Scientific Committee on Health, Environmental and Emerging Risks
SCHEER

Opinion on electronic cigarettes

The SCHEER adopted this Opinion by written procedure on 16 April 2021.

ABSTRACT

Following a request from the Commission, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) reviewed the most recent scientific and technical information on electronic cigarettes. The SCHEER was asked to focus only on health impacts compared to non-smoking.

The SCHEER concludes that on health effects

a) For users of electronic cigarettes

1. The overall weight of evidence is **moderate** for risks of local irritative damage to the respiratory tract of users of electronic cigarette due to the cumulative exposure to polyols, aldehydes and nicotine. However, the overall reported incidence is low.
2. The overall weight of evidence for risks of long-term systemic effects on the cardiovascular system is **moderate**.
3. The overall weight of evidence for risks of carcinogenicity of the respiratory tract due to long-term, cumulative exposure to nitrosamines and due to exposure to acetaldehyde and formaldehyde is **weak to moderate**. The weight of evidence for risks of adverse effects, specifically carcinogenicity, due to metals in aerosols is **weak**.
4. The overall weight of evidence for risks of other long-term adverse health effects, such as pulmonary disease CNS and reprotoxic effects based on the hazard identification and human evidence, is **weak,** and further consistent data are needed.
5. To date, there is **no specific data** that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure.
6. The overall weight of evidence for risks of poisoning and injuries due to burns and explosion, is **strong**. However, the incidence is low.

b) For second-hand exposed persons

1. The overall weight of evidence is **moderate** for risks of local irritative damage to the respiratory tract mainly due to exposure to glycols.
2. The overall weight of evidence for risks of systemic cardiovascular effects in second-hand exposed persons due to exposure to nicotine is **weak to moderate**.
3. The overall weight of evidence for **carcinogenic risk** due to cumulative exposure to nitrosamines is **weak to moderate**.

Electronic cigarettes are relatively new in terms of exposure to humans. More research is needed, in particular on long-term health effects.

Regarding the role of electronic cigarettes as a gateway to smoking/the initiation of smoking, particularly for young people, the SCHEER concludes that there is moderate evidence that electronic cigarettes are a gateway to smoking for young people. There is **strong** evidence that nicotine in e-liquids is implicated in the development of addiction and that flavours have a relevant contribution for attractiveness of use of electronic cigarette and initiation.

Regarding the role of electronic cigarettes in cessation of traditional tobacco smoking, the SCHEER concludes that there is **weak** evidence for the support of electronic cigarettes' effectiveness in helping smokers to quit while the evidence on smoking reduction is assessed as **weak to moderate**.

Keywords: Electronic cigarettes, e-cigarettes, e-liquid, health impacts, risk assessment, initiation, gateway, cessation, scientific opinion, SCHEER

Opinion to be cited as:
SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Scientific Opinion on electronic cigarettes, 16 April 2021.

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

The SCHEER members:

Peter Hoet
Rodica Mariana Ion
Renate Krätke (Rapporteur)
Demosthenes Panagiotakos
Ana Proykova
Emanuela Testai (Chair)
Theo Vermeire

External experts:

Urmilla Nair
Reinskje Talhout
Constantine Vardavas

Additional SCHEER Members were appointed to check for the application of the principle described in the Weight of Evidence (WoE) memorandum thus contributing to the Opinion:

Teresa Borges (WoE)
Theodoros Samaras (WoE)

All Declarations of Working Group members are available at the following webpage:
https://ec.europa.eu/transparency/regexpert/index.cfm

About the Scientific Committees (2016-2021)

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). The Scientific Committees review and evaluate relevant scientific data and assess potential risks. Each Committee has top independent scientists from all over the world who are committed to work in the public interest.

In addition, the Commission relies upon the work of other Union bodies, such as the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHEER

This Committee, on request of Commission services, provides opinions on questions concerning health, environmental and emerging risks. The Committees addresses questions on:
- health and environmental risks related to pollutants in the environmental media and other biological and physical factors in relation to air quality, water, waste and soils.
- complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health, for example antimicrobial resistance, nanotechnologies, medical devices and physical hazards such as noise and electromagnetic fields.

SCHEER members

Roberto Bertollini, Teresa Borges, Wim de Jong, Pim de Voogt, Raquel Duarte-Davidson, Peter Hoet, Rodica Mariana Ion, Renate Krätke, Demosthenes Panagiotakos, Ana Proykova, Theo Samaras, Marian Scott, Emanuela Testai, Theo Vermeire, Marco Vighi, Sergey Zacharov

Contact:
European Commission
DG Health and Food Safety
Directorate C: Public Health
Unit C2 – Health information and integration in all policies
L-2920 Luxembourg
SANTE-C2-SCHEER@ec.europa.eu

ISSN ISBN
doi ND

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.
http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

TABLE OF CONTENTS

1. SUMMARY

The Commission mandated the SCHEER to assess the most recent scientific and technical information on electronic cigarettes. The aim of this scientific Opinion is to feed into the Commission's reporting obligations under Article 28 of the Tobacco Products Directive 2014/40/EU (TPD) and also to help the Commission in assessing the potential need for legislative amendments under the Directive or other regulatory/enforcement measures. The SCHEER was asked to focus only on health impacts compared to non-smoking.

The Opinion addresses the role of electronic cigarettes, focussing into potential impacts on the EU context, in relation to:

1. their use and adverse health effects (i.e. short- and long-term effects) risks associated with their technical design and chemical composition (e.g. number and levels of toxicants) and with the existing EU regulatory framework (e.g. nicotine concentration and limits)

2. their role as a gateway to smoking/the initiation of smoking (particularly focusing on young people)

3. their role in cessation of traditional tobacco smoking

To address the terms of reference of this Opinion, the SCHEER compiled information mainly from review articles published between January 2015 and April 2019, as well as relevant primary sources and literature beyond this period. In addition, the SCHEER used reports by other organisations on this topic, and information provided by the Commission. In order to evaluate the health risks related to the use of electronic cigarettes, the SCHEER follows different lines of evidence, i.e. information on exposure of users and second-hand exposed persons, hazards of ingredients in the aerosol and information from human experience as well as from epidemiological studies. The SCHEER weighs the evidence for every line considered and provide an overall risk assessment based on all lines. The SCHEER weighs the evidence of its assessment according to the five levels: strong, moderate, weak, uncertain or not possible.

1. The SCHEER is of the opinion that chemicals present in the aerosol are mainly responsible for possible health effects for users of electronic cigarettes. Electronic-cigarette aerosol is composed of droplets containing chemicals that can have different origin: i) from e-liquids (propylene glycol, glycerol, nicotine, water, flavourings, preservatives); ii) formed by chemical reaction or thermal decomposition in the heating element of some constituents or solvent carriers (e.g. aldehydes, free radicals and reactive oxygen species, furans, acetic acid); iii) originating from the device (e.g. metals). Carrier liquids and nicotine were almost completely aerosolised, and their concentrations in the aerosol are therefore determined nearly entirely by the power output of the aerosoliser and the behaviour of the user. The ingredients are considered and assessed by the SCHEER independently from their origin.

