and functional activity of the body as a whole (191); in fact the animals seemed to thrive somewhat better than the control groups, as judged by weight gain and increase in red blood cells and haemoglobin content. Another old experimental study exposed rabbits to 10% propylene glycol inhalations and found that there was a minimal alteration of the ultrastructure of the ciliated cells in the airways. The action of propylene glycol was manifested chiefly in the goblet cells, which rapidly discharged their mucus (192). A recent industry-sponsored review found that none of the glycols reviewed presented evidence of carcinogenic, mutagenic or reproductive/developmental toxicity potential to humans, and that the propylene glycols present a very low risk to human health (193°). Another newer study conducted by the tobacco industry exposed dogs and rats for 28 days and concluded that propylene glycol aerosol could be administered safely in humans (194°). However, in the rats there was ocular and nasal irritation and laryngeal squamous metaplasia. In dogs the study found decreases in haemoglobin but no apparent tissue toxicity of the lung, liver and kidney (194°).

Newer experimental studies with propylene glycol have shown an increased number of goblet cells in the respiratory tract and nasal haemorrhaging (195), irritation to the upper respiratory tract and squamous metaplasia of the epiglottis following exposure at concentrations present in e-cigarettes (196). Volunteers exposed to propylene glycol mist for one minute developed ocular and airway irritation and a few reacted with slight airway obstruction and increased self-rated severity of dyspnea (197). Long-term exposure to propylene glycol has been found to exacerbate and/or induce multiple allergic symptoms in children (198). A study with electronic shisha pens (e-cigarettes designed to mimic a water pipe) showed that already after one puff, the concentrations
of propylene glycol and glycerol are sufficiently high to potentially cause irritation of the airways (199). When used in high doses or for prolonged periods, propylene glycol toxicity can occur. Reported adverse effects in paediatric patients include central nervous system toxicity, hyposmolarity, haemolysis, cardiac arrhythmia, seizures, agitation and lactic acidosis (200). One e-cigarette study found that the highest levels of carbonyls in e-cigarettes were observed in vapours generated from propylene glycol-based solutions, compared with a 50:50 solvent with glycerine (10).

Glycerine is used in food as a humectant and as a solution carrier in flavours. Glycerine is considered generally safe for oral intake (201), but the same considerations apply as for propylene glycol when inhaling it. Ethylene glycol, associated with pronounced toxicological risks (202), has been found to replace glycerol/propylene glycol in several brands (37). Diethylene glycol, associated with pronounced toxicological risks, has been detected in small quantities in very few studies (22, 65*).

Nicotine. Almost all regular users report that they use e-cigarettes with nicotine (203), with levels in ecigarette users (204) almost as much as in smokers (205), and higher than in nicotine replacement therapy users (206). It is well established that nicotine is highly addictive (207, 208). More than 60% of smokers wish to quit because they do not like being dependent (209), and switching to e-cigarettes does not break the nicotine addiction.

Nicotine is referred to by some health professionals as harmless, and a meta-analysis found no increased risk of serious adverse events, after 12 months or less (210). To our knowledge, only one study has investigated the health effects of long-term pure nicotine or nicotine replacement therapy use, finding no increase in the risk of cancer after 12 years (211). Others do not share this view. However, nicotine has significant biologic activity: in the central nervous system nicotine stimulates the release of important neurotransmitters and hormones (212), and in the peripheral system it stimulates the release of catecholamines, with effects such as vasoconstriction, increase in heart rate and myocardial contractility (213). In vitro evidence points to possible direct carcinogenic and genotoxic effects of nicotine (214–221). Human and animal data support that nicotine exposure during periods of developmental vulnerability has multiple adverse health consequences, including impaired fetal brain and lung development, and altered development of cerebral cortex and hippocampus in adolescents (222). Animal studies (the applicability to human beings may be questioned) suggest that nicotine accelerates atherosclerosis (213), reduces sperm quality (223), promotes growth of cancer cells and the proliferation of endothelial cells, and reduces the responsiveness of several cancers to chemotherapy (214, 224–227), and fetal and neonatal nicotine exposure leads to widespread adverse postnatal physical and mental health consequences (228–230). Epidemiological evidence for such an effect of nicotine is still unavailable. While being on the “high priority” list for evaluation by the WHO International Agency for Research on Cancer, nicotine has so far not been classified by the agency.
Intentional (231) and non-intentional poisoning occurs. Poison centres are receiving many calls regarding e-fluid (213, 232); mostly, exposures have resulted in minimal toxicity (e.g. vomiting, nausea, tachycardia) (109), but a case of fatal nicotine poisoning in a child has been reported (233).

The fatal dose of nicotine is unclear but has in adults been estimated at 30 to 60 mg, while for young children it is estimated at only 10 mg (234).

**Flavours.** Flavour ingredients are an essential part of e-liquids. A recent study concluded that concentrations of some flavour chemicals in e-cigarette fluids are sufficiently high for inhalation exposure by vaping to be of toxicological concern, and almost half of the tested products on the United States market were more than 1% by weight flavour chemicals (69). Many of the studies in this review have found flavours to be associated with potential harm (5, 35*, 66, 69, 84, 89, 90, 96, 118, 235, 236†). As with propylene glycol it is important to note that “generally recognized as safe” applies only to oral intake. None of the primary safety assessment programmes for flavours, including the GRAS programme sponsored by the Flavour and Extract Manufacturers Association of the United States (FEMA), has evaluated flavour ingredients for use in products other than human food. A FEMA GRAS™ status for the use of flavour ingredients in food does not mean that these flavour ingredients are safe for use in e-cigarettes (237). Diacetyl, a food sweetener, was approved as completely safe for oral intake but it turned out that workers exposed to inhalation of diacetyl during food manufacturing frequently had airway obstruction and this was caused by a rare lung disease, bronchiolitis obliterans, later popularly named as “popcorn lung” (238). Diacetyl has in a recent study been found in 75% of the samples (83).

The potentially tempting effect of candy-like tastes on youths should also be kept in mind. Finally, flavours are also known to affect the stability of products, and flavours may impact nicotine concentrations (239).
4. General considerations

4.1 General considerations of quality of studies and other research challenges

The research field is new and very challenging. Serious methodological problems were identified:

1. The core problem is that any research only applies to the specific e-cigarette brand, model and batch tested, with no certainty that the findings will apply to other or future brands, models or batches. E-cigarettes are subject to very frequent modifications; there are currently approximately 500 brands and 8000 flavours, and with the third generation of e-cigarettes (the “mods”), and the fourth, consumers have even more choices to customize their own e-juices.

2. Studies sponsored or conducted by the tobacco industry have severe conflicts of interest. Studies sponsored or performed in collaboration with e-cigarette manufacturers also have a conflict of interest that might influence the results, the presentation of results or the conclusions. In general, most studies with severe conflicts of interest (as identified at the start of the reference list) found less or no potentially harmful effects from substances than studies without conflict of interest. Therefore, we must carefully consider whether these can be trusted.

3. Studies investigating fluid do not take into account that e-cigarettes can generate new compounds (e.g. formaldehyde, acetaldehyde and acrolein) that did not exist in the original solution – generally produced via oxidation of the glycols through heating, thereby underestimating the risks of vaping.

4. More than 80 compounds have been identified in e-cigarette aerosols and we lack knowledge of possible interactions between all these chemicals. A compound found in a harmless concentration might interact with other compounds of low concentration creating harmful effects.

5. There are no “standard vaping machines” or standards for testing of ingredients in e-cigarettes, so studies are difficult to compare. E-cigarette use topography
is significantly different than smoking \((154)\). When vaping, vapers are sucking harder and have longer puffing duration, approximately double that of smoking, especially if the fluid content in the cartridge is low \((240)\). Therefore, the real uptake of harmful substances might be underestimated when testing on e-cigarette naive volunteers or standard smoking machines. Also, studies show that there are significant variations in puffing topography among users of various ecigarette models \((241)\), that production of harmful substances is influenced by battery voltage output \((10)\), vaporizer \((22)\) and e-liquid levels left \((37)\), and that pH may influence the doses of nicotine delivered to users \((85)\) – this complicates the research even more.

6. Human experiments were mostly based on very short-term exposure, for example vaping for a few minutes – not reflecting real-life exposure and thereby underestimating negative long-term effects.

7. Some animal studies might have overexposed the animals, thereby overestimating negative health effects. Also, it is important to remember that health effects in animal studies do not always apply to humans.

8. Some studies might have overheated fluid when generating vapour, thereby overestimating negative health effects.

9. Studies of adverse events are seriously biased by selection bias. Those based on new vapers probably overestimate harm, whereas those based on regular vapers probably underestimate harm.

Studies identifying negative health effects of vaping, or identifying high concentrations of harmful substances, have been targets of intense, sometimes even aggressive critique. In some cases it might be correct that there have been methodological problems causing overestimation of risk. However, it seems very unlikely that all of the many studies identifying increased risk of negative health effects by e-cigarette use should be poor science.

### 4.2 General health risk considerations

#### 4.2.1 Impact of the diversity of products

While a smoker smoking a conventional cigarette of one brand has more or less the same risk as another smokers who smokes a conventional cigarette of another brand, a consumer vaping one e-cigarette might have a completely different risk than another consumer vaping another e-cigarette. First, there are approximately 500 different brands and 8000 different flavours \((242)\). Second, the risk seems to depend not only on the brand and batch of ecigarette or efluid, but also on the flavour, the heating of the e-cigarette, how dirty or worn the ecigarette is, the vaper, the vaporizer, and factors still unknown. As an example, a study found that two apparently identical vaporizers made by the same manufacturer and filled with the
same e-liquid yielded formaldehyde concentrations in vapour that differed by a factor of > 25 (22). Therefore, it is not meaningful to speak of risk of e-cigarettes as risk of one product. Box 8 summarizes some higher risks that have been identified in studies.

### Box 8. Higher risk as identified in studies

- Some brands
- Some flavours
- High voltage devices
- Second half of a vaping period
- Overheating
- “Dripping”
- “Dry puff” conditions
- The state of the heating element
- The vaporizer
- Vehicle/carrier: ethylene glycol, propylene glycol

#### 4.2.2 Dual use

Replacing a very harmful product with a less harmful product is the logic idea behind the “harm reduction strategy”.

The rationale for “harm reduction”

However, as the large majority of e-cigarette users, almost 80% (243–247), do not quit smoking when they switch to e-cigarettes, but instead continue with dual use, reductions in harm can hardly be expected.

The reality
Those who have not reduced their tobacco intake but supplement with e-cigarettes will have an increased risk of harm. But even those who substantially reduce their consumption will probably not have a (substantial) health benefit. Evidence from large cohorts shows that even a halving of daily intake of number of cigarettes or more does not reduce all-cause mortality, incidence of cardiovascular disease or smoking-related cancer/cancer mortality (248–253), but reductions in lung cancer risk have been found in two studies (252, 254).

Substantial reductions in number of conventional cigarettes are not reported in dual users. One study reported that there was no change in conventional cigarette consumption after one year (255), 86% did not cut back substantially in another study (256), yet another study concluded that e-cigarette use is not linked with lower smoking quantity (257), and a 12-month cohort study of more than 200 dual users found a reduction of only approximately five conventional cigarettes per day (156). A study found that compared to single-product users, dual users puffed and smoked more, were more likely to smoke a conventional cigarette when they first woke up, and used products with higher nicotine levels compared to exclusive e-cigarette users. Taken together, these findings suggest that dual users are more addicted to nicotine (245).

We have extremely little evidence on health effects of combined vaping and smoking. Some positive health effects have been described: a retrospective study describing pulmonary changes in eight dual users who had substantially reduced their tobacco consumption to a mean of less than four conventional cigarettes per day showed significant improvement in lung function after 12 months (117). An observational study found that after four weeks of dual use (n=17) there was a reduction in conventional cigarette intake followed by a reduction in carbon monoxide, cotinine, creatinine and a main metabolite of acrolein (potentially carcinogenic) (136), but dual users had 3 times higher levels of the metabolite of acrolein than quitters.

On the other hand, there are findings indicating harm. The largest study (n > 45 000) is a population-based survey performed in randomly selected schools in China, with a 95% participation rate, so it is representative for a general population of adolescents. Those with dual use reported slightly more respiratory symptoms than smokers who were not using e-cigarettes. Analyses were adjusted for potential confounders, there were few cases and the difference was not significant (146). A 12-month cohort study of more than 200 dual users found no significant improvement in health (156). A case report describes a possible case of reversible cerebral vasoconstriction syndrome in a young healthy dual user who switched from 60 conventional cigarettes per day to use of 20 conventional cigarettes per day combined with e-cigarette use (169). A prospective study found that those who switched to e-cigarettes and completely quit smoking reported only health improvements, whereas the dual use group reported both positive and negative symptoms (160). Long-term follow-up studies in non-selected populations are urgently needed. An eventual interaction (“cocktail effect”) between smoking and vaping would be a worst-case scenario.
4.3 Other general risk considerations

Most studies have compared e-cigarettes with conventional cigarettes and it can be questioned whether this reference is the correct to use:

A conventional cigarette is the most harmful legal product that exists and everything will seem “harmless” compared with it. Also, by searching for harmful ingredients found in conventional cigarettes we may neglect or overlook other ingredients of potential harm (e.g. glycols, flavours, metals, rubber, silicone, ceramics and yet unknown ingredients), as the e-cigarette is a radically different product. Are we comparing apples with pears?

Many of the harmful substances detected were identified at very low concentrations but we are dealing with intense and chronic exposure. Values below the threshold limit do not necessarily protect against the health effect of (for example) 300 daily inhalations (24) over decades – harm might accumulate over years and decades, as with conventional cigarettes. Further, the presence of, for example, 10 substances below the official threshold limit values may add up in a synergic way, and the safety of the combination of substances (“cocktail effect”) has not been evaluated. Also, long-term inhalation of a warm aerosol may increase the risk of tuberculosis, as observed in smoking (258).
5. Conclusions

1. Even though no firm conclusions can be drawn on the safety of e-cigarettes there is an increasing body of evidence indicating harm.

2. Due to the many methodological problems, the many studies with severe conflicts of interest, the inconsistencies and contradictions in results, the relatively few high-quality studies, the rapidly changing designs of the product and the lack of long-term follow-up, it seems very premature to perform calculations for how harmful vaping is compared with smoking, and much is still left to subjective interpretation.

3. It is not meaningful to speak of risk of vaping of e-cigarettes as risk of one product, as the risk seems to depend not only on the brand and batch, but also on, for example, the preferred flavour, the heating of the e-cigarette, the vaporizer, how dirty or worn the e-cigarette is, the method of vaping, and factors still unknown.

4. In a simple product-to-product comparison most e-cigarettes are probably less, and some products may even be much less, harmful than conventional cigarettes, but as the large majority of e-cigarette users continue to smoke, the health risks of dual use must be taken into account in assessment of the harm of vaping.

5. We have almost no evidence on the health effects of dual use of e-cigarettes and conventional cigarettes.

6. For ex-smokers and never smokers, use of e-cigarettes will increase the risk of harm on health.

7. Negative health effects should be expected from the pulmonary system but adverse effects from (for example) the cardiovascular system and a carcinogenic effect cannot be ruled out either.

8. E-cigarettes are highly addictive and there is insufficient evidence on the safety of long-term use of nicotine.
9. Comparing risk of vaping with the risk of (for example) drinking coffee is misleading.

10. Systematic high-quality research is urgently needed, especially on health effects of dual use.

Box 9 summarizes some of the findings causing concern.

<table>
<thead>
<tr>
<th>Box 9. Some of the findings causing concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Findings causing concern include the following:</td>
</tr>
<tr>
<td>• substantial levels of nanoscale particles</td>
</tr>
<tr>
<td>• detectable levels of many different toxic materials</td>
</tr>
<tr>
<td>• recent large sample toxicity assessment: none of the tested products were totally free of potentially toxic compounds and some liquids showed particularly high ranges of chemicals</td>
</tr>
<tr>
<td>• presence of diacetyl (causing “popcorn lung”) found in most flavoured samples</td>
</tr>
<tr>
<td>• cytotoxicity, oxidative stress and inflammation found in most in vitro studies</td>
</tr>
<tr>
<td>• dysregulation of gene expression</td>
</tr>
<tr>
<td>• DNA strand breakage</td>
</tr>
<tr>
<td>• urinary toxicant and carcinogen metabolites found in vapers</td>
</tr>
<tr>
<td>• toxicants found in exhaled vapour</td>
</tr>
<tr>
<td>• airway obstruction in human experimental studies</td>
</tr>
<tr>
<td>• airway inflammation, asthma and chronic obstructive pulmonary disease development in animal studies</td>
</tr>
<tr>
<td>• impaired pulmonary antimicrobial defences in animal study</td>
</tr>
<tr>
<td>• interaction with radon</td>
</tr>
</tbody>
</table>
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for Drug Evaluation and Research, Department of Health and Human Services, Food and Drug Administration; 2009.


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# Overview of studies investigating the effect of electronic cigarettes and health

Table 1. Studies investigating the content of fluid or vapor of electronic cigarettes and in-vitro experiments where cells were exposed to fluid/vapor/vapor extract (n=105*).

For details in methodology and results please see appendix 2.

<table>
<thead>
<tr>
<th>Name of first author</th>
<th>Reference Year</th>
<th>Conflict of interest</th>
<th>Reference product</th>
<th>Fluid/ Vapor/ other</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen JG [2]</td>
<td>2015</td>
<td>No</td>
<td>No</td>
<td>◦Vapor</td>
<td>-Findings confirm the presence of diacetyl (causing bronchiolitis obliterans/“pop-corn lungs”) and other high priority flavoring chemicals in flavored compounds in EC</td>
</tr>
<tr>
<td>Aug A [3]</td>
<td>2014</td>
<td>No</td>
<td>CC</td>
<td>◦Fluid</td>
<td>-EC have immediate and profound adverse effects on the metabolomic state of primary human bronchial epithelial cells similar to those seen with CS condensate</td>
</tr>
<tr>
<td>Bahl V [4]</td>
<td>2012</td>
<td>No</td>
<td>No</td>
<td>◦Fluid</td>
<td>-Approx. one third of samples were highly cytotoxic to human embryonic stem cells and mouse neural stem cells</td>
</tr>
<tr>
<td>Behar RZ [8]</td>
<td>2014</td>
<td>No</td>
<td>No</td>
<td>◦Fluid</td>
<td>-Cinnamon flavorings in refill fluids are linked to cytotoxicity</td>
</tr>
<tr>
<td>Bertholon JF [9]</td>
<td>2013</td>
<td>No</td>
<td>CC and water pipe</td>
<td>◦Vapor</td>
<td>-Contrary to CC smoke, which has a half-life in air of 19 to 20 minutes, the half-life of EC is very short and risk of passive “smoking” exposure from EC is modest</td>
</tr>
<tr>
<td>Brot L [10]</td>
<td>2015</td>
<td>No</td>
<td>CC extract and PPG</td>
<td>◦Vapor extract</td>
<td>-Results suggest that the intestinal epithelium inflammatory response is not altered by exposure to vapor from EC</td>
</tr>
<tr>
<td>Bush D [13]</td>
<td>2014</td>
<td>▲25</td>
<td>CC and no use</td>
<td>◦Nicotine on surface</td>
<td>-Using EC indoors leads to significantly less third-hand exposure to nicotine compared to smoking</td>
</tr>
<tr>
<td>Cameron JM [14]</td>
<td>2013</td>
<td>No</td>
<td>No</td>
<td>◦Fluid</td>
<td>-Large variability in nicotine concentrations was found</td>
</tr>
</tbody>
</table>

1 Results of studies influenced by the tobacco industry are marked with an asterisk (*) in the paper.
2 Studies funded by e cigarette manufacturers or performed in collaboration with the e cigarette industry are labelled with a chevron (▲) in the paper.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Type</th>
<th>Fluid &amp; Vapor</th>
<th>Findings &amp; Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervellati F [18] 2014</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>Exposure to EC vapors is far less toxic than exposure to CC smoke</td>
</tr>
<tr>
<td>Chausse P [19] 2015</td>
<td>No</td>
<td>No</td>
<td>Heating of EC</td>
<td>It is possible for a 3.3 V EC to obtain the power of a 5 V EC, with risk of dissemination of formaldehyde</td>
</tr>
<tr>
<td>Cheah NP [20] 2012</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td>Contained nicotine even though they claimed to be nicotine free. Significant difference in the nicotine content across EC with same label, brand-to-brand and cartridge-to-cartridge variations. Polycyclic aromatic hydrocarbons and TSNAs compounds were not found</td>
</tr>
<tr>
<td>Chen L [22] 2015</td>
<td>No</td>
<td>CC smoke extract</td>
<td>Vapor extract</td>
<td>Preliminary evidence that e-vapor exposure may alter platelet functions associated with cardiovascular disease progression</td>
</tr>
<tr>
<td>Colard S [24] 2015</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>The exposure of bystanders to nicotine in the exhaled aerosol is not at levels that would be expected to cause health concerns</td>
</tr>
<tr>
<td>Costigan S [27] 2015</td>
<td>No</td>
<td>No</td>
<td>Risk assessment</td>
<td>Presents an approach to risk assessment of in-going flavoring ingredients in e-liquid and potential thermal breakdown and reaction products in the aerosol</td>
</tr>
<tr>
<td>Costigan S [26] 2014</td>
<td>No</td>
<td>No</td>
<td>Risk assessment</td>
<td>Presents a contact sensitization and risk assessment model</td>
</tr>
<tr>
<td>Cox C [28] 2015</td>
<td>No</td>
<td>No</td>
<td>Vapor</td>
<td>The majority of EC produce very high levels of acetaldehyde and formaldehyde. High levels of these cancer-causing chemicals are produced even by some EC without nicotine</td>
</tr>
<tr>
<td>Czogala J [30] 2014</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>Using EC in indoor environments may involuntarily expose non-users to nicotine but not to toxic tobacco-specific combustion products</td>
</tr>
<tr>
<td>Davis B [31] 2015</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td>Nicotine concentration labeling on electronic cigarette refill products was often inaccurate but showed improvement recently in products from one company</td>
</tr>
<tr>
<td>El-Hellani A [38] 2015</td>
<td>No</td>
<td>No</td>
<td>Fluid and vapor</td>
<td>Nicotine partitioning varies considerably across commercial EC liquids and these differences can persist when the liquids are vaped.</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Study Type</td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Etter JF [41]</td>
<td>2013</td>
<td>Fluid</td>
<td>Half of the liquids analyzed contained up to five times the maximum amount of impurities specified in the European Pharmacopoeia.</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [47]</td>
<td>2015</td>
<td>Vapor</td>
<td>Diacetyl and acetyl propionyl chemicals associated with respiratory disease when inhaled were found in a large proportion of sweet-flavored EC liquids, many of them exposing users to higher than safety levels.</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [46]</td>
<td>2015</td>
<td>Fluid</td>
<td>Natural Extract of Tobacco liquids contained higher levels of phenols and nitrates, but lower levels of acetaldehyde compared to conventional EC liquids. All EC liquids contained far lower levels of the tobacco-derived toxins compared to CC.</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [52]</td>
<td>2015</td>
<td>Vapor</td>
<td>Levels of daily exposure from EC use are significantly lower compared to acceptable exposure from inhalational medications and by orders of magnitude lower than the regulatory limits for daily occupational exposure.</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [45]</td>
<td>2015</td>
<td>Fluid and vapor</td>
<td>Minimal levels of tobacco specific nitrosamines were found in the liquid samples.</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [49]</td>
<td>2015</td>
<td>Vapor</td>
<td>Study indicates that some EC samples have cytotoxic properties on cultured cardiomyoblasts, but sign less compared to CC. For EC extracts produced by high-voltage and energy, viability was reduced.</td>
<td></td>
</tr>
<tr>
<td>Feng Y [54]</td>
<td>2015</td>
<td>Vapor</td>
<td>The results indicate that EC-droplets, being more hygroscopic than CC smoke particles, tend to grow larger in maximum size in a typically highly humid environment.</td>
<td></td>
</tr>
<tr>
<td>Fernández E [55]</td>
<td>2015</td>
<td>Vapor</td>
<td>ECs used under real-life conditions emit toxicants, including PM$_{2.5}$, although these are notably lower than those from CC.</td>
<td></td>
</tr>
<tr>
<td>Fouco FC [59]</td>
<td>2013</td>
<td>Vapor</td>
<td>Particle number distribution modes of the EC-generated vapor were similar to the CC. ECs were found to be a major particle source, which can lead to significantly high deposition in vapers.</td>
<td></td>
</tr>
<tr>
<td>Geiss O [60]</td>
<td>2014</td>
<td>Vapor</td>
<td>Relatively high concentrations of PPG and glycerol could be quantified in the air of the chamber tests.</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Outcome</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>---------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Goniewicz ML [65] 2013</td>
<td>▲ 5</td>
<td>CC</td>
<td>Fluid and vapor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>There is very little risk of nicotine toxicity from major EC brands in the United Kingdom.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nicotine concentration in e-liquid is not well related to nicotine in vapor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>None of the tested products reached nicotine concentrations as high as CC</td>
<td></td>
</tr>
<tr>
<td>Goniewicz ML [66] 2013</td>
<td>▲ 3</td>
<td>Medicinal nicotine inhalator, CC</td>
<td>Vapor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toxic compounds: metals, carbonyls and volatile organic compounds were found in almost all EC, but much lower levels than in CC smoke</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vapor of some EC contains traces of carcinogenic nitrosamines</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exposure to carcinogenic formaldehyde comparable with CC smoking</td>
<td></td>
</tr>
<tr>
<td>Goniewicz ML [67] 2013</td>
<td>▲ 4</td>
<td>No</td>
<td>Vapor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vapor contains nicotine, but EC brands and models differ in their efficacy and consistency of nicotine vaporization</td>
<td></td>
</tr>
<tr>
<td>Goniewicz ML [68] 2015</td>
<td>▲ 17</td>
<td>No</td>
<td>Fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Most of the analysed samples had no significant discrepancies in labelled nicotine concentrations and contained low nicotine levels. Some products labelled as ‘nicotine-free’ had detectable levels of nicotine</td>
<td></td>
</tr>
<tr>
<td>Goniewicz ML [64] 2015</td>
<td>▲ 21</td>
<td>No</td>
<td>Vapor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study indicates that there is a risk for third-hand exposure to nicotine from EC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Third-hand exposure levels differ depending on the surface and EC brand</td>
<td></td>
</tr>
<tr>
<td>Hadwiger ME [69] 2010</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Presence of unapproved active pharmaceutical ingredients</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nicotine-free products contained nicotine</td>
<td></td>
</tr>
<tr>
<td>Hahn H [70] 2014</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>From all compounds tested, only nicotine reached exposures that fall into a high risk category</td>
<td></td>
</tr>
<tr>
<td>Han S [71] 2015</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Compounds that may originate from tobacco, solvents or other sources, such as TSNAs, solanesol, VOCs, PAHs, phenolic compounds, and carbonyl compounds were all found with different levels and detection frequencies</td>
<td></td>
</tr>
<tr>
<td>Herrington JS [74] 2015</td>
<td>No</td>
<td>No</td>
<td>Fluid and aerosol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Formaldehyde, acetaldehyde, acrolein, and siloxanes were found in the aerosol profiles; however, these compounds were never present in the solutions</td>
<td></td>
</tr>
<tr>
<td>Higham [75] AJ 2014</td>
<td>No</td>
<td>No</td>
<td>Vapor extract</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In vitro study shows that EC exposure causes an inflammatory response from neutrophils and macrophages</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The effects are similar to those caused by CC</td>
<td></td>
</tr>
</tbody>
</table>
# A systematic review of health effects of electronic cigarettes