   There is strong evidence that exposure to nicotine from electronic cigarettes is highly variable and depends on product characteristics and that there is substantial evidence that nicotine intake from electronic cigarette devices among experienced adult electronic cigarette users can be comparable to that from combustible tobacco cigarettes. A very high variability is confirmed also for the exposure to other aerosol constituents. The SCHEER considers exposure of electronic cigarette users to be sufficiently characterised for risk assessment.

Second-hand exposure may be to exhaled air following a puff. The reported concentrations of aerosol ingredients are orders of magnitude lower than those reported for exposure of electronic cigarette users.
The hazard profiles for some relevant ingredients like nicotine and its derivates are well known, with strong weight of evidence. However, for a large number of other chemicals, the weight of evidence for their hazard profiles is moderate or weak, there lacking harmonised hazard classification (CLP), especially via inhalation, the relevant route of exposure.

Acute effects reported for electronic cigarette users are mouth/throat irritation, and cough, but the overall incidence is low. The weight of evidence is moderate. There are also cases of i) poisoning from accidental ingestion of liquid nicotine, ii) injuries due to burns and explosions. For both, poisoning and injuries, the evidence for the intrinsic capability to cause health problems is strong, but the incidence is quite low.

Overall, there is moderate, but growing level of evidence from human data suggesting that electronic cigarette use has harmful health effects, especially but not limited to the cardiovascular system. However, more studies, in particular on long-term health effects, are needed.

With regard to human data on effects associated to second-hand exposure, the weight of evidence to date is weak to moderate, mainly due to the limited database. There exists a complete paucity of evidence regarding the acute and long-term effects on cardiovascular and other health outcomes in children and adolescents. Therefore, further research is needed on whether children and adolescents are at greater risk than adults of being adversely affected by regular second-hand exposure to electronic cigarettes within their home environments.

2. Electronic cigarettes are rapidly becoming a new trend among adolescents and the number of users doubled from 2012 to 2017 (7.2-14.6%) in the EU. Among the general adult in Europe the prevalence of current electronic cigarette use ranged from 0.2% to 27%. Amongst young adults, curiosity was the most frequently reported reason for initiating the use of electronic cigarettes, while reasons for continuing to use electronic cigarettes were various. Young non-users perceive the electronic cigarette as a cool and fashionable product that mimics the smoking routine and is judged to be rather safe to use.

   It has to be noted, that many of the studies published on this topic are dealing with data from the US. Products on the US market may differ considerably from those sold in the EU and conclusions drawn for the US may not be directly transferable to the EU. Nevertheless, trends may also spill over and developments outside the EU should not be disregarded.

   Regarding flavours, consistent evidence was found that flavours attract both youth and adults to use electronic cigarettes. Flavours decrease harm perceptions and increase willingness to try and initiate use of electronic cigarettes. Adolescents consider flavour the most important attribute in these products and were more likely to initiate using through flavoured electronic cigarettes. Among adults, electronic cigarette flavours increase product appeal and are a primary reason for many adults to use the product.

   The most popular flavour of electronic cigarette is fruit flavour (47%), followed by tobacco flavour (36%), menthol or mint (22%) and candy flavour (18%). Examples of preferred food-related tastes and odours for young people included cherry, candy, strawberry, orange, apple and cinnamon. Non-smokers in particular prefer coffee and menthol flavours. Overall, consumers preferred flavoured electronic cigarettes, and such preference varied with age groups and smoking status.

Nicotine-containing e-liquids have a stimulating effect on the reward system within the brain, which is implicated in the development of addiction. Whereas flavours are added to increase product liking, addictive substances such as nicotine play a role in motivation and influence the reward system through mechanisms of learning and wanting.

Weak evidence exists regarding a positive interaction between menthol flavour and nicotine strength. Typical nicotine absorption from a conventional cigarette is 1 mg (range 0.3–2 mg), with blood nicotine levels ranging from an average of 15 to 30 ng/mL. Studies of electronic cigarette use have revealed that, depending on duration of use and user puffing topography, serum levels of nicotine can be as high with electronic cigarette use as with use of a conventional cigarette. It is also interesting to note that a modified version of a popular pod device with a large US-market share is now on the EU market, with technological adjustments. This product type compensates for the lower nicotine levels in the liquid, and the increased aerosolisation results in nicotine delivery per puff approximately equal to the American original using high nicotine levels in the liquid. This suggests similar addictiveness potential of the enhanced European version and the original American product.

Some data available from the US indicate that the prevalence of electronic cigarette use is increasing in children and adolescents. Health effects of electronic cigarette use in this population are mainly due to nicotine, but are also associated with the particular flavour ingredients (including menthol) and which are most often preferred by this population group.

Overall, the SCHEER is of the opinion that there is moderate evidence that electronic cigarettes are a gateway to smoking for young people. There is strong evidence that nicotine in e-liquids is implicated in the development of addiction and that flavours have a relevant contribution for attractiveness of use of electronic cigarette and initiation.

3. In the EU, research has indicated that from current and former smokers, the number of those who had ever attempted to quit without assistance increased from 70.3% in 2012 to 74.8% in 2017 and to 76% in 2020. During this timeframe, use of electronic cigarettes for smoking cessation increased (3.7% to 9.7% to 11%). The use of pharmacotherapy (14.6% to 11.1% to 13%) and the use of smoking cessation services (7.5% in 2014 to 5.0% in 2017 to 6% in 2020) did not show clear trends. Notably, the differences in cessation methods across European Member States were associated with the existence of comprehensive national smoking cessation policies. Recent data on quitting activity, including quit attempts, intention to quit, and use of cessation assistance among a cohort of smokers from eight European countries, indicated that ever use of an electronic cigarette as a smoking cessation device in the last quit attempt differed substantially across different European Member States, ranging from 5% in Spain to 51.6% in England – highlighting the differences across the EU.

   From recent reviews, there is evidence that electronic cigarettes help smokers to stop smoking in the long term compared with placebo electronic cigarettes. However, the small number of trials, low event rates and wide confidence intervals around the estimates result in weak evidence by GRADE standards regarding the support of electronic cigarettes' effectiveness in helping smokers to quit while the evidence on smoking reduction is assessed as weak to moderate.

2. MANDATE FROM THE EU COMMISSION SERVICES

The Tobacco Products Directive 2014/40/EU (TPD)[1] lays down rules for tobacco and related products placed on the EU market. It aims to improve the functioning of the internal market for tobacco and related products, while ensuring a high level of health protection for European citizens. Article 20 of the Tobacco Products Directive introduces for the first time a comprehensive regulatory framework for electronic cigarettes with a focus on safety, quality, consumer protection and collection of information. It also sets out requirements for nicotine containing liquid, including the prohibition of certain additives. Under Article 28, the European Commission has been tasked with reporting to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions on the application of the Directive by 20 May 2021. Further, the Commission shall be 'assisted by scientific and technical experts in order to have all the necessary information at its disposal' and the report shall indicate, 'elements of the Directive which should be reviewed or adapted in the light of scientific and technical developments'. Article 28 also further emphasises that the Commission shall pay special attention to electronic cigarettes (e-cigarettes) and the report shall be followed by proposals for amending the Directive. E-cigarettes are recent products on the EU market and evidence concerning their potential risks and benefits is emerging. While some work has been carried out outside of the EU[2,3], research performed in a European context and focused on EU policy needs is still limited. At this stage, the Commission and Member States are monitoring scientific evidence, user profiles and market developments regarding all types of e-cigarettes. Open questions particularly include the role of e-cigarettes in relation to their use and adverse health effects (i.e.; short- and long-term effects), their role as a gateway to smoking / the initiation of smoking (particularly focusing on young people), their role in harm reduction / cessation of traditional tobacco smoking, as well as risks associated with their chemical composition (e.g.; number and levels of toxicants). E-cigarettes and Article 20 of the Tobacco Products Directive Article 20 of the TPD sets down a number of safety and quality requirements for nicotine-containing e-cigarettes and the relevant nicotine-containing liquid intended for the consumer market. These consumer e-cigarettes may be disposable, rechargeable with a cartridge or refillable by means of refill containers containing e-liquid. Manufacturers and importers must notify their products to Member State competent authorities (Article 20(2)). This notification must include information on ingredients and emissions, toxicological data, information on nicotine doses and uptake, and a description of the device and production processes. Manufacturers must also submit sales data and information on consumer preferences annually to Member States (Article 20(7)).

Manufacturers and importers must collect information on suspected adverse effects on human health and take immediate corrective action if they believe their products to be unsafe (Article 20(9)). The TPD contains provisions on the ingredients that can be used in e-cigarettes and sets limits on the amount of nicotine that can be sold in consumer electronic cigarettes and refill containers (Article 20(3)). E-liquids must not contain more than 20mg/ml nicotine (Article 20(3)(b)), tanks and cartridges must not be larger than 2ml, and refill containers must not be larger than 10ml (Article 20(3)(a)). Refill containers and electronic cigarettes must also be child-resistant and tamper-proof, and sold with instructions for use and health warnings (Article 20 paragraphs 3(g), 4(a) and (b)). Cross-border advertising and sponsorship of e-cigarettes is not allowed (Article 20(5)) and Member States may choose to prohibit cross-border distance sales in the same manner as

[1] https://ec.europa.eu/health/sites/health/files/tobacco/docs/dir_201440_en.pdf
[2] http://nationalacademies.org/hmd/Reports/2018/public-health-consequences-of-e-cigarettes.aspx
[3] https://www.nap.edu/resource/24952/012318ecigaretteConclusionsbyOutcome.pdf

for tobacco products (Article 20(6)). The regulation of flavours, local advertising and age limits are left to Member States.

2.1. Terms of Reference

The main purpose of the scientific opinion is to assist the Commission in assessing the most recent scientific and technical information on e-cigarettes. Findings presented in the scientific opinion will feed into the Commission's reporting obligations under Article 28 of the TPD and also help the Commission in assessing the potential need for legislative amendments under the Directive or other regulatory/enforcement measures. The assessment should include and address the role of e-cigarettes, looking into potential impacts on the EU context, in relation to:

- their use and adverse health effects (i.e.; short- and long-term effects) risks associated with their technical design and chemical composition (e.g.; number and levels of toxicants) and with the existing EU regulatory framework (e.g. nicotine concentration and limits)

- their role as a gateway to smoking / the initiation of smoking (particularly focusing on young people)

- their role in cessation of traditional tobacco smoking

While drawing-up the scientific opinion, the committee should take into consideration the most recent and up-to-date scientific evidence and technical developments and, as appropriate, the existing provisions concerning e-cigarettes under the TPD (in particular Article 20(3)) and the evolution of new products on the market. The scientific opinion should address considerations relevant both at individual level and at a population level, from a public health perspective.

2.2. Deadline

Article 28 report needs to be submitted to the EU Parliament by 20 May 2021. In this respect the SCHEER should deliver the final Opinion in September/October 2020 at the latest.

3. SCIENTIFIC OPINION

To address the terms of reference of this Opinion, the SCHEER compiled information mainly from review articles published between January 2015 and April 2019 as well as relevant primary sources and literature beyond this period. In addition, the SCHEER used reports by other organisations on this topic, and information provided by the Commission. The SCHEER weighs the evidence of its assessment according to five levels strong, moderate, weak, uncertain or not possible. The SCHEER was asked to focus only on health impacts compared to non-smoking.

The SCHEER concluded the following:

1. Use of electronic cigarettes and adverse health effects associated with their technical design and chemical composition and with the existing EU regulatory framework.

Electronic cigarettes consist of a mouthpiece, a tank or a cartridge for e-liquid, and an

atomizer. The atomizer has a wicking material that delivers liquid to a battery-powered heating coil. The e-liquid, upon heating, forms an aerosol inhaled by the user. Most e-liquids contain the organic solvents propylene glycol and glycerol, along with nicotine, flavouring molecules, and/or various other additives, in various proportions. These substances affect nicotine delivery, appeal and ease of product use, influencing the individual preferences that may play a role in use patterns.

There are currently five generations of electronic cigarettes on the EU market, but innovations rapidly make their route to the customers. It is noted that products as well as liquids used differ in the EU and the US, with the US allowing higher nicotine concentrations than the limit of 20 mg/ml nicotine set by the TPD in EU.

Regarding e-liquid composition, the SCHEER focusses in this Opinion on i) nicotine, ii) carriers (e.g. glycerol and propylene glycol) considered of high importance and present with high frequency at high levels and iii) ingredients present in more than 10% of products tested with a median amount > 1 mg or present in less than 10% of products tested but with a median amount of >10 mg, according to lists of the most common ingredients of e-liquids that have been compiled by competent authorities. The great majority of chemicals other than nicotine and carriers (e.g. glycerol and propylene glycol) are flavourings. The categories containing the highest number of e-liquids were fruit (34%) and tobacco (16%).

In order to evaluate the health risks related to the use of electronic cigarettes, the SCHEER follows different lines of evidence. For the risk assessment, the exposure and the hazard profile of major aerosol constituents are described. The SCHEER considers also human data on health impacts on users of electronic cigarettes from epidemiological studies or clinical trials. The SCHEER is of the opinion that chemicals present in the aerosol are mainly responsible for possible health effects for users of electronic cigarettes. Further potential health effects associated with the use of electronic cigarettes are poisoning from ingestion of liquid nicotine, particularly by young children, as well as injuries due to burns and explosions.