This report was prepared at the request of WHO Prevention of Noncommunicable Diseases. The findings, interpretations, and conclusions expressed in this work do not necessarily reflect the views of WHO.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Ingestion Form(s)</th>
<th>Inhalation Form(s)</th>
<th>Other Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Husari A [78]</td>
<td>2015</td>
<td>No</td>
<td>Fluid and vapor</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Hutzler C [79]</td>
<td>2014</td>
<td>No</td>
<td>Fluid and vapor</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Ingebrethsen BJ [80]</td>
<td>2012</td>
<td>No</td>
<td>Vapor</td>
<td>Particle diameters and particle number conc. as in CC smoke</td>
</tr>
<tr>
<td>Jensen RP [81]</td>
<td>2015</td>
<td>No</td>
<td>Fluid and vapor</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kavalkakis MP [82]</td>
<td>2015</td>
<td>No</td>
<td>Fluid</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kienhus AS [83]</td>
<td>2015</td>
<td>No</td>
<td>Fluid and vapor</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kim H-J [84]</td>
<td>2015</td>
<td>No</td>
<td>Fluid</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kim S [85]</td>
<td>2015</td>
<td>No</td>
<td>Fluid</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kim YH [86]</td>
<td>2015</td>
<td>No</td>
<td>Fluid</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kirschner R [87]</td>
<td>2015</td>
<td>No</td>
<td>Fluid, aerosol, vapor</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
<tr>
<td>Kosmider L [88]</td>
<td>2014</td>
<td>No</td>
<td>Glycerin, PPG, mixture of both</td>
<td>CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations.</td>
</tr>
</tbody>
</table>
### A systematic review of health effects of electronic cigarettes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kubica P [89] 2014</td>
<td>No No</td>
<td>Fluid</td>
</tr>
<tr>
<td>Laugesen M [91] (2 versions) 2009</td>
<td>CC</td>
<td>Fluid and vapor</td>
</tr>
<tr>
<td>Laugesen M 2008</td>
<td>CC</td>
<td>Fluid</td>
</tr>
<tr>
<td>Laugesen M [90] 2008</td>
<td>CC</td>
<td>Fluid and vapor</td>
</tr>
<tr>
<td>Laugesen M [92] 2015</td>
<td>CC</td>
<td>Vapor</td>
</tr>
<tr>
<td>Lauterbach JH [94] 2012</td>
<td>CC</td>
<td>Vapor</td>
</tr>
<tr>
<td>Lauterbach JH [95] 2012</td>
<td>CC</td>
<td>Vapor</td>
</tr>
<tr>
<td>Lerner CA [98] 2015</td>
<td>No No</td>
<td>Liquid and vapor</td>
</tr>
<tr>
<td>Lerner CA [97] 2015</td>
<td>No CC</td>
<td>Vapor</td>
</tr>
<tr>
<td>Lisko JG [100] 2015</td>
<td>No No</td>
<td>Fluid</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Type</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Long GA [101] 2014</td>
<td>◆ ▲ 23</td>
<td>CC</td>
</tr>
<tr>
<td>Maloney JC [102] 2015</td>
<td>◆ ▲ 37</td>
<td>No</td>
</tr>
<tr>
<td>Manigrasso M [104] 2015</td>
<td>No</td>
<td>CC</td>
</tr>
<tr>
<td>Manigrasso M [103] 2015</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Marco E [106] 2015</td>
<td>No</td>
<td>CC</td>
</tr>
<tr>
<td>Martinez RE [109] 2015</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>McAuley TR [110] 2012</td>
<td>▲ 11</td>
<td>CC</td>
</tr>
<tr>
<td>Misra M [115] 2014</td>
<td>◆ ▲ 19</td>
<td>Medicinal nicotine product</td>
</tr>
<tr>
<td>Neilson L [118] 2015</td>
<td>◆ ▲ 22</td>
<td>CC</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>CC/VE</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>O’Connell G [120]</td>
<td>2015</td>
<td>No</td>
</tr>
<tr>
<td>Palpant NJ [122]</td>
<td>2015</td>
<td>CC</td>
</tr>
<tr>
<td>Papousek R [124]</td>
<td>2014</td>
<td>CC (cigar)</td>
</tr>
<tr>
<td>Park S [125]</td>
<td>2014</td>
<td>CC</td>
</tr>
<tr>
<td>Pellegrino RM [126]</td>
<td>2012</td>
<td>CC</td>
</tr>
<tr>
<td>Romagna G[132]</td>
<td>2013</td>
<td>CC</td>
</tr>
<tr>
<td>Romagna G [133]</td>
<td>2012</td>
<td>CC</td>
</tr>
<tr>
<td>Ruprecht AA [135]</td>
<td>2014</td>
<td>CC</td>
</tr>
<tr>
<td>Saffari [136]</td>
<td>2014</td>
<td>CC</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Methodology</td>
</tr>
<tr>
<td>---------------------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Samways B [138]</td>
<td>2014</td>
<td>No</td>
</tr>
<tr>
<td>Sancilio S [139]</td>
<td>2015</td>
<td>No</td>
</tr>
<tr>
<td>Shivalingappa P</td>
<td>2015</td>
<td>Room-air controls</td>
</tr>
<tr>
<td>Scheffler S [140]</td>
<td>2015</td>
<td>CC</td>
</tr>
<tr>
<td>Schober W [141]</td>
<td>2014</td>
<td>No vaping</td>
</tr>
<tr>
<td>Schripp T [142]</td>
<td>2013</td>
<td>CC</td>
</tr>
<tr>
<td>Schweitzer KS [143]</td>
<td>2015</td>
<td>CC</td>
</tr>
<tr>
<td>Stepanov I [146]</td>
<td>2014</td>
<td>No</td>
</tr>
</tbody>
</table>
| Talih S [148]       | 2015 | No          | Vapor         | Direct dripping of e-liquids may involve greater exposure to volatile aldehyde due to the potentially higher temperatures; may expose users to increased volatile aldehyde levels relative to conventional EC and even relative to CC, for a given nicotine yield.
<table>
<thead>
<tr>
<th>Study</th>
<th>Status</th>
<th>Country</th>
<th>Study Type</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talio MC [149] 2015</td>
<td>No</td>
<td>Fluid</td>
<td></td>
<td>In all studied samples, lead contents in EC liquids were in the same order as in CC.</td>
</tr>
<tr>
<td>Tayyarah R [150] 2015</td>
<td>▲ 20</td>
<td>CC</td>
<td>Vapor</td>
<td>The deliveries of harmful and potentially harmful constituents tested for EC products were similar to the study air blanks rather than to deliveries from CC smoke.</td>
</tr>
<tr>
<td>Theophilus E [151] 2014</td>
<td>▲ 30</td>
<td>CC</td>
<td>Vapor</td>
<td>EC (Brand: VUSE) aerosol was not cytotoxic whereas CC smoke was cytotoxic.</td>
</tr>
<tr>
<td>Tierney PA [153] 2015</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td>The concentrations of some flavor chemicals EC fluids are sufficiently high for inhalation exposure by vaping to be of toxicological concern. Almost half of the tested products on the US market were more than 1% by weight flavors chemicals.</td>
</tr>
<tr>
<td>Trehy ML [154] 2011</td>
<td>No</td>
<td>CC</td>
<td>Fluid</td>
<td>Some products were found to contain high conc. of nicotine when labeled not to contain nicotine. The actual amount of nicotine delivered is likely to be highly variable. Transfer of rimonabant and aminotadalafil to the vapor phase is low. Impurity level is lower than for CC.</td>
</tr>
<tr>
<td>Uchiyama S [156] 2013</td>
<td>No</td>
<td>No</td>
<td>Vapor</td>
<td>EC generate incidentally carbonyls. In some cases they are generated with extremely high concentrations.</td>
</tr>
<tr>
<td>Uryupin AB [157] 2013</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td>The main components of mixtures were non-tobacco products.</td>
</tr>
<tr>
<td>Vargas Trassiera C [164] 2015</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>The increase in the attached Potential Alpha Energy Concentration was higher for the EC than for traditional CC. The aerosol from EC operates as a carrier of the radon progeny and, as a consequence it decreases the &quot;plate out&quot; of the radon daughter.</td>
</tr>
<tr>
<td>Varlet V [165] 2015</td>
<td>▲ 31</td>
<td>No</td>
<td>Fluid</td>
<td>None of the products under scrutiny were totally exempt of potentially toxic compounds. A minority of liquids, especially those with flavorings, showed particularly high ranges of chemicals.</td>
</tr>
<tr>
<td>Study</td>
<td>Supports</td>
<td>Study Type</td>
<td>Study Points</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Visser W [166] 2015</td>
<td>No</td>
<td>CC</td>
<td>Fluid and vapor</td>
<td>The toxic substance-related health risks associated with the use of CC are far greater than those associated with EC, nevertheless, daily use of e-cigarettes is not without health risks. E.g., the concentration of formaldehyde can be up to 3 times higher in EC vapor than in tobacco smoke.</td>
</tr>
<tr>
<td>Westenberger B [168] (FDA) 2009</td>
<td>No</td>
<td>Medicinal nicotine inhalator</td>
<td>Fluid</td>
<td>Diethylene glycol in one cartridge. Detectable levels of carcinogens and toxic chemicals.</td>
</tr>
<tr>
<td>Willershausen I [169] 2014</td>
<td>No</td>
<td>Phosphate-buffered saline</td>
<td>Fluid</td>
<td>This in vitro study demonstrated that menthol additives of EC have a harmful effect on human periodontal ligament fibroblasts. The menthol-flavored liquid caused a highly significant reduction of cell migration.</td>
</tr>
<tr>
<td>Williams M [170] 2013</td>
<td>No</td>
<td>CC</td>
<td>Fluid and vapor</td>
<td>Harmful or potentially harmful elements detected. Aerosol: significant amounts of tin and other metals, silicate beads, and nanoparticles, mostly higher than or equal to corresponding conc's in CC smoke. Fluid with tin particles was cytotoxic.</td>
</tr>
<tr>
<td>Wu Q [171] 2014</td>
<td>No</td>
<td>No</td>
<td>Fluid</td>
<td>Findings strongly suggest the deleterious health effects of EC in the airways of young people. Promotes proinflammatory cytokine IL-6 production and Human rhinovirus infection in primary human airway epithelial cells.</td>
</tr>
<tr>
<td>Yu V [173] 2015</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>At biologically relevant doses, vaporized EC liquids induce increased DNA strand breaks and cell death, and decreased clonogenic survival in both normal epithelial and head and neck squamous cell carcinoma cell lines independently of nicotine content.</td>
</tr>
<tr>
<td>Zervas E [174] 2014</td>
<td>No</td>
<td>Ambient air</td>
<td>Vapor</td>
<td>EC liquids generate nano-particles; 300-3000 more than ambient air.</td>
</tr>
<tr>
<td>Zhang Y [175] 2013</td>
<td>No</td>
<td>CC</td>
<td>Vapor</td>
<td>CC produce more particles initially, but particle counts converge to a similar scale as the aerosols condense. EC and CC produce aerosols having generally similar particle sizes.</td>
</tr>
</tbody>
</table>
Four of these studies are also/partly mentioned in Table 3/Appendix 5 on animal experimental studies [98] [122] [143] [78].

Three studies [101, 106, 133] could as well have been described in Table 2/Appendix 4, human experimental studies.

CC = conventional cigarette
EC = electronic cigarette
FDA = US Food and Drug Administration
PPG = propylene glycol

Conflicts of interest – Conflicts of interest of each study should be assessed individually.

▲ 1: MLG received research funding from manufacturer of medicinal products for smoking cessation. AS received research funds and travel expenses from manufacturer of ECs.

▲ 2: JFE: reimbursed by manufacturer of e-liquids for travels. EZ and SS: employed by manufacturer of medicinal products for smoking cessation.

▲ 3: MLG: research funding from manufacturer of medicinal products for smoking cessation. NB: consultant for manufacturers of medicinal products for smoking cessation.

▲ 4: MLG: research funding from manufacturer of medicinal products for smoking cessation.

▲ 5: all received research funding and/or performed provided consultancy for manufacturer of medicinal products for smoking cessation.

❖ ▲ 6: Study funded by tobacco company. Two of three authors affiliate to this tobacco company.

▲ 7: MLG received research funding from manufacturer of medicinal products for smoking cessation. AS received research funds and travel expenses from manufacturer of ECs.

◆ ▲ 8: Manufacturers of both EC and CC funded the study. ML is cited as one of 5 most influential persons in the EC industry, http://ecigarettereviewed.com/top-5-most-influential-people-in-the-electronic-cigarette-industry/

❖ ▲ 9: Research contract with manufacturer of EC. See also CI #8

◆ ▲ 10: No conflict stated, but JHL affiliates to Lauterbach & Associates - a consulting firm that specializes in providing contract scientific affairs and regulatory support to the tobacco industry. Also see CI#8 for ML.

▲ 11: Study sponsored by National Vapers Club and EC vendors. Subsequent to data-collection SB became part owner of EC company.

❖ ▲ 12: Study funded by EC company.

▲ 13: study funded by crowd funding in vaper community. A volunteer vaper is acknowledged for assistance with fund raising. Some of the studies by KF and VV were performed using funds provided to the institution by EC companies.

◆ ▲ 14: A small number of KF’s and VV’s studies on electronic cigarettes were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. Enthalpy Analytical is a for-profit CRO and provides testing for the EC industry but did not receive any compensation for this study. MM was working at Enthalpy Analytical at the time of the study but is currently employed by a tobacco company.

▲ 15: The authors declare no conflict of interest. A small minority of the studies by KF and VV were
performed using unrestricted funds provided to Onassis Cardiac Surgery Center by EC companies.

16: Some of the studies by K.F. and V.V. were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. EC manufacturer is thanked for free equipment.

17: MLG reports a grant from a manufacturer of smoking cessation drugs, outside the submitted work; AS reports personal fees from eSmoking Institute, Poland, and nonfinancial support from a manufacturer of EC.

18: Agencies which sold some of the tested EC contributed to expenses of testing.

19: Authors are employees of tobacco company which also manufactures EC.

20: Authors are employees of tobacco company which also manufactures EC.

21: MLG received a research grant from a manufacturer of smoking cessation medications.

22: Authors are employees of tobacco company which also manufactures EC.

23: Authors are employees of tobacco company which also manufactures EC.

24: Authors are employees of tobacco company which also manufactures EC.

25: MLG received a research grant from manufacturer of smoking cessation medication, outside scope of this work.

26: All authors are employees of tobacco company. The work in this paper was supported by tobacco company.

27: Some of the studies by KEF and VV were performed using funds provided to the institution by EC companies.

28: Partly sponsored by Altria group which is parent company for tobacco company.

29: Some of the studies by KEF and VV were performed using funds provided to the institution by EC companies. This study was funded in part by the Greek Association of E-cigarette Businesses (SEEHT) - the sponsor funded the expenses of the laboratory. The study was investigator-initiated and investigator-driven.

30: Authors are employees of tobacco company which also manufactures EC.

31: JFE was reimbursed by a manufacturer of e-liquids for traveling to London and to China, but he received no honoraria for these meetings aimed at mutual information. Some of the other studies performed by KF used unrestricted funds provided to research center by e-cigarette companies.

32: Authors are employees of tobacco company which also manufactures EC.

33: Nothing is stated but previous study by RG was funded by EC company. Some of the studies by KEF were performed using funds provided to the institution by EC companies.

34: None stated. Previous study was founded by manufacturers of both EC and CC. ML is cited as one of 5 most influential persons in the EC industry.

35: Study was joint funded by a manufacturer of non-tobacco products (a company set up in 2010 by tobacco company which also manufactures EC) and by tobacco company which also manufactures EC, and the authors are full time employees.

36: Study was joint funded by a manufacturer of non-tobacco products (a company set up in 2010 by tobacco company which also manufactures EC).

37: Authors are employees of tobacco company which also manufactures EC.
### Table 2. Human experimental studies reporting health effects (n=32).

For details in methodology and results please see appendix 3.

<table>
<thead>
<tr>
<th>Name of first author</th>
<th>Conflict of interest</th>
<th>Reference product</th>
<th>Method Exposure</th>
<th>Numbers of participants</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballbé M [5] 2014</td>
<td>No</td>
<td>CC or room air</td>
<td>-Observational study with non-smokers</td>
<td>54 non-smoker volunteers from homes with smokers, EC users, control homes</td>
<td>-Non-smokers passively exposed to EC vapor absorb approx. as much nicotine as when exposed to smoke from CC</td>
</tr>
<tr>
<td>Battista L[7] 2013</td>
<td>No</td>
<td>CC</td>
<td>-Experimental study</td>
<td>12 regular users of EC</td>
<td>-EC inhalation produces the same patho-physiological cardiovascular effects of CC smoking</td>
</tr>
<tr>
<td>Chorti M [23] 2012</td>
<td>No</td>
<td>CC</td>
<td>-Volunteers in CC group smoked 2 CC</td>
<td>15 EC naive heavy-smokers</td>
<td>-Passive but not active EC vaping resulted in short-term lung obstruction and increased cotinine</td>
</tr>
<tr>
<td>Colbyl H [25] 2015</td>
<td>No</td>
<td>0 mg nicotine EC</td>
<td>-Experimental study</td>
<td>13 subjects (not described)</td>
<td>-Study suggests that nicotine, when acutely inhaled via EC does not impair the cerebral pressure-flow relationship</td>
</tr>
<tr>
<td>Czogala J [29] 2012</td>
<td>No</td>
<td>CC</td>
<td>-A repeated measures design</td>
<td>42 EC naive daily smokers</td>
<td>-Slight non-sign elevation in diastolic blood pressure, pulse and carboxyhemoglobin</td>
</tr>
<tr>
<td>Dawkins L [33] 2013</td>
<td>▲ ▲</td>
<td>0 mg nicotine EC</td>
<td>-Within-subjects design</td>
<td>20 EC naive smokers</td>
<td>-EC can effectively deliver nicotine to impact on cognitive performance; improved time-based memory</td>
</tr>
</tbody>
</table>

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### A systematic review of health effects of electronic cigarettes

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<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Nicotine</th>
<th>Exposure</th>
<th>Design</th>
<th>Main Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawkins L [34]</td>
<td>2012</td>
<td>0 mg</td>
<td>CC</td>
<td>No</td>
<td>Improved nicotine withdrawal impaired memory</td>
<td></td>
</tr>
<tr>
<td>Dispenegast P [36]</td>
<td>2015</td>
<td>0 mg</td>
<td>CC</td>
<td>No</td>
<td>*6 EC naive smokers</td>
<td></td>
</tr>
<tr>
<td>Eisenberg T [37]</td>
<td>2010</td>
<td>No</td>
<td>CC</td>
<td>No</td>
<td>*30 healthy non-smokers</td>
<td></td>
</tr>
<tr>
<td>et al. [40]</td>
<td>2005</td>
<td>No</td>
<td>CC</td>
<td>No</td>
<td>Saliva sampling in current vapers</td>
<td></td>
</tr>
<tr>
<td>Farsalinos KE [43]</td>
<td>2012</td>
<td>▲5 CC</td>
<td>CC</td>
<td>▲5</td>
<td>Hemodynamic measurements + echocardiogram at baseline and after smoking/vaping</td>
<td></td>
</tr>
<tr>
<td>Ferrari M [55]</td>
<td>2014</td>
<td>No</td>
<td>CC</td>
<td>No</td>
<td>Saliva sampling in current vapers</td>
<td></td>
</tr>
<tr>
<td>Flouris AD [57]</td>
<td>2013</td>
<td>No</td>
<td>CC</td>
<td>No</td>
<td>Repeated measures controlled study</td>
<td></td>
</tr>
</tbody>
</table>

- **NICOTINE LEVELS**: 0 mg
- **EXPOSURE**: CC
- **DESIGN**: Randomized cross-over design
- **Main Findings**: Slight elevation in diastolic blood pressure but no effect on cardiac function in experienced EC users

**Note**: The table provides a summary of the findings from various studies, highlighting the effects of electronic cigarettes on different physiological measures. Further details and specific findings are elaborated in the respective references.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Exposure</th>
<th>Participants</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flouris AD [58] 2012</td>
<td>No CC</td>
<td>Three experimental sessions; active and passive exposure. Exposure: 2 CC within 30 min or 'a number of puffs' within 30 min.</td>
<td>15 EC naive smokers and 15 never-smokers</td>
<td>Acute active and passive vaping did not influence complete blood count indices in smokers and never smokers</td>
</tr>
<tr>
<td>Gennimata S [61] 2012</td>
<td>No CC</td>
<td>Exposure: vaping for 10 minutes</td>
<td>8 never smokers and 24 EC naive smokers</td>
<td>Short-term exposure caused immediate airway obstruction</td>
</tr>
<tr>
<td>Hecht SS [73] 2014</td>
<td>No CC</td>
<td>Urine sampling in current vapers who had not smoked CC for at least 2 months</td>
<td>28 current EC vapers</td>
<td>Urinary toxicant and carcinogen metabolites were significantly lower in EC users than in CC smokers. Some EC users had levels of total NNAL higher than when exposed to second hand smoking</td>
</tr>
<tr>
<td>Marini S [108] 2014</td>
<td>No CC</td>
<td>Experimental study. Exposure: 4 puffs</td>
<td>25 smokers</td>
<td>Similar effect on human airways, and same particle dose received with smoking and vaping</td>
</tr>
<tr>
<td>McRobbie H [114] 2015</td>
<td>▲ 9 CC</td>
<td>Experimental study. Exposure: free use of EC as smoking cessation aid, 4 weeks observation</td>
<td>40 adult smokers wanting to stop smoking</td>
<td>After 4 weeks: in dual users, EC use significantly reduced exposure to CO and acrolein because of a reduction in smoke intake</td>
</tr>
<tr>
<td>Palamidas A [121] 2014</td>
<td>No CC</td>
<td>Experimental study. Exposure: Gr.A: vaping in 10 min</td>
<td>70 volunteers (27 with asthma/COPD). Smokers+ never smokers</td>
<td>Increased airway resistance and a concomitant decrease in specific airway conductance</td>
</tr>
<tr>
<td>Papaseit [123] 2014</td>
<td>No CC</td>
<td>Randomized and crossover controlled trial. Exposure: 2 sessions; 10 puffs in 5 min./1 CC</td>
<td>6 EC naive regular CC smokers</td>
<td>EC use produces a moderate increase in vital parameters—increases in heart rate, diastolic and systolic arterial pressure</td>
</tr>
<tr>
<td>Polosa R [128] * 2014</td>
<td>▲ 10 CC</td>
<td>Retrospective review of changes in lung function and asthma control. Exposure: 6 and 12 months follow-up</td>
<td>18 smoking asthmatics who switched to regular EC use</td>
<td>Study indicates that regular use of EC to substitute smoking is associated with objective and subjective improvements in asthma outcomes</td>
</tr>
<tr>
<td>Popa C [131] 2015</td>
<td>No CC</td>
<td>Experimental study. Exposure: 2 sessions of 10 min with vaping or smoking</td>
<td>5 current CC smokers and 5 current EC vapers</td>
<td>Increased oxidative stress after vaping but lower than after smoking</td>
</tr>
</tbody>
</table>
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### Experimental Study: Vaping in 10 min
- **62 volunteers**, non-smokers + smokers: 28 with COPD/asthma
  - **Increased heart rate** and symptoms like cough and sore throat

- **64 volunteers**, non-smokers
  - **Increased heart rate**, palpitations and a decrease in SpO2
  - **Increased in oxygen saturation**, no changes in blood pressure and pulse rate, cough worse/improved
  - **Pleural increased in some but decreased in more**

- **15 smokers switched to EC, 2 drop-outs**
  - **No changes in plasma nicotine and heart rate**
  - **No increase in CO**

### Experimental Study: Switch to EC Vaping in 2 weeks
- **15 smokers switched to EC, 2 drop-outs**
  - **Increase in oxygen saturation**, no changes in blood pressure and pulse rate
  - **Cough worse/improved**
  - **Phlegm increased in some but decreased in more**

### Repeated-measures controlled study
- **32 EC naive heavy smokers**
  - **Increase in heart rate**

### Repeated-measures controlled study
- **20 EC naive heavy smokers**
  - **Increase in heart rate**

### Experimental study: Two exposure scenarios from Day 1 to Day 11: half-hour controlled administration and one hour ad lib use
- **38 EC naive daily smokers included, withdrew: 14, included in analyses: 23**
  - **Significantly increased blood pressure and heart rate after use of several EC products**
  - **EC: less exposure of nicotine and thereby less cardiovascular effects compared to CC smoking**

### Tsikrika S [155]
2014
- **No**
- **No**
- **No**
- **Increased heart rate and symptoms like cough and sore throat**

### Vakali S [158]
2014
- **No**
- **No**
- **No**
- **Increased heart rate, palpitations and a decrease in SpO2**

### Vansickel A [161]
2010
- **No**
- **CC**
- **No**
- **Increase in heart rate**

### Vansickel A [162]
2012
- **No**
- **CC**
- **No**
- **Increase in heart rate**

### Vardavas CI [163]
2012
- **No**
- **EC with cartridge removed**
- **No**
- **Increase in heart rate**

### Yan XS [172]
2015
- **▲ 7 CC**
- **▲ 7 CC**
- **▲ 7 CC**
- **Increase in heart rate**

### van Staden SR [159]
2013
- **▲ 4 No**
- **No**
- **No**
- **No**

### Vardavas CI [163]
2012
- **No**
- **EC with cartridge removed**
- **No**
- **Increase in heart rate**

### Vansickel A [162]
2012
- **No**
- **CC**
- **No**
- **Increase in heart rate**

### Tsikrika S [155]
2014
- **No**
- **No**
- **No**
- **Increased heart rate and symptoms like cough and sore throat**

### Vakali S [158]
2014
- **No**
- **No**
- **No**
- **Increased heart rate, palpitations and a decrease in SpO2**

### Vansickel A [161]
2010
- **No**
- **CC**
- **No**
- **Increase in heart rate**

### Vansickel A [162]
2012
- **No**
- **CC**
- **No**
- **Increase in heart rate**

### Vardavas CI [163]
2012
- **No**
- **EC with cartridge removed**
- **No**
- **Increase in heart rate**

### Yan XS [172]
2015
- **▲ 7 CC**
- **▲ 7 CC**
- **▲ 7 CC**
- **Increase in heart rate**

---

80 A systematic review of health effects of electronic cigarettes
This study could as well have been placed in appendix 3 showing adverse events [128]

EC= electronic cigarette  
CC= conventional cigarette  
total NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and its glucuronides

**Conflicts of interest** - Conflicts of interest of each study should be assessed individually.

- ▲ 1: Study was funded and supported by manufacturer of EC. LD has received funding to speak at research conferences and benefits in kind from EC companies.
- ▲ 2: KD has a collaborative relationship with manufacturer of EC who provided free supplies of the EC for the study
- ▲ 3: KD has a collaborative relationship with manufacturer of EC who provided free supplies of the EC for the study
- ▲ 4: EC manufacturer sponsored the EC used in study
- ▲ 5: Some of the studies by KF and VV were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. Some of the studies by KF were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. Other studies by GR have been sponsored by EC company.
- ▲ 7: employees in tobacco company which also manufactures EC
- ▲ 8: No stated, but some of the studies by KF were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. KF has a website “Ecigarette Research Advocate Group” which represents a strictly positive view on EC and provides several links to vapor clubs.
- ▲ 9: HR is Clinical Director at The Dragon Institute (research-based training, studies on the latest changes in the health industry etc.); reports receiving commercial research grant from manufacturer of smoking cessation medication; and has received speakers’ bureau honoraria from manufacturers of smoking cessation medication. MLG reports receiving commercial research grant from manufacturer of smoking cessation medication. PJ has received speakers’ bureau honoraria from and is a consultant/advisory board member for the manufacturers of stop-smoking medications. No potential conflicts of interest were disclosed by the other authors
- ▲ 10: RP has received grant support, has served as a speaker and has served as a consultant for anti-asthma drug manufacturers and has received payment for developing educational presentations and being a consultant for manufacturer of smoking cessation medication; he has also served as a consultant for EC distributor. JBM has received honoraria for speaking and financial support to attend meetings/advisory boards from anti-asthma drug manufacturers
### Table 3. Animal experimental studies reporting health effects (n=11*)

For details in methodology and results please see appendix 4.

<table>
<thead>
<tr>
<th>Name of first author Reference Year</th>
<th>Conflict of interest ▲ =Yes</th>
<th>Reference product</th>
<th>Animal type and number</th>
<th>Exposure</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraghty P [62] 2014</td>
<td>No</td>
<td>Phosphate-buffered saline; Vehicle</td>
<td>A/J mice. 4 Cohorts of mice (n=8 per group)</td>
<td>Exposed for 1 hour/day, 5 days a week for 4 months by a small animal nebulizer</td>
<td>Study shows that longer-term exposure of EC causes asthma and emphysema</td>
</tr>
<tr>
<td>Husari A [78] 2015</td>
<td>No</td>
<td>Room air or CC smoke</td>
<td>Four-month male C57BL/6J mice</td>
<td>Exposed for 6h/day for 3 days</td>
<td>Despite higher exposure conditions, EC exhibited less toxic effects on lungs of experimental animals than CC smoke</td>
</tr>
<tr>
<td>Lerner CA [98] 2015</td>
<td>No</td>
<td>No</td>
<td>Eight weeks old wild type C57BL/6J mice</td>
<td>* Mice were exposed to sidestream EC vapor for 5 h per day for 3 days (acute exposure) in inhalation chambers</td>
<td>EC inhalation have an impact on cellular oxidative stress, redox imbalance, and lung inflammation, in vitro in lung cells and in vivo in lungs</td>
</tr>
<tr>
<td>Lim [99] 2014</td>
<td>No</td>
<td>CC</td>
<td>24 five-week-old female BALB/c mice</td>
<td>Diluted solution was intra-tracheally instilled to Ovalbumin−sensitized mice two times a week for 10 weeks</td>
<td>Suggest that the inhalation of EC solutions can function as an important factor to exacerbate the allergy-induced asthma symptoms</td>
</tr>
<tr>
<td>McGrath-Morrow S [112] 2015</td>
<td>No</td>
<td>Room air</td>
<td>Timed pregnant C57BL/6J mice and their neonatal pups</td>
<td>Neonatal mice were exposed to EC vapor or room air for 9 days of life or kept in room air</td>
<td>EC emissions +nicotine during the neonatal period can adversely impact weight gain +Exposure to EC with nicotine caused diminished alveolar cell proliferation and a modest impairment in postnatal lung growth</td>
</tr>
<tr>
<td>Palpant NJ [122] 2015</td>
<td>No</td>
<td>CC extract</td>
<td>Wild-type zebrafish (Danio rerio)</td>
<td>Zebrafish embryos were exposed from the onset of differentiation (day 0) and added fresh at every media change</td>
<td>Study indicate a negative effect of EC on heart development in vitro and in vivo +Impact of EC on heart development seems to be the consequence of other components than nicotine</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Conditions</td>
<td>Experimental Details</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>---------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Ponzoni L</td>
<td>2015</td>
<td>CC or room air</td>
<td>183 Male BALB/c mice; one month old; Exposed three 30-min sessions/day for 7 weeks in inhalation chambers</td>
<td>EC vapor induces addiction-related neurochemical, physiological and behavioural alteration, independent of nicotine</td>
<td></td>
</tr>
<tr>
<td>Salturk Z</td>
<td>2015</td>
<td>Room air</td>
<td>16 Female Wistar albino rats; Exposed to EC vapor for 1 hour/day for 4 weeks in inhalation chambers</td>
<td>EC vapor exposed animals developed more frequently hyper- and metaplasia in the larynx than non-exposed animals; non-significant differences (small study)</td>
<td></td>
</tr>
<tr>
<td>Schweitzer KS</td>
<td>2015</td>
<td>Saline</td>
<td>C57Bl/6 mice (4-mo-old females); Exposure: nebulized and harvested immediately, or harvested after either 30 min or 24 h.</td>
<td>It is anticipated that long-term EC use will include dose-dependent sustained oxidative stress and inflammatory lung damage with limitation of endothelial repair</td>
<td></td>
</tr>
<tr>
<td>Smith D</td>
<td>2015</td>
<td>PPG without nicotine + Room air</td>
<td>C57BL/6J mice (pregnant + male offspring); Exposed to 2.4% nicotine in PPG or 0% nicotine /PPG once a day from gestational day 15 until delivery. + 14 days from postnatal day 2 through 16</td>
<td>Male mice exhibited increased levels of activity when exposed to vapor containing nicotine during late prenatal and early postnatal life - indicating that nicotine exposure from EC may cause persistent behavioral changes</td>
<td></td>
</tr>
<tr>
<td>Sussan TE</td>
<td>2015</td>
<td>Room air</td>
<td>Male C57BL/6 (age 8 weeks) mice; Exposure: via a whole-body exposure system for 1.5 h, twice per day for 2 weeks. One hour after final exposure: infected intra-nasally with S. Pneumoniae bacteria or Influenza A virus</td>
<td>Exposure to EC vapor induced oxidative stress and moderate inflammatory response - Significant impairment in bacterial clearance in lungs + Enhanced susceptibility to influenza infection, based on increased percent weight loss, mortality, and viral titer</td>
<td></td>
</tr>
</tbody>
</table>

*Four of these studies are also/partly mentioned in Table 3/Appendix 5 on animal experimental studies [98] [122] [143] [78]|

EC= electronic cigarette  
CC= conventional cigarette  
PPG= propylene glycol
Table 4. Studies reporting adverse events  
(n=31)

For details in methodology and results please see appendix 5.