Electronic-cigarette aerosol is composed of droplets containing chemicals that can have different origins: from e-liquids (propylene glycol, glycerol, nicotine, water, flavourings, preservatives); formed by chemical reaction or thermal decomposition in the heating element of some of constituents or solvent carriers (e.g. aldehydes, free radicals and reactive oxygen species, furans, acetic acid); originating from the device (e.g. metals). Carrier liquids and nicotine were almost completely aerosolised, and their concentrations in the aerosol are therefore determined nearly entirely by the power output of the aerosoliser and the behaviour of the user. The ingredients are considered and assessed by the SCHEER independently from their origin.

Exposure assessment

In order to assess the quantities of chemicals consumers are exposed to when using electronic cigarettes, specific information on consumer behaviour was collected regarding the frequency of use, number of puffs, puff duration, puff volume and puff interval.

Electronic cigarette users tend to take longer puffs and have longer use bouts than combustible cigarette users. Average puff duration ranges from 1.8-5.9 seconds, average inter-puff interval 11-38, average puff volume 48-134 ml. Note that there is diversity in test subjects, test products, and test methods. A large number of devices and liquids are available on the market and new ones are frequently added. There is also large variation in individual exposures due to the variability in concentrations in the inhaled aerosol, the duration of exposure, the frequency of exposure events (electronic cigarette use sessions) and the frequency of inhalation during sessions of electronic cigarette use. This is a great challenge for the exposure assessment for users of electronic cigarettes and for those exposed to exhaled air from these users (second-hand exposure).

Based on laboratory simulation, a 10-puff session would result in 2.5–72.5 mg e-liquid inhaled, with 37–69% of aerosol being <4 µm in size (highly respirable). For e-liquid containing 20 mg/mL nicotine, this would be an intake of 0.08–1.45 mg nicotine/session.

There is strong evidence that exposure to nicotine from electronic cigarettes is highly variable and depends on product characteristics as well as individual nicotine use patterns; there is substantial evidence that nicotine intake from electronic cigarette devices among experienced adult electronic cigarette users can be comparable to that from combustible tobacco cigarettes.

A very high variability is confirmed also for the other aerosol constituents. In spite of the high overall variability of results, caused by unstandardised experimental settings and expressed by the large ranges reported, the quality and the consistency of the composition data is judged to be medium to high. The weight of evidence for external exposure assessment for users of electronic cigarettes is judged to be moderate to strong. The highest uncertainty is related to the proper distinction of realistic versus dry puff conditions and the corresponding carbonyl concentrations.

The weight of evidence for the characterisation of use behaviour[4] for users of electronic cigarettes is judged to be moderate to strong. The highest uncertainty is related to differences between individuals and types of devices as well as to the proper distinction of realistic versus dry puff conditions[5] and the corresponding carbonyl concentrations. Exposure of electronic cigarette users is considered to be sufficiently characterised for risk assessment.

Electronic cigarette use induces relatively high concentrations of ultrafine particles (<100 nm), the exposure level of ultrafine particles of the mainstream aerosol can reach up to 4x 10^9 particles/cm³. Still insufficient information is available on the particle size and size distribution. Due to the lack of characterisation data of particles generated by electronic cigarette use, it is not possible to weigh the evidence concerning the nature of these different fractions. No clear data can be found on whether the particle fractions detected are liquid or solid and whether these particles contain other contaminants (e.g. metal). Due to the scarce data, nanoparticles are not taken into account in the final risk assessment of electronic cigarettes use by the SCHEER.

Individuals may be exposed second-hand to exhaled air following a puff. The compounds identified in exhaled air of electronic cigarette users include particulate matter, nicotine, glycerol, propylene glycol, formaldehyde and acetaldehyde, volatile organic compounds (VOCs), metals and, in rare case, polycyclic aromatic hydrocarbons (PAH). The reported concentrations are orders of magnitude lower for all these substances than for those reported for exposure of electronic cigarette users. Data on second-hand exposure are reported in different units and related to highly different exposure scenarios, device designs, topography, and liquid compositions. The consistency of the data is judged to be medium. The weight of evidence for second-hand exposure assessment is judged to be moderate. The highest uncertainty is related to the comparison of concentrations in indoor air due to the highly different exposure scenarios and the scarcity of data.

Hazard profiles and health effects

The hazard profiles of nicotine and its derivates (e.g. nitrosamines), some VOCs, thermal degradation or reaction products, and metals deriving from the device, are known and reported, with strong weight of evidence, in the Opinion. The adverse effects of nicotine on

[4] For details see section 6.5.1.
[5] These occur when the coil runs dry, which results in a strong burnt flavour.

the cardiovascular system appear particularly relevant for the SCHEER conclusions on the use of electronic cigarettes. However, besides nicotine, a large number of other chemicals, which are also used as additives in the traditional cigarette and other tobacco products, are present in e-liquids and in the aerosol. These ingredients can be toxic, with different target organs and mechanisms involved, but the weight of evidence is moderate or weak, since for most of them there is not a classification to clearly identify their hazards, and the toxicological profile has not been fully investigated, e.g. for many of them the toxicity following inhalation is unknown, and it is equally uncertain if they form degradation products in the conditions of use.

The health impacts of electronic cigarette's use are still difficult to establish due to the lack of long-term data from epidemiological studies or clinical trials. However, the most recent World Health Organization (WHO)[6] report noted that, electronic cigarettes "pose risks to users and non-users", but "There is insufficient evidence to quantify this risk and the long-term effects of exposure are unknown".

Both potential acute effects and long-term effects were considered by the SCHEER. However, acute effects/intoxications due to misuse or counterfeit products were not considered within the current mandate.

Acute mouth/throat irritation and coughing related to electronic cigarette use are reported, but the overall incidence is low. The effects are probably not related to the nicotine content. However, for these acute health effects, the weight of evidence is moderate.

Another potential health effect associated with the use of electronic cigarettes is poisoning from accidental ingestion of liquid nicotine, particularly by young children (reported symptoms include vomiting, tachycardia, headache). When associated to high nicotine concentrations in e-liquid, severe toxicity may result in neurological and neuromuscular harm, respiratory failure and even death. For these reasons, it is important that e-liquids containers are characterised by a child-proof fastening and opening mechanism.

Additionally, electronic cigarette use can be the cause of injuries due to burns and explosions, which have been reported and predominantly attributed to the malfunction of lithium-ion batteries. The pattern and severity of electronic cigarette related injuries depend on the status of the device (charging, in-use, stored) and its positioning relative to the user (e.g. in the victim's mouth, in very close proximity to his/her face, or in a pocket). For both poisoning and injuries due to burns and explosion, the evidence for the intrinsic capability to cause health problems is strong, but the incidence is quite low: only few case reports are available and the notifications to the Rapid Alert System for dangerous non-food products are limited. Therefore, the risk is expected to be low.. However, a lack of notifications is not necessarily an indicator of good safety.

Although electronic cigarettes are relatively new in terms of exposure to humans, and more research is needed over a longer period of time, there is large scientific body of studies indicating that electronic cigarette use can pose various health risks to the user, whereas there are also studies suggesting that there is not enough evidence to suggest an increase in long-term cardiovascular or lung disease risk as a result of nicotine exposure from either NRT or electronic cigarettes (Price and Martinez, 2020).

Overall, there is a moderate, but growing level of evidence from human data suggesting that electronic cigarette use has harmful health effects, especially but not limited to the cardiovascular system. However, more studies, in particular on long-term health effects, are needed.

6

With regard to human data on effects associated to second-hand exposure, the weight of evidence to date is weak, due to the limited database. There exists a complete paucity of evidence regarding the acute and long-term effects on cardiovascular and other health outcomes in children and adolescents. Therefore, further research is needed on whether children and adolescents are at greater risk than adults of being adversely affected by regular second-hand exposure to electronic cigarettes within their home environments.

Risk assessment and overall weight of evidence

The daily exposure to aerosol from an electronic cigarette is a compilation of multiple peak exposures with irregular time intervals. Starting from the same total inhaled daily dose it is hardly comparable with exposure scenarios for the general population (continuous exposure of 24 hours per day). Because the available hazard information, often based on animal experiments, will mostly be obtained with an exposure regimen that also will significantly differ from the electronic cigarette use scenario, a direct comparison of exposure and hazard characteristics will generally not be correct and will be affected by a high degree of uncertainty. As a consequence, risks can not be properly assessed using health-based guidance values (HBGVs), which are not suitable to cover peak air concentrations reached during a puff (around two orders of magnitude higher than the inhaled concentration of the general population), followed by non-exposures between electronic cigarette smoking sessions. As a pragmatic alternative, the Margin of Exposure (MoE) approach may be applied with a minimal factor of 100 required for non-carcinogenic effects.

Because of the wide variability in the individual exposure parameters (duration, frequency, etc.) to ingredients in liquids and aerosols, the quantitative exposure assessment for second-hand exposure was based on aerosol analysis data obtained from pre-defined exposure scenarios for daily users and on exhaled air. In the risk assessment, these were compared to suitable Points of Departure (PoD) from animal experiments or, in the case of second-hand exposure, to health-based limit values for the general population. Metals and flavours were not included in this quantitative analysis because the calculated risk factors were based on exposure conditions (continuous pattern) not applicable to electronic cigarette users. The use topography information used for this assessment was derived from scientific literature and was supplemented with market survey data on the frequency and nature of electronic cigarette use.

Overall assessment for electronic cigarette users

Based on the exposure assessment (Section 6.5.2), the hazard identification (Section 6.5.3), the human health impacts (Section 6.5.4) and the risk assessment (Section 6.5.5), the SCHEER concludes for exposure of electronic cigarette users that:

- The overall weight of evidence is **moderate** for risk of local irritative damage to the respiratory tract of electronic cigarette users due to the cumulative exposure to polyols, aldehydes and nicotine. The lines of evidence are the following:
    - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.
    - These substances are all identified as irritants (strong weight of evidence)
    - In cohort studies, mouth and throat irritation, dissipating over time, was the most frequently reported adverse effect in electronic cigarette users. The overall reported incidence was low (moderate weight of evidence).
    - The model studies revealed low MoEs for irritative effects for individual chemicals and these will be even lower in an additive approach. It is noted that nicotine salts are less irritating. With regard to the risk calculation on aldehydes: formaldehyde, acrolein and diacetyl were present in concentrations sufficient for potential damage to the respiratory tract for heavy users (moderate weight of evidence).

- The alveolar concentrations of nicotine calculated in the model studies are higher than or comparable to effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour (moderate weight of evidence).

- The overall weight of evidence for risk of long-term systemic effects on the cardiovascular system is **moderate**. The lines of evidence are the following:
  - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.
  - There is strong evidence regarding the cardiovascular effects of nicotine – based on increase of heart rate, hypertension and vascular calcification (strong weight of evidence).
  - The level of evidence regarding the cardiovascular effects of nicotine contained in electronic cigarettes and the related pathophysiological mechanisms is considered from moderate to strong.
  - The absorbed doses of nicotine calculated in the model studies are higher than effect levels in studies with human volunteers exposed repeatedly to nicotine vapour showing cardiovascular effects (moderate weight of evidence).
  - Based on human evidence, there is moderate weight of evidence for cardiovascular effects triggered by nicotine however, the weight of evidence related to long-term effects is weak due to lack of longitudinal studies and taking into account the possible substance mixture effects in e-cigarettes (e-liquids/aerosols.

- The overall weight of evidence for risk of respiratory tract carcinogenicity due to long-term, cumulative exposure to nitrosamines and due to exposure to acetaldehyde and formaldehyde is **weak to moderate**. The lines of evidence are the following:
  - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.
  - Nitrosamines, formaldehyde and acetaldehyde have been identified as genotoxic and carcinogenic (strong weight of evidence).
  - In the model calculations, exposure to the nitrosamines increased the calculated risk of tumour development in the respiratory tract, especially, in heavy users. If TSNA is present in the e-liquids, it is assumed that this risk will increase due to cumulative exposure to these chemicals (moderate weight of evidence).
  - The formaldehyde-induced damage to the respiratory epithelium is a precursor to tumour formation and may be exacerbated by the presence of acetaldehyde, acrolein and diacetyl (weak weight of evidence).
  - The human evidence is very limited and does not allow a conclusion (weak weight of evidence).

- The weight of evidence for risk of adverse effects from the metals in aerosols. specifically carcinogenicity, is **weak**. This conclusion is mainly based on the comparison between measured exposure levels in aerosols and health-based guidance values (weak weight of evidence).

- Based on the hazard identification and human evidence, the overall weight of evidence for risks of other long-term adverse health effects such as pulmonary disease, CNS and reprotoxic effects, is **weak,** and further consistent data are needed.

- The overall carcinogenic risk of substances condensed on particulate matter from electronic cigarettes was found to be below 10^{-5}.

- To date, there is no consistent data that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure.

- The concentrations of aldehydes resulting from flavourings are considered too low to add substantially to the already apparent cumulative risk to the respiratory tract from the aldehydes generated in the electronic cigarette and from polyols and nicotine. The weight of evidence is **weak** due to the absence of inhalation toxicological data and specific risk assessments.

- The overall weight of evidence for poisoning and injuries due to burns and explosion is **strong**. However, the incidence is low. Therefore, the risk is expected to be low.

On risks for second-hand exposure

Based on the exposure assessment (Section 6.5.2), the hazard identification (Section 6.5.3), the hazard assessment (Section 6.5.4) and the risk assessment (Section 6.5.5), the SCHEER concludes that:

- The overall weight of evidence is **moderate** for risk of local irritative damage to the respiratory tract. The lines of evidence are the following:
    - Moderate weight of evidence for second-hand exposure
    - This irritation is mainly due to exposure to glycols. Glycols are identified as irritants (strong weight of evidence).
    - The model studies revealed low MoEs for irritative effects from propylene glycol (moderate weight of evidence).
    - The assessment of second-hand nicotine exposure do not point at a risk for respiratory irritation (moderate weight of evidence).
    - Second-hand exposure of bystanders to glycerol, propylene glycol or aldehydes is negligible or orders of magnitude lower than for electronic cigarette users (moderate weight of evidence).