<table>
<thead>
<tr>
<th>Name of first author Reference Year</th>
<th>Conflict of interest ▲=Yes</th>
<th>Type of study RCT=randomised controlled trial</th>
<th>Number of participants Evt. duration of follow-up</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| Adriens K [1] 2015                 | No                        | RCT                                         | 48 volunteers not willing to quit. 3 sessions over two months: vaped/smoked for 5 min | • EC users reported more benefits in prospective study  
• Dual use group reported positive and negative symptoms |
| Bartram A [6] 2015                 | No                        | Case report                                 | A 55-year-old healthy man; quit and switched to EC | • EC use was found to be associated with a florid lichenoid reaction |
| Bullen C [11] 2013                | ▲ 7                       | RCT                                         | 657 participants randomized to nicotine-EC (n=289), placebo EC (n=295) or nicotine patch (n=73) for 13 weeks | • A higher number and proportion of adverse events occurred in the nicotine EC group than in the patches group; however, there was no evidence of an association with study product, and the event rate was not significantly different |
| Bullen C [12] 2010                | ▲ 1                       | Single blind randomised cross-over trial    | 40 adult dependent smokers of 10 or more CC per day. | • Nausea and mouth and throat irritation were common  
• Less common: aching jaws, vertigo, feeling high, palpitations |
| Camus M[15] 2014                   | No                        | Case report                                 | A 49-year-old woman with colitis ulcerosa        | • Patient presented with a “smoking-dependent form” of colitis ulcerosa, which recurred nearly immediately after replacing CC smoking by nicotine containing EC |
| Caponetto P [16] 2013             | ▲ 2                       | Prospective observational study             | 14 smokers with schizophrenia not intending to quit 12-months | • Positive and negative symptoms of schizophrenia were not increased after smoking reduction/cessation in patients using EC  
• AE (cough, nausea, throat irritation, headache) declined over time |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Study Type</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caponetto P [17] 2013</td>
<td>▲ 2</td>
<td>RCT</td>
<td>300 smokers not intending to quit over 12-months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AE as cough, dry mouth, shortness of breath, and headache declined over time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Small reduction in CO compared with reduction in number CC</td>
</tr>
<tr>
<td>Chen IL [21] 2013</td>
<td>No</td>
<td></td>
<td>Adverse events reported to U.S. FDA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Online survey</td>
<td>Approximately half of all tobacco-related AE reports since late 1980ies concern EC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Many reports of AE and SAE - There is not necessarily a causal relationship between AEs reported and EC use, as some AEs could be related to pre-existing conditions or due to other causes not reported</td>
</tr>
<tr>
<td>Dawkins L [35] 2013</td>
<td>▲ 3</td>
<td></td>
<td>Online survey</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1349 users of EC (218 current smokers + 1123 ex-smokers + 4 never smokers)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Respondents (most had quit smoking) reported few negative symptoms and many positive health effects with EC - Majority state: it feels healthier and use improved cough</td>
</tr>
<tr>
<td>Etter JF [39] 2010</td>
<td>▲ 4</td>
<td>Survey</td>
<td>A survey of users</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>81 respondents ever users of EC (72 daily users, 63% recently quit smoking CC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Respondents reported more positive than negative effects with EC: many reported positive effects on the respiratory system, which were probably associated with stopping smoking</td>
</tr>
<tr>
<td>Farinha H [42] 2015</td>
<td>No</td>
<td></td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>66-year old female patient, heavy smoker- had stopped smoking and initiated EC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A case of probable association between EC use and lingua villosa nigra is reported</td>
</tr>
<tr>
<td>Farsalinos KE [50] 2013</td>
<td>▲ 11</td>
<td></td>
<td>Interviews with vapors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>111 experienced EC users who had switched from CC to EC use for at least 1 month</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Side effects were mild and temporary - The vast majority of participants reported better exercise capacity and improved olfactory and gustatory senses</td>
</tr>
<tr>
<td>Farsalinos KE [48] 2013</td>
<td>▲ 9</td>
<td>Case report</td>
<td>32 old male patient with idiopathic chronic neutrophilia. Then, quit smoking with EC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Despite daily use of EC, the beneficial effects of smoking cessation on idiopathic chronic neutrophilia were maintained</td>
</tr>
<tr>
<td>Farsalinos [51] 2014</td>
<td>▲ 10</td>
<td>Survey</td>
<td>19,414 EC regular users world wide - Median use: 10 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Side effects were minor and health benefits were substantial, especially for those who completely substituted smoking with EC use</td>
</tr>
<tr>
<td>Gillen S [63] 2015</td>
<td>No</td>
<td>Case report</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A 1 day old boy born at full term- Mother: vaping EC in pregnancy and during labor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antenatal exposure to EC vapor might be a possible etiology to total colonic necrotizing enterocolitits in a new born child</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Study Type</td>
<td>Number of Participants</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Heavner K [72] 2010</td>
<td>▲ 5</td>
<td>Online survey</td>
<td>303 users of EC</td>
</tr>
<tr>
<td>Hua M [76] 2013</td>
<td>No</td>
<td>Online search</td>
<td>481 vapors</td>
</tr>
<tr>
<td>Hureaux J [77] 2014</td>
<td>No</td>
<td>Case report</td>
<td>A 43 year old patient with smoking-related COPD and lung adenocarcinoma</td>
</tr>
<tr>
<td>Lee S [96] 2013</td>
<td>No</td>
<td>Case report</td>
<td>A 35-year old man with history of pan-ulcerative colitis which began after smoking cessation + EC use</td>
</tr>
<tr>
<td>Manzoli L [105] 2015</td>
<td>No</td>
<td>Prospective cohort study</td>
<td>Adults (30–75 years); 236 EC vapers, 491 CC smokers, and 232 dual smokers</td>
</tr>
<tr>
<td>Maridet C [107] 2015</td>
<td>No</td>
<td>Case report</td>
<td>A 52-year-old woman</td>
</tr>
<tr>
<td>McCauley L [111] 2012</td>
<td>No</td>
<td>Case report</td>
<td>A patient</td>
</tr>
<tr>
<td>McQueen A [113] 2011</td>
<td>No, 1,</td>
<td>Interviews with vapors</td>
<td>13 vapors</td>
</tr>
<tr>
<td>Monroy AE [116] 2012</td>
<td>No</td>
<td>Case report</td>
<td>70 year old woman, smoking history: 40 pack-years. Undergone hip-arthroplasty</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Sample</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>--------------</td>
<td>--------</td>
</tr>
<tr>
<td>Munoz A [117] 2015</td>
<td>No</td>
<td>Survey in a smoking cessation clinic</td>
<td>64 ever-users of EC</td>
</tr>
<tr>
<td>Polosa R [127] 2011</td>
<td>▲ 6</td>
<td>Prospective study</td>
<td>40 smokers not intending to quit</td>
</tr>
<tr>
<td>Polosa R [129] 2013</td>
<td>▲ 6</td>
<td>Prospective observational study</td>
<td>23 smokers not intending to quit (5 not using EC at follow-up)</td>
</tr>
<tr>
<td>Thota D [152] 2014</td>
<td>No</td>
<td>Case report</td>
<td>A 20-year-old healthy man</td>
</tr>
<tr>
<td>Vannier S [160] 2014</td>
<td>No</td>
<td>Case report</td>
<td>A 39-year-old healthy man switched from 60 CC/day to dual use of 20 CC/day + EC</td>
</tr>
<tr>
<td>Wang MP [167] 2015</td>
<td>No</td>
<td>Population-based survey</td>
<td>45,128 students; 95% of all invited</td>
</tr>
</tbody>
</table>
EC=electronic cigarette  
CC=conventional cigarette  
AE= adverse events  
SEA = serious adverse events  

Conflicts of interest - Conflicts of interest of each study should be assessed individually.  
▲ 1: This project was funded by EC manufacturer. The study sponsors supplied the ECs used in the trial and funded the trial. The trial design conduct, analysis and interpretation of results were conducted independently of the sponsors. HM has received honoraria for speaking at research symposia and received benefits in kind and travel support from, and has provided consultancy to the manufacturers of smoking cessation medications. ML acted as contract manager with the sponsor, manufacturer of ECs. MG has provided consultancy to the manufacturers of smoking cessation medications  
▲ 2: RP has received lecture fees and research funding from manufacturers of stop smoking medications. He has served as a consultant for manufacturers of smoking cessation medications and the distributor EC used.  
▲ 3: LD has a collaborative relationship with manufacturer of EC and received funds to attend academic conferences. E-manufacturer reviewed and approved content of questionnaire and set up links from their websites.  
▲ 4: JFE was previously consultant for manufacturer of smoking cessation medications  
▲ 5: Study was funded and supported by manufacturer of EC and manufacturer is co-author. All other authors are employed at University of Alberta, which is financially supported by a large smokeless tobacco manufacturer. CVP advises on tobacco harm reduction and is compensated for this work.  
▲ 6: RP has received lecture fees from manufacturer of EC and has been serving as a consultant for manufacturer of EC. Manufacturer of the EC supplied product, technical and consumer support  
▲ 7: ML, via his company Health New Zealand, previously did research funded by an EC manufacturer. CB and HM have done research on ECs funded by Health New Zealand, independently of EC manufacturer. HM has received honoraria for speaking at research symposia, has received benefits in kind and travel support from, and has provided consultancy to, the manufacturers of smoking cessation drugs. NW has provided consultancy to the manufacturers of smoking cessation drugs, received honoraria for speaking at a research meeting and received benefits in kind and travel support from a manufacturer of smoking cessation drugs. JW has provided consultancy to the manufacturers of smoking cessation medications.  
▲ 8: CB has undertaken research on e-cigarettes funded by Health NZ (funded by e-cig manufacturer), independently of e-cigarette manufacturer. NW has provided consultancy to the manufacturers of smoking cessation drugs, received honoraria for speaking at a research meeting and received benefits in kind and travel support from a manufacturer of smoking cessation drugs  
▲ 9 to 11: “No” stated, but some of the other studies performed by KF used unrestricted funds provided to research center by e-cigarette companies. KEF has a website “E-cigarette Research Advocate Group” which represents an unambiguously positive view on EC and provides several links to vapor clubs  
π, 1: AMQ acknowledges the support of the organizers and attendees at vapers’ meeting where recruitment took place
Reference List


19. Chausse P, Naughton G, utheil F. Electronic Cigarettes. The resistance value of the heating filament could be the key to lung toxicity (Comment). Chest 2015; 148


on lung function [Abstract]. Abstracts/ Toxicology Letters 2012; 211S:45


53. Farsalinos KE, Voudris V, Poulas K. E-cigarettes generate high levels of aldehydes only in ‘dry puff’ conditions. Addiction 2015;


68. Goniewicz ML, Lee L. Electronic cigarettes are a source of thirdhand exposure to nicotine. Nicotine Tob.Res. 2015; 17:256-258


70. Hahn J, Monakhova YB, Hengen J, Kohl-Himmelseher M, Schussler J, Hahn H, Kuballa T,


91. Laugesen M. Ruyan E-cigarette Bench-top tests [Abstract]. Society for Research on Nicotine and Tobacco (SRNT) 2009;


94. Lauterbach JH, Laugesen M. Comparison of toxicant levels in mainstream aerosols generated by Ruyan® electronic nicotine delivery systems (ENDS) and conventional cigarette products [Abstract]. The Toxicologist 2012; 126:
95. Lauterbach JH, Laugesen M, Ross BB. Suggested protocol for estimation of harmful and potentially harmful constituents in mainstream aerosols generated by electronic nicotine delivery systems (ENDS) [Abstract]. *The Toxicologist* 2012; 126:


6-month pilot study. BMC.Public Health 2011; 11:786


This report was prepared at the request of WHO Prevention of Noncommunicable Diseases. The findings, interpretations, and conclusions expressed in this work do not necessarily reflect the views of WHO.


Annexes with search strategies and detailed description of studies
Annexes with search strategies and detailed description of studies

Annex 1. Systematic search in databases showing number of articles found (Identified/screened (title)/screened (abstract)/eligible)

**First search:** all studies published before 2 September 2013.

<table>
<thead>
<tr>
<th>Search word</th>
<th>PubMed</th>
<th>EMBASE</th>
<th>Cinahl</th>
<th>+ Other Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>Identified/screened (title)/screened (abstract)/eligible</td>
<td>#</td>
<td>Identified/screened (title)/screened (abstract)/eligible</td>
</tr>
<tr>
<td>Electronic cigarette</td>
<td>1</td>
<td>342/93/36/36</td>
<td>5</td>
<td>98/96/30/7</td>
</tr>
<tr>
<td>Electrically heated cigarette</td>
<td>2</td>
<td>34/22/22/22</td>
<td>9</td>
<td>31/31/22/0</td>
</tr>
<tr>
<td>E-cigarette</td>
<td>4</td>
<td>71/55/21/3</td>
<td>10</td>
<td>67/59/20/0</td>
</tr>
<tr>
<td>ENDS and cigarette</td>
<td>3</td>
<td>63/9/1/0</td>
<td>7</td>
<td>65/14/1/0</td>
</tr>
<tr>
<td>Electronic nicotine delivery system</td>
<td>8</td>
<td>3/3/0/0</td>
<td>6</td>
<td>8/8/1/0</td>
</tr>
<tr>
<td>Electronic nicotine delivery device</td>
<td>11</td>
<td>20/20/8/1</td>
<td>12</td>
<td>6/6/4/0</td>
</tr>
<tr>
<td>E-liquid</td>
<td>19</td>
<td>1/1/0/0</td>
<td>20</td>
<td>8/1/0/0</td>
</tr>
<tr>
<td>Total number: Identified/screened (title)/screened (abstract)/eligible</td>
<td>534/203/88/62</td>
<td>283/215/78/7</td>
<td>38/36/11/0</td>
<td>8/7</td>
</tr>
</tbody>
</table>

# Search number
1. Update:

PubMed: studies published between 2 September 2013 and August 5 2014.

EMBASE: studies published in 2013/2014


<table>
<thead>
<tr>
<th>Search word</th>
<th>PubMed</th>
<th>EMBASE</th>
<th>Cinahl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>Identified/screened (title)/screened (abstract)/eligible</td>
<td>#</td>
</tr>
<tr>
<td>Electronic cigarette</td>
<td>1</td>
<td>683/165/26/16</td>
<td>8</td>
</tr>
<tr>
<td>Electrically heated cigarette</td>
<td>2</td>
<td>0/0/0/0</td>
<td>9</td>
</tr>
<tr>
<td>E-cigarette</td>
<td>4</td>
<td>127/121/16/1</td>
<td>10</td>
</tr>
<tr>
<td>ENDS and cigarette</td>
<td>3</td>
<td>21/11/0/0</td>
<td>11</td>
</tr>
<tr>
<td>Electronic nicotine delivery system</td>
<td>5</td>
<td>13/13/3/1</td>
<td>12</td>
</tr>
<tr>
<td>Electronic nicotine delivery device</td>
<td>6</td>
<td>5/4/4/0</td>
<td>13</td>
</tr>
<tr>
<td>E-liquid</td>
<td>7</td>
<td>6/5/1/0</td>
<td>14</td>
</tr>
<tr>
<td>Total number: Identified/screened (title)/screened (abstract)/eligible</td>
<td><strong>855/319/51/18</strong></td>
<td><strong>437/328/43/7</strong></td>
<td><strong>70/69/3/0</strong></td>
</tr>
</tbody>
</table>

In total INCLUDED in first published review [127], based on 2 searches: 68 + 8 identified elsewhere= 76

Annexes – 2
2. Update:
+Filter: Search field=title or title/abstract (starting with step: screened by title)
PubMed: studies published between 5 August 2014 and 7 July 2015.
Finally, search #1 to #7 was repeated; in PubMed only: studies published between 7 July 2015 and 26 Nov 2015. Search field=title

<table>
<thead>
<tr>
<th>Search word</th>
<th>PubMed</th>
<th>EMBASE</th>
<th>Cinahl</th>
<th>+ Other source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>Identified/screened (title)/screened (abstract)/eligible</td>
<td>#</td>
<td>Identified/screened (title)/screened (abstract)/eligible</td>
</tr>
<tr>
<td>Electronic cigarette</td>
<td>1</td>
<td>229/69/38/36 52/16/13/10</td>
<td>9</td>
<td>211/40/27/8 75/17/17/1</td>
</tr>
<tr>
<td>Electrically heated cigarette (not searched, is non-combustible CC)</td>
<td>2</td>
<td>0/0/0/0</td>
<td>10</td>
<td>+ Other source</td>
</tr>
<tr>
<td>E-cigarette</td>
<td>3</td>
<td>211/40/27/8 75/17/17/1</td>
<td>11</td>
<td>+ Other source</td>
</tr>
<tr>
<td>ENDS and cigarette</td>
<td>4</td>
<td>14/1/1/0 0/0/0/0</td>
<td>12</td>
<td>Found in searched articles/included after reading of article</td>
</tr>
<tr>
<td>Electronic nicotine delivery device</td>
<td>5</td>
<td>9/2/1/0 1/1/0/0</td>
<td>13</td>
<td>+ Other source</td>
</tr>
<tr>
<td>Electronic nicotine delivery system</td>
<td>6</td>
<td>7/1/1/1 0/0/0/0</td>
<td>14</td>
<td>+ Other source</td>
</tr>
<tr>
<td>E-liquid</td>
<td>7</td>
<td>16/7/6/0 1/1/1/1</td>
<td>15</td>
<td>+ Other source</td>
</tr>
<tr>
<td>E-juice (new)</td>
<td>8</td>
<td>3/1/0/0 1/1/1/1</td>
<td>16</td>
<td>Found in searched articles/included after reading of article</td>
</tr>
<tr>
<td>Total number: Screened (title)/screened (abstract)/eligible/included</td>
<td>489/121/74/45 99/5/31/12</td>
<td>232/323/0/0</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

# Search number

In total identified at 2. Update of search: 88 + 11 from elsewhere =99
In total INCLUDED: 76 from first search and first update + 99 from second update= 175
**Annex 2. Studies investigating the content of fluid or vapor of electronic cigarettes and in-vitro experiments** where cells were exposed to fluid/vapor/vapor extract (n=105*). Detailed version.

<table>
<thead>
<tr>
<th>Name of first author. Reference Year</th>
<th>Conflict of interest ▲ △ = Yes ✤ = Tobacco industry ✓ = EC industry</th>
<th>Relevant for passive exposure to EC □ = Yes</th>
<th>Type of product(s) Reference (ref) product</th>
<th>Fluid/vapor/ nicotine on surface Aim</th>
<th>Methods</th>
<th>Results</th>
<th>Method problems/ weaknesses</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen JG [2] 2015</td>
<td>No</td>
<td></td>
<td>-51 types of flavored EC sold by leading brands and flavors appealing to youth Ref: no</td>
<td>-Vapor • Aim: to determine if the flavoring chemical diacetyl, and two other high-priority flavoring chemicals 2,3-pentanedione, and acetoain, are present in a ECs</td>
<td>-Air stream was captured and analyzed for total mass of diacetyl, 2,3-pentanedione, and acetoain, according to OSHA Method 1012</td>
<td>-At least one flavoring chemical was detected in 47 of 51 unique flavors tested • Diacetyl: detected above the laboratory limit of detection 39 of the 51 flavors tested, ranging from &lt; limit of qualification to 239 µg/EC • 2,3-pentanedione and acetoain: detected in 23 and 46 of the 51 flavors tested at concentrations up to 64 and 529 µg/EC, respectively</td>
<td>-Possible that samples did not fully reflect the total chemical content if liquid remained in the EC at the time the sampler was turned off; underestimate of chemical content</td>
<td>-Findings confirm the presence of diacetyl (causing bronchiolitis obliterans “pop-corn lungs”) and other high priority flavoring chemicals in flavored compounds in EC</td>
</tr>
<tr>
<td>Aug A [3] 2014</td>
<td>No</td>
<td></td>
<td>“strong/high” AIRSmoke EC liquid condensate</td>
<td>Fluid • Aim: to assess the impact of EC exposure on the metabolome of primary human bronchial epithelial cells (HBEc) and evaluate the effect of an antioxidant glutathione analogue UPF1 on the changes</td>
<td>Human bronchial epithelial cells, differentiated at air-liquid interface, were exposed to EC liquid of CC smoke condensate for 1h, followed by treatment with 0-10 µM UPF1 for 1-12 h. Cell lysates were analysed on an AB Q-Trap 3200 mass spectrometer</td>
<td>Exposure to EC: a rapid shift of the HBEc metabolomic state, followed by a delayed approach to the initial state by 12 h. • The changes caused by EC occurred at similar direction with those produced by CC smoke condensate in 54.4%, 70.1%, 84.4%, 52.3% and 58.8% of signals at 1, 2, 5, 7 and 13 h, respectively • The effect of EC on the metabolites was stronger than that of CC smoke condensate in 38.0%, 56.5%, 79.2%, 63.3% and 49.1% of the signals at 1, 2, 5, 7 and 13 h, respectively • UPF1 diminished the metabolomics derangements in the EC-stimulated cells with its maximal effect being at 5 h</td>
<td>Tested • Use of fluid, not vapor</td>
<td>-EC have immediate and profound adverse effects on the metabolomic state of primary human bronchial epithelial cells similar to those seen with CSC</td>
</tr>
<tr>
<td>Bahl V [4] 2012</td>
<td>No</td>
<td>-35 different refill fluid samples from 4 major US brands</td>
<td>Refill fluids • Aim: test cytotoxicity of fluid • Human embryonic stem cells (hESc)</td>
<td>-Human embryonic stem cells (hESc) • Humectants: non-cytotoxic for all cells • 15 samples were moderately</td>
<td>-Vapors were performed at a maximum conc. of</td>
<td>-Possible that samples did not fully reflect the total chemical content if liquid remained in the EC at the time the sampler was turned off; underestimate of chemical content</td>
<td>-Approx. one third of samples were highly cytotoxic to hESc and</td>
<td></td>
</tr>
</tbody>
</table>

1 Results of studies influenced by the tobacco industry are marked with an asterisk (*) in the paper.

2 Studies funded by e cigarette manufacturers or performed in collaboration with the e cigarette industry are labelled with a chevron (▲) in the paper.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Conditions</th>
<th>Methods</th>
<th>Results/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertholon JF [9]</td>
<td>2013</td>
<td>No</td>
<td>- One brand: la Cigarette CT model ZenAttitude, 16 mg nicotine - Reference: CC, Gauloise, and water pipe</td>
<td>- Vapor: Inhaled: Measure aerosol particle sizes in three streams; inhaled by the user(S1), released by the device itself (S2)and, exhaled by the user (S3) - Electrostatic low-pressure impactor (ELPI), giving particle size distributions in real time and calculating median diameters, D50, and dispersion - 26% of the total vapor would deposit, of which 14% would reach the alveoli - These data are close to those found with CC. - The half-life in air of the S3 stream was 11 seconds due to a rapid evaporation - The EC vapor, as measured here, is made of particles bigger than those of CC and water pipe aerosols</td>
</tr>
<tr>
<td>Brot L [10]</td>
<td>2015</td>
<td>No</td>
<td>- Unknown EC brand, containing PPG - Ref: CC smoke extract; solvent: PPG - Vapor extract - Aim: to compare the impact of the EC with that of CC on inflammatory response in an epithelial intestinal cell culture model - The intestinal inflammatory response was evaluated using a human intestinal epithelial cell line model (HT29), transfected with bacterial LPS - Cells exposed to vapor showed inflammatory response comparable to control cells and significantly lower than those treated with CC smoke extracts. - Inflammatory response was greatly elevated in cells exposed to CC smoke, as measured by IL-8 release (pg/mg protein)</td>
<td>- Unknown single brand - Results suggest that the intestinal epithelium inflammatory response is not altered by exposure to vapor from EC</td>
</tr>
<tr>
<td>- No ref product</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>No.</td>
<td>Ref.</td>
<td>Sample size</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>-----</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Bush D [13]</td>
<td>2014</td>
<td>▲25</td>
<td>Θ</td>
<td>Unknown brands</td>
</tr>
<tr>
<td>Cameron JM [14]</td>
<td>2013</td>
<td>No</td>
<td></td>
<td>7 types of e-liquids</td>
</tr>
<tr>
<td>Cervellati F [18]</td>
<td>2014</td>
<td>No</td>
<td></td>
<td>Cloud-smoke (balsamic flavors with or without nicotine)</td>
</tr>
<tr>
<td>Chausse P [19]</td>
<td>2015</td>
<td>No</td>
<td></td>
<td>No ECs tested</td>
</tr>
</tbody>
</table>

**Table Notes:**
- ▲: Vapour, Smart Smoke, Labeled brands: vendors in USA.
- □: No ref product
- □: No ECs tested
- □: Heating of EC
- □: No ECs tested

**Results:**
- Nicotine on surfaces in households
- Households of 8 EC users (50-500 puffs daily), 6 CC smokers (5-40 cigarettes per day), and 8 non-users of nicotine-containing products
- Three surface wipe samples were taken from the floor, wall and window
- Nicotine was extracted and analyzed using gas chromatography
- Half of the EC users' homes had detectable levels of nicotine on surfaces whereas nicotine was found in all of the tobacco cigarette smokers' homes
- The levels of nicotine in ECs users' homes were almost 200 times lower than the levels detected in CC smokers homes (average concentration 7.7 ± 17.2 vs. 1303 ± 2676 g/m², p < 0.05)
- There was no significant difference in the amount of nicotine in homes of EC users and non-users (p > 0.05)
- Only test of fluids
- Nicotine level estimated in 5 samples
- Large variability in nicotine concentrations was found
- Exposure to EC vapors is far less toxic than exposure to CC smoke
<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>No Testing</th>
<th>Products Tested</th>
<th>Cartridges</th>
<th>Vapor Extracts</th>
<th>Platelets</th>
<th>Duration of Exposure</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheah NP [20]</td>
<td>2012</td>
<td>No</td>
<td>-20 variants of EC - cartridges</td>
<td>Cartridges</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Products confiscated from the Immigration and Checkpoints authority, Singapore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No ref product</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nicotine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Humectants: PPG, glycerol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Organic solvent extraction followed by detection by chromatography with flame ionisation detector.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Each compound was identified using the same instrument with mass spectrometer detection.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 products: contained &gt;100 mg of PPG per cartridge (max. 1320 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 products: contained a very high level of glycerol (max. 339 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 products: actual nicotine content did not correspond to the amount reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 products: contained nicotine even though they claimed to be nicotine free</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polycyclic aromatic hydrocarbons and tobacco-specific nitrosamine compounds were not found</td>
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<td>Tested only one batch of liquid per brand/model</td>
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<td>Not vapor</td>
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<td>Presence of a high amount of glycols (PPG and glycerol) in great quantities</td>
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<td></td>
<td>Contained nicotine even though they claimed to be nicotine free</td>
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<td>Significant difference in the nicotine content across EC with same label, brand-to-brand and cartridge-to-cartridge variations</td>
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<td></td>
<td>Polycyclic aromatic hydrocarbons and tobacco-specific nitrosamine compounds were not found</td>
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</tbody>
</table>

| Cheah NP [20]       | 2012 | No         | EC of unknown brand, with nicotine of different conc |
|                     |      |            | Ref: CC smoke extract |
|                     |      |            | Vapor extracts |
|                     |      |            | Aim: to elucidate if the exposure to physiologically relevant levels of e-vapor can alter platelet functions |
|                     |      |            | Exposed platelets to vapor extracts |
|                     |      |            | Exposure time? |
|                     |      |            | Platelet aggregation was enhanced |
|                     |      |            | For the e-juice formulations with the highest concentration of nicotine, this enhancement mirrored the effects of mainstream and sidestream tobacco smoke extracts |
|                     |      |            | Altered platelet aggregation was partially induced by an up-regulation of CD42b |
|                     |      |            | Adhesion potential of platelets was also enhanced via an up-regulation of CD41a and CD62P, respectively |
|                     |      |            | Platelets were more likely to participate in coagulation based reactions, suggesting an enhancement of the coagulation cascade |
|                     |      |            | Unknown brand |
|                     |      |            | Unknown duration of exposure |
|                     |      |            | Study illustrates preliminary evidence that e-vapor exposure may alter platelet functions associated with cardiovascular disease progression |

| Colard S [24]       | 2015 | No Testing | No specific product tested |
|                     |      |            | Vapor |
|                     |      |            | Aim: to calculate whether the aerosol exhaled following the use of EC has implications for the quality of air breathed by bystanders |
|                     |      |            | Mathematical models based on empirical emissions data and basic assumptions Simulation model of the cumulative effect of vaping over time |
|                     |      |            | The maximum concentration of nicotine the bystander will be exposed to over the working day is approximately 1.8 µg/m³ (workplace exposure limit for nicotine: 500 µg/m³ over 8 h in the workplace) |
|                     |      |            | The model showed good agreement with the published values of indoor air nicotine concentration |
|                     |      |            | Calculations were based on published studies performed by persons with conflict of interest |
|                     |      |            | Not real-life measurements |
|                     |      |            | The exposure of bystanders to nicotine in the exhaled aerosol is not at levels that would be expected to cause health concerns |

| Costigan S [27]     | 2015 | None       | None |
|                     |      |            | A flavor ingredient screening and risk assessment process flow |
|                     |      |            | A flow of ingredients that can be helpful when there is a lack of data on local and systemic toxicity is the toxicological threshold of concern (TTC) |
|                     |      |            | Suggests use of toxicological threshold of concern (TTC). A TTC |
|                     |      |            | No testing of fluid/vapor |
|                     |      |            | Presents an approach to risk assessment of in-going flavoring ingredients in e-liquid and potential thermal breakdown and reaction products in the aerosol |

Annexes – 7
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Type</th>
<th>Aim</th>
<th>Methodology</th>
<th>Findings</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costigan S [26]</td>
<td>2014</td>
<td>None</td>
<td>Aim: To assess in an evaluation approach model if flavour ingredients have the potential to induce contact sensitization (delayed “Type IV” hypersensitivity)</td>
<td>A flavor ingredient screening and risk assessment process flow</td>
<td>The approach developed here applies both to single ingredients and to components of naturals In example Geraniol 1% is not below 1000ppm but has no sensitization potential and the sensitizer level is supportable Isoeugenol 3% is not below 1000ppm and has sensitization potential and the sensitizer level is not supportable</td>
<td>Calculations only, no testing Presents a contact sensitization and risk assessment model</td>
</tr>
<tr>
<td>Cox C [28]</td>
<td>2015</td>
<td>No</td>
<td>97 EC (15 disposable, 32 cartridge, 50 refillable) from 24 EC companies, including the leading US brands</td>
<td>Tested in independent testing laboratory that is accredited by the American Association for Laboratory Accreditation and that has been testing both cigarettes and EC for many years Standard smoking machines that simulate how consumers use the products</td>
<td>Formaldehyde exposures up to 473 times the Proposition 65 safety level and acetaldehyde exposures up to 254 times the safety level 21 of the 24 EC companies had at least one product that produced high levels acetaldehyde and/or formaldehyde, in violation of California’s consumer protection law, Proposition 65 Even nicotine-free EC produced high levels of both chemicals One nicotine-free EC produced acetaldehyde exposures &gt;13 times safety level and formaldehyde exposures &gt; 74 times the safety threshold</td>
<td>No reference Levels and methods not shown in detail The majority of EC produce very high levels of acetaldehyde and formaldehyde High levels of these cancer-causing chemicals are produced even by some EC without nicotine</td>
</tr>
<tr>
<td>Czogala J  [30]</td>
<td>2014</td>
<td>▲ 1</td>
<td>3 models of EC (high, medium, low nicotine), popular brands in Poland: (a) Colins Age with Camel High cartomizer, (b) Dekang 510 Pen with SGC Regular cartridge, and (c) Mild M201 Pen with Marlboro cartridge</td>
<td>Exposure chamber Study 1: A smoking machine and controlled exposure conditions Study 2: Compared secondhand exposure with e-cigarette vapor and tobacco smoke generated by 5 dual users</td>
<td>Air concentrations of nicotine ranged from 0.82 to 6.23 µg/m³. The average concentration of nicotine resulting from CC was 10 times higher than from EC (31.60 ± 6.91 vs. 3.32 ± 2.49 µg/m³, respectively; p = .008) The mean concentration of PM2.5 from CC was 7 times higher than from EC (819.3 ± 228.6 vs. 151.7 ± 86.8 µg/m³, respectively; p = .008). Both studies : VOCs: only toluene was detected No changes in CO concentration after use of EC</td>
<td>Tested only 3 brands Measured a limited number of chemicals Assessed concentrations of several markers in the air but not serum concentrations in people exposed to secondhand vapor Using EC in indoor environments may involuntarily expose nonusers to nicotine but not to toxic tobacco-specific combustion products</td>
</tr>
<tr>
<td>Davis B  [31]</td>
<td>2015</td>
<td>No</td>
<td>71 EC refill fluids and 1 do-it-yourself product</td>
<td>Fluid</td>
<td>High-performance liquid chromatography</td>
<td>35 of 54 nicotine-containing fluids had quantified nicotine concentrations that deviated by more than ± 10%</td>
</tr>
</tbody>
</table>

**Aim:** To test to evaluate the performance of one or both of two cancer-causing chemicals, acetaldehyde and formaldehyde in EC and compare with California’s consumer protection law, Proposition 65

**Methodology:** Tested in independent testing laboratory that is accredited by the American Association for Laboratory Accreditation and that has been testing both cigarettes and EC for many years

**Findings:**Formaldehyde exposures up to 473 times the Proposition 65 safety level and acetaldehyde exposures up to 254 times the safety level

21 of the 24 EC companies had at least one product that produced high levels acetaldehyde and/or formaldehyde, in violation of California’s consumer protection law, Proposition 65

Even nicotine-free EC produced high levels of both chemicals

One nicotine-free EC produced acetaldehyde exposures >13 times safety level and formaldehyde exposures > 74 times the safety threshold

**Additional Information:** No reference Levels and methods not shown in detail

The majority of EC produce very high levels of acetaldehyde and formaldehyde

High levels of these cancer-causing chemicals are produced even by some EC without nicotine

**Aim:** To assess in an evaluation approach model if flavour ingredients have the potential to induce contact sensitization (delayed “Type IV” hypersensitivity)

**Methodology:** A flavor ingredient screening and risk assessment process flow

**Findings:** The approach developed here applies both to single ingredients and to components of naturals In example Geraniol 1% is not below 1000ppm but has no sensitization potential and the sensitizer level is supportable

Isoeugenol 3% is not below 1000ppm and has sensitization potential and the sensitizer level is not supportable

**Additional Information:** Calculations only, no testing Presents a contact sensitization and risk assessment model

**Aim:** To test to evaluate the performance of one or both of two cancer-causing chemicals, acetaldehyde and formaldehyde in EC and compare with California’s consumer protection law, Proposition 65

**Methodology:** Tested in independent testing laboratory that is accredited by the American Association for Laboratory Accreditation and that has been testing both cigarettes and EC for many years

**Findings:**Air concentrations of nicotine ranged from 0.82 to 6.23 µg/m³. The average concentration of nicotine resulting from CC was 10 times higher than from EC (31.60 ± 6.91 vs. 3.32 ± 2.49 µg/m³, respectively; p = .008) The mean concentration of PM2.5 from CC was 7 times higher than from EC (819.3 ± 228.6 vs. 151.7 ± 86.8 µg/m³, respectively; p = .008).