- The overall weight of evidence for risk of systemic cardiovascular effects in second-hand exposed persons due to exposure to nicotine is **weak to moderate**. The lines of evidence are the following:
    - Moderate weight of evidence for second-hand exposure.
    - Heart rate and blood pressure effects were identified as hazards for nicotine (strong weight of evidence).
    - In the model calculations, the worst case MoEs for cardiovascular effects are low (moderate weight of evidence).
    - There exists a complete paucity of human evidence regarding the acute and long-term effects on cardiovascular and other health outcomes in children and adolescents (weak weight of evidence).

- The overall weight of evidence for a carcinogenic risk due to cumulative exposure to TSNAs and to substances on particulate matter is **weak to moderate**. The lines of evidence are the following:
    - Moderate weight of evidence for second-hand exposure.
    - Nitrosamines have been identified as genotoxic and carcinogenic (strong weight of evidence).
    - The MoEs calculated for the carcinogenic risk from TSNAs are low (moderate weight of evidence). If TSNA is present in the e-liquids. it is assumed that this risk will increase due to cumulative second-hand exposure to these chemicals.
    - The excess lifetime carcinogenic risk of substances on particulate matter in second-hand aerosol from electronic cigarettes was found to be below 10^{-7} (moderate weight of evidence). Human evidence is lacking (weak weight of evidence).

Further research is needed into whether children and adolescents are at a higher risk of adverse health effects than adults when regularly subjected to second-hand exposed within their home environments.

2. Role of electronic cigarettes as a gateway to smoking/the initiation of smoking, particularly for young people

Electronic cigarettes are rapidly becoming a new trend among adolescents and the number of users increased from 7.2% in 2012, to 11.6% in 2014 to 14.6% in 2017 in the EU. According to the 2020 Eurobarometer, 14% of the respondents have at least tried electronic cigarettes and 2% use them regularly. Indeed, 25% of young people (aged 15-24) have at least tried e-cigarettes, compared with 8% of the oldest respondents (aged 55 or over). Notably, among the 15–24-year-olds who were ever users of electronic cigarettes, 16.9% transitioned to regular users, however the rate of transition between experimentation and regular use was higher in other age groups. According to Eurobarometer 2020, 59% of users of electronic cigarettes are dual users.

A more recent review on the prevalence of electronic cigarette uses among the general adult in Europe concluded that the prevalence of current electronic cigarette use ranged from 0.2% to 27%, everuse ranged from 5.5% to 56.6% and daily use ranged from 1% to 2.9%. It also showed a higher prevalence of electronic cigarette use among males, adolescents and young adults, smokers of conventional cigarettes, and former smokers. In 2014, across the European Member States, having ever used electronic cigarettes was 5.75 times more likely among 18–24-year-olds compared to those >55 years of age, however, adolescents were less likely to be regular user than those aged ≥55 years (16.9% vs. 38.1%).

Among adolescents, older age, male gender, conventional smokers, peer influence, daily smoking, and heavier smoking are the most common characteristics of electronic cigarette users. Amongst young adults aged 18-25, curiosity was the most frequently reported reason for initiating the use of electronic cigarettes. Reasons for continuing to use electronic cigarettes were various. The continued use of electronic cigarettes could be either a means to replicate smoking habits, or a way for a different and personalized use of nicotine by inhalation. Overall, reasons for using electronic cigarettes in young adults vary. While adults' perceptions and reasons for electronic cigarette use are often related to smoking cessation, youth like the novelty of the product. Young non-users perceive the electronic cigarette as a cool and fashionable product that mimics the smoking routine and is judged to be rather safe to use. In general, perceived benefits reported include avoidance of smoking restrictions, the product being cool and fashionable, having health benefits, lower costs compared to cigarettes, positive experiences (mimics smoking routine, enjoyable taste, throat hit, weight control, increases concentration), safety of use, social acceptability, and perceived benefits for second-hand exposed persons. Regarding product type, especially pod devices have become a more socially acceptable alternative to combustible cigarettes among adolescents and young adults as a result of (1) sleek designs, (2) user-friendly functions, (3) less aversive smoking experiences, (4) desirable flavours, and (5) the ability to be used discreetly in places where smoking is forbidden.

It has to be noted that many of the studies published on this topicdeal with data from the US. Products on the US market may differ considerably from those from the EU and conclusions drawn for the US may not be directly transferable to the EU. Nevertheless, trends may also spill over and developments outside the EU should not be disregarded.

In a meta-analysis of cohort studies mainly reflecting the US-situation that assessed initial use of electronic cigarettes and subsequent cigarette smoking including 17 389 adolescents and young adults, the ages ranged between 14 and 30 years at baseline, and 56.0% were

female. The pooled probabilities of cigarette smoking initiation were 30.4% for baseline ever electronic cigarette users and 7.9% for baseline never electronic cigarette users. The pooled probabilities of past 30-day cigarette smoking at follow-up were 21.5% for baseline past 30-day electronic cigarette users and 4.6% for baseline non-past 30-day electronic cigarette users. Although the studies had different survey methods, sample sizes, age groups and differed in follow up. They were supported by similar results from other studies. On the antipode, however are a number of studies that indicate that exposure to electronic cigarette use may not be directly related to smoking uptake among youth. In the US a decline in past 30-day smoking prevalence between 2014-2017 was reported, which coincides with the timeframe of electronic cigarette proliferation in the US.

Regarding flavours, consistent evidence was found that flavours attract both youth and adults to use electronic cigarettes. Flavours decrease the perception of harm and increase willingness to try and initiate use of electronic cigarettes. Adolescents consider flavour the most important electronic cigarette attribute in trying electronic cigarettes and were more likely to initiate using through flavoured electronic cigarettes. Among adults, electronic cigarette flavours increase product appeal and are a primary reason for many adults to use the product. Most young people start off by trying flavoured electronic cigarettes when they first start using these devices. These flavours enhance the appeal of electronic cigarettes by creating sensory perceptions of sweetness and coolness and masking the aversive taste of nicotine. Most e-liquid brands are available in a variety of youth-appealing flavours, ranging from fruits, desserts, candy, and soda to traditional tobacco. The number of available e-liquid flavours exceeded 7500 in 2014 and is still increasing. Forty-three main flavour categories have been found in literature, e.g. tobacco, menthol, mint, fruit, bakery/dessert, alcohol, nuts, spice, candy, coffee/tea, beverages, chocolate, sweet flavours, vanilla, and unflavoured. The 2020 Eurobarometer reports that the most popular flavour of electronic cigarette is fruit flavour (48%), followed by tobacco flavour (36%), menthol or mint (30%) and chocolate or candy flavour (20%). Alcohol flavoured electronic cigarettes are the least popular, favoured by only 4% of respondents. The older the e-cigarette users, the more likely they were to prefer tobacco-flavoured e-cigarettes: 56% of those aged 55 or more give this answer, compared with 22% of those aged between 15 and 24. The reverse is true for fruit-flavoured e-cigarettes: three quarters of those aged 15-24 mention this flavour, compared with 18% of the oldest cohort. The youngest users are also the most likely to mention menthol or mint flavour (46%, compared with 25-27% among older users) and candy flavours (30%, compared with 10-23%). Sweet preference in children and adolescents is higher than in adults. Examples of preferred food-related tastes and odours for young people included cherry, candy, strawberry, orange, apple and cinnamon. Several flavours (candy and fruit flavours) were associated with decreased harm perception, while tobacco flavour was associated with increased harm perception. Tobacco products in flavours preferred by young people may impact tobacco use and initiation, while flavours preferred by adults may impact product switching or dual use. Non-smokers in particular prefer coffee and menthol flavours. Overall, consumers preferred flavoured electronic cigarettes, and such preference varied with age groups and smoking status.

Nicotine-containing e-liquids have a stimulating effect on the reward system within the brain, which is implicated in the development of addiction. Whereas flavours are added to make the products more appealing addictive substances such as nicotine play a role in motivation and influence the reward system through mechanisms of learning and wanting. Specific to youth, nicotine addiction and dependence leading to lifelong tobacco use is a major concern when considering electronic cigarette use. Consumer preference for nicotine strength and types depends on smoking status, electronic cigarette use history, and gender. Non-smokers and inexperienced electronic cigarette users tend to prefer no-nicotine or low-nicotine electronic cigarettes, while smokers and experienced electronic cigarette users prefer medium- and high- nicotine electronic cigarettes. Weak evidence exists regarding a positive interaction between menthol flavour and nicotine strength. Typical nicotine absorption from a conventional cigarette is 1 mg (range 0.3–2 mg), with blood nicotine levels ranging from an average of 15 to 30 ng/mL. Studies of electronic

cigarette use have revealed that, depending on duration of use and user puffing topography, serum levels of nicotine can be as high with electronic cigarette use as with use of a conventional cigarette. It is also interesting to note that a modified version of a popular pod device with a large US-market share is now on the EU market, with technological adjustments. This product type compensates for the lower nicotine levels in the liquid, and the increased aerosolisation results in nicotine delivery per puff approximately equal to the American original using high nicotine levels in the liquid. This suggests similar addictiveness potential of the enhanced European version and the original American product.

Overall, the SCHEER is of the opinion that there is **moderate** evidence that electronic cigarettes are a gateway to smoking for young people. In addition, there is strong evidence that nicotine in e-liquids is implicated in the development of addiction. There is **strong** evidence that flavours have a relevant contribution for attractiveness of use of electronic cigarette and initiation too.

3. Role of electronic cigarettes in cessation of traditional tobacco smoking.

In the EU, research has indicated that among current and former smokers, the number of those who had ever attempted to quit without assistance increased from 70.3% in 2012 to 74.8% in 2017, to 76% in 2020. During this timeframe, use of electronic cigarettes for smoking cessation increased (3.7% to 9.7% to 11%). The use of pharmacotherapy (14.6% to 11.1% to 13%) and the use of smoking cessation services (7.5% in 2014 to 5.0% in 2017 to 6% in 2020) did not show clear trends. Notably, the differences in cessation methods across European Member States were associated with the existence of comprehensive national smoking cessation policies. Recent data on quitting activity, including attempts to quit, intention to quit, and use of cessation assistance among a cohort of smokers from eight European countries indicated that use of electronic cigarettes as a smoking cessation device in the last quit attempt differed substantially across different European Member States, ranging from 2% in Portugal to 21% in Ireland – highlighting the differences across the EU.

Taking into account data from cohort studies and randomised control trials, the weight of evidence for smoking cessation is weak and for smoking reduction, it is weak to moderate. There is evidence that nicotine containing electronic cigarettes help smokers to stop smoking in the long term compared with placebo electronic cigarettes (nicotine free). However, the small number of trials, low event rates and wide confidence intervals around the estimates result in low evidence by GRADE standards regarding the support of electronic cigarettes' effectiveness in helping smokers to quit.

4. METHODOLOGY

The SCHEER, on request of Commission services, provides scientific opinions on questions concerning health, environmental and emerging risks. The scientific assessments carried out should always be based on scientifically accepted approaches, and be transparent with regard to the data, methods and assumptions that are used in the risk assessment process. They should identify uncertainties and use harmonised terminology, where possible, based on internationally accepted terms. In its scientific work, the SCHEER relies on the Memorandum on weight of evidence and uncertainties (SCHEER, 2018), i.e. the search for relevant information and data for the SCHEER comprises of identifying, collecting and selecting possible sources of evidence in order to perform a risk assessment and/or to answer the specific questions being asked. For each line of evidence, the criteria of validity, reliability and relevance need to be applied and the overall quality has to be assessed.

To address the terms of reference of this Opinion, the Commission library service performed a literature search until April 2019. The search terms used are listed in Annex 4. This search resulted in 3 715 articles published. To cope with this amount of scientific publications, the members of the working group agreed to firstly use review articles published between 1 January 2015 and April 2019 for this Opinion. If necessary, the primary sources were also used, as well as further articles of importance published after April 2019 until 26 October 2020 (end of the public consultation). In addition, the SCHEER made use of reports by other organisations on this topic, as well as on information provided by the Commission.

Many publications used by the SCHEER reflect the situation of the US market. Although, the products as well as the liquids used differ frequently between Europe and the US (e.g. with US allowing higher nicotine concentrations with respect to the limit of 20mg/ml nicotine set by TPD in Europe), the SCHEER uses data describing the US market if necessary and tries to draw conclusions for Europe wherever possible. The SCHEER is aware that this Opinion is related to a fast-developing market with new product types brought to the market within short time periods. In the view of the SCHEER, it is important not to disregard the development in non-European regions, as trends may also spill over to the EU, even if new products have to be adapted to the requirements of the EU legislation (i.e. regarding maximum nicotine content).

5. TERMINOLOGY

The aerosol (mist, emission) produced by an electronic cigarette is commonly but inaccurately called vapour (Bertholon, 2013). The term vapour is a misnomer due to the fact that the aerosol generated by electronic cigarettes has both a particulate and gas phase (Orellana-Barrios *et al.*, 2015). An aerosol is a colloidal suspension of particles dispersed in air or gas. The consumption of an electronic cigarette is often described as "vaping". The SCHEER does not use this term, as it may imply, that the consumption of electronic cigarettes are a "healthy" alternative to cigarette smoking and consumers may misperceive risks associated with the use of electronic cigarettes. The SCHEER prefers to use the neutral "use (users) of electronic cigarette".

6. RATIONALE

6.1 Introduction/Definition

Electronic cigarettes (also known as e-cigarettes) are designed for heating and converting a solution usually containing nicotine and flavouring chemicals dissolved in propylene glycol and/or glycerin (liquid) into an inhalable aerosol (Breland *et al.*, 2017). Electronic cigarettes are defined as products that can be used for the consumption of a nicotine-containing aerosol via a mouthpiece, or any component of that product, including a cartridge, a tank and the device without cartridge or tank.