Both studies: VOCs: only toluene was detected No changes in CO concentration after use of EC

**Additional Information:** Tested only 3 brands Measured a limited number of chemicals Assessed concentrations of several markers in the air but not serum concentrations in people exposed to secondhand vapor

Using EC in indoor environments may involuntarily expose nonusers to nicotine but not to toxic tobacco-specific combustion products

**Aim:** To assess in an evaluation approach model if flavour ingredients have the potential to induce contact sensitization (delayed “Type IV” hypersensitivity)

**Methodology:** A flavor ingredient screening and risk assessment process flow

**Findings:**The approach developed here applies both to single ingredients and to components of naturals

In example Geraniol 1% is not below 1000ppm but has no sensitization potential and the sensitizer level is supportable

Isoeugenol 3% is not below 1000ppm and has sensitization potential and the sensitizer level is not supportable

**Additional Information:** Calculations only, no testing Presents a contact sensitization and risk assessment model
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Country</th>
<th>Methodology</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Farsalinos KE [46] 2015</td>
<td>▲ 13</td>
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<td>Etter JF [41] 2013</td>
<td>▲ 2</td>
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<td>▲ 2</td>
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<tr>
<td>El-Hellani A [38] 2015</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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</tbody>
</table>

### References

- **Farsalinos KE [46] 2015**
  - 159 sweet-flavored samples from 36 manufacturers and retailers in 7 countries
  - 3 liquids were prepared by dissolving
  - Vapor
  - A modified version of the High Performance Liquid Chromatography (HPLC) carbonyl compound analysis
  - DA and AP in 74.2% of the samples
  - DA and AP in 74.2% of the samples
  - Sweet flavors only
- **Etter JF [41] 2013**
  - Prefilled EC cartridges of the Vapor for Life, V2, Green Smoke, Apollo, Bull Smoke, Halo, G6, Bluewater, and Blu brands in various nicotine concentrations were procured from US Internet vendors as were samples of EC liquid refill solutions: My Freedom Smoke Do It Yourself (100 mg/mL)
  - Fluid and vapor
  - A solvent extraction method for determining total nicotine and its partitioning in EC liquids and aerosols by gas chromatography
  - Most of the nicotine was in the free-base form, with aerosols exhibiting higher free-base nicotine fraction than the parent liquids
  - Apparent pH was found to correlate with nicotine partitioning and can provide a useful indirect measure when chromatography is unavailable
- **El-Hellani A [38] 2015**
  - Prefilled EC
  - Fluid and vapor
  - Refill fluids
  - E-liquids diluted with ammonia solution
  - With each brand: some differences between the duplicates
- **Annexes**
  - Half of the liquids analyzed contained up to five times the maximum amount of impurities specified in the European Pharmacopoeia
  - The nicotine content in the samples generally corresponded to the labels on the bottles
  - No clinical evidence indicating that calculated cut-off level set by
| Farsalinos KE | ✦ 14 | 21 samples (10 conventional EC liquids and 11 Natural Extract of Tobacco (NET) liquids) were obtained from the US and Greek market | − Fluids
  − Aim: to evaluate nicotine levels and the presence of tobacco-derived toxins in tobacco flavored conventional EC liquids and NET liquids
  − Nicotine levels were measured and compared with labelled values
  − The levels of tobacco-derived chemicals were compared with literature data on CC products
  − 12 samples had nicotine levels within 10% of the labelled value
  − TSNAs were present in all samples at ng/mL levels.
  − Total TSNAs and nitrate were present at levels 200–300 times lower in NET liquids; Flavourart RY4 = 40 ng/mL.
  − Nitrates were present at levels mostly in NET liquids; Flavourart RY4 = 40 ng/mL.
  − Acetaldehyde was present predominantly in conventional liquids; liquid AtmosLab RY69=20 ng/mL.
  − Formaldehyde was detected in almost all EC liquids at trace-levels.
  − Phenols were present in trace amounts, mostly in NET liquids, compared to CC liquids. |
| Farsalinos KE [45] 2015 | | | − Not vapor
  − Inter-batch variability not tested
  − Compares levels in EC liquid with level of CC smoke
  − Compares 1 ml EC liquid with 1 gram CC
  − Formaldehyde and acetaldehyde are formed during the heating process of EC – underestimation of true exposure! |
| Farsalinos KE [52] 2015 | ✦ 15 | Two studies were found in the literature, measuring metals emitted to the aerosol from 13 EC products | − Literature study
  − A batch of 13 EC products was tested including the amount of metal emissions based on findings from the published literature
  − Literature study only
  − Products tested were used for the first time during the study sessions – but there might be a change in the stability and related metal emissions after some days of use
  − Some safety limits are for occupational exposure |
| Farsalinos KE [44] 2015 | ✦ 27 | Three 100-puff sets were trapped in filter | − Only NAB was found at trace levels in two commercial liquids (1.2 and 2.3 ng/g), while the third |
| | | Inhalation (1 ng nicotine/ml) of tobacco flavor, Greek EC | − Study was not designed to detect whether the |
| | | | − The levels of daily exposure from EC use are significantly lower compared to acceptable exposure from inhalational medications and by orders of magnitude lower than the regulatory limits for daily occupational exposure |
| | | | − Natural Extract of Tobacco liquids contained higher levels of phenols and nitrates, but lower levels of acetaldehyde compared to conventional EC liquids |
| | | | − All EC liquids contained far lower (by 2–3 orders of magnitude) levels of the tobacco-derived toxins compared to CC |

Annexes – 10
<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Page</th>
<th>Text</th>
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<tbody>
<tr>
<td>2013</td>
<td>Farsalinos KE</td>
<td>29</td>
<td>• 20 EC liquid samples (17 tobacco flavors, 3 sweet or fruit flavors), 4 samples produced by using cured tobacco leaves 1.set: lithium battery (eGo), a 2.2-Ohms atomizer (510 T) and a tank-type cartridge 2.set: variable-voltage device (Lavatube), total energy 9.2 watts  • Ref: 1.&quot;base&quot; liquid sample (50% glycerol/50% propylene glycol, with no nicotine or flavorings) 2. CC Marlboro, 0.8 mg nicotine  • Vapor  • Aim: to evaluate the cytotoxic potential of the vapor of on cultured myocardial cells  • Cytotoxicity was tested according to the ISO 10993-5 standard  • CC smoke was produced according to ISO 3308 method  • The extracts, undiluted (100%) and in four dilutions were applied to myocardial cells (H9c2); percent-viability was measured after 24 h incubation. According to ISO 10993-5, viability of &lt;70% was considered cytotoxic  • Three EC extracts (produced by tobacco leaves) were cytotoxic at 100% and 50% extract conc.  • One (&quot;Cinnamon-Cookies&quot; flavour) was cytotoxic at 100% conc.  • For EC extracts produced by high-voltage and energy, viability was reduced but no sample was cytotoxic according to ISO 10993-5 definition  • Cell survival was not associated with nicotine conc. of EC liquids  • CC smoke extract was cytotoxic at extract conc. &gt;6.25% Inhibitory conc. 50 was &gt;3 times lower in CC smoke extract compared to the worst-performing EC vapour extract.  • Are the EC extracts comparable to CC smoke extract?  • Study indicates that some EC samples have cytotoxic properties on cultured cardiomyoblasts  • Cytotoxicity was mainly observed in samples produced by using tobacco leaves  • All EC vapor extracts were significantly less cytotoxic compared to CC smoke extract  For EC extracts produced by high-voltage and energy, viability was reduced</td>
</tr>
<tr>
<td>2015</td>
<td>Feng Y [54]</td>
<td>28</td>
<td>• Hypothetical EC vapor and CC smoke  • Vapor  • Aim: to provide fundamental understanding of the dynamics and transport of aerosols from an EC in and idealized tubularG3–G6 respiratory tract model  • A computational model has been developed that includes the effects of hygroscopic growth as well as evaporation from multicomponent aerosol droplets  An experimentally validated computational fluid-particle dynamics (CF-PD) model is  • Due to the combined multicomponent evaporation/condensation effects, all EC-droplets will undergo size-changes  Vaporization/condensation of a droplet will be influenced by its initial temperature for a negligible time duration after the droplet has been released from the inlet  After the droplet temperature quickly approaches the ambient temperature, water vapor start to condensate at the droplet surface, leading to hygroscopic growth, i.e., droplet-size  • Computer simulation model, not human experiment  • The results indicate that EC-droplets, being more hygroscopic than CC smoke particles, tend to grow larger in maximum size in a typically highly humid environment</td>
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<td>Author</td>
<td>Year</td>
<td>Type</td>
<td>Notes</td>
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<tr>
<td>Fernández E</td>
<td>2015</td>
<td>No</td>
<td>+ Unknown</td>
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<tr>
<td>Fouco FC</td>
<td>2013</td>
<td>No</td>
<td>+ 2 rechargeable models A and B) and one disposable model (C)  - 4 liquid flavors, liquid nicotine contents (low, medium, high)  - Reference: CC Marlboro, 0.8 mg nicotine  - Tested few brands  - Few brands</td>
</tr>
<tr>
<td>Geisse O</td>
<td>2014</td>
<td>No</td>
<td>+ Two ‘second generation’ refillable EC  - Type A and type B EC were equipped with a 280 mAh and 180 mAh battery, respectively  - Two refill liquids: “traditional”= approximately equal parts of PPG and glycerol as a base and 10% water. ‘Velvet’ consisted of only glycerol (80%) and water (20%)  - Each with three</td>
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</table>

Annexes – 12
<table>
<thead>
<tr>
<th>Gomiewicz ML [65] 2013</th>
<th>▲ 5</th>
<th>different amounts of nicotine</th>
<th>5 UK brands (6 products) with high internet popularity, high and extra high nicotine content&lt;br&gt;Ref product: CC</th>
<th>Fluid and vapor&lt;br&gt;Aim: determine the nicotine content in fluid and vapor and estimate the safety and consistency of nicotine delivery across batches&lt;br&gt;Gas chromatography with the Thermionic Specific Detector</th>
<th>The nicotine content of cartridges within the same batch varied by up to 12% relative standard deviation&lt;br&gt;Mean difference between different batches of the same brand ranged from 1% to 20% for five brands and 31% for the sixth&lt;br&gt;The puffing schedule vaporized 10–81% of the nicotine&lt;br&gt;The nicotine delivery from 300 puffs ranged from approx. 2 mg to 15 mg and was not related significantly to the variation of nicotine content in e-liquid (r = 0.06, P = 0.92).&lt;br&gt;Tested few brands&lt;br&gt;There is very little risk of nicotine toxicity from major EC brands in the United Kingdom.&lt;br&gt;Variation in nicotine concentration in the vapor from a given brand is low.&lt;br&gt;Nicotine concentration in e-liquid is not well related to nicotine in vapor&lt;br&gt;None of the tested products reached nicotine concentrations as high as CC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gomiewicz ML [66] 2013</td>
<td>▲ 3</td>
<td>12 brands of EC&lt;br&gt;Most popular brands in Poland&lt;br&gt;Ref product: Medicinal nicotine inhalator Nicorette 10 mg and CC (not tested, used from other reference)</td>
<td>Vapor&lt;br&gt;Aim: test content of four groups of potentially toxic and carcinogenic compounds: 15 carbonyls, 11 volatile organic compounds, 2 nitrosamines, 12 heavy metals</td>
<td>Vapours: using a modified smoking machine.&lt;br&gt;The selected toxic compounds were extracted from vapours into a solid or liquid phase&lt;br&gt;Analysed with chromatographic and spectroscopic methods</td>
<td>Detected in EC:&lt;br&gt;4 carbonyls (formaldehyde (2.0–56.1 µg), acetaldehyde (1.1–13.6 µg), O-methylbenzaldehyde (1.3–7.1 µg) and acrolein (0.7–41.9 µg) and 2 volatile organic compounds (toluene (0.2–6.3 µg), and p-m-xylene) identified in almost all EC.&lt;br&gt;In 9 vapors: Both nitrosamines, NNN (0.8–4.3 ng), and NNK (1.1–28.3 ng), identified&lt;br&gt;In all vapors: 3 metals, cadmium (0.01–0.22 µg), nickel (0.11–0.29 µg) and lead (0.03–0.57 µg) identified Nicorette inhalator: Trace amounts of cadmium, nickel, lead, formaldehyde, acetaldehyde and O-methylbenzaldehyde were detected&lt;br&gt;No volatile organic compounds</td>
</tr>
<tr>
<td>Gomiewicz ML [67] 2013</td>
<td>▲ 4</td>
<td>16 EC&lt;br&gt;15 most popular brands in Poland, UK and USA&lt;br&gt;20 cartridges and 15 nicotine refill solutions&lt;br&gt;Paired each tested EC with cartridges of same brand and same batch and series&lt;br&gt;No ref product</td>
<td>Vapor&lt;br&gt;Aim: test efficacy and consistency of various EC in converting nicotine to vapor&lt;br&gt;Vapors: generated using an automatic smoking machine&lt;br&gt;Nicotine was absorbed in a set of washing bottles with methanol and analyzed with gas chromatography&lt;br&gt;Three samples of each refill solution</td>
<td>The total level of nicotine in vapor generated by 20 series of 15 puffs varied from 0.5 to 15.4 mg.&lt;br&gt;Most of the analyzed ECs effectively delivered nicotine during the first 150–180 puffs.&lt;br&gt;On an average, 50%–60% of nicotine from a cartridge was vaporized&lt;br&gt;High consistency between the results of one product tested in both studies</td>
<td>The puffing profile used may not reflect actual user puff topography–actual doses of toxicants inhaled by EC users might be higher&lt;br&gt;Small number of samples from each product</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Products</td>
<td>Study Design</td>
<td>Results</td>
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</tbody>
</table>
| Gomiewicz ML [64] | 2015 | ▲ 17 | ▲ -32, 29 and 30 e-liquids purchased between 2013 and 2014 from locations in the United States (US), South Korea, and Poland, respectively  
▲ Fluid  
▲ Aims: to test nicotine concentrations measured using gas chromatography with a nitrogen–phosphorus detector (GC-NPD, Agilent, USA).  
▲ Modified standard NIOSH 2551 method for determination of nicotine in air  
▲ Significant discrepancies (>20%) in the labelled nicotine concentrations in 19% of analysed e-liquids.  
▲ US: nicotine concentration varied from 0 to 36.6 mg/mL. Traces of nicotine were found in 3 products labelled as ‘nicotine free’.  
▲ South Korea: two-thirds of products did not contain detectable amounts of nicotine. Nicotine concentration in other products varied from 6.4 ± 0.7 to 150.3 ± 7.9 (labelled as ‘pure nicotine’) mg/mL.  
▲ Poland: nicotine concentration varied from 0 to 24.7 ± 0.1 mg/mL.  
▲ Tested only one batch of liquid  
▲ Most of the analysed samples had no significant discrepancies in labelled nicotine concentrations and contained low nicotine levels  
▲ Some products labelled as ‘nicotine-free’ had detectable levels of the substance  
▲ Quality of the products may differ across countries |}

| Gomiewicz ML [68] | 2015 | ▲ 21 | ▲ -3 products, with different flavors based on their popularity:  
▲ Go reusable tank system  
▲ Ecto Cooler liquid, 24 mg/ml nicotine, orange and tangerine flavor or Bubblegum eJuice, 32 mg/ml nicotine  
▲ 801-T nicotine + Ecto Cooler liquid, 24 mg/ml nicotine, orange and tangerine flavor  
▲ Blu disposable, 20–24 mg nicotine, classic tobacco flavor  
▲ No reference  
▲ Vapor  
▲ Aims: to investigate whether nicotine from EC can be deposited on various surfaces  
▲ Released 100 puffs from each product directly into an exposure chamber  
▲ Surface wipe samples were taken from 5 indoor 100 cm² surfaces (window, walls, floor, wood, and metal) pre- and post-release of vapors  
▲ Nicotine was extracted from the wipes and was analyzed using gas chromatography  
▲ 3 of the 4 experiments showed significant increases in the amount of nicotine on all five surfaces.  
▲ The floor and glass windows had the greatest increases in nicotine  
▲ The average amount of nicotine deposited on a floor during each experiment was 205 µg/m² and varied from limit of quantitation to 550 µg/m²  
▲ Small sample size  
▲ Short term exposure  
▲ Controlled laboratory settings, not real life  
▲ Did not investigate the effect of exhaled vapors by the users but simulated exposure conditions  
▲ Study indicates that there is a risk for third-hand exposure to nicotine from EC  
▲ Third-hand exposure levels differ depending on the surface and EC brand |}

| Hadwiger ME [69] | 2010 | No | ▲ -3 Cartridges + 2 refill liquids labeled as containing Cialis  
▲ -3 Cartridges + 2 refill liquids labeled as containing Rimonabant  
▲ -Labeled with nicotine content  
▲ -No ref product  
▲ -Cartridges and refill liquids  
▲ -Aim: test the presence of unapproved active pharmaceutical ingredients  
▲ -A high-pressure liquid chromatography-diode array detection and multi-mode ionization tandem mass spectrometry method  
▲ -Products advertised as containing Cialis did not contain tadalafil, rather they contained amino-tadalafil.  
▲ -Products advertised as containing rimonabant, did contain rimonabant and a significant amount of an oxidative impurity of rimonabant  
▲ -Products advertised as containing no nicotine, did contain nicotine  
▲ -Tested only one batch of liquid  
▲ -The used method was inadequate for resolution of certain nicotine impurities  
▲ -Not vapor  
▲ -Presence of unapproved active pharmaceutical ingredients added  
▲ -Presence of undisclosed degradation of advertised ingredients  
▲ -Nicotine-free products contained nicotine |}

| Hahn H [70] | 2014 | No | ▲ -54 samples  
▲ -Liquids (n = 20) submitted for official medicines and tobacco control purposes  
▲ -Samples suspected of  
▲ -Fluid  
▲ -Aim: to test the compounds contained  
▲ -NMR spectroscopy  
▲ -Risk assessment was based on probabilistic exposure estimation and comparison with toxicological  
▲ -18 from 23 samples were confirmed as nicotine-free. In one EC liquid nicotine was not detected while being declared on the labelling. Major compounds: glycerol, propylene glycol, and ethylene glycol  
▲ -Fluid only  
▲ -Used thresholds for oral exposure – not for inhalation  
▲ -From all compounds tested, only nicotine reached exposures that fall into a high risk category  
▲ -Solvents with more favourable toxicological profiles should be used | Annexes – 14
Annexes – 15

containing illegal or unusual substances, tobacco and beverage flavour
◦ All varieties of declared nicotine content
◦ No ref product

thresholds using the margin of exposure (MOE) approach
◦ Furthermore, 1,3-propanediol, thujone and ethyl vanillin were detected
◦ The average exposure for daily users was estimated as 0.38 mg/kg bw/day for nicotine, 8.9 mg/kg bw/day for glycerol, 14.5 mg/kg bw/day for 1,2-propanediol, 2.1 mg/kg bw/day for ethylene glycol, and below 0.2 mg/kg bw/day for the other compounds. The MOE was below 0.1 for nicotine, but all other compounds did not reach MOE values below 100 except ethylene glycol and 1,2-propanediol instead of ethylene glycol and 1,2-propanediol, which may fall into a risk category

<table>
<thead>
<tr>
<th>Han S [71]</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 refill solutions for 17 brands on the Chinese market</td>
<td></td>
</tr>
<tr>
<td>Fluid</td>
<td></td>
</tr>
</tbody>
</table>
- Aim: to develop methods and to assess the levels of eight groups of compounds |
- The total mass% of propylene glycol and glycerol in most refill solutions ranged from 80%–97% |
- Triethylene glycol was detected in one sample and menthol was found in 16 samples including in samples that were not labeled as “mint”. |
- The labeled concentrations of nicotine of the 25 samples were not consistent with, and were in most cases lower than the measured concentrations |
- The concentrations of nicotine in samples that were labeled at the same “strength” (eg, HIGH, MIDDLE, or LOW) differed significantly among brands |
- Selected groups of compounds including TSNAs, solanesol, VOCS, PAHs, phenolic compounds, and carbonyl compounds were all detectable, with varying levels and detection frequencies |
- Only refills analysed, should also be vapor |
- Methods failed to separate positional isomers |
- Glycol and glycerol constitute the major ingredients of most refill solutions, and also indicated the necessity for clearly and accurately labeling nicotine content of e-liquids |
- Compounds that may originate from tobacco, solvents or other sources, such as TSNAs, solanesol, VOCS, PAHs, phenolic compounds, and carbonyl compounds were all found with different levels and detection frequencies

<table>
<thead>
<tr>
<th>Herrington JS [74]</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four commercially available EC (first generation) were chosen from the “Best E-Cigarettes of 2014”</td>
<td></td>
</tr>
<tr>
<td>Fluid and aerosol</td>
<td></td>
</tr>
</tbody>
</table>
- Aim: evaluating e-cigarette solutions and their resultant aerosol for potential differences |
- Multi-sorbent thermal desorption (TD) tube |
- Gas chromatography (GC) mass spectrometry (GC–MS) method |
- Detectable levels of >115 VOCS and semivolatile organic compounds (SVOCs) from a single 40 mL puff |
- Solution profiles produced upwards of 64 unidentified and identified (somewhat tentatively) constituents and aerosol profiles produced upwards of 82 compounds. |
- Distinct analyte profiles between liquid and aerosol samples |
- Formaldehyde, acetaldehyde, acrolein, and siloxanes were found in the aerosol profiles; however, these |
- First generation EC only |
- Fluid profiles produced upwards of 64 unidentified and identified constituents, and aerosol profiles produced upwards of 82 compounds |
- Formaldehyde, acetaldehyde, acrolein, and siloxanes were found in the aerosol profiles; however, these compounds were never present in the solutions |
- The aerosolization process
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study Design</th>
<th>Materials/Methods</th>
<th>Findings</th>
<th>Relevance</th>
</tr>
</thead>
</table>
| Higham AJ | 2014 | No | Unknown | - Vapor extract from six healthy non-smokers were exposed to EC vapor extract for 6 hr.  
- Alveolar macrophages isolated from resected lung tissue from three ex-CC smokers exposed to vapor extract for 24 hr.  
- ELISA  
- Zymography  
- EC exposure to cells: Increased MMP-9 and CXCL8 release with the maximal effect observed at an optical density (OD) of 0.003  
- Increase in MMP-9 gelatinase activity and increased p38 MAPK activation  
- Neutrophil shape change, and dual CD11b and CD66b expression increased in response to vapor extract treatment compared to untreated cells  
- Increase in CXCL8 release from alveolar macrophages | - In vitro study only  
- In vitro study shows that EC exposure causes an inflammatory response from neutrophils and macrophages  
- The effects are similar to those caused by CC |
| Husari A | 2015 | No | Pre-filled V4L CoolCart (strawberry flavor, 3.5 Ohm, 18 mg/mL labeled nicotine concentration) cartomizer cartridges, connected to an automatically actuated 4.2 V Vapor Titan Soft Touch battery Ref: CC smoke | - Pre-filled V4L CoolCart (strawberry flavor, 3.5 Ohm, 18 mg/mL labeled nicotine concentration) cartomizer cartridges, connected to an automatically actuated 4.2 V Vapor Titan Soft Touch battery Ref: CC smoke | - Both EC and CC smoke extracts reduced cell proliferation, however, CC smoke exhibited effects at lower concentrations |
| Hutzler C | 2014 | No | 28 liquids of seven manufacturers purchased in Germany  
- 10 liquids were declared “free-of-nicotine” Reference: no | - Fluid and vapor  
- Aim: to analyze content of e-fluids  
- Gas chromatography method, in conjunction with a flame ionization detector (GC–FID)  
- Standardized machine smoking protocol to mimic human smoking behavior, Borgwaldt RM20H smoking machine  
- 7 out of 10 liquids declared as nicotine-free were identified containing nicotine in the range of 0.1–15 µg/ml.  
- In 18 liquids, no declaration regarding nicotine was provided by the manufacturers – 16 contained nicotine.  
- Ethylene glycol replaced glycerol and propylene glycol in 5 brands  
- Coumarin and acetamide detected  
- Significant amounts of formaldehyde, acetaldehyde and propionaldehyde were only found at 150 °C by headspace GC–MS analysis  
- High amounts of aldehydes can be reached - comparable or even higher as in CC - in the last part of the Eden  
- Overheating?  
- Many ECs labeled as ‘nicotin free’ contained nicotine  
- Release of aldehydes is strongly enhanced in the second half of the vaping period  
- The occurrence of aldehydes seems to be associated with lower liquid levels within the cartridges, leading to an increased air flow - could promote overheating of the wire | - Many ECs labeled as ‘nicotin free’ contained nicotine  
- Release of aldehydes is strongly enhanced in the second half of the vaping period  
- The occurrence of aldehydes seems to be associated with lower liquid levels within the cartridges, leading to an increased air flow - could promote overheating of the wire |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Authors</th>
<th>Sample Type</th>
<th>Description</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingebritsen BJ [80] 2012</td>
<td>No</td>
<td>Rechargeable EC and a non-rechargeable EC</td>
<td>Vapor</td>
<td>Particle size distribution of aerosols produced by EC was measured in an undiluted state by a spectral transmission procedure and after high dilution with an electrical mobility analyzer</td>
<td>Particle diameters of average mass in the 250–450 nm range and particle number conc. in the 109 particles/cm3 range, the same as in previous CC smoke studies</td>
</tr>
<tr>
<td>Jensen RP [81] 2015</td>
<td>No</td>
<td>Unknown commercial e-liquid vaporized with the use of a “tank system” EC featuring a variable voltage battery</td>
<td>Vapor</td>
<td>Aerosolized liquid was collected in an NMR spectroscopy tube</td>
<td>At low voltage (3.3 V): did not detect the formation of any formaldehyde-releasing agents (estimated limit of detection, approximately 0.1 µg per 10 puffs) At high voltage (5.0 V): a mean (±SE) of 380±90 µg per sample (10 puffs) of formaldehyde was detected as formaldehyde-releasing agents Extrapolating from the results at high voltage, an EC user vaping at a rate of 3 ml per day would inhale 14.4±3.3 mg of formaldehyde per day in formaldehyde-releasing agents</td>
</tr>
<tr>
<td>Kavvalakis MP [82] 2015</td>
<td>No</td>
<td>EC-liquid samples, produced by 13 companies obtained from the Greek market</td>
<td>Fluid</td>
<td>Gas and liquid chromatography–mass spectrometry</td>
<td>Details on accuracy of measurement are described The measured concentrations of nicotine correlated with the theoretical concentrations as reported by the manufacturers An analog relation between the concentration of the glycerol and of propylene glycol was noticed 141 volatile flavors detected Nitrosamines and PAHs were not detected in any sample</td>
</tr>
<tr>
<td>Kienhus AS [83] 2015</td>
<td>No</td>
<td>Disposable, nicotine-free shisha-pens (3 strawberry, 1 apple and 1 grape) bought in a local store</td>
<td>Fluid and vapor</td>
<td>Gas chromatography analysis on a Varian GC 3900/FID</td>
<td>Main components: propylene glycol and glycerol (54%/46%). One puff (50 to 70 mL) resulted in exposure of propylene glycol and glycerol of 430 to 603 mg/m3 and 348 to 495 mg/m3, respectively. Exposure concentrations were higher than the points of departure for airway irritation based on a human study and a rat study Few samples Differences between studies and the actual exposure (e.g. differences in duration of exposure and differences between animals and human scan)</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>No</td>
<td>Products and/or Refill</td>
<td>Aim</td>
<td>Method</td>
</tr>
<tr>
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</tr>
<tr>
<td>Kim H-J [84] 2013</td>
<td>No</td>
<td>105 refill liquid brands from 11 EC companies in South Korea</td>
<td>Refill liquids</td>
<td>Aim: test for carcinogenic compounds and conc. of four TSNAs - NNN - NNK - NAB - NAT</td>
<td>A liquid chromatography–tandem mass spectrometric method - Solid-phase extraction and liquid–liquid extraction were compared to each other to select the optimum cleanup method</td>
</tr>
<tr>
<td>Kim S [85] 2015</td>
<td>No</td>
<td>32 liquid refill products (17 Korean domestic, 15 imported) and one pure nicotine product at 6 different EC retail stores in Seoul between May and June 2014</td>
<td>Fluid</td>
<td>Aim: to examine the level of heterogeneity of contents of the labels and discrepancy of the nicotine content between that indicated on the label and the actual values for EC liquid refill products in South Korea</td>
<td>Analysed at the Roswell Park Cancer Institute, Buffalo, NY, USA by a blinded analyst using gas chromatography with a thermionic specific detector</td>
</tr>
<tr>
<td>Kim YH [86] 2015</td>
<td>No</td>
<td>Θ</td>
<td>EC device (Korea) and an EC solution without nicotine (Korea)</td>
<td>Fluid, vapor, and aerosol</td>
<td>Mass change tracking approach - TD-GC-MS system</td>
</tr>
</tbody>
</table>

**Notes:**
- TSNAs: volatile organic carcinogenic compounds
- TSNAs levels-NNN may be produced from nitrosation of nicotine converted from nicotine
- Refill liquid nicotine from a separate bottle = uncontrolled or inaccurate dose of nicotine
- Only one of each product
- Almost all fluids contained carcinogenic compounds, TSNAs
- High maximum conc. of total TSNAs
- Great variability in content of the four measured TSNAs
- There is no standardization of EC liquid labelling
- The labels did not accurately reflect the content
- The measured nicotine concentration was significantly lower than the labeled nicotine concentrations
- One product labeled 'pure nicotine' raises concerns, since it may be poisonous to consumers, especially to children
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Methodology</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirschner R [87] 2013</td>
<td>No</td>
<td>- 6 samples of e-liquids with different flavors</td>
<td>- Fluid: Aim: to test content of nicotine and compare with declared content - Dissolved in methanol: Analyzed with liquid chromatograph mass spectrometer - Isotope dilution method - All bottles contained nicotine 14.8 to 87.2 mg/ml - Measured concentration of nicotine differed from declared by up to 50% - No undeclared ingredients identified - Alkaline pH</td>
</tr>
<tr>
<td>Kosmider L [88] 2014</td>
<td>▲ 7</td>
<td>- Ten kinds of commercially available e-liquids- nicotine concentration 18 to 24 mg/ml - Vapors were generated using three different battery voltages: 3.2, 4.0, and 4.8 V - Reference: pure glycerin, pure propylene glycol, or a mixture of both solvents (50:50) - Vapor: Aim: to evaluate how various product characteristics, including nicotine solvent and battery output voltage, affect the levels of 12 carbonyls in EC vapor - 1 ml of each e-liquid was collected and 10 clearomizers of the same type were refilled 24 hr before aerosol generation. - Each clearomizer was used only for one e-liquid - Vapors from ECs were generated using the automatic smoking machine Palacbot (2 series of 15 puffs with a 5-min interval) - Formaldehyde and acetaldehyde were found in 8 of 13 samples. - The highest levels of carbonyls were observed in vapors generated from PPG-based solutions. - Increasing voltage from 3.2 to 4.8 V resulted in 4 to over 200 times increase in formaldehyde, acetaldehyde, and acetone levels. - The levels of formaldehyde in vapors from high-voltage device were in the range of levels reported in tobacco smoke. - Puffing topography may affect levels of carbonyls released from different ECs. - There are some discrepancies between puffing regime used in this study and the results of clinical studies. - This finding suggests that in certain conditions ECs might expose their users to the same or even higher levels of carcinogenic formaldehyde than CC smoke. - High-voltage EC may expose users to high levels of carbonyl compounds. - Vapors from EC contain toxic and carcinogenic carbonyl compounds. - Both solvent and battery output voltage significantly affect levels of carbonyl compounds in EC vapors</td>
<td></td>
</tr>
<tr>
<td>Kubica P [89] 2014</td>
<td>No</td>
<td>- 37 samples from different producers of popular EC were purchased on the local market - The labels did not contain any information about carbohydrate content Ref: no</td>
<td>- Fluid: Aim: to test high performance liquid chromatography in hydrophilic interaction liquid chromatography mode and tandem mass spectrometry for fast and simple determination of sucrose and other saccharides in - Q-Trap 4000 triplequadrupole mass spectrometer from Applied Biosystems with electrospray ionization in negative mode, using Analyst® 1.5.2. - The chromatographic separation was done using an Ascentis Express OH5 column - It was possible to determine the presence of sucrose and other saccharides such as fructose, glucose, maltose and lactose. - Only sucrose was found in all samples of e-liquids. - The detection limit of sucrose was 0.73 µg/g, and the sucrose content ranged from 0.76 to 72.93 µg/g (chocolate flavor) - The harmful effect of sucrose is hypothesized. - Sucrose was found in all samples of e-liquids; the presence of sucrose in EC may be a source of aldehydes and organic acid. - The source of sucrose in EC is unknown (flavor/taste additives or a contaminant from the production process?)</td>
</tr>
<tr>
<td>Date</td>
<td>Title</td>
<td>Description</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| 2009 | Laugesen M [91] (abstract in 2 versions) | - Ryan EC 16 mg nicotine  
  - Ref: CC. 4 different: NZ Holiday regular and mild, Marlboro Red regular, Canadian regular brands  
  - Aims: test toxic emissions and nicotine dose and measure particle size  
  - Selection of 59 toxicants for testing of mist was based on published priority lists, e.g. from WHO, of CC smoke toxicants  
  - Smoke tests by ISO smoking machine  
  - Liquid and mist tested by different laboratories and methods (detailed)  
  - Particle size distribution measured  
  - A score for toxic emissions: CC=100-134, EC=0  
  - Mercury detected in trace quantity, 0.17 ng per EC  
  - Nicotine per puff: CC 48-103 (max puffing intensity), EC=9-10.  
  - Not tested: acetaldehydes (shortage of reagent), hydrazine, chlorinated dioxans, oxides of nitrogen and urethane  
  - Particle size: 0.04 microns. Smoke from CC: >0.15 microns (measured on a different instrument)  
  - Tested only one brand/(batch?)  
  - Only a score for toxic emissions presented, not individual toxins  
  - Tobacco smoke measure on a different instrument  
  - Tested by ISO smoking machine, no human puffing behaviour  
  - Very low operating temperature  
  - In the version from April: Acetaldehyde both mentioned as present but also as not tested.  
  - Very low score for toxic emissions (based on >50 toxicants)  
  - Small particle size  
  - Mercury detected  
  - Nicotine dose and particle size too small to ensure deposition in the alveoli/bronchioles and rapid nicotine absorption as in cigarette smoking |
| 2008 | Laugesen M [93] | - Ryan EC 16, 11, 6 and 0 mg nicotine  
  - Ref: for CO measurement: CC  
  - Fluid  
  - Aims: test toxic emissions and nicotine dose, safety for bystanders (by CO in exhaled breath) and risk of microorganisms  
  - Risk of microorganisms tested as aerobic plate count 35° in one unused and one repeatedly used cartridge  
  - VOC: Acetaldehyde= 9.4 ppm Benzen= 1.5 ppm, Acrolein = 0.49 ppm. Other VOCs< LOQ  
  - CO: in EC =1.5, compared to 9-14 in exhaled breath of CC smoker  
  - Smoke toxicants as butadiene and acrylonitrile <0.3 ppm  
  - Labeling of nicotine= actual content  
  - No tendency for microorganisms to grow in the liquid  
  - Metal (n=8) all <1 ppm, not a risk  
  - TSNAs= 8 ng/g, same as nicotine gum. CC smoke=500 ng/g  
  - MAO inhibition= no sign. effect  
  - Tested only one brand  
  - No detailed description of test methods  
  - Acetaldehyde, benzene, acrolein and TSNAs detected at low levels  
  - Metals, CO and other VOCs at lower limits than detection |
| 2008 | Laugesen M [90] | - Ruyan® EC with different nicotine content 0 to 16 mg  
  - Ref: CC  
  - Fluid and vapor  
  - Aims: to test the safety of the Ruyan® EC  
  - Use of different measurements methods  
  - GC- Mass Spectrograph  
  - SIFT- Mass Spectrograph  
  - Head Space Solid-Phase Micro-Extraction  
  - Selected Ion Flow Tube and Mass Spectrograph  
  - TSNAs, found only in CC, were not found in the Ruyan® EC liquid except at trace quantity (Average TSNAs 3.9 ng/cartridge): 1200 times less than in 20 CC  
  - Absence of a MAO inhibitor effect: EC has no detectable addictive potential beyond that of nicotine  
  - Compounds identified: propylene glycol, ethyl alcohol, nicotine, acetaldehyde, pyridine, and acetone  
  - Acetaldehyde and acrolein found in  
  - Tested only one brand  
  - The composition of the cartridge liquid is not hazardous to health  
  - After a revised formulation from 2007 to 2008: acetaldehyde, acrolein, benzene and cresols in EC decreased, or not measurable |
- CO measurement: 48 volunteer smokers. A non-smoker, not exposed to passive smoking: 20 inhalations of EC head space measurements
- After a revised formulation: acetaldehyde, acrolein, benzene and cresols decreased, or not measurable
- PAH carcinogens found in CC smoke are not detectable in the EC liquid. PAHs that were detected are not rated as carcinogens by IARC.
- No arsenic, antimony, cadmium, chromium, cobalt, copper, lead, manganese or nickel detected
- No gamma-emitting nucleotides were found to be above the detection limit
- No increase in CO

| Laugesen M [92] 2015 | ▲ 18 | - 14 EC brands with tobacco flavour available in New Zealand (8 from China, 6 from UK and USA) purchased via internet
- Vapor (mainstream aerosol)
- Aim: to analyse EC brands available in New Zealand for nicotine content and toxicant yield ratings (toxic aldehydes and glycols)
- Health Canada standards smoking machine (70 ml puff, 3 s puff duration, 10 s interval)
- High-performance liquid chromatography with ultra-violet detection
- Gas chromatography
- Mean aldehydes in vapor were 73% lower than in ref-EC Ryan from 2008
- 100 times less formaldehyde, 2800 times less acetaldehyde, 200 times less acrolein than CC
- DEG and MEG below detection level
- Mean nicotine level has increased since 2008
- Differences between labeled and actual nicotine level
- Tested one batch
- Tested by smoking machine, not = human puffing behaviour
- EC available in New Zealand in 2013 exposed users to higher nicotine levels than in older brand
- Far lower levels of toxicant than in CC and older EC brand

| Lauterbach JH [94] 2012 | ▲ 10 | - Ryan classic V8
- Ref: Marlboro KS and very low tar 1.2 mg CC
- Vapor (mainstream aerosol)
- Aim: test toxic emissions and nicotine dose
- ISO standards smoking machine (35 ml puff, 2 s puff duration, 60 puff interval)
- Of 62 CC toxicants 37 were measurable in the very low tar CC and 11 in EC vapor (acetaldehyde 1.39 µg, formaldehyde 0.37 µg. Estimated relative toxicant emission scores: 0.4 for EC, 55 for very low tar CC and 137 for Marlboro KS CC
- Mercury present at trace level
- 3 TSNs (NNN, NNK, and NAT) present at trace level - much lower than CC
- Low nicotine level 0.06 mg (compared with 1.02 in CC)
- Tested only one brand
- Tested by ISO smoking machine, not = human puffing behaviour
- Acetaldehyde, formaldehyde, TSNs and mercury detected
- Compared to CC level of toxins and carcinogens were reduced by >90%

| Lauterbach JH [95] 2012 | ▲ 10 | - Not described
- Ref: US-blend full flavor CC KS
- Vapor (mainstream aerosol)
- Aim: to suggest standard testing conditions and chemical and
- ISO standards smoking machine (35 ml puff, 2 s puff duration, 60 puff interval) for EC and Health Canada Intensive Smoking Protocol (55 ml puff, Tar=11 mg/l, formaldehyde= 11 µg/l, acetaldehyde= 21 µg/l, acrolein= 3 µg/l, NNN= 5 ng/l, NAT= 3 ng/l, NAB= 0.6 ng/l, NNK= 2 ng/l, traces of benz(a)pyrene, benzene, total HCN, 1,3 butadiene, acrylonitrile, o- cresol, diethyl glycol
- TSNs (NNN, NNK, NAB and NAT)
- No description of brand/number of batches
- TSNAs, tar, formaldehyde, acetaldehyde, acrolein, and other toxins found in vapor
- Most toxicants were reduced by over 98% compared with CC
toxicological properties of aerosol 2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC present at trace level - much lower than CC * This testing approach can detect toxins in mainstream aerosol that would be missed by other analytical approaches

Lisko JG [100] 2015

No 2 devices: refillable eGO Vision, Blu disposable E-liquids: Blu, Drip, Encore, ROC Juice, Upstate Vape, Vaper drops, Vapor dudes Different flavours; tobacco, cinnamon, menthol and fruits

Lerner CA [98] 2015

No Liquid and vapor assay: vapor/smoke produced by smoking machine, levels of OX/ROS were determined using 2, 7′-di-chlorofluorescein diacetate fluorogenic probe

Liquid and vapor assay: vapor/smoke produced by smoking machine, levels of OX/ROS were determined using 2, 7′-di-chlorofluorescein diacetate fluorogenic probe

Lerner CA [97] 2015

No Rechargeable Blu EC (7 batteries and 17 cartomizer) used over a 24 h Period

Rechargeable Blu EC (7 batteries and 17 cartomizer) used over a 24 h Period

Liskos JG [100] 2015

No 36 e-liquids brands from 4 manufacturers

Fluid pneumonia: to evaluate the chemical

Analysis of nicotine content with 2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC

2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC

2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC

2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC

2 sec puff duration, 30 s puff interval, 100% blocking of filter ventilation) for CC

36 e-liquids brands from 4 manufacturers

-36 e-liquids brands from 4 manufacturers

-36 e-liquids brands from 4 manufacturers

-36 e-liquids brands from 4 manufacturers

-36 e-liquids brands from 4 manufacturers

Annexes – 22
<table>
<thead>
<tr>
<th>Brand</th>
<th>Study</th>
<th>Subjects</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
</table>
| Long GA [101] 2014 | eCigs Classic Tobacco Disposable | 20 current EC vapers and 10 smokers with a stable preference for one of the 3 specified products (≥6 months) | - 20 current EC vapors and 10 smokers with a stable preference for one of the 3 specified products (≥6 months)  
- Each subject used their preferred product (~ nine sessions; 3 replicates per subject in the 3 analyte classes)  
- Conducted in a 40 m² conference room  
- 3 cigarettes /max. of 99 puffs per session  
- Vacuum-assisted filter pad capture system | - Total phenolic content in exhaled EC aerosol: not distinguishable from exhaled breath blanks  
- Total phenolics in exhaled CC-smoke were significantly greater than in exhaled EC aerosol and exhaled breaths  
- Total carbonyls in exhaled EC aerosols were not distinguishable from exhaled breaths or room air blanks  
- Total carbonyls in exhaled CC smoke was significantly greater than in exhaled EC aerosols, exhaled breath and room air blanks  
- Large individual differences in phenols in exhaled aerosol. E.g. one EC vaper had high acetaldehyde levels |
| Maloney JC [102] 2015 | MarkTen® prototype EC with and without menthol | 185 panelists in Study 1 and 145 panelists in Study 2 | - Only formaldehyde was detected above the LOQ of the analytical methods used, however these levels were overlapping the range of the background levels (6-8 µg/m² with background levels 5-7 µg/m²)  
- EC does not produce airborne levels of chemical ingredients (e.g. menthol, nicotine, propylene glycol, glycerol or total suspended particulates) above the limit of quantitation of the standard industrial hygiene sampling and analytical methods used in this study | - Studies do not represent ad libitum use  
- Standards not designed for inhalation  
- Indoor vaping of MarkTen® prototype EC does not produce chemical constituents at quantifiable levels or background levels using standard industrial hygiene collection techniques and analytical methods |
<table>
<thead>
<tr>
<th>Manigrasso M [104] 2015</th>
<th>No</th>
<th>Unknown EC brand; rechargeable, commercial model comprising of a tank system and a 14 mg mL−1 nicotine. Ref: CC with 0.8 mg nicotine</th>
<th>Vapor • Aim: to estimate size segregated doses from EC aerosols as a function of the airway generation number in lung lobes • Condensation Particle Counter and a Fast Mobility Particle Sizer spectrometer • Mainstream aerosol measurements were performed for puffs of 2-s duration • Particle deposition in the human respiratory system: Multiple-Path Particle Dosimetry model (MPPD v2.1, ARA 2009)</th>
<th>7.7 x 10^9 particles (DTot) with a surface area of 3.6 x 10^6 mm^2 (STot), and 3.3 x 10^9 particles with a surface area of 4.2 x 10^6 mm^2 were deposited in the respiratory system for the EC and CC, respectively. • Total regional doses, in head and lobar tracheobronchial and alveolar regions, ranged from 2.7 x 10^7 to 1.3 x 10^8 particles and 1.1 x 10^7 to 5.3 x 10^7 particles, for the electronic and conventional cigarettes, respectively. • Total regional doses in the right-upper lung lobe: about twice that found in left-upper lobe and 20% greater in right-lower lobe than the left-lower lobe.</th>
<th>One brand only • Not tested on humans</th>
<th>High dose - more than double the dose compared to CC- of 10^8 particles are deposited in the lung • In the tracheobronchial and alveolar regions, a single puff delivers total regional doses that represent 40% and 30% of the daily dose of a no-smoking Italian. • The lobar bronchi and right lung lobes represent sites where effects of the aerosol from EC may be more likely to occur.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manigrasso M [103] 2015</td>
<td>No</td>
<td>Unknown EC brand; rechargeable, commercial model comprising of a tank system and 8 different e-liquids in terms of nicotine content and flavor. No ref</td>
<td>Vapor • Aim: to give a contribution to fill the gap between source emission and related health effects providing dosimetry data useful to estimate both acute and long-term effects of the aerosols delivered by EC. • Condensation Particle Counter and a Fast Mobility Particle Sizer spectrometer • Mainstream aerosol measurements were performed for puffs of 2-s duration • Particle deposition in the human respiratory system: Multiple-Path Particle Dosimetry model (human lung model) • Particle number concentrations varied between 3.26 x 10^6 and 4.09 x 10^6 part cm^-3 for e-liquids without nicotine and between 5.08 x 10^6 and 5.29 x 10^6 part cm^-3 for e-liquids with nicotine. • No flavor effects were detected on particle concentration data. • Particle size distributions: unimodal with modes between 107-165 nm and 165-255 nm, for number and volume metrics, respectively. • Averagely, 6.25 x 10^6 particles were deposited in respiratory tree after a single 2-s puff. • Highest deposition densities and mean layer thickness of EC liquid on the lung epithelium were estimated at lobar bronchi.</td>
<td>Unknown EC • Not tested on humans</td>
<td>Human lung model: EC are a source of extremely high particle doses in the human respiratory system. • 10^7 particles were deposited in the respiratory tree after a single 2-s puff, approximately 30% of the daily doses of a non-smoking individual.</td>
<td></td>
</tr>
<tr>
<td>Marco E [106] 2015</td>
<td>No</td>
<td>θ</td>
<td>2 types EC: disposable (Type 1 e-cigarette) or rechargeable (Type 2 e-cigarette) Ref: CC, blend type American tobacco cigarettes with filters, low nicotine content (0.6 mg), low tar (8 mg)</td>
<td>Smoke/vapor or exhaled breath were collected in BioVOCs. VOCs were then desorbed in Tenax cartridges which were subsequently analyzed by thermal desorption coupled to gas chromatography–mass spectrometry. • Vapor of EC: mainly composed of PPG and glycerin, nicotine and related products such as miosmine and nicotyline. • Exhaled breath of vapers: chromatographic peaks of PPG and glycerin were absent, and there was decrease of the peaks corresponding to nicotine and related compounds, indicating that they remained in the respiratory system. • Two main peaks in the chromatograms from exhaled breath.</td>
<td>Contamination? All volunteers were asked to smoke CC and both types of EC • Only 2 types of EC</td>
<td>Comparison of the concentrations between smoke and equivalent exhaled breath illustrated the incorporation of higher burdens of VOCs in the smokers than in EC vapers.</td>
</tr>
</tbody>
</table>
were those corresponding to acetone and isoprene which likely represent endogenous sources. In addition, benzene, toluene and 2,5-dimethylfuran were also found.

- Results from disposable EC were very similar to those from rechargeable EC
- CC smoke and smokers breath contained numerous VOCs

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>N</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Martinez RE | 2015 | No | • Three e-liquids were tested:
  1) an unflavored solution in PPG
  2) an unflavored solution in PPG and VG
  3) a flavored solution in PPG and VG

  • Vapor
  • Aim: to test for nicotyrine, a nicotine analog that could impede nicotine metabolism
  • Thermal Desorption Aerosol Gas Chromatograph
  • A heating duration experiment determined the nicotyrine to nicotine ratio (NNR) in particle phase as a function of the duration of e-cig activation
  • An aging experiment determined the NNR in e-liquids and vapor

  • Nicotine and nicotyrine were quantified in all 3 e-liquids and aerosols; NNR is higher in the aerosol when PPG only is used in the e-liquid
  • Duration of EC activation was inversely related to NNR (NNR = 0.04 with 3-s activation, 0.26 with 0.5 s)
  • Aging influenced both e-liquid NNR and aerosol NNR
  • On average, the e-liquid NNR increased from 0.03 at 11 days after opening to 0.08 after 60 days
  • For similar heating durations, aerosol NNR increased from 0.05 at 11 days to 0.23 after 60 days
  • Storage conditions had little effect on NNR

  • Few liquid, only one batch
  • VG only, unflavored solution not tested

  • E-cig aerosols have variable nicotyrine quantities
  • Aerosol nicotyrine to nicotine ratio depends on vaping technique and time elapsed since the e-liquid was exposed to air
  • Aerosolized nicotyrine could facilitate nicotine absorption, inhibit the metabolism of nicotine, and reduce a user’s urge to smoke

| McAuley TR | 2012 | ▲ 11 | • 12 new cartomisers were filled with e-liquid from 4 different bottles
  • 4 popular e-liquid brands, tobacco flavored and the highest commonly used level of nicotine
  • Ref. CC (Marlboro Red)

  • Vapor
  • Aim: test for six different types of pollutants:
  • 4 TSNAs: NNN, NNK, NAB, NAT
  • PAHs
  • Glycols: PPG, DEG
  • VOCs
  • Carboxyls (formaldehyde, acrolein, acetaldehyde)

  • E-liquids were vaporized in two sets of experiments by generic 2-piece ECs
  • Modified smoking machine connected with polyethylene glove bags
  • Risk analyses were conducted based on dilution into a 40 m³ room and standard toxicological data

  • CC smoke particle number conc. was an order of magnitude higher than the highest conc. of any e-liquid (2963 ± 3122, liquid C vs. 21,352 ± 50,414)
  • Average VOC conc.s: below the limit of detection with exception of ethylbenzene, benzene, toluene, and m/p xylenes
  • For most carboxyls: low conc., with some exceptions, such as acetone, formaldehyde, and acetaldehyde
  • Most PAHs: below the limit of detection
  • TSNAs: typically found at lower levels than tobacco smoke
  • Nicotine levels were also significantly higher in CC smoke than in the e-liquid vapor

  • Cross-contamination with smoke
  • Particle count from vapor uncertain; could not be replicated in phase II due to instrumental problems
  • Total air emission conc.s for many pollutants were found to be very low, also in CC smoke
  • Excess Lifetime Cancer Risks values for mainstream CC smoke samples were low, did not include

  • Ethylbenzene, benzene, toluene, and m/p xylenes acetone, formaldehyde, and acetaldehyde detected
  • TSNAs: typically found at lower levels than tobacco smoke
  • Conc. of pollutants were generally orders of magnitude lower than in CC smoke

Annexes – 25
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Reference</th>
<th>Nicotine and Menthol Formulation</th>
<th>Methodology</th>
<th>Results</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misra M</td>
<td>2014</td>
<td>[115]</td>
<td>-blu EC glycerol-based e-liquids, with and without nicotine and two market leader flavors (Classic Tobacco and Magnificent Menthol), Ref: 1. CC Kentucky Reference 3R4F, 1R5F and Marlboro Gold), 2.smokeless tobacco products (Marlboro Snus, Copenhagen Snuff) 3) NRT product (Nicorette Lozenge)</td>
<td>-Fluid and vapor Aim: to test toxicity of EC liquids; smokeless tobacco products; a NRT lozenge product; and of pad-collected particulate matter from freshly-generated CC smoke and EC vapor</td>
<td>- In all assays, exposures with EC liquids and collected aerosols, at the doses tested, showed no significant activity when compared to CC -Presence of nicotine and flavors, at the levels tested, did not induce any cytotoxic, genotoxic or inflammatory effects -No significant IL-8 release was observed for most of the products, with the exception of the blu MM-no nicotine, blu MM-High and blu CT-no nicotine treatments which resulted in higher IL-8 release only at extremely high doses of 6.9–13.8 mg/mL</td>
<td>-One brand only -Did not use cell systems that are most sensitive to EC vapor</td>
</tr>
<tr>
<td>Neilson L</td>
<td>2015</td>
<td>[118]</td>
<td>-NJOY Bold 4.5% nicotine and NJOY Menthol 3.0% nicotine Ref: 3R4F CC</td>
<td>-Vapor Aim: to develop physiologically relevant test methods to analyse potential irritant effects to the respiratory tract caused by EC aerosols</td>
<td>-CC smoke reduced cell viability in a time dependent manner to 12% at 6 h -EC vapor showed no such decrease in cell viability and displayed similar results to that of the untreated air controls</td>
<td>-Two brands only -Tested by smoking machine, not human puffing behaviour</td>
</tr>
<tr>
<td>O’Connell G</td>
<td>2015</td>
<td>[120]</td>
<td>❁</td>
<td>- Disposable ‘closed system EC: Puritane Ref: No</td>
<td>-Indoor air *Aim: to measure volatile organic compounds (including nicotine and low molecular weight carbonyls), polycyclic aromatic -5 male volunteers: 3 current vapers + 2 non-smokers/vapers *Exposure: 165 min. ad libitum vaping session in a closed room (38.5 m3), real-life setting</td>
<td>Concentration in the indoor air during consumption of EC: -No increase in nicotine -Glycerol: &lt;350 µg/m3 which is below the UK WEL of 10,000 µg/m3 -PPG: 203.6 µg/m3 which is below the UK WEL of</td>
</tr>
</tbody>
</table>

* EC liquids and vapor does not produce any meaningful toxic effects in four widely-applied in vitro test systems, in which the conventional cigarette smoke preparations are markedly cytotoxic and genotoxic.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study Design</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palpant NJ</td>
<td>2015</td>
<td>No</td>
<td>Vapor from EC cartridge (South Beach Smoke, Tobacco Classic, Full Flavored, 16 mg nicotine/cartridge) Ref: smoke from University of Kentucky, 3R4F Research grade CC</td>
</tr>
<tr>
<td>Papousek R</td>
<td>2014</td>
<td>No</td>
<td>1. Disposable EC with a Marlboro flavor 2. Refillable EC with flavored refill liquids (cherry or Turkish) Ref: cigar</td>
</tr>
<tr>
<td>Park S</td>
<td>2014</td>
<td>No</td>
<td>EC of unknown type Ref: CC smoke</td>
</tr>
</tbody>
</table>

**Hydrocarbons, tobacco-specific nitrosamines and trace metal levels in the air before, during and after EC use in a typical small office meeting room**

- Total volatile organic compounds (TVOCs): 379.8 µg/m³; UK Building Regulations: 8 h average: 300 µg/m³
- No measurable increase in any of 16 PAHs during the vaping period (all <1.25 µg/m³)
- Metals: <1.0 µg/m³ for antimony, arsenic, barium, cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, selenium and zinc; <2.0 µg/m³ for aluminium, beryllium, silver and thallium, and <10 µg/m³ for phosphorus; all below UK WEL
- No increase in N′-nitrosornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosodentatonabine (NAT) and N′-nitrosodentabasine (NAB)

**Palpant NJ [122] 2015**
- Vapor from EC cartridge (South Beach Smoke, Tobacco Classic, Full Flavored, 16 mg nicotine/cartridge)
- Aim: to determine the impact of EC and CC on heart development in vitro and in vivo
- Human embryonic stem cells
- Undifferentiated RUES2 female line
- Both EC and CC exposure resulted in decreased expression of cardiac transcription factors in cardiac progenitor cells, suggesting a persistent delay in differentiation
- In definitive human cardiomyocytes, both EC and CC treated samples showed reduced expression of sarcomeric genes such as ML2v and MYL6
- Cells differentiated in purified nicotine were not significantly different on the basis of all endpoints compared to control samples
- One brand only
- Study indicate a negative effect of EC on heart development in vitro and in vivo
- The finding that nicotine treatment alone recapitulated untreated controls indicates that the impact of EC on heart development is the consequence of other components

**Papousek R [124] 2014**
- 1. Disposable EC with a Marlboro flavor
- 2. Refillable EC with flavored refill liquids (cherry or Turkish) Ref: cigar
- Aim: to describe a fast and simple procedure for simultaneous determination of both acrylamide and acrolein under standard conditions
- Gas chromatography–mass spectrometry (GC–MS) method
- The derivatization of acrylamide and acrolein was carried out by a bromination method with elemental bromine
- Acrolein was found in all tested samples
- Acrolein was detected only in smoke from cigar–side-stream smoke contained a significant amount [2.40 and 1.52 µg (cig. eq.)–1].
- Few brands
- Tested by smoking machine, not = human puffing behaviour
- Acrolein, a compound with toxic and potentially and mutagenic effects was found in all tested samples

**Park S [125] 2014**
- EC of unknown type Ref: CC smoke
- Aim: to assess the impact of EC exposure on the carcinogenic potential of:
- Enhanced colony growth in the H3mut-P53/KRAS cells following a 10-day treatment with the high nicotine EC- and CC-conditioned media compared to the untreated and low nicotine treatment groups
- One brand only
- Preliminary analyses indicate the observed EC-specific gene expression changes were concordantly changed following CC-conditioned media exposure.
<table>
<thead>
<tr>
<th>Study</th>
<th>No</th>
<th>Types of E-liquids</th>
<th>Description</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellegrino RM [126] 2012</td>
<td>No</td>
<td>2 types of Italian brand</td>
<td>-One with and one without nicotine Ref: CC (nicotine 0.8mg/tar 10 mg)</td>
<td>-E-liquid and vapor -Aim: test for toxicity during a &quot;smoking&quot; simulation -Quali-quantitative determination of the aromatic mixture and the vapor content -E-liquid: -Gas-chromatography/ mass-spectrometry -Vapor: modified smoking machine, vapor collected -Indoor emission of PM: laser operated aerosol mass analyser -PPG and VG together: &gt;90% of the total ingredients. Other ingredients detected in trace levels. -Vapor: 11 and 10 substances found in +nicotine+nicotine EC: major compound is PPG and VG -PM in vapor: fine + ultrafine particles: density ratio compared with CC 6-21 lower Total PM: 15 times lower from EC than CC -Tested only 2 brands -Tested only one batch of liquid per brand -PPG and VG are major ingredients – other ingredients = traces -PM in vapor: fine + ultrafine particles -PM emissions are significantly lower than in CC smoke</td>
</tr>
<tr>
<td>Romagna G [133] 2013</td>
<td>✔</td>
<td>21 commercially available e-liquids with different flavouring</td>
<td>-Manufactured by same manufacturer, Italy Ref: CC (1mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide)</td>
<td>-Vapor -Aim: test for in vitro cytotoxicity of vapor extract and to compare it with the cytotoxicity of CC smoke extract -Vapor: e-liquid evaporated and extracted in culture medium. -CC extract from one cig. was produced -The extracts, undiluted and in five dilutions were applied to cultured murine fibroblasts (3T3) -Viability was measured -Only &quot;Coffee&quot; exhibited a cytotoxic effect; this was observed at the highest extract conc. only -All e-liquids: the range of fibroblast viability was 88.5–117.8% at 3.125%, 86.4–115.3% at 6.25%, 85.8–111.7% at 12.5%, 78.1–106.2% at 25%, 79.0–103.7% at 50% and 51.0–102.2% at 100% extract -Conc. of CC extract: significant cytotoxicity at extract conc. &gt;12.5% -Tested only one brand -Tested only one batch of liquid per brand -Too low CC exposure? -Fibroblasts, are normally not in direct contact with vapor -Vapor from 1 out of 21 EC liquids examined had cytotoxic effects on cultured fibroblast -CC: significantly higher cytotoxicity</td>
</tr>
<tr>
<td>Romagna G [134] 2012</td>
<td>✇</td>
<td>E-liquid (FlavourArt), nicotine concentration 11 mg/ml Ref: CC, 0.6mg nicotine</td>
<td>-Room air -Aim: to identify and quantify the chemicals released on a closed environment from the use of EC -60m3 closed-room -Two sessions: 5 smokers and 5 users of EC. Both sessions lasted 5 h. total organic carbon (TOC), toluene, xylene, carbon monoxide (CO), nitrogen oxides (NOx), nicotine, acrolein, poly- -During the sessions: EC session, 1.6 ml of liquid was consumed, 17.6mg of nicotine: CC: 19 cigarettes were smoked, 11.4mg of nicotine -EC: TOC =0.73 mg/m3 and glycerin=72 µg/m3. No toluene, xylene, CO, NOx, nicotine, acrolein or PAHs were detected on room air during the e-CIG session -CC: TOC=6.66mg/m3, toluene=1.7 µg/m3. -Two brands only -Preliminary assessments -Several harmful substances from smoke were not detected in air either -Preliminary assessment: vaping does not produce detectable amounts of toxic and carcinogenic substances in the air of an enclosed space</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>No.</td>
<td>Experimental Details</td>
<td>Aims</td>
<td>Results</td>
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<td>-------</td>
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</tr>
<tr>
<td>Rubenstein DA [135] 2015</td>
<td>No</td>
<td>- NJoy, OneJoy Traditional Flavor, 1.2% and 1.8% nicotine - eGo, OKC Vapes, Desert Sands Flavor with 0 mg, 12 mg or 18 mg nicotine - Pure nicotine 50 nM - Ref: Marlboro 100s (16 mg tar and 1.2 mg nicotine)</td>
<td>- Vapor aroma - Immortalized Kupffer cells (from Sprague-Dawley Rats)</td>
<td>- No sign difference between EC and CC samples for zinc (Zn), nickel (Ni) and silver (Ag) - Despite the 10-fold decrease in the total exposure to particulate elements in EC compared to normal cigarettes, specific metals (e.g. Ni and Ag) still displayed a higher emission rate from EC</td>
</tr>
<tr>
<td>Ruprecht AA [136] 2014</td>
<td>No</td>
<td>- Elips Serie C, Tank System (Ovale Europe Srl), refilled with and without 16 mg nicotine Reference: CC, popular brand - 50 m3 office</td>
<td>- Vapor - PM mass as PM1, PM2.5, PM7, PM10, total suspended particles (TSP) measured by use of pre-calibrated Aerocet, Model 531 - UFP by condensation particle counter, Model 3007 concentrations - Measure of urban background pollution</td>
<td>- EC generated consistently less PM of all measured sizes than CC - This difference was particularly evident for the nicotine-refilled device, which showed only marginal PM production in its sidestream smoke, while the EC without nicotine showed low but present production of all PM</td>
</tr>
<tr>
<td>Saffari [137]A 2014</td>
<td>No</td>
<td>- Elips Serie C, Tank System (without nicotine) Ref: a widely used brand of normal CC (i.e. tobacco-containing)</td>
<td>- Particle phase of EC emission - PM volume of 48 m³</td>
<td>- No sign difference between EC and CC samples for zinc (Zn), nickel (Ni) and silver (Ag) - Despite the 10-fold decrease in the total exposure to particulate elements in EC compared to normal cigarettes, specific metals (e.g. Ni and Ag) still displayed a higher emission rate from EC</td>
</tr>
</tbody>
</table>
| Samways B [139] 2014 | ▲ 32 | -4 commercially available disposable, non-refillable and non-rechargeable 2 with menthol 4.5 or 3% nicotine Ref: no | -Vapor  
-\textit{Aim:} to assess the suitability of QCMs as an \textit{in vitro} dosimetry tool for EC aerosols, using the Vitrocell® VC 10 Smoking Robot. Product durability before battery depletion, and how this relates to \textit{in vitro} dose was also investigated  
-\textit{Four QCMs} (Vitrocell® Systems, Vitrocell® Systems) smoked 4 \textit{EC}  
-\textit{QCMs} read real-time aerosol particle deposition at a resolution of \textit{10 ng/cm²/second}  
-\textit{Ten repeats per product}  
-\textit{Aerosol mass deposition} ranged from 40.71 – 88.95µg/cm², 24.20 – 71.77µg/cm², 73.84 – 111.23µg/cm² and 32.12 – 128.98µg/cm² for Product A, Product A Menthol, Product B and Product B Menthol  
-\textit{Menthol products} produced less mass in comparison to their higher nicotine concentration, non-mentholated equivalents, despite lasting similar durations before exhaustion  
-\textit{Deposited aerosol mass} varied greatly from repeat experiments with all products  
-Unknown brand  
-Deposited aerosol mass varied greatly from repeat experiments with all products  
-\textit{Variability of aerosol cellular dose} \textit{in vitro} needs to be taken into consideration for future \textit{in vitro} studies |
| Sancilio S [140] 2015 | No | -Two cartridge solutions (nicotine content 0 and 24 mg/ml, respectively) from Halo Company containing propylene glycol, glycerin, and natural artificial flavorings Ref: no | -Vapor and fluid  
-\textit{Aim:} to investigate the effects of the liquids of EC on human gingival fibroblasts and to compare the effects of nicotine-containing fluid to the fluid itself  
-\textit{Cells} were treated with different concentrations for different times (0–72 h)  
-\textit{Cytotoxicity: MTT assay}  
-\textit{Apoptosis occurrence and Bax expression: flow cytometry}  
-\textit{Reactive oxygen species (ROS) production: fluorescence optical microscopy}  
-\textit{Metabolic activity} was reduced in a time- and dose-dependent manner  
-Both nicotine-containing and nicotine-free fluids induced an increased ROS production after 24 h, along with an increased Bax expression. \textit{Apoptosis occurrence} after 48 h of exposure  
-Extreme toxicity for concentrations higher than 1 mg/mL just after 24 h  
-The cytotoxicity exerted on human gingival fibroblasts by EC fluids is not entirely ascribable to nicotine  
-One brand only  
-Findings indicated that EC fluids induce an oxidative stress and early and late apoptosis, with a major extent in nicotine-treated samples, but present anyway in the samples treated with nicotine-free fluids |
| (Chandramani Shivalingappa P [145] 2015 | No | -Unknown EC 2.5 mg or 7.5 mg Ref: room-air controls | -Vapor  
-\textit{Aim:} to quantify the impact of \textit{EC} on proteostasis and to evaluate if short-term effects of \textit{EC} exposure  
-\textit{Beas2b cells} exposed for 1, 3 and 6 h  
-\textit{Immunoblotting}  
-\textit{Fluorescence microscopy} and \textit{immunoprecipitation}  
-\textit{Vapor induced protein-aggregation can activate oxidative stress, apoptosis (caspase-3/7) and senescence (p<0.01) as compared to controls}  
-\textit{Sign increase in accumulation of total polyubiquitinated-proteins} with time-dependent decrease in proteosomal-activities of vapor  
-Unknown brand  
-Exposure not sufficiently described  
-One brand only?  
-EC vapor exposure induces proteostasis/autophagy impairment leading to oxidative stress, apoptosis, and senescence that can be ameliorated by an autophagy inducer  
-EC vapor-induced |
modulate mechanisms known to be involved in CC induced COPD emphysema

| Schoffler S  [141] | No | 1) E-liquid with or without nicotine 2) carrier substances PPG and glycerol | - Vapor  
- Aim: to test toxicological effects of EC vapor and pure carrier substances  
- Primary human bronchial epithelial cells (NHBE) of two different donors  
- Smoking robot  
- CULTEX® RFS compact module  
- 24 h post-exposure: cell viability and oxidative stress levels  
- Toxicological effects of EC vapor and the pure carrier substances, whereas the nicotine concentration did not have an effect on the cell viability  
- The viability of cells exposed to mainstream CC smoke was 4.5–8 times lower and the oxidative stress levels 4.5–5 times higher than those of EC vapor exposed cells, depending on the donor  
- The pure carrier substances PPG and glycerol exhibited toxicological effects  

 exposure as compared to control  
- Even minimal exposure (1 hr) induces vasocon containing protein (p<0.001), sequestosome-1/p62 (aberrant-autophagy marker; p<0.05) and aggresome formation  
- Inhibition of protein synthesis by 6 hr cyclohexamide (50 µg/ml) treatment sign (p<0.01) alleviates vapor-induced (1 hr) aggresome-bodies  

- Experimental dose of EC, not necessarily reflecting real-life exposure  
- Short term exposure  
- The number of puffs taken was not identical for CC and EC/carrier substance- adjusted by multiplying the results  

- Toxicological effects of EC vapor and the pure carrier substances, whereas the nicotine concentration did not have an effect on the cell viability  

Schober W  [142]  

2014**  

| No | Θ | - Red Kiwi, without and with 18 mg nicotine  
- Reference: no vaping  

- Indoor air  
- Aim: to measure inner and outer exposure assessment of EC emissions in terms of PM, particle number concentrations, VOC, PAH, carbonyls, and metals under real-life conditions  
- Room size: 18 m² and its volume: 45 m³  
- In 6 vaping sessions 9 volunteers (occasional smokers) consumed EC with and without nicotine in a thoroughly ventilated room for two hours.  
- Monitored effects on FeNO release and urinary metabolite profile of the subjects  

- Room size: 18 m² and its volume: 45 m³  
- In 6 vaping sessions 9 volunteers (occasional smokers) consumed EC with and without nicotine in a thoroughly ventilated room for two hours.  
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- Room size: 18 m² and its volume: 45 m³  
- In 6 vaping sessions 9 volunteers (occasional smokers) consumed EC with and without nicotine in a thoroughly ventilated room for two hours.  
- Monitored effects on FeNO release and urinary metabolite profile of the subjects  

- Substantial amounts of 1,2-propanediol, glycerine and nicotine were found in the gas-phase, as well as high concentrations of PM2.5 (mean 197 µg/m3)  
- PAH in indoor air increased by 20% to 147 ng/m³  
- Aluminum showed a 2.4-fold increase  
- Particle number concentrations ranged from 48,620 to 88,386 particles/cm³ (median), with peaks at diameters 24–36 nm  
- FeNO increased in 7 of 9 individuals  
- Urine: 3-HPMA, the mercapturic acid metabolite of the pyrolysis product acrolein, was elevated after nicotinic vaping  
- The nicotine content of the liquids varied and was 1.2-fold higher than stated  

- Tested one brand only  
- Underestimation due to EC-naive volunteers?  

- EC are not emission-free and their pollutants could be of health concern for users and secondhand smokers  
- In particular, ultrafine particles formed from supersaturated 1,2-propanediol vapor can be deposited in the lung  
- Aerosolized nicotine from EC seems capable of increasing the release of the inflammatory signaling molecule NO upon inhalation  
- Whether effects also occur in passive smokers, is uncertain.  

Schripp T  [143]  

2013**  

| No | -3 types of e-liquids  
- 2 apple-and one tobacco flavored  
- With nicotine or  

- Vapor  
- Determination of the release of VOC and (ultra)fine  
- Near-to-real-use conditions; a volunteering smoker/vaper in an  

-1,2-propanediol: detected in the chamber atmosphere - below the limit of determination  

- High amount of 1,2-propanediol  

- Evaporation under the sampling conditions?  

- High amount of 1,2-propanediol in the exhaled air  
- Emissions of aerosols and Annexes – 31
| Schweitzer KS [144] 2015 | No | Nicotine solutions | Vanilla, Kentucky Prime, and nicotine-free Kentucky Prime EC used to generate vapor: iClear 16 Ref: filtered research-grade CC (2R4F) or nicotine-free CC (1R5F) | Fluid and vapor 
Aim: to investigate the contribution of nicotine in CS or EC to lung endothelial injury | Cell cultures: Primary rat lung endothelial cells (RLEC) and human bronchial epithelial cells (Beas-2B) Primary mouse lung endothelial cells (MLEC) Primary human microvascular cells-lung derived (HMVEC-LBI) **Animal experiments 
Exposed to nicotine, EC solution, or condensed EC vapor (1–20 mM nicotine) or to nicotine free CC smoke extract or EC solutions >NMR, mass spectrometry and gas chromatography 
Electric cell-substrate impedance sensing | Nicotine-independent effects of EC solutions as endothelial barrier dysfunction were noted, which may be attributable to acrolein, detected along with PPG, glycerol, and nicotine in both EC solutions and vapor 
Detected acrolein not only in condensed vapor, but also in all EC solutions tested; heating was not a necessary 
Although nicotine at sufficient concentrations to cause endothelial barrier loss did not trigger cell necrosis, it markedly inhibited cell proliferation. | Experimental dose of EC, not necessarily reflecting real-life exposure 
Short term exposure | Prominent components in the gas-phase: 1,2- propanediol, 1,2,3-propanetriol, diacetin, flavorings, and traces of nicotine 
Passive vaping must be expected from the consumption of ECs 
The aerosol size distribution alters in the human lung and leads to an exhalation of smaller particles |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Stepnov I [147] 2015 | No | Nicotine | Green Smoke, NJOY, V2, Blu 
No nicotine, low, medium and high nicotine 
Regular tobacco taste and menthol | Fluid 
Aim: to study the pH in EC | To measure pH, the contents of each cartridge were removed, extracted with 10 mL ultrapure water, and the pH of the | pH of EC cartridge content ranges widely, from 4.78 to 9.60, depending on the brand and nicotine level 
While pH of nicotine-free cartridges is generally neutral or even slightly acidic, over 50% of nicotine- | Tested fluid only | ECs with the same nicotine content, but different pH, may deliver different doses of nicotine to users 
Most of the tested brands have basic pH - the long-term effect of chronic eco- |
<table>
<thead>
<tr>
<th>Reference: no</th>
<th>Reference: CC</th>
<th>Reference: no</th>
<th>Reference: no</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Direct drip atomizer + eGo-T battery (Joyetech), PPG-based liquid (Liquid Express, WaterMelon Chill, 0 or 18 mg/mL nicotine concentration) Ref: no</td>
<td>4 Refill liquids: Tobacco USA Mix (18 mg nicotine), Cappuccino (12 mg nicotine), Ice Mint (0 mg nicotine), Tobacco Winston (11 mg nicotine) Ref: CC</td>
<td>4 Three blu eCigs products and two SKYCIG products (most popular) Ref: CC (Marlboro Gold Box, and Lambert &amp; Butler Original and Menthol products) and ambient air</td>
<td></td>
</tr>
<tr>
<td>Aerosol •Aim: to investigate whether “dripping” e-liquids directly onto a heater coil can produce significant levels of non-nicotine toxicant emissions</td>
<td>Fluid •Aim: to develop a new environmental friendly methodology based on fluorescent signal enhancement of rhodamine B dye for lead traces quantification in EC and measure lead in EC</td>
<td>Vapor •Aim: to test for harmful and potentially harmful constituent in EC vapor</td>
<td></td>
</tr>
<tr>
<td>Aerosols were machine-generated from an NHALER 510 Atomizer = direct drip atomizer •High-performance liquid chromatography-mass spectrometry •Heater coil temperatures were measured using an infrared camera</td>
<td>Fluorescent signal enhancement of rhodamine B dye, using a preconcentration step based on the coacervation phenomenon</td>
<td>ISO 17025 accredited analytical methods were used •Health Canada Test Method T-115 Tested for: delivery of major ingredients and for select constituents (carbon monoxide (CO), carbonyls, phenolics, volatile organic compounds (volatiles), metals, tobacco-specific</td>
<td></td>
</tr>
<tr>
<td>Depending on the condition, volatile aldehyde emissions, including formaldehyde, greatly exceeded values previously reported for conventional EC and CC, both per puff and per unit of nicotine yield •Increasing the inter-drip interval resulted in greater volatile aldehyde emissions, and lower total particulate matter and nicotine yields •Maximum heater coil temperature ranged from 130°C to more than 350°C</td>
<td>In all studied samples, lead contents in EC liquids were in the same order as in CC •The proposed methodology showed to be an alternative environmental friendly, simple, economical, rapid, and precise for determination of lead traces</td>
<td>Aerosol nicotine for EC samples was 85% lower than nicotine yield for the CC •Mainstream CC smoke delivered approximately 1500 times more harmful and potentially harmful constituents tested when compared to EC aerosol or to puffing room air were estimated as &lt;5% of threshold limit value.</td>
<td></td>
</tr>
<tr>
<td>One brand •One puffing topography regimen •Some portion of the measured volatile aldehyde yields may have been present at the outset •There may be significant quantities of volatile aldehyde (particle phase) that was trapped on the sampling filter pad</td>
<td>Not vapor</td>
<td>Two brands •One puffing topography regimen •Puff procedure = real life?</td>
<td>Lead contents in EC liquids were in the same order as in CC •Direct dripping of e-liquids apart from its clear implications for drug abuse liability, may also involve greater exposure to volatile aldehyde due to the potentially higher temperatures attained in the atomizer •May expose users to increased volatile aldehyde levels relative to conventional EC and even relative to CC, for a given nicotine yield</td>
</tr>
<tr>
<td>One brand</td>
<td></td>
<td></td>
<td>Digestive tract exposure is not known</td>
</tr>
</tbody>
</table>

**Annexes – 33**
| Theophilos E | 2014 | ◆ ▲ 30 | EC VUSE | Ref: different commercial EC and CC | Vapor (Mainstream aerosol) VUSE aerosol was generated using the VitroCell® VC10® aerosol exposure system and cells were exposed at the air–liquid interface | Aerosol was collected using a machine puffing regimen (55 ml puff volume/30 s inter-puff interval/3 s puff duration) and either bell shaped or square wave puffing profiles | Individual constituent yields, chromatographic profiling, and in vitro data for commercial VUSE products tested under the conditions of these studies indicated that: (1) VUSE aerosol was chemically significantly less complex than mainstream smoke from CC and (2) consistent with the simpler aerosol chemistry, VUSE aerosol was not cytotoxic (i.e., IC50 could not be derived) whereas CC smoke was cytotoxic (IC50 was derived). | Only abstract available – not possible to see details, values or brands of other EC |
| Tierney PA | 2015 | No | 30 flavored fluids | -BLU and NJOY, disposable-cartridge, in five flavours; tobacco, menthol, vanilla, cherry and coffee and refill bottles in five other confectionary flavors (chocolate/cocoa, grape, apple, cotton candy and bubble gum) | Flavors | Gas chromatography (Agilent DB-5MS UI)/mass spectrometry | Flavored products do not typically list the levels of specific flavor chemicals present, and most do not identify the major flavor chemicals present | Not vapor |
| Trehy ML | 2011 | No | A random sampling of 4 of US suppliers of cartridges, refills, and EC devices | Cartridges, refill e-liquid, and vapor | Aim: determine | Sample extracts of the products were analyzed using a validated gradient HPLC | One manufacturer: some cartridges labeled as containing nicotine, did not contain nicotine and some cartridges labeled as not containing nicotine, did contain nicotine | Puff procedure = real life? |

**Annexes – 34**
<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Samples</th>
<th>Method</th>
<th>Compounds</th>
<th>Findings</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uchiyama S 2013</td>
<td>No</td>
<td>365 EC</td>
<td>Vapor: to measure carbonyl compounds in EC</td>
<td>Carboxyl compounds in EC vapor mist were measured using coupled silica cartridges evolved with</td>
<td>9 of the 13 brands generated various carbonyl compounds</td>
<td>EC generate incidentally carboxyls. In some cases they are generated with extremely high concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography/</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>electrospray spectrometry + 1H and 13C NMR spectroscopy</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uryupin AB [158] 2013 (</td>
<td>No</td>
<td>?</td>
<td>E-fluids: study the composition of fluids</td>
<td>One and two-dimensional homonuclear 1H and 13C NMR spectroscopy</td>
<td>Samples differed sharply in water content</td>
<td></td>
</tr>
<tr>
<td>Russian original paper from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vargas Trassier C [165] 2015</td>
<td>No</td>
<td>Θ</td>
<td>Vapor (side-stream vapor): characterizing the interaction between</td>
<td>Walk-in radon chamber inner volume of 150 m³: 4 tests were carried out in the radon chamber. Three</td>
<td>Increase of the Potential Alpha-Energy Concentration (PAEC) due to the radon decay products</td>
<td>The increase in the attached Potential Alpha Energy Concentration was higher for the EC than for traditional CC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>radon with aerosol both from EC and CC</td>
<td>of them were made generating aerosol from e-cigarette at different radon concentration</td>
<td>attached to aerosol for higher particle number concentrations. This varied from 7.47 ±</td>
<td>Therefore, the aerosols from EC operate as a carrier of the radon progeny and, as a consequence it decreases the “plate out” of the radon daughter</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Radon gas obtained by natural emanation from the underneath soil</td>
<td>0.34 MeV L⁻¹ to 12.6 ± 0.26 MeV L⁻¹ (69%) for the EC</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Notes:
- "Ref: CC" indicates the study was referenced to a CC.
- "Ref: none" indicates no reference was provided.
- "Cartridge contents vary sign. from one cartridge to another" indicates variability among cartridges.
- "No reference" indicates no specific reference is given for the study.
- "Rechargeable EC" indicates the EC is rechargeable.
- "Increased" indicates an increase was observed.
- "Decreased" indicates a decrease was observed.
- "No change" indicates no significant change was observed.
- "Different" indicates differences were observed.
- "Impurity level is lower than for CC" indicates the impurity level is lower for EC compared to CC.
- "Increase of the Potential Alpha Energy Concentration (PAEC) due to the radon decay products attached to aerosol for higher particle number concentrations." indicates the increase in PAEC due to radon decay products attached to aerosol.
- "The increase in the attached Potential Alpha Energy Concentration was higher for the EC than for traditional CC." indicates the EC has a higher increase in PAEC compared to traditional CC.

Annexes – 35
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>No</th>
<th>-42 models from 14 popular brands purchased on the Internet in 2013</th>
<th>-183 e-liquids available on the Dutch market chosen on the basis of their popularity, their flavors and their nicotine content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visser W</td>
<td>2015</td>
<td>31</td>
<td>Flavors and their popularity, their chosen on the basis of their presence on the Dutch market</td>
<td>Fluids and vapor</td>
</tr>
<tr>
<td>Varlet V</td>
<td>2015</td>
<td></td>
<td>- Particle number concentration and particle size distribution: Potential Alpha Energy (PAEC) Concentration</td>
<td>- Presence of VOCs and TSNAs was investigated in a sample group of 60 liquids</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Radon activity concentration: Alpha Guard Professional Radon Monitor</td>
<td>- Presence of VOCs and TSNAs was investigated in a sample group of 60 liquids</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- These growths still continue for long time after the combustion, by increasing the exposure risk</td>
<td>- The radon progeny, in presence of aerosol, tends to attach to airborne particles. Therefore, the particles emitted by cigarettes (CC and EC) operate like carrier of the radon or thoron progeny</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- The radon progeny, in presence of aerosol, tends to attach to airborne particles. Therefore, the particles emitted by cigarettes (CC and EC) operate like carrier of the radon or thoron progeny</td>
<td>- The toxic substance-related health risks associated with the use of CC are far greater than those associated with EC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Nevertheless, daily use of e-cigarettes is not without health risks</td>
<td>- Concentrations of most relevant substances in vapor from e-liquids are lower or much lower than that in smoke</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- The concentration of formaldehyde can be up to 3</td>
<td>- The concentration of formaldehyde can be up to 3</td>
</tr>
</tbody>
</table>

**Annexes**
max. concentration of 1.6 µg/ml.  
- The flavorant diacetyl: present in 34 liquids, with the highest concentration 5591 µg/ml  
- Almost all samples contained other aldehydes and ketones, sometimes in high concentrations, probably due to use as flavorants  
- 2 of the liquids were found to have a measurable concentration of VOCs: 9.5 µg/ml of benzene and 0.58 µg/ml toluene.  
- In 15 liquids, a measurable quantity of one or more TSNAs was present, the highest concentration detected being 80 ng/ml  
- Various metals were found in extremely varied concentrations  
- Concentrations of cadmium, lead, nickel and arsenic are considerably lower than in smoke.  
- Chromium concentrations are comparable to smoke.  
- Further 150 substances were detected, many of them flavorants.  
- Many substances will pass into the vapor unchanged, while others will decompose under the influence of heat during vaping.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Cartridges</th>
<th>Cartridges Aim</th>
<th>Cartridges Analysis</th>
<th>Cartridges Detected</th>
<th>Cartridges Not vapor</th>
</tr>
</thead>
</table>
| (FDA) Westenberger BJ [169] 2009                                     | No     | 2 samples of EC and components from leading US brands, 18 cartridges, various flavours, +/- nicotine | 2 samples of EC and components from leading US brands, 18 cartridges, various flavours, +/- nicotine | A sparging apparatus and headspace GC analysis were used to stimulate actual use of products. Repeated testing. Diethylene glycol presence was confirmed with proton NMR. Nicotine quantification by methanol extraction and a acetonitrile/phosphoric acid in water extraction | Detected: Diethylene glycol in one cartridge at 1%  
- Certain tobacco-specific nitroamines in half of the sample  
- Tobacco specific impurities (anabasine, myosmine, beta-nicotrine) in the majority  
- Large variability in nicotine concentrations was found within cartridges with same label  
- Low nicotine in No-nicotine cartridges, in all, except one  
- One High-nicotine cartridge delivered twice as much nicotine as by an inhalation product for smoking cessation | Not vapor  
- Diethylene glycol in one cartridge  
- Detectable levels of carcinogens and toxic chemicals |

Willershausen I [170] 2014  
- Two samples of EC and components from leading US brands, 18 cartridges, various flavours, +/- nicotine  
- Ref: Nicotrol inhaler 10 mg for smoking cessation  
- Aim: test the content of nicotine and presence of tobacco constituents  
- A sparging apparatus and headspace GC analysis were used to stimulate actual use of products. Repeated testing. Diethylene glycol presence was confirmed with proton NMR. Nicotine quantification by methanol extraction and a acetonitrile/phosphoric acid in water extraction  
- Detected: Diethylene glycol in one cartridge at 1%  
- Certain tobacco-specific nitroamines in half of the sample  
- Tobacco specific impurities (anabasine, myosmine, beta-nicotrine) in the majority  
- Large variability in nicotine concentrations was found within cartridges with same label  
- Low nicotine in No-nicotine cartridges, in all, except one  
- One High-nicotine cartridge delivered twice as much nicotine as by an inhalation product for smoking cessation  
- Not vapor  
- Diethylene glycol in one cartridge  
- Detectable levels of carcinogens and toxic chemicals  
- This in vitro study demonstrated that menthol additives of EC have a harmful effect on human
| Williams M [171] 2013 | No | 22 cartomizers from a leading manufacturer  
Purchased from one manufacturer on four different occasions over a two year period  
Ref: CC (Marlboro brand) | -Cartomizers (fluid + aerosol)  
Aim: test for structural and elemental contents, cytotoxicity, and aerosol emissions  
-Light and electron microscopy, cytotoxicity testing, x-ray microanalysis, particle counting, and inductively coupled plasma optical emission spectrometry  
-Apparent electrophoretic movement of the cartomizer fluid towards the battery, deposition of tin particles on the inner and outer fibers, and burning of the inner fibers  
-Fluid with and without particles inhibited human pulmonary fibroblasts (hPF) survival at a dose of 1%  
-Fluid with tin particles inhibited both attachment and proliferation of hPF dose dependently  
-One puff of cartomizer aerosol contained numerous particles (mainly tin, silver, nickel and aluminum)  
-Nano particles in vapor (<100 nm): tin, chromium, and nickel  
-Silicon, calcium, aluminum, and magnesium- the most abundant elements in vapor  
-Lead and chromium conc’s in aerosols: within the range of CCs, while nickel was about 2–100 times higher than in CC  
-Room air contained relatively few particles; small end of the size range  
-Tested one brand only | -A total of 22 elements were identified in EC aerosol, and three of these elements (lead, nickel, and chromium) appear on the FDA’s ‘‘Harmful and potentially harmful chemicals’’ list  
-Aerosol: significant amounts of tin and other metals, silicate beads, and nanoparticles  
-Conc’s of most elements in aerosol were higher than or equal to corresponding conc’s in CC smoke  
-Cytotoxicity: cartomizer fluid containing tin particles inhibited attachment and survival of hPF  
-Metals in aerosol: from poor solder joints, wires, other metal components  
-Silicate particles: from the fiberglass wicks  
-Evidence of use/presale testing prior to packaging | periodontal ligament fibroblasts  
The menthol-flavored liquid caused a highly significant reduction of cell migration |
| Wu Q [172] 2014 | No | InnoVapor tobacco-flavored e-liquid without nicotine or with 18 mg/ml of nicotine Ref:no | -Fluid  
Aim: to determine if EC use alters human young subject airway epithelial functions such as inflammatory response and innate immune defense against respiratory viral (i.e., human  
-Experimental study  
Lung cells (normal hTBE cells from the tracheas and bronchi) from organ donors (8–10 years old) whose lungs were not suitable for transplantation cells were treated with medium, tobacco-  
-E-fluid did not decrease primary human airway epithelial cell viability  
-Nicotine-free e-liquid promoted IL-6 production and Human rhinovirus infection -addition of nicotine into e-liquid further amplified the effects  
-E-fluid inhibited the expression of SPLUNC1 (an important antimicrobial protein in airways against various bacterial infections) in primary human airway epithelial cells  
-Tested one brand only | -Findings strongly suggest the deleterious health effects of EC in the airways of young people  
-EC promotes proinflammatory cytokine IL-6 production and Human rhinovirus infection in primary human airway epithelial cells  
-EC inhibits the expression of SPLUNC1, a host | periodontal ligament fibroblasts  
The menthol-flavored liquid caused a highly significant reduction of cell migration |
<table>
<thead>
<tr>
<th>No.</th>
<th>Authors</th>
<th>Year</th>
<th>Reference</th>
<th>EC Type</th>
<th>Vapor generator</th>
<th>Preparation</th>
<th>Exposures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu V [174]</td>
<td>No</td>
<td>V2 and Vaping</td>
<td>Vapor extract</td>
<td>Aim: to evaluate the cytotoxicity and genotoxicity of short- and long-term EC vapor exposure on a panel of normal epithelial and head and neck squamous cell carcinoma (HNSCC) cell lines</td>
<td>Experiments were performed both in normal and cancer cells</td>
<td>Cells were treated with vapor extract for periods ranging from 48 h to 8 weeks</td>
<td>Both brands produced a significant induction of DNA double-strand breaks in human epithelial cell line as compared to the untreated control, with foci number increased by up to 1.5-fold in nicotine-free EC-treated cells and up to 3-fold in nicotine-containing EC-treated cells. Extract led to the highest number of DNA double-strand breaks in human epithelial cell line and head and neck squamous cell carcinoma cell lines, but were not significantly higher than V2 nic 1%</td>
<td>Tested one brand only</td>
</tr>
<tr>
<td>Zervas E [175]</td>
<td>No</td>
<td>7 different EC fluids, ± nicotine 1.2%, ± flavor 2% or 5%</td>
<td>Vapor</td>
<td>Aim: to study direct particle emission of EC liquids</td>
<td>Scanning Mobility Particle Sizer (SMPS) in order to determine the number and size of particles inhaled by e-cigs users</td>
<td>EC emit $10^5$ - $10^7$ particles with a size distribution peaked at 10-20nm &amp; 100-500nm and a median diameter of 200-400nm</td>
<td>Unknown brand</td>
<td>EC liquids generate nanoparticles; 300-3000 more than ambient air</td>
</tr>
<tr>
<td>Zhang Y [176]</td>
<td>No</td>
<td>Bloog MaxX Fusion EC</td>
<td>Vapor</td>
<td>Aim: test for basic physical characteristics of aerosols produced by a smoking machine</td>
<td>Aerosol generated by a smoking machine</td>
<td>Stable peak diameters of particles reach steady state with gas phase content</td>
<td>Tested only two types of liquid</td>
<td>Tested only two types of liquid</td>
</tr>
</tbody>
</table>

**Annexes – 39**
*Four of these studies are also/partly mentioned in Table 3/Annex 5 on animal experimental studies [98] [122] [144] [78]

Three studies [101, 106, 134] could as well have been described in Table 2/Annex 4, human experimental studies

| AP         | acetyl propionyl                  |
| EC         | electronic cigarette              |
| CC         | conventional cigarette            |
| CO         | carbonmonoxide                    |
| Conc.      | concentration                     |
| DA         | diacetyl                          |
| DEG        | diethylene glycol                 |
| HPHC       | harmful and potentially harmful constituents |
| hESC       | human embryonic stem cells        |
| mNSC       | mouse neural stem cells           |
| hPF        | human pulmonary fibroblasts       |
| LOQ        | limit of quantification           |
| LOD        | lower limit of detection          |
| MEG        | monoethylene glycol               |
| MOE        | Margin of exposure approach; toxicological threshold. MOE < 10 is judged to pose "high risk", while MOE < 100 are judged as "risk" |
| NNN        | N’-nitrosonornicotine             |
| NNK        | 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone |
| NAB        | N’-nitrosoanabasine               |
| NAT        | N’-nitrosoanatabine               |
| NET        | natural extract of tobacco, extracts of cured tobacco leaves produced by a process of solvent extraction and steeping |
| NO         | nitric oxide                      |
| NRT        | nicotine replacement therapy      |
| OX/ROS     | oxidants or reactive oxygen species |
| PA         | acetyl propionyl                  |
| PAH        | polyaromatic hydrocarbon          |
| PM         | particular matter                 |
| PPG        | propylene glycol                  |
| ROSA       | reactive oxygen species           |
| TSNAs      | tobacco specific nitrosamines     |
| UFP        | ultra fine particles              |
| UPF1       | 4-methoxy-L-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine) |
| VG         | vegetable glycerin                |

20%-27%, with the remainder exhaled - CC deposition is slightly higher at 25%-35%
VOCs = volatile organic compounds

**Conflicts of interest** - Conflicts of interest of each study should be assessed individually.

▲ 1: MLG received research funding from manufacturer of medicinal products for smoking cessation. AS received research funds and travel expenses from manufacturer of ECs

▲ 2 JFE: reimbursed by manufacturer of e-liquids for travels. EZ and SS: employed by manufacturer of medicinal products for smoking cessation

▲ 3 MLG: research funding from manufacturer of medicinal products for smoking cessation. NB: consultant for manufacturers of medicinal products for smoking cessation

▲ 4 MLG: research funding from manufacturer of medicinal products for smoking cessation

▲ 5: all received research funding and/or performed provided consultancy for manufacturer of medicinal products for smoking cessation

◆ ▲ 6: Study funded by tobacco company. Two of three authors affiliate to this tobacco company.

▲ 7: MLG received research funding from manufacturer of medicinal products for smoking cessation. AS received research funds and travel expenses from manufacturer of ECs

◆ ▲ 8: Manufacturers of both EC and CC funded the study. ML is cited as one of 5 most influential persons in the EC industry, [http://ecigarettreviewed.com/top-5-most-influential-people-in-the-electronic-cigarette-industry/](http://ecigarettreviewed.com/top-5-most-influential-people-in-the-electronic-cigarette-industry/)

◆ ▲ 9: Research contract with manufacturer of EC. See also CI #8

◆ ▲ 10: No conflict stated, but JHL affiliates to Lauterbach & Associates - a consulting firm that specializes in providing contract scientific affairs and regulatory support to the tobacco industry. Also see CI #8 for ML

▲ 11: Study sponsored by National Vapers Club and EC vendors. Subsequent to data-collection SB became part owner of EC company

◆ ◆ ▲ 12: Study funded by EC company

▲ 13: study funded by crowd funding in vaper community. A volunteer vaper is acknowledged for assistance with fund raising. Some of the studies by KF and VV were performed using funds provided to the institution by EC companies

◆ ▲ 14: A small number of KF’s and VV’s studies on electronic cigarettes were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. Enthalpy Analytical is a for-profit CRO and provides testing for the EC industry but did not receive any compensation for this study. MM was working at Enthalpy Analytical at the time of the study but is currently employed by a tobacco company

▲ 15: The authors declare no conflict of interest. A small minority of the studies by KF and VV were performed using unrestricted funds provided to Onassis Cardiac Surgery Center by EC companies.

▲ 16: Some of the studies by K.F. and V.V. were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. EC manufacturer is thanked for free equipment

▲ 17: MLG reports a grant from a manufacturer of smoking cessation drugs, outside the submitted work; AS reports personal fees from eSmoking Institute, Poland, and nonfinancial support from a manufacturer of EC

▲ 18: Agencies which sold some of the tested EC contributed to expenses of testing

◆ ◆ ▲ 19: authors are employees of tobacco company which also manufactures EC

◆ ◆ ▲ 20: authors are employees of tobacco company which also manufactures EC

▲ 21: MLG received a research grant from a manufacturer of smoking cessation medications

◆ ◆ ▲ 22: authors are employees of tobacco company which also manufactures EC

◆ ◆ ▲ 23: authors are employees of tobacco company which also manufactures EC

◆ ◆ ▲ 24: authors are employees of tobacco company which also manufactures EC

▲ 25: MLG received a research grant from manufacturer of smoking cessation medication, outside scope of this work

Annexes – 41
26: All authors are employees of tobacco company. The work in this paper was supported by tobacco company.

27: Some of the studies by KEF and VV were performed using funds provided to the institution by EC companies.

28: partly sponsored by Altria group which is parent company for tobacco company.

29: Some of the studies by KEF and VV were performed using funds provided to the institution by EC companies. This study was funded in part by the Greek Association of E-cigarette Businesses (SEEHT) - the sponsor funded the expenses of the laboratory. The study was investigator-initiated and investigator-driven.

30: authors are employees of tobacco company which also manufactures EC.

31: JFE was reimbursed by a manufacturer of e-liquids for traveling to London and to China, but he received no honoraria for these meetings aimed at mutual information. Some of the other studies performed by KF used unrestricted funds provided to research center by e-cigarette companies.

32: authors are employees of tobacco company which also manufactures EC.

33: nothing is stated but previous study by RG was funded by EC company. Some of the studies by KEF were performed using funds provided to the institution by EC companies.

34: None stated. Previous study was founded by manufacturers of both EC and CC. ML is cited as one of 5 most influential persons in the EC industry.

35: Study was joint funded by a manufacturer of non-tobacco products (a company set up in 2010 by tobacco company which also manufactures EC) and by tobacco company which also manufactures EC, and the authors are full time employees.

36: Study was joint funded by a manufacturer of non-tobacco products (a company set up in 2010 by tobacco company which also manufactures EC)

37: authors are employees of tobacco company which also manufactures EC.
### Annex 3. Human experimental studies reporting health effects (n=32)

<table>
<thead>
<tr>
<th>Name of first author</th>
<th>Conflict of interest</th>
<th>Passive exposure to EC</th>
<th>Type of product(s)</th>
<th>Method</th>
<th>Exposure</th>
<th>Numbers of participants</th>
<th>Aim of study / Outcome measure</th>
<th>Results</th>
<th>Weakness</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballbé M [5] 2014</td>
<td>No</td>
<td>Θ</td>
<td>-PPG-based liquids: Totally Wicked, Puff, and Free Life Ref: no</td>
<td>Observational study with non-smokers Exposure: real-use conditions with passive exposure to EC or CC for one week Control group: no exposure</td>
<td>54 non-smoker volunteers from different homes: 25 living at home with conventional smokers, 5 living with nicotine EC users, and 24 from control homes (not using EC or CC) Aim: to characterize passive exposure to nicotine from e-cigarettes' vapor and conventional cigarettes' smoke at home among non-smokers under real-use conditions</td>
<td>*The airborne markers: statistically higher in CC-homes than in EC-homes (5.7 times higher). *Concentrations of urine and saliva cotinine in non-smokers exposed to CC smoke or EC vapor were statistically similar (only 2 and 1.4 times higher respectively). *Control homes: no exposure</td>
<td>Very small sample of EC homes Potential exposure to smoke/vapor in other places than at home possible (but exposure was also registered by detailed questionnaire)</td>
<td>Non-smokers passively exposed to EC vapor absorb approx. as much nicotine as when exposed to smoke from CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Battista L. [7] 2013</td>
<td>No</td>
<td>EC of unknown type Ref: CC</td>
<td>Experimental study Exposure: vaping of own EC at the usual concentration of nicotine (4 to 9 mg/ml) in 4 min.</td>
<td>* 12 regular users of EC Aim: to investigate the acute hemodynamic effects of nicotine</td>
<td>*CO increased and systemic vascular resistances decreased after 2 and 4 minutes * Diastolic BP and mean arterial pressure increased at 4 minutes. Oxygen saturation did not change</td>
<td>*Selected regular users? *Low-moderate nicotine content in EC</td>
<td>EC inhalation produces the same pathophysiological cardiovascular effects of CC smoking</td>
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</tbody>
</table>

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3 Results of studies influenced by the tobacco industry are marked with an asterisk (*) in the paper.

4 Studies funded by e cigarette manufacturers or performed in collaboration with the e cigarette industry are labelled with a chevron (^) in the paper.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>N</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chorti M [23]</td>
<td>2012</td>
<td>No</td>
<td>-Unknown (probably same as in Flouris AD 2012) Ref: -Unlit CC -Lit own brand CC -Experimental study -Exposure: Volunteers in CC group smoked 2 CC -Volunteers in EC group puffed 1 EC -15 heavy-smokers -Aim: assess acute impact of active and passive EC and CC smoking on the pulmonary function tests -FEV1, FEV1/FVC, FEF25-75, FeNO, CO -Active EC vaping: no sign change in lung function but sign increase in cotinine -Exposure to EC vapor (passive vaping): FEV1/FVC ratio was reduced and cotinine increased -CC smoking sign decreased lung function, FeNO and increased CO and cotinine -Only one brand of EC -EC naïve participants -Stronger pulmonary reaction with passive than active vaping indicates insufficient inhalation -Small study -Passive but not active EC vaping resulted in short-term lung obstruction and increased cotinine</td>
</tr>
<tr>
<td>Colby H [25]</td>
<td>2015</td>
<td>No</td>
<td>-Unknown label, 18 mg nicotine -Ref: same EC, 0 mg nicotine -Experimental study -Volunteers inhaled vapor 18 mg or 0 mg nicotine on separate days (randomized) -Non-invasive measurements -Oscillatory lower body negative pressure (OPLBNP) between 0 and -60mmHg was applied for 20 cycles at 0.05 Hz and 0.1 Hz -13 subjects -Aim: to explore if acute inhalation of EC vapor would impair cerebral blood flow in response to variations in arterial pressure. -Heart rate, mean middle cerebral velocity, Mean arterial pressure and cerebral oxygen saturation were similar at baseline in the two groups. Mean arterial pressure and cerebral oxygen saturation very low frequency power and low frequency power were higher under the placebo condition (p= 0.03-0.06) -Cross-spectral analysis in the low and very low frequency revealed that gain between mean arterial pressure - mean middle cerebral velocity was similar (p= 0.128) -Small study -Unknown brand -Unknown intensity and duration of exposure -No information on volunteers: smokers, vapers, non-smokers? -Study suggests that nicotine, when acutely inhaled via EC does not impair the cerebral pressure-flow relationship</td>
</tr>
<tr>
<td>Czogala J [29]</td>
<td>2012</td>
<td>No</td>
<td>-MILD model M201, 14 mg nicotine -Ref: CC, L&amp;M Blue Label, 0.7 mg nicotine, 8 mg tar -Experimental study -Two sessions. 1. session: smoking of CC, 2. session 7 days after the 1.: vaping of EC -Sessions preceded by 12 hours abstinence of smoking and coffee -Exposure: 5 min of smoking/vaping -42 healthy adult daily smokers -Aim: evaluate the hemodynamic effect -Blood pressure, COHb, heart rate -EC: slight elevation in diastolic blood pressure (2%), pulse and COHb – non-sign. changes -CC: sign elevation in systolic and diastolic blood pressure, COHb and pulse -Only one brand of EC -EC naïve participants -Slight non-sign elevation in diastolic blood pressure, pulse and COHb</td>
</tr>
<tr>
<td>Dawkins L [32]</td>
<td>2013</td>
<td>▲ 1</td>
<td>-SKYCIG 18 mg/ml nicotine -Experimental study -A repeated measures design -Experimental sessions after 12 hours of abstinence -Exposure: 1) Ten puffs 2) 1 hour ad lib use -14 regular EC users - using at least one 18 mg nicotine cartridge per day). -Smokers or ex-smokers -Aim: to explore the effect of EC on blood nicotine, tobacco withdrawal symptoms, AE and urge to smoke -Plasma nicotine concentration: mean maximum of 13.91 ng/ml by the end of the ad lib puffing period. -Very low level of the total mean AE score: 13 (max. =200). -Light-headedness showed the highest mean, followed by throat irritation, dizziness, Salvation, mouth irritation. 21 different negative symptoms reported. -Only one brand of EC -Selected regular users who probably tolerate EC and have positive experiences -AE were pre-defined symptoms, no spontaneous reporting -Low reporting of AE in regular users. Most frequent: light-headedness, throat irritation and dizziness</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Study Design</td>
<td>Sample Description</td>
</tr>
<tr>
<td>--------------------</td>
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<tr>
<td>Dawkins L [33]</td>
<td>2013</td>
<td>-Experimental study &lt;br&gt;-Within-subjects design &lt;br&gt;-Experimental sessions after 8-10 hours of abstinence, completed two experimental sessions under nicotine (18 mg) and placebo (0 mg) EC conditions &lt;br&gt;-Exposure: 10 min. ad lib use</td>
<td>-20 smokers &lt;br&gt;-Aim: measure prospective memory: Desire to smoke, The Cambridge Prospective Memory Test, Mood and Physical Symptoms Scale &lt;br&gt;-Improved time-based but not event-based prospective memory &lt;br&gt;-Reduced desire to smoke and tobacco withdrawal symptoms</td>
</tr>
<tr>
<td>Dawkins L [34]</td>
<td>2012</td>
<td>-Experimental study &lt;br&gt;-Mixed experimental design &lt;br&gt;-Abstinence of 1-2 hours. &lt;br&gt;-Exposure: 5 min. ad lib use</td>
<td>-86 EC naïve smokers &lt;br&gt;-Aim: memory tests &lt;br&gt;-Letter Cancellation and Brown-Peterson Working Memory Tasks, performed by 60</td>
</tr>
<tr>
<td>Dicpinigaitis PV [36]</td>
<td>2015</td>
<td>-Disposable EC Blu, Classic Tobacco flavor, 20-24 mg nicotine &lt;br&gt;-EC containing EC</td>
<td>-30 healthy nonsmokers &lt;br&gt;-Subgroup: 8 &lt;br&gt;-Use: to evaluate the effect of a single exposure to EC vapor on cough reflex sensitivity &lt;br&gt;-Cough reflex sensitivity (Subjects were not aware that the EC being evaluated in the second phase of the study did not contain nicotine)</td>
</tr>
<tr>
<td>Eisenberg T [37]</td>
<td>2010</td>
<td>-‘NPRO’, 16 mg nicotine cartridge, or ‘Hydro’, 16 mg nicotine cartridge. ≈ Menthol or regular flavor</td>
<td>-16 smokers &lt;br&gt;-Aim: evaluate the hemodynamic effect, heart rate</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Study Design</td>
<td>Participants</td>
</tr>
<tr>
<td>-----------</td>
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<td>--------------</td>
</tr>
<tr>
<td>Etter JF [40]</td>
<td>2011</td>
<td>No randomized trials</td>
<td>36 current users of EC</td>
</tr>
<tr>
<td>Farsalinos K [51]</td>
<td>2012</td>
<td>▲ 8</td>
<td>36 smokers and 40 EC users</td>
</tr>
<tr>
<td>Farsalinos K [43]</td>
<td>2014</td>
<td>▲ 6</td>
<td>108 healthy participants; 51 smokers, and 57 daily EC users who had stopped smoking</td>
</tr>
<tr>
<td>Farsalinos KE [53]</td>
<td>2015</td>
<td>▲ 5</td>
<td>7 experienced vapers blinded to set up of each atomizer</td>
</tr>
</tbody>
</table>

**Annexes – 46**
<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Country</th>
<th>Participants</th>
<th>Design</th>
<th>Aim</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Ferrari M [56]</td>
<td>Italy</td>
<td>No</td>
<td>Experimental study – cross over design?</td>
<td>- Experimental study with flavor, low dose nicotine or no nicotine?</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>Flouris AD [57]</td>
<td>Greece</td>
<td>No</td>
<td>Experimental study</td>
<td>- Giant, Nobacco with ‘‘tobacco taste’’, nicotine 11 mg/ml</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Flouris AD [58]</td>
<td>Greece</td>
<td>No</td>
<td>Experimental study</td>
<td>- Nobacco with ‘‘tobacco taste’’, nicotine 11 mg/ml</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- Experienced vapers took 4-s puffs at 6.5 watts (W), 7.5W, 9W and 10W power levels with both atomizers and were asked to report whether dry puffs were generated.
- Power levels associated with normal and dry puff conditions. Atomizers were attached to a smoking machine and aerosol was trapped.
- Puffs were found at all power levels with A1 (up to 11.3 µg for formaldehyde, 4.5 µg for acetaldehyde and 1.0 µg for acrolein) and at 6.5W and 7.5W with A2 (up to 3.7 µg for formaldehyde, 0.8 µg for acetaldehyde and 1.3 µg for acrolein). The levels were increased by 30 to 250 times in dry puff conditions (up to 344.6 µg for formaldehyde, 206.3 µg for acetaldehyde and 210.4 µg for acrolein, P<0.001).
- Acetone was detected only in dry puff conditions (up to 22.5 µg).
- Few vapers strongly unpleasant taste.
- Vapers will avoid dry puff conditions.

**Aim:**
- To assess the impact of active and passive EC and CC smoking on lung function, fraction of exhaled CO and nitric oxide on lung function, fraction of exhaled NO.
- To evaluate the acute effects of EC on lung function in smokers and never smokers.
- Small study.
- EC naïve participants.

**Notes (Continued):**
- Experienced vapers took dry puff conditions (up to 22.5 µg).
- Few vapers strongly unpleasant taste.
- Vapers will avoid dry puff conditions.

**Exposure:**
- 30 min. of smoking or vaping.
- 15 smokers and 15 never-smokers.
- 10 smokers and 10 non-smokers.
- EC and CC generated similar (p=0.001) effects on serum cotinine levels after active (60.6±34.3 versus 61.3±36.6 ng/ml) and passive (2.4±0.9 versus 2.6±0.6 ng/ml) smoking.
- Neither a brief session of active EC smoking (indicative: 3% reduction in FEV1/FVC) nor a 1 h passive EC smoking (indicative: 2.3% reduction in FEV1/FVC) significantly affected the lung function (P<0.001).
- Active (indicative: 7.2% reduction in FEV1/FVC; p<0.001) but not passive (indicative: 3.4% reduction in FEV1/FVC; P=0.005) CC smoking undermined lung function.
- No effect of active EC smoking on FeNO.

**EC effects:**
- EC and CC with normal smoking, and active EC vaping.
- EC and CC with normal smoking, and active EC vaping.
- EC and CC with normal smoking, and active EC vaping.

**Cotinine levels:**
- 15 smokers and 15 never-smokers.
- Smokers reporting previous use of EC were excluded.
- Aim: to assess the impact of the short term exposure on lung function, fraction of exhaled CO and nitric oxide.

**Cotinine levels:**
- Use of EC: sign decrease in FEF75% (61.6±18.7 vs. 55.4±17.7, p=0.04) in smokers.
- Use of EC without nicotine: no immediate adverse physiologic effects after short-term use in the non-smokers and a small effect on FEF75% in the smokers group.
- Smoking: sign increase in fraction of exhaled CO, sign decrease in FEV1 and FEF75%, while no significant changes were observed in fractional exhaled Nitric Oxide.

**EC effects:**
- EC and CC generated similar (p=0.001) effects on serum cotinine levels after active (60.6±34.3 versus 61.3±36.6 ng/ml) and passive (2.4±0.9 versus 2.6±0.6 ng/ml) smoking.
- Neither a brief session of active EC smoking (indicative: 3% reduction in FEV1/FVC) nor a 1 h passive EC smoking (indicative: 2.3% reduction in FEV1/FVC) significantly affected the lung function (P<0.001).
- Active (indicative: 7.2% reduction in FEV1/FVC; p<0.001) but not passive (indicative: 3.4% reduction in FEV1/FVC; P=0.005) CC smoking undermined lung function.
- No effect of active EC smoking on FeNO.

**Cotinine levels:**
- 15 smokers and 15 never-smokers.
- Smokers reporting previous use of EC were excluded.
- Aim: to evaluate the acute effect of active and passive EC and CC smoking on lung function, fraction of exhaled CO and nitric oxide.

**Cotinine levels:**
- EC and CC with normal smoking, and active EC vaping.
- EC and CC with normal smoking, and active EC vaping.
- EC and CC with normal smoking, and active EC vaping.

**Cotinine levels:**
- EC and CC generated similar (p=0.001) effects on serum cotinine levels after active (60.6±34.3 versus 61.3±36.6 ng/ml) and passive (2.4±0.9 versus 2.6±0.6 ng/ml) smoking.
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- Active (indicative: 7.2% reduction in FEV1/FVC; p<0.001) but not passive (indicative: 3.4% reduction in FEV1/FVC; P=0.005) CC smoking undermined lung function.
- No effect of active EC smoking on FeNO.

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- Neither a brief session of active EC smoking (indicative: 3% reduction in FEV1/FVC) nor a 1 h passive EC smoking (indicative: 2.3% reduction in FEV1/FVC) significantly affected the lung function (P<0.001).
- Active (indicative: 7.2% reduction in FEV1/FVC; p<0.001) but not passive (indicative: 3.4% reduction in FEV1/FVC; P=0.005) CC smoking undermined lung function.
- No effect of active EC smoking on FeNO.
<table>
<thead>
<tr>
<th>Study</th>
<th>Authors</th>
<th>Year</th>
<th>No</th>
<th>Treatment</th>
<th>Participants</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gennimata S. A.</td>
<td>[61] 2012</td>
<td>Abstract</td>
<td>No</td>
<td>Unknown</td>
<td>Experimental study; Exposure: vaping for 10 minutes</td>
<td>32 consecutive subjects, 8 never smokers and 24 smokers (11 with normal spirometry, and 13 patients with COPD and asthma) - Define the acute effects of an EC on respiratory functions in healthy subjects and smokers with and without chronic airway obstruction</td>
</tr>
<tr>
<td>Hecht SS</td>
<td>[73] 2014</td>
<td>No</td>
<td>21 different from US market</td>
<td>Current vapers: Current vapers who had not smoked CC for at least 2 months provided urine samples which were analyzed by validated methods for a suite of toxicant and carcinogen metabolites. Levels were compared to those found in CC smokers from three previous studies</td>
<td>- Urine sampling in current vapers - Current vapers who had not smoked CC for at least 2 months provided urine samples which were analyzed by validated methods for a suite of toxicant and carcinogen metabolites. Levels were compared to those found in CC smokers from three previous studies</td>
<td>- Levels of 1-HOP, total NNAL, 3-HPMA, 2-HPMA, HPMPMA, and SPMA were significantly lower in the urine of EC users compared to CC smokers - 4 EC users had higher than expected levels of total NNAL, albeit lower than typically seen in smokers - Levels of nicotine and cotinine were significantly lower in EC users compared to CC smokers in one study but not in another</td>
</tr>
<tr>
<td>Martini S</td>
<td>[108] 2014</td>
<td>No</td>
<td>A tobacco flavor e-liquid (low + high nicotine) - Ref: CC 0.8 mg nicotine</td>
<td>- Experimental study; Exposure: asked to smoke a CC and to vape an EC (with and without nicotine), and an EC without liquid (control session). Three puff profiles made up of four consecutive puffs with a 30-s inter puff interval were performed for each test</td>
<td>- 25 smokers - Aims: to compare the short-term respiratory effects due to the inhalation of EC and CC-generated mainstream aerosols through the measurement of the exhaled nitric oxide (eNO)</td>
<td>- The mean eNO variations measured after each smoking/vaping session were equal to 3.2 ppb, 2.7 ppb and 2.8 ppb for EC without nicotine, with nicotine, and for CC, respectively. - Total particle number concentrations in the mainstream resulted equal to 3.5±0.4×10^6, 5.1±0.1×10^6, and 3.1±0.6×10^6 part. cm^-3 for EC without nicotine, with nicotine, and for CC, respectively. - Alveolar doses for a resting subject were estimated equal to 3.8×10^3, 5.2×10^3 and 3.5×10^3 ppm</td>
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<td></td>
<td>Only one brand of EC</td>
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<td></td>
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<td></td>
<td>Short-term exposure caused immediate airway obstruction</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Type</td>
<td>Details</td>
<td>Conclusion</td>
<td>Notes</td>
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</tr>
<tr>
<td>McRobbie H</td>
<td>2015</td>
<td>Experimental study</td>
<td>- Green Smoke EC (labeled 2.4% nicotine), a first-generation &quot;cig-a-like&quot; device Ref: no - Experimental study: Exposure: at target quit date participants were provided with their EC and received instructions on its use. Instructed to use EC ad lib - Received standard withdrawal-oriented behavioral support x 2 - 40 adult smokers wanting to stop smoking, recruited through advertisements in free newspapers - Excluded: women who were pregnant or breast-feeding, smokers with any current serious illness, and those who had used EC for more than one week in the past After 4 weeks of EC: - Use: 33 participants were using EC, 16 (48%) were abstinent (CO-validated) from smoking during the previous week (EC only users), and 17 (52%) were &quot;dual users.&quot; - Sign reduction in CO in EC-only users (–12 ppm) and dual users (–12 ppm). Cotinine levels: declined, but to a lesser extent - Mean 3-HPMA (primary metabolite of acrolein) levels: decreased 1.28 ng/mg creatinine in EC-only users and by 1.47 ng/mg creatinine in dual users - Tested one brand only - Longer follow-up needed if dual users can maintain significant reduction in smoking - In dual users, EC use significantly reduced exposure to CO and acrolein because of a reduction in smoke intake</td>
<td>- Test one brand only - EC naive participants - Short term exposure</td>
<td>- The present study supports our preliminary results showing increased airway resistance and a concomitant decrease in specific airway conductance. - These changes might be due to the vaporizing liquid but not to the inhaled nicotine per se.</td>
<td></td>
</tr>
<tr>
<td>Papaseit [123]</td>
<td>2014</td>
<td>Randomized and crossover controlled trial</td>
<td>- Nhos 16 mg/mL nicotine second-generation EC Ref: CC Marlboro. - Randomized and crossover controlled trial: Exposure: nicotine 0.8 mg/cig was administered in two successive doses separated by an interval of 1 h: baseline, 10 puffs in 5 minutes (equivalent to smoking one CC), 55-min of rest period, 10 puffs and a 55-min of rest period - 6 healthy male regular CC smokers who were abstinent from nicotine use for 12 h - Nicotine produced increases in heart rate, diastolic and systolic arterial pressure immediately after administration, being more intense after CC than EC use - Temperature and pupil diameter was not consistently changed</td>
<td>Tested one brand only - EC naive participants - Short term exposure</td>
<td>EC use produces a moderate increase in vital parameters</td>
<td></td>
</tr>
<tr>
<td>Polosa R</td>
<td>2014</td>
<td>Retrospective review of changes in lung function and asthma control</td>
<td>- EC of unknown type - Retrospective review of changes in lung function and asthma control: Exposure: self-selected switch from smoking to - 18 smoking asthmatics who switched to regular EC use (10 EC only, 8 dual use, all dual users smoked ≤5 conventional cigarettes/day)</td>
<td>Significant improvements in spirometry data, asthma control and airway hyper-responsiveness - Dual users smoked 3.9 CC p. day only. They also had sign. improvement in lung function</td>
<td>Selected regular users who probably tolerate EC and have positive - Study indicates that regular use of EC to substitute smoking is associated with objective and subjective improvements.</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>Brand</td>
<td>Type</td>
<td>Study Design</td>
<td>Exposure</td>
<td>Aim</td>
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<tr>
<td>--------</td>
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</tr>
<tr>
<td>Popa C</td>
<td>2015</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Experimental study</td>
<td>2 sessions of 10 min with vaping or smoking</td>
<td>-10 volunteers, 5 current CC smokers and 5 current EC vapers&lt;br&gt;- CO, laser-photocoustic spectrometry&lt;br&gt;- Aim: to examine the ethylene changes at different time intervals in the exhaled breath composition of EC vapers and CC smokers, before and after vaping/smoking</td>
</tr>
<tr>
<td>Tsikrika S</td>
<td>2014</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Experimental study</td>
<td>Gr. A: vaping EC with 11mg nicotine in 10 min&lt;br&gt;Gr. B: same, but reduced to nicotine to 0mg nicotine</td>
<td>-62 volunteers&lt;br&gt;-10 non-smokers/52 smokers: Gr. A: vaping EC with 11mg nicotine in 10 min</td>
</tr>
<tr>
<td>Vakali S</td>
<td>2014</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Experimental study</td>
<td>Gr. A: vaping 10 min, EC with 11mg nicotine Gr. B: same, but 0mg nicotine</td>
<td>-64 volunteers&lt;br&gt;-Gr. A: 12 never-smokers and 29 smokers&lt;br&gt;-Gr. B: 14 never-smokers and 9 smokers</td>
</tr>
<tr>
<td>van Staden SR</td>
<td>2013</td>
<td>eGo</td>
<td>A single group within-subject design</td>
<td>Experimental study</td>
<td>Switch from smoking to EC vaping in 2 weeks</td>
<td>-15 smokers switched to EC, 2 drop-outs&lt;br&gt;-Aim: determine the effects of EC on arterial and venous COHb levels and evaluate participants’ perception on their health</td>
</tr>
</tbody>
</table>
| Vansickel AR [162] 2010 | No | ECs: "NPRO" 18 mg nicotine cartridge  
"Hydro" 16mg nicotine cartridge  
Ref:  
- Own brand CC  
- Sham= unlit CC  
- Experimental study  
- Repeated-measures controlled study  
- Refrained from smoking in 12 hours  
- 4 Latin-square ordered conditions  
- Exposure: two, 10-puff EC bouts  
- 32 healthy smokers of at least 15 cig  
- Aim: describe clinical laboratory methods that could be used to characterize EC users' nicotine and CO exposure, cardiovascular response  
- EC or sham conditions, pre- and post administration:  
  - No significant changes in plasma nicotine  
  - No significant changes in heart rate  
  - No significant changes in CO level  
  - No reporting of "lightheaded" and "dizzy" within the first five minutes following the first administration  
| EC or sham conditions, pre- and post administration:  
  - No significant changes in plasma nicotine  
  - No significant changes in heart rate  
  - No significant changes in CO level  
  - No reporting of "lightheaded" and "dizzy" within the first five minutes following the first administration  
| Only one brand of EC  
| Short experiment duration  
| Only one type of EC  
| Small study  
| No changes in plasma nicotine and heart rate  
| No increase in CO  

| Vansickel AR [163] 2012 | No |  
- "Vapor King" + "WOW Cowboy"  
- Ref: own brand CC  
- Experimental study  
- 4 within-subject sessions  
- Exposure: six 10-puff bouts - separated by 30-mins  
- 20 healthy smokers of at least 15 cig  
- Aim: abuse liability assessment of EC in current CC smokers  
- Plasma nicotine, cardiovascular response, and subjective effects  
- After 5 minutes:  
  - Mean plasma nicotine increased from a pre-administration level of 2.2 (SD=0.78) ng/ml to 7.4 (SD=5.1) ng/ml (4 bouts of 10 puffs needed)  
  - Heart rate increased from a pre-administration average of 67.5 (SD: 6.2) bpm to 75 (SD: 8.3) bpm  
  - No adverse events  
| Short experiment duration  
| EC naïve participants  
| Few puffs of EC  
| Only one type of EC  
| Small study  
| Increase in heart rate  

| Vardavas CI [164] 2012 | No |  
- NOBACCO EC + NOBACCO MLB-MED filter cartridge 11 mg nicotine  
- Ref: control group inhaled with cartridge removed  
- Experimental study  
- Exposure: ad lib use for 5 min  
- 30 healthy smokers of at least 5 pack years (10 volunteers were in both control and experimental group)  
- Aim: assess acute impact on the pulmonary function tests and F ENO, impedance, respiratory resistance  
- Statistically significant decrease in F ENO and an increase in impedance by 0.04 kPa/(L/s) (P = .003), respiratory resistance at 5 Hz by 0.04 kPa/(L/s) (P = .003), at 10 Hz by 0.034 kPa/(L/s) (P = .008), at 20 Hz by 0.043 kPa/(L/s) (P = .007), and overall peripheral airway resistance (beta , 0.042 kPa/[L/s], P = .024), after using an EC  
| Only one brand of EC  
| EC naïve participants  
| Lack of proper control group  
| Overlap of control and experiment group  
| 5 min vaping only  
| Small study  
| Immediate adverse effects on the airways after short-term use that are similar to some of the effects seen with tobacco smoking  
| Usage was associated with increased flow resistance even though spirometry-assessed lung function was deemed normal |
Yan XS [173] 2015

| EC | 2 commercial products that contain 16 mg/mL nicotine, 3 non-commercial products that contain 24 g/mL nicotine |
| EC | Flavors: Classic Tobacco or Menthol, Glycerin and/or PPG based |
| EC | Ref: CC; Marlboro Gold King Size 0.8 mg nicotine |

- **Experimental study**
  - Two exposure scenarios from Day 1 to Day 11: half-hour controlled administration and one hour ad lib use
  - 38 healthy EC-naive daily smokers included from start, 14 withdrew, 23 included in analyses
  - Aim: to characterize EC users’ exposure to nicotine, and to investigate the acute effects of EC on the hemodynamic measurements (blood pressure and heart rate) in comparison with the effects of regular smoking

- **Significantly increased blood pressure and heart rate after use of several EC products**
  - Especially diastolic blood pressure was increased by EC use - comparable to increase in CC smoking
  - Use of EC had no impact on the exhaled CO levels
  - Nicotine plasma concentrations after 1.5 h: significantly lower in the users of EC than of CC
  - The combination of glycerin and propylene glycol as the vehicle facilitated delivery of more nicotine than glycerin alone

- **Only one brand of EC**
  - 1 person missing (38-14=24) – what happened?
  - EC-naive daily smokers = low nicotine exposure in EC users and under-estimation of real effect in current vapers
  - Drop-outs not described
  - Small short-term study

*This study could as well have been placed in annex 3 showing adverse events [129]*

**EC** = electronic cigarette
**CC** = conventional cigarette
**SAE** = serious adverse event
**AE** = adverse events
**COHb** = carboxyhemoglobin
**CO** = Carbon monoxide
**COHb** = carboxyhemoglobin
**CBC** = complete blood count
**HPHC** = harmful and potentially harmful constituents
**HMPMA** = 3-hydroxy-1-methylpropyl mercapturic acid
**F ENO** = Fraction of exhaled nitric oxide
**FEV1**
**FEV1/FVC**
**FEF25-75**
**ECG** = electrocardiography
**HDL** = High-density lipoprotein
**HMPMA** = 3-Hydroxy-1-methylpropyl mercapturic acid
**hs-CRP** = High-sensitivity
**IL-6** = interleukin-6
**MHBMA** = monohydroxybutenyl mercapturic acid
**MPO** = myeloperoxidase
**NEq** = Nicotine equivalents
**o-TOL** = o-Toluidine
**ox-LDL** = low-density lipoprotein
**PPG** = propylene glycol

- **Significantly increased blood pressure and heart rate after use of several EC products**
- **The studied EC delivered less exposure of nicotine and thereby less cardiovascular effects compared to CC smoking**

Annexes – 52
**Conflicts of interest** - Conflicts of interest of each study should be assessed individually.

- ▲ 1: Study was funded and supported by manufacturer of EC. LD has received funding to speak at research conferences and benefits in kind from EC companies.
- ▲ 2: KD has a collaborative relationship with manufacturer of EC who provided free supplies of the EC for the study.
- ▲ 3: KD has a collaborative relationship with manufacturer of EC who provided free supplies of the EC for the study.
- ▲ 4: EC manufacturer sponsored the EC used in study.
- ▲ 5: Some of the studies by KF and VV were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies.
- ▲ 6: Some of the studies by KF and VV were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. Other studies by GR have been sponsored by EC company.
- ▲ 7: employees in tobacco company which also manufactures EC.
- ▲ 8: No stated, but some of the studies by KF were performed using unrestricted funds provided to the Onassis Cardiac Surgery Center by EC companies. KF has a website “Ecigarette Research Advocate Group” which represents a strictly positive view on EC and provides several links to vapor clubs.
- ▲ 9: HR is Clinical Director at The Dragon Institute (research-based training, studies on the latest changes in the health industry etc.); reports receiving commercial research grant from manufacturer of smoking cessation medication; and has received speakers’ bureau honoraria from manufacturers of smoking cessation medication. MLG reports receiving commercial research grant from manufacturer of smoking cessation medication. PJ has received speakers’ bureau honoraria from and is a consultant/advisory board member for the manufacturers of stop-smoking medications. No potential conflicts of interest were disclosed by the other authors.
- ▲ 10: RP has received grant support, has served as a speaker and has served as a consultant for anti-asthma drug manufacturers and has received payment for developing educational presentations and being a consultant for manufacturer of smoking cessation medication; he has also served as a consultant for EC distributor. JBM has received honoraria for speaking and financial support to attend meetings/advisory boards from anti-asthma drug manufacturers.
### Annex 4. Animal experimental studies reporting health effects (n=11*).

<table>
<thead>
<tr>
<th>Name of first author</th>
<th>Reference Year</th>
<th>Conflict of interest ▲</th>
<th>Relevance for passive exposure to EC (Yes= ▲)</th>
<th>Type of product(s) Type/number of animal</th>
<th>Method Exposure Reference groups</th>
<th>Aim of study/ Outcome measure</th>
<th>Results</th>
<th>Weakness</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraghty P</td>
<td>2014</td>
<td>No</td>
<td></td>
<td>A/J mice (n=8 per group) 1. EC liquid (American eLiquid Store) 18 mg/ml nicotine in 50% PPG/50% VG 2. EC liquid, 36 mg/ml nicotine in 50% PPG/50% VG</td>
<td>Exposure by a small animal nebulizer. Exposed for 1 hour/day, 5 days a week for 4 months Reference: 1. Nebulized phosphate-buffered saline (PBS), 2. Vehicle (50% PPG/50% VG),</td>
<td>Aim: to assess the safety and lung effects of e-cigarettes</td>
<td>Exposure to EC vapor with nicotine increased lung cytokine and protease expression, mucin staining in the airways, caspase 3/7 activity in the tissue and TUNEL staining in the lung parenchyma. Exposure to EC vapor induced emphysema and airway hyper-reactivity while the vehicle had no effect</td>
<td>Few animals in each group, One brand, Relatively short daily exposure</td>
<td>Animal study shows that longer-term exposure of EC causes asthma and emphysema</td>
</tr>
<tr>
<td>Husari A</td>
<td>2015</td>
<td>No</td>
<td></td>
<td>Four-month maleC57BL/6J mice Pre-filled V4L</td>
<td>Smoke generator, mixing/conditioning chamber and &quot;nose-only&quot; rodent exposure</td>
<td>Aim: to investigate the effects of EC aerosol and CC smoke in an animal</td>
<td>Wet-to-dry ratio was higher in CC when compared to EC but sign higher in EC than in control group</td>
<td>The aerosol constituents and size</td>
<td>Despite higher exposure conditions, EC exhibited less</td>
</tr>
</tbody>
</table>
Lerner CA  
[98] 2015  
No  
Blu EC (Classic tobacco flavor; 16 mg nicotine)  
- Eight weeks old wild type C57BL/6J mice  
- Mice were exposed to side-stream EC vapor for 5 h per day for 3 days (acute exposure) in inhalation chambers  
- No reference group  
- Aim: to investigate if exposure to EC vapor results in measurable oxidative and inflammatory responses in the lung  
- Exposure to EC vapor caused lung inflammation and pro-inflammatory response  
- MCP-1, a potent macrophage chemotactic cytokine was significantly increased  
- Levels of IL-6, IL-1β and IL-13 were significantly increased  
- Increased pro-inflammatory cytokines and diminished lung glutathione levels which are critical in maintaining cellular redox balance  
- Short term exposure  
- One brand  
- Few animals  
- EC inhalation have an impact on cellular oxidative stress, redox imbalance, and lung inflammation, in vitro in lung cells and in vivo in lungs

Lim HB  
[99] 2014  
No  
- Z-company, 16 mg/ml nicotine  
- 24 Five-week-old female BALB/c mice  
- Normal group (n = 8) given drinking water  
- Ovalbumin (OVA)-sensitized group (n = 8)  
- OVA sensitized EC treated group (n = 8)  
- Exposure: cartridge solution of EC was diluted 50 times and 100 µl of the diluted solution was intratracheally instilled two times a week for 10 weeks  
- Aim: to investigate the effects of an EC solution on allergen related asthmatic airway inflammation (AI) and airway hyper-responsiveness (AHR), when it is delivered by intra-tracheal route in mice  
- No remarkable changes in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase enzymes in serum  
- Increased infiltration of inflammatory cells including eosinophils, into airways from blood, aggravated the asthmatic AI and AHR, and stimulated the production of cytokines such as interleukin (IL)-4, IL-5 and IL-13, and OVA-specific IgE production  
- Fluid not vapor  
- Few animals  
- Single brand  
- Experimental dose of EC, not necessarily reflecting real-life exposure - Intra-tracheally installed EC solution instead of inhalation of vapor  
- EC inhalation can function as an important factor to exacerbate the allergy-induced asthma symptoms

McGrath-Morrow S  
[112] 2015  
No  
Joytech 510-T EC with 510-T tank cartridges, atomizer and auto switch battery; Liquid: 0% and 1.8% nicotine solution with no flavoring  
- Neonatal mice were exposed to EC vapor or room air  
  - The size of the chamber was 13.5 cm x 9 cm x 8.7 cm  
  - 1) group: 1.8% nicotine PPG or 0% nicotine PPG once a day for days 1 and 2 of life then twice a day from days 3 to 9 of life  
  - 2) Control: kept in  
- Aim: to determine if neonatal exposure to EC emissions would lead to impaired postnatal lung growth and systemic nicotine absorption  
- Outcome: weight gain, postnatal alveolar growth and systemic  
- Mice exposed to 1.8% nicotine/PPG had a 13.3% decrease in total body weight compared to room air controls  
- Decreased mean weight in the 0% nicotine/PPG mice compared to room air controls suggest that nicotine alone did not entirely account for the lower weights  
- Plasma cotinine levels were found to be elevated in neonatal mice exposed to EC  
- Short term study  
- Single brand  
- Experimental dose of EC, not necessarily reflecting real-life exposure - Impaired lactation in the mother  
- EC emissions (with or without nicotine) during the neonatal period can adversely impact weight gain  
- Exposure to EC with nicotine cause detectable levels of...
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Treatment Details</th>
<th>Experimental Setup</th>
<th>Aim</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palpant NJ</td>
<td>2015</td>
<td>- Wild-type zebrafish (Danio rerio)</td>
<td>- Zebrafish embryos were exposed to either control, EC extract or CC extract</td>
<td>Aim: to determine the impact of EC and CC on heart development in vitro and in vivo.</td>
<td>- Exposure to both types of cigarettes resulted in broad, dose-dependent developmental defects coupled with severe heart malformation, pericardial edema and reduced heart function. - CC are more toxic than EC at comparable nicotine concentrations.</td>
</tr>
<tr>
<td>Pouzoni L</td>
<td>2015</td>
<td>- 183 Male BALB/c mice; one month old</td>
<td>- Inhalation chambers (22 cm wide x 40 cm long x 20 cm high) connected to Rodent Ventilator</td>
<td>Aim: to compare the effects of CC smoke and EC vapor containing the same amount of nicotine on mice</td>
<td>- Second-hand exposure to EC vapor or CC smoke led to similar brain cotinine and nicotine levels, urine cotinine levels up-regulation of α4β2 nicotinic acetylcholine receptors in different brain areas. - EC and CC had different effects on body weight, food intake, and the signs of mecamylamine-precipitated and spontaneous withdrawal episodic memory and emotional responses.</td>
</tr>
<tr>
<td>Salturk Z</td>
<td>2015</td>
<td>- 16 Female Wistar albino rats</td>
<td>- Exposure: The study group was exposed to EC vapor for 1 hour/day for 4 weeks in inhalation chambers (30 x 40 x 50 cm)</td>
<td>Aim to examine the vocal folds of rats exposed to EC vapor (histopathologically by hematoxylin and eosin staining and immunohistochemically by Ki67 staining)</td>
<td>- Squamous metaplasia was detected in 4/8 rats in the study group but in only 1/8 rat in the control group; not significant (P = 0.106)</td>
</tr>
<tr>
<td>Schweitzer</td>
<td></td>
<td>- C57Bl/6 mice (4-)</td>
<td>- Exposed to nicotine, EC</td>
<td>Aim: to investigate acute</td>
<td>- Nicotine and EC extracts</td>
</tr>
<tr>
<td>Ref.</td>
<td>Year</td>
<td>Treatment</td>
<td>Exposure</td>
<td>Effect</td>
<td>Control</td>
</tr>
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<tr>
<td>KS [144]</td>
<td>2015</td>
<td>Male C57BL/6 (age 8 wks) mice</td>
<td>-Nicotine solutions (Vanilla, Kentucky Prime) and nicotine-free Kentucky Prime EC used to generate vapor: Joyetech 510 T tank, atomizer, cartridges, with 510-T EC or nicotine-free CC (1R5F)</td>
<td>-Exposure: via a whole-body exposure system for 1.5 h, twice per day for 2 weeks</td>
<td>-EC exposed mice:</td>
</tr>
<tr>
<td>Smith D [146]</td>
<td>2015</td>
<td>Timed-pregnant C57BL/6f mice</td>
<td>-Exposed to 2.4% nicotine in PPG or 0% nicotine /PPG once a day for gestational day 15 until delivery.</td>
<td>-Aim: to determine if exposure to EC nicotine vapors during late prenatal and early postnatal life altered behavior in adult mice</td>
<td>-Adult male mice exposed to 2.4% nicotine /PPG vapors had significantly more head dips in the zero maze test and higher levels of rearing activity in the open field test compared to 0% nicotine /PPG exposed mice and untreated controls.</td>
</tr>
<tr>
<td>Susan TE [148]</td>
<td>2015</td>
<td>Male C57BL/6 (age 8 wks) mice</td>
<td>-Nicotine solutions (Vanilla, Kentucky Prime) and nicotine-free Kentucky Prime EC used to generate vapor: Joyetech 510 T tank, atomizer, cartridges, with 510-T EC or nicotine-free CC (1R5F)</td>
<td>-Exposure: via a whole-body exposure system for 1.5 h, twice per day for 2 weeks</td>
<td>-EC exposed mice:</td>
</tr>
</tbody>
</table>

**Notes:**
- 

**Aim:** to determine whether nicotine in EC evaporates or condenses to produce a vapor which mimics the inhalation of nicotine by humans.
- 

**Control:** filtered air
- 

**Effect:** caused rapid oxidative and nitrooxidative stress observed in the bronchoalveolar lavage fluid and plasma as well as a trend toward greater neutrophil lung inflammation at 24 h following inhalation as measured by the relatively less sensitive method of bronchoalveolar lavage fluid cytospins, rather than intravital microscopy.
air exposure, but decreased Th1 and Th17 cytokine levels

*Four of these studies are also/partly mentioned in Table 3/Annex 5 on animal experimental studies [98] [122] [144] [78]

EC = electronic cigarettes
CC = conventional cigarettes
PPG = propylene glycol
VG = vegetable glycerin
IL-6 = Interleukin 6
Th = T-helper cells
## Annex 5. Studies reporting adverse events (n=31)

<table>
<thead>
<tr>
<th>Name of first author</th>
<th>Conflict of interest</th>
<th>Type of product(s)</th>
<th>Type of study</th>
<th>Participants</th>
<th>Symptoms reported</th>
<th>Symptoms</th>
<th>Weakness/strength Association between EC and symptoms?</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adriens K [1] 2015</td>
<td>No</td>
<td>“Joyetech eGo-C” and the “Kanger T2-CC”; 30 mL bottles of tobacco-flavored e-liquid (Dekang “Turkish Blend”), containing 18 mg/mL of nicotine</td>
<td>Prospective study; randomized controlled smoking reduction trial with three arms three lab 3 sessions (over two months): vaped/smoked for five minutes</td>
<td>48 volunteers not willing to quit</td>
<td>EC group reported only positive symptoms /improvements, dual use group reported positive and negative</td>
<td>The control group reported more complaints about CC than the EC groups about using EC</td>
<td>Only two brands</td>
<td>EC users reported more benefits in prospective study</td>
</tr>
<tr>
<td>Bartram A [6] 2015</td>
<td>No</td>
<td>Unknown but high content of PPG</td>
<td>Case report</td>
<td>A 55-year-old healthy man; drank 40 units of alcohol/week and smoked 30CC/day, and but quit and switched to EC</td>
<td>8-week history of ulceration on the right buccal mucosa associated with white patches throughout the mouth and lower lip after he started to use EC</td>
<td>Examination: a typical appearance of lichen planus with white reticular patterned striae on the oral mucosa and the lower lip</td>
<td>One patient</td>
<td>EC use was found to be associated with a florid lichenoid reaction</td>
</tr>
<tr>
<td>Bullen C [11] 2013</td>
<td>▲ 7</td>
<td>Elusion + 16mg or 0 mg nicotine</td>
<td>Prospective study; randomized controlled smoking cessation trial</td>
<td>Total 657 participants were randomized to nicotine-EC (n=289), no-nicotine/placebo EC (n=295) or nicotine patch (n=73) for 13 weeks</td>
<td>AE= 107 participants in the nicotine EC group (137 events); 96 participants in the patches group (119 events); 26 participants in the EC placebo group (36 events)</td>
<td>The difference between the AE rates in the nicotine EC group and patches</td>
<td>Only one brand</td>
<td>A higher number and proportion of adverse events occurred in the nicotine EC group than in the patches group; however, there was no evidence of an association</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Design</td>
<td>Participants</td>
<td>Outcomes</td>
<td></td>
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</tr>
<tr>
<td>Bullen C</td>
<td>1</td>
<td>Single blind randomised repeated measures cross-over trial</td>
<td>40 adult dependent smokers of 10 or more CC per day.</td>
<td>Most frequently reported AE: mouth and throat irritation; statistically significantly more frequent than with inhalator (p&lt;0.001).</td>
<td></td>
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</tr>
<tr>
<td>Camus M</td>
<td>No</td>
<td>Case report</td>
<td>A 49-year-old woman with colitis ulcerosa</td>
<td>Patient restarted smoking 9 months after colitis ulcerosa diagnosis while symptoms were still present, stopped any medication and went into clinical remission within a few days.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caponetto P</td>
<td>2</td>
<td>Prospective 12-months observational study</td>
<td>14 smokers with schizophrenia smoking ≥20 CC per day and not intending to quit</td>
<td>Most frequent AE: Nausea, throat irritation, headache (all 14%) and dry cough 29%. AE diminished substantially by week-24.</td>
<td></td>
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</tr>
</tbody>
</table>

RuyanV8, 16 mg nicotine or 0 mg capsules

≤Ref: Nicorette nicotine inhalator or usual CC

≤SAE events: death (n=1, in nicotine EC group), life threatening illness (n=1, in nicotine EC group), admission to hospital (12% of all events in nicotine EC group, 8% in patches group, and 11% in placebo EC group), persistent or significant disability or incapacity, congenital abnormality, medically important (6% of all events in nicotine EC group, 4% in patches group, and 3% placebo EC group)

No serious AE in any groups were related to product use with study product, and the event rate was not significantly different
<table>
<thead>
<tr>
<th>Study</th>
<th>#</th>
<th>Study groups</th>
<th>Participants</th>
<th>Adverse Events</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caponetto P [17] 2013</td>
<td>▲ 2</td>
<td>3 Study groups: Categoria 7.2 mg nicotine for 12 weeks, Categoria 7.2 mg nicotine for 6 weeks and 5.4 mg for 6 weeks, Ref: Categoria without nicotine</td>
<td>300 smokers not intending to quit</td>
<td>AE: Sign. reduction in frequency of cough, dry mouth, shortness of breath, and headache was observed in all three study groups (p&lt;0.001)</td>
<td>Only one brand of EC, comparison with other smoking cessation products not possible, high drop-out rate could be caused by AE, no information on how many/which AE were estimated to be causally associated with EC, many reports of AE and SAE, there is not necessarily a causal relationship between AEs reported and EC use, as some AEs could be related to pre-existing conditions or due to other causes not reported.</td>
</tr>
<tr>
<td>Chen IL [21] 2013</td>
<td>No</td>
<td>Unknown</td>
<td>Approximately half of all tobacco-related AE reports since late 1980ies concern EC</td>
<td>Of the 47 reports on ECs, 8 reported SAE, SAE reported: hospitalization for illnesses such as pneumonia, congestive heart failure, disorientation, seizure, hypotension, possible aspiration pneumonia, second-degree burns to the face (product exploded in consumer's mouth), chest pain and rapid heartbeat, possible infant death secondary to choking on EC cartridge, and loss of vision requiring surgery.</td>
<td>No information on how many/which AE were estimated to be causally associated with EC.</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Sample</td>
<td>Methodology</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>-------</td>
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<td>----------</td>
<td></td>
</tr>
<tr>
<td>Dawkins L [35] 2013</td>
<td>3</td>
<td>TECC and Totally Wicked E-Liquid</td>
<td>Online survey + Users of the two most popular brands in UK + EC users’ nature, use of EC, effects of EC</td>
<td>Shortness of breath, abdominal pain, pleurisy, blurry vision, and sleepy/tired. 74% reported they had not smoked for weeks/months since using the EC. The most common was throat irritation, followed by mouth irritation. &lt;16% reported experiencing any degree of effect, &lt;3% reported a high level of AE. Only two brands of EC. Respondents (most had quit smoking) reported few negative symptoms (mouth and throat irritation) and many positive health effects with EC. Majority state: it feels healthier and use improved cough.</td>
<td></td>
</tr>
<tr>
<td>Etter JF [39] 2010</td>
<td>4</td>
<td>Sixteen different brands, most frequent: Janty, Joye, Sedansa</td>
<td>A survey of users</td>
<td>81 respondents ever users of EC who indicated the most used brand + 72 daily users, 63% recently quit smoking CC</td>
<td>Positive and negative symptoms. EC positive symptoms, 134: improved breathing and reduced cough and expectoration, fewer sore throats, improved health and physical fitness, improved sleep, smell and sense of taste. EC negative symptoms, 61: dry mouth and throat, vertigo, headache or nausea, weight gain. Self-reports. Selected vapers, probably more motivated to quit smoking, slightly less dependent on tobacco, and more highly educated. Respondents reported more positive than negative effects with EC: many reported positive effects on the respiratory system, which were probably associated with stopping smoking.</td>
</tr>
<tr>
<td>Farinha H [42] 2015</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Case report</td>
<td>66-year-old female patient, heavy smoker and coffee drinker, with hypertension and history of depression. She had stopped tobacco smoking and initiated EC a few weeks before</td>
<td>1 negative symptom. Presented with an asymptomatic black discoloration of the tongue she noted that day, no other sign associated. The diagnosis of lingua villosa nigra was established. She stopped using the EC and started smoking again and the lesions started disappearing spontaneously in less than one week. The lesions worsened when she began using EC again. Time association. Symptoms reversed when patient stopped using EC and worsened when she started again. A case of probable association between EC use and lingua villosa nigra is reported.</td>
</tr>
<tr>
<td>Farsalinos KE [49] 2013</td>
<td>▲ 11</td>
<td>Second or third generation EC</td>
<td>Interviews with vapors (32 visitors to a hospital + 81 members of consumers’ internet)</td>
<td>111 experienced EC users who had completely substituted smoking with EC use for at least 1 month</td>
<td>Positive and negative symptoms. 42% had quit during the first month of using ECs. Reported AE: throat irritation (27%) cough (14%), gastrointestinal discomfort/epigastric burning (7%), palpitations (5%), headache, sleepiness, sleeplessness, atypical chest pain, gum and nose bleeding. Selected vapors; those who tolerate EC, have a regular use and experience positive changes they want to share. Those who had persistent AE had quit. Side effects were mild and temporary. The vast majority of participants reported better exercise capacity and improved olfactory and gustatory senses.</td>
</tr>
<tr>
<td>Author</td>
<td>N</td>
<td>EC type</td>
<td>Study Design</td>
<td>Findings</td>
<td>Use</td>
</tr>
<tr>
<td>-----------------</td>
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<td>------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Farsalinos KE</td>
<td>9</td>
<td>One unknown brand</td>
<td>Case report</td>
<td>* 32 old male smoking patient with idiopathic chronic neutrophilia</td>
<td>* After 6 months of smoking cessation, laboratory examination showed normalized leukocyte count and C-reactive protein levels, confirmed immediately by a second laboratory and by repeated tests after 1 and 2 months</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td>* Then, quit smoking with EC</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>* A positive effect</td>
<td></td>
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<tr>
<td>Farsalinos KE</td>
<td>10</td>
<td>EC of unknown type</td>
<td>Survey</td>
<td>* 19,414 EC regular users world wide</td>
<td>* One case</td>
</tr>
<tr>
<td>[50] 2014</td>
<td></td>
<td></td>
<td></td>
<td>* Median use: 10 months</td>
<td>* Time association between smoking cessation and relieved chronic idiopathic neutrophilia</td>
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<td></td>
<td>* Positive and negative symptoms</td>
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<td>* 60% reported AE</td>
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<td></td>
<td>* Most common AE: sore/dry mouth and throat; side effects were mild and in most cases were subsequently resolved</td>
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<td></td>
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<td></td>
<td></td>
<td>* Participants experienced significant benefits in physical status and improvements in pre-existing disease conditions</td>
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<td></td>
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<td></td>
<td></td>
<td>* Being former smoker was independently associated with positive effects in health and improvements in disease conditions</td>
<td></td>
</tr>
<tr>
<td>Gillen S</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Case report</td>
<td>* A 1 day old boy born at full term</td>
<td>* Selected vapers; those who tolerate EC , have a regular use and experience positive changes they want to share</td>
</tr>
<tr>
<td>[63] 2015</td>
<td></td>
<td></td>
<td></td>
<td>* Negative symptoms from two organ systems</td>
<td>* Those who had persistent AE had quit use</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>* Mother had been consistently vaping EC throughout the pregnancy from 30-50 times per day. During the time of active labor, she vaped EC approx. 50-70 times</td>
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<td>* Admitted for abdominal distention and respiratory distress.</td>
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<td></td>
<td>* Physical exam: a distended abdomen with upper abdominal tenderness</td>
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<td></td>
<td>* Abdominal X-rays: extensive pneumatosis intestinalis without free-air</td>
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<td></td>
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<td></td>
<td>* Intraoperative findings: the ascending, transverse, and descending colon had patchy areas of superficial necrosis</td>
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<td></td>
<td>* A suction rectal biopsy: ruled out Hirschsprung’s disease as a possible etiology of profound and isolated colonic necrotizing enterocolitis</td>
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<td></td>
<td></td>
<td></td>
<td>* Time association</td>
<td></td>
</tr>
<tr>
<td>Heavner K</td>
<td>5</td>
<td>Products sold by Online</td>
<td>303 users of EC</td>
<td>* Most had replaced CC by EC</td>
<td>* Selected vapers;</td>
</tr>
<tr>
<td>[5]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>* Respondents reported</td>
</tr>
<tr>
<td>Year</td>
<td>Manufacturer</td>
<td>Survey</td>
<td>Positive Symptoms</td>
<td>Health Improvements</td>
<td>Comments</td>
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</tr>
<tr>
<td>2010</td>
<td>one EC manufacturer</td>
<td>survey</td>
<td>- Positive symptoms</td>
<td>- Better health (94%), cough (98%), exercise ability (88%), sense of smell (82%), sense of taste (77%)</td>
<td>those who tolerate EC, have a regular use and experience positive changes they want to share - Those who had persistent AE had quit use</td>
</tr>
<tr>
<td>2013</td>
<td>Hua M [76]</td>
<td>No</td>
<td>- Many different</td>
<td>- Improvements in health, especially general health and cough by replacing CC with EC</td>
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<td>- Online search</td>
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<td>- 481 vapors</td>
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<td>- 429 (405 different symptoms)</td>
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<td>- 78 positive, 326 negative, 1 neutral</td>
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<td></td>
<td>- Health effects were broadly distributed: 10 organ systems (eg, respiratory, neurological) and two anatomical regions (chest and mouth/throat)</td>
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<td></td>
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<td></td>
<td>- Respiratory, mouth/throat, neurological, and sensory had the most symptoms</td>
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<td></td>
<td>- Mouth and throat had most negative symptoms</td>
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<td>- A significant number of health effects appeared in the digestive, muscular/skeletal, and integumentary systems</td>
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<td>- 34% of the individuals had negative effects in more than one system - such as the circulatory and neurological systems</td>
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<tr>
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<td></td>
<td></td>
<td>- Few individuals had positive effects in more than one system</td>
<td></td>
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<tr>
<td>2014</td>
<td>Hureaux J [77]</td>
<td>No</td>
<td>- La dynamique and two ‘e-liquids’ Kentucky (19 mg/mL of nicotine) and Eastern (19 mg/mL of nicotine)</td>
<td>- EC use can have wide ranging positive and negative effects</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Case report</td>
<td>- Respiratory, mouth/throat, neurological, and sensory had the most symptoms associated with them</td>
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<tr>
<td></td>
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<td></td>
<td>- A 43 year old patient with history of stage II smoking-related COPD + primary lung adenocarcinoma with an isolated brain metastasis treated by radiotherapy, lobectomy and chemotherapy - under surveillance for 7 months</td>
<td>- Users with negative symptoms often reported more than one symptom-interactions were often seen between systems</td>
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<tr>
<td></td>
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<td></td>
<td>- Negative pulmonary symptoms</td>
<td>- Positive effects usually occurred singly and most frequently affected the respiratory system</td>
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<tr>
<td></td>
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<td></td>
<td>- After 48 h use of EC: onset of cough with whitish secretions and subsequently developed progressive breathlessness on minimal exertion</td>
<td>- Selection bias: probably new vapors that experience negative AE they want to discuss</td>
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<td></td>
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<td>- Severe dyspnoea with mixed ventilatory disorder are primarily suggestive of bronchiolitis</td>
<td>- Some symptoms occurred during EC use, such as “metal taste in mouth”</td>
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<td>- After having stopped for 48 h: marked improvement of cough, sputum and breathlessness.</td>
<td>- Others occurred just after use, such as “choking after use”</td>
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<tr>
<td></td>
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<td></td>
<td>- After 7 days, all symptoms had completely resolved with no treatment</td>
<td>- Selection bias: probably new vapors that experience negative AE they want to discuss</td>
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<td></td>
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<td></td>
<td>- One patient</td>
<td>- Reversibility of symptoms after cessation of EC</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>- Time association</td>
<td>- A patient who presented with subacute bronchial toxicity associated with deterioration of pulmonary function tests after starting use of EC</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Time association registered by health professional</td>
<td>- It is impossible to formally conclude on the causal role of the EC in the onset of the clinical features despite the observed temporal correlation</td>
<td></td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>EC Type</td>
<td>Study Design</td>
<td>Participants</td>
<td>Outcome</td>
</tr>
<tr>
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<td>---------</td>
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<tr>
<td>Lee S [96] 2013</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Case report</td>
<td>35-year old man with 1½ year history of pan-ulcerative colitis which began 4 weeks after smoking cessation</td>
<td>Mayo score decreased from 8 to 2; Fecal calprotectin decreased from 424 to 25 µg/g</td>
</tr>
<tr>
<td>Manzoli L [105] 2015</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Prospective cohort study</td>
<td>Adults (30–75 years); 236 EC vapers, 491 CC smokers, and 232 dual smokers (overall response rate 70.8%)</td>
<td>At 12 month follow-up: although significant, a minimal increase from baseline in self-rated health score was observed among vapers only (+0.3±1.5; p = 0.013)</td>
</tr>
<tr>
<td>Maridet C [107] 2015</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Case report</td>
<td>52-year-old woman</td>
<td>Itchy erythematous dermatitis on the right hand that had started 8 months previously</td>
</tr>
<tr>
<td>McCauley L [111] 2012</td>
<td>No</td>
<td>EC of unknown type</td>
<td>Case report</td>
<td>7-month history of dyspnoea, productive cough and subjective fevers</td>
<td>Diagnosed with exogenous lipid pneumonia (chronic inflammatory reaction to the deposition of lipid)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Type</th>
<th>Methodology</th>
<th>Findings</th>
<th>Notes</th>
</tr>
</thead>
</table>
| McQueen A [113] 2011 | No ☐, 1 | EC’s of unknown type | Interviews with vapors | °13 vapors  
°Positive symptoms  
°Improved sense of taste and smell, ability to be physically active, and less coughing and breathlessness  
°Improved quality of life  
°Few persons  
°Selected vapors; those who tolerate EC, have a regular use and experience positive changes they want to share  
°Time association not investigated | °Improved self-reported health and quality of life |
| Monroy AE [116] 2012 | No | One unknown brand | Case report | °70 year old woman, smoking history: 40 pack-years.  
°Undergone total hip arthroplasty; infected hematoma  
°1 negative symptom  
°3 asymptomatic episodes of atrial fibrillation with rapid ventricular response  
°Normal cardiac enzyme levels  
°No episodes of atrial fibrillation after she stopped using EC | °One case  
°Self-reported  
°Pt. recalled that use of EC had preceded each episode  
°Time association  
°Possible association between use of EC and atrial fibrillation |
| Munoz A [117] 2015 | No | Unknown brands | Survey in a smoking cessation clinic | °64 ever-users of EC  
°Benefits from smoking cessation: less coughing, improved breathing and better physical fitness reported by 60%  
°Selections bias possible  
°Health improvements by use of EC cannot be distinguished from health improvements of quitting smoking | °Health improvements by use of EC -in those who had quit -are reported |
| O’Brien B [119] 2015 | 8 | Elusion + 16mg or 0 mg nicotine | Prospective study; randomized controlled smoking cessation trial | °Mentally ill volunteers  
°86 (13%) of the total 657 participants in study [11] reported using ≥1 medication associated with mental illness  
°In persons with mental illness: adverse event counts relative to the number of participants were similar (these were not subject to statistical testing due to small numbers)  
°No serious study-related adverse events were noted in any group  
°Only one brand  
°Time association  
°No selection bias  
°Small numbers  
°Sub-study of study[11] - not powered to detect differences | °Persons with mental illness seem to tolerate EC |
| Polosa R [128] 2011 | 6 | One Italian brand (‘Categoria’) | Prospective 6 month pilot study | °40 smokers not intending to quit  
°Negative symptoms  
°The most frequently reported adverse events: mouth irritation (21%), throat irritation (32%), and dry cough (32%)  
°Symptoms commonly reported at the beginning of the study waned spontaneously | °Primarily mouth/throat and respiratory symptoms  
°No SAE |
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polosa R</td>
<td>2013</td>
<td>6</td>
<td>Different brands</td>
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<tr>
<td></td>
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<td></td>
<td>A 24-month prospective observational study</td>
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<td></td>
<td>23 smokers not intending to quit (5 not using EC at one year follow-up)</td>
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<td></td>
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<td>Negative symptoms</td>
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<td>Mouth irritation, throat irritation, and dry cough were most common and reported in 9–13% at 24 months</td>
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<td></td>
<td></td>
<td></td>
<td>Headache 4%</td>
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<td></td>
<td></td>
<td></td>
<td>No SAE</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Slight increase in mouth irritation and dry cough over time</td>
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<td></td>
<td>Time association registered by health professional</td>
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<td></td>
<td>Persistent mouth/throat and respiratory symptoms after one year of use</td>
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<td></td>
<td></td>
<td></td>
<td>No SAE</td>
</tr>
<tr>
<td>Thota D</td>
<td>2014</td>
<td>No</td>
<td>EC of unknown type</td>
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<td></td>
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<td></td>
<td>Case report</td>
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<td>A 20-year-old healthy man with no history of exposure to any pulmonary irritants (other than EC)</td>
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<td></td>
<td>Negative pulmonary symptoms</td>
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<td>3 days of persistent cough, shortness of breath, and facial flushing</td>
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<td>Symptom cluster began 1 h after smoking an EC</td>
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<td>Tachycardia, tachypnea, mild leukocytosis, 2.0% eosinophils</td>
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<td>X-ray: “subtle diffuse patchy reticulo-nodular opacities”</td>
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<td>A chest CT scan: bilateral diffuse infiltrates</td>
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<td>Bronchoscopy: many white blood cells with eosinophilia in the lavage</td>
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<td></td>
<td>No infectious etiologies</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Treated with 60 mg of prednisone - discharged from the hospital with improvement in his symptoms</td>
</tr>
<tr>
<td>Vannier S</td>
<td>2014</td>
<td>No</td>
<td>EC of unknown type</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Case report</td>
</tr>
<tr>
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<td>A 39-year-old healthy man switched from 60 CC/day to dual use of 20 CC/day + EC (due to wish to quit)</td>
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<td>Daily severe thunderclap headaches, after 7 days: two seizures</td>
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<td>Magnetic resonance imaging (MRI) of the brain: a posterior reversible encephalopathy syndrome(PRES)</td>
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<td>Multiple cerebral artery irregularities with alternations of segmental multifocal constrictions</td>
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<td>One patient</td>
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<td></td>
<td></td>
<td>Time association</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Reversibility of symptoms after cessation of EC?</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Possible case of acute eosinophilic pneumonitis</td>
</tr>
<tr>
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<td></td>
<td>If seeing a patient in the with pulmonary symptoms after use of EC, acute eosinophilic pneumonitis should be considered in the differential</td>
</tr>
</tbody>
</table>

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Polosa R [130] 2013

Thota D [153] 2014

Vannier S [161] 2014
| Wang MP [168] 2015 | No | -EC of unknown type | -Population-based survey in schools | -75 randomly selected schools in Hong Kong | -45,128 students | -Approx. 12 to 18 years old | -Paper published negative symptoms from respiratory system | -There was a higher prevalence of respiratory symptoms in EC users regardless of smoking status | -Overall, EC-use was significantly associated with respiratory symptoms (OR, 1.28; 95% CI, 1.06-1.56) in analyses adjusted for sex, age, perceived family affluence, secondhand smoke exposure, and school clustering effect | -The corresponding ORs (95% CIs) were 2.06 (1.24-3.42) in never-smokers, 1.39 (1.14-1.70) in ever-smokers, and 1.40 (1.02-1.91) in ex-smokers | -Positive but non-significant associations were observed in experimenters (OR, 1.09; 95% CI, 0.66-1.80) and current smokers (OR, 1.15; 95% CI, 0.81-1.62) | -Current smoking was defined as smoking at least once in the last 30 days | -Current EC use was use of EC in the past 30 days | -Unknown EC consumption (brand, intensity, duration) | -The first evidence of an association between e-cigarette use and respiratory symptoms in never- and ever-smoking adolescents, which is consistent with findings from other laboratory and adult studies on short-term adverse respiratory functions |

**EC**= electronic cigarette  
**CC**= conventional cigarette  
**AE**= adverse events  
**SEA**= serious adverse events
Conflicts of Interest - Conflicts of interest of each study should be assessed individually.

▲ 1: This project was funded by EC manufacturer. The study sponsors supplied the ECs used in the trial and funded the trial. The trial design conduct, analysis and interpretation of results were conducted independently of the sponsors. HM has received honoraria for speaking at research symposia and received benefits in kind and travel support from, and has provided consultancy to the manufacturers of smoking cessation medications. ML acted as contract manager with the sponsor, manufacturer of ECs. MG has provided consultancy to the manufacturers of smoking cessation medications

▲ 2: RP has received lecture fees and research funding from manufacturers of stop smoking medications. He has served as a consultant for manufacturers of smoking cessation medications and the distributor EC used.

▲ 3: LD has a collaborative relationship with manufacturer of EC and received funds to attend academic conferences. E-manufacturer reviewed and approved content of questionnaire and set up links from their websites.

▲ 4: JFE was previously consultant for manufacturer of smoking cessation medications

▲ 5: Study was funded and supported by manufacturer of EC and manufacturer is co-author. All other authors are employed at University of Alberta, which is financially supported by a large smokeless tobacco manufacturer. CVP advises on tobacco harm reduction and is compensated for this work.

▲ 6: RP has received lecture fees from manufacturer of EC and has been serving as a consultant for manufacturer of EC. Manufacturer of the EC supplied product, technical and consumer support

▲ 7: ML, via his company Health New Zealand, previously did research funded by an EC manufacturer. CB and HM have done research on ECs funded by Health New Zealand, independently of EC manufacturer. HM has received honoraria for speaking at research symposia, has received benefits in kind and travel support from, and has provided consultancy to, the manufacturers of smoking cessation drugs. NW has provided consultancy to the manufacturers of smoking cessation drugs, received honoraria for speaking at a research meeting and received benefits in kind and travel support from a manufacturer of smoking cessation drugs. JW has provided consultancy to the manufacturers of smoking cessation medications.

▲ 8: CB has undertaken research on e-cigarettes funded by Health NZ (funded by e-cig manufacturer), independently of e-cigarette manufacturer. NW has provided consultancy to the manufacturers of smoking cessation drugs, received honoraria for speaking at a research meeting and received benefits in kind and travel support from a manufacturer of smoking cessation drugs

▲ 9 to 11: “No” stated, but some of the other studies performed by KF used unrestricted funds provided to research center by e-cigarette companies. KEF has a website “E-cigarette Research Advocate Group” which represents an unambiguously positive view on EC and provides several links to vapor clubs

π, 1: AMQ acknowledges the support of the organizers and attendees at vapers’ meeting where recruitment took place
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Annexes – 80


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171. Williams M, Villarreal A, Bozhilov K, Lin S, Talbot P. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PLoS.One. 2013; 8:e57987

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