The term electronic cigarette refers to a variety of evolving devices and there are various types of electronic cigarettes on the market, including disposable and refillable versions in different designs, and these devices and their contents are rapidly developing. Electronic cigarettes are also available under other names like vapes, vape pens, vaping products, mods, pod mods, electronic nicotine delivery systems (ENDS) or alternative nicotine delivery devices (ANDs).

Despite their current variety in shape and form, electronic cigarettes are devices used to inhale an aerosol received by heating of a liquid that may contain nicotine and/or other chemicals. Electronic cigarettes were originally developed in China in 2003.

This Opinion is restricted to the terms of references given by the European Commission. Therefore, the SCHEER Opinion focuses only on health impacts compared to non-smoking. It covers electronic cigarette products complying with the TPD. Electronic cigarettes not containing nicotine are not addressed in this Opinion. Other devices defined as "novel tobacco products" under the TPD, such as heated tobacco, are also not addressed in this Opinion. The SCHEER is aware of cases of adverse events caused by misuse of electronic cigarette products or by ingredients (e.g. vitamins or hallucinogenic drugs) not allowed in e-liquids in the EU. These cases are not part of the current mandate.

6.2. Design Features

Electronic cigarettes consist of a mouthpiece, a tank or a cartridge for e-liquid, a battery and an atomizer. The atomizer design is especially important because it affects the performance of the electronic cigarette and what transfers into the aerosol. The atomizer has a wicking material that delivers liquid to a battery-powered heating coil. The e-liquid, upon heating, forms an aerosol inhaled by the user. Most e-liquids contain the organic solvents propylene glycol and glycerol, along with nicotine, different flavours, and/or various other additives (Pisinger and Dossing, 2014) (see also 6.4, table 2), in various combinations. They affect nicotine delivery, appeal, and ease of product use influencing the individual preferences that may play a role in use patterns (Glasser *et al.*, 2017).

When heated, the volatile liquid generates the characteristic aerosol associated with electronic cigarette use (Wang *et al.*, 2019). The heating process is important as the temperature and components of the atomizer may influence the chemicals that transfer into the aerosols (Visser *et al.,* 2014 and 2015; see also table 3). Some of these chemicals are toxic and could produce adverse health effects (Behar *et al*., 2018).

The early devices looked like a conventional cigarette, often including a small light on the tip that lit when the user puffed. The basic processes of aerosol transformation (dynamics) upon inhalation, also indicating the need for the accurate determination of the size of droplets in the inhaled electronic-cigarette-aerosol, is considered in the review paper (Sosnowski, 2018). Electronic cigarettes are either "closed" (not intended to be refilled with liquid nor their battery or atomizer can be replaced by the user) or are "open", meaning that they can be refilled and often allow users to select and replace some ingredients, resulting in a high number of different products including increased nicotine yields (Breland *et al.*, 2017). It is important to stress that the e-cigarettes should contain safety features that protect against overvoltage and overheating, which is a challenge with the open systems.

There are currently five generations of electronic cigarettes (Glasser *et al.*, 2017; Farsalinos *et al.*, 2014; Strongin, 2019; Williams and Talbot, 2019):

1. The first-generation models, e.g., the "cig-alike" devices, bear the greatest physical resemblance to traditional cigarettes. They afford the least amount of user control over heating and other variables, though newer models can come with refillable cartridges. Nicotine delivery is not as efficient as compared to newer devices.

2. Second-generation models are larger, enable voltage adjustment by users (ca. 3.0–6.0 V), and have higher-capacity lithium-ion rechargeable batteries.

3. Third-generation electronic cigarettes have larger batteries that are removable and are charged externally. The tanks contain more e-liquid that is heated at higher temperatures and afford user control over both voltage and wattage. Electronic cigarette users can also modify (rebuild) third-generation electronic cigarette atomizers. These models often contain sub-ohm resistance heating coils that aid users in generating relatively large aerosol volumes.

4. Fourth-generation electronic cigarettes enable control over the temperature of the heating coil. Later generation models can be used at much higher power levels (e.g., >200 W) as compared to most earlier devices (ca. <15 W).

5. The latest innovation are electronic cigarettes that use changeable, nicotine salt-based liquid cartridges and temperature regulation to produce an aerosol as an alternative to traditional cigarettes (Strongin, 2019). This type of electronic cigarette does not fall into any of the four generation classifications, but rather is part of a new genre called pod-mods.

Like with first-generation devices, pod-mods do not afford control over power levels or customization of device components; users only choose among the available flavoured liquids. What sets them apart is the relatively small size and specific design with a striking resemblance to USB flash drives. The fact that this type of electronic cigarettes contains nicotine salts, which reduces throat irritation and results in high peak levels of nicotine, similar to those of a tobacco cigarette, enables users to consume higher levels of nicotine compared to the vast majority of other brands. These electronic cigarettes have cornered a large US market share and and are particulary popular among teens. This electronic cigarette brand started entering the EU market in Q2 of 2018 and since Q1 of 2019, it has been available in almost all European Member States. Although the trend needs to be monitored, in the EU the nicotine content has to be lower than in the US, in line with the TPD restrictions.

The fact that there are hundreds of electronic cigarette brands with varied configuration of nicotine delivery available in the market makes collation of data on health effects more difficult for generation of scientific evidence (Chakma *et al.*, 2019). In addition, it has to be noted, that many electronic cigarette users also mix their e-liquids themselves (Do It Yourself, DIY), which then may not comply with the requirements set out in the TPD.

6.3 European Regulatory Framework

In Europe, a high level of public health protection is taken into account when regulating these products. In addition, Member States have the possibility to implement stricter regulations at national level. However, electronic cigarettes not containing nicotine do not fall under the TPD.

The TPD includes several requirements for electronic cigarettes. In order to enable Member States to carry out their surveillance and control tasks, manufacturers and importers of electronic cigarettes and refill containers are required to submit a notification of the relevant products before they are placed on the market (EU-CEG). EU-CEG is an IT system for the manufacturers and importers to submit information to EU Member States on electronic cigarettes and their refills to comply with Tobacco Products Directive 2014/40/EU. Within this reporting system, manufacturers and importers comply to the reporting obligations established by Commission Implementing Decision (EU) 2015/2183 establishing a common notification format for electronic cigarettes and refill containers and report amongst others on product design and on product chemical composition (see TPD 20(2)). Information to be provided includes a list of all ingredients contained in, and emissions resulting from, the use of the product, including quantities thereof; toxicological data regarding the product's ingredients and emissions, including when heated, referring in particular to their effects on the health of consumers when inhaled. This data should also take into account, inter alia, any addictive effect and include; and information on the nicotine doses and uptake when consumed under normal or reasonably foreseeable conditions.

The amount of information within the system may have significant utility in future product risk assessments. The reporting of new products across European Member States was

extensive leading to thousands of new product submissions and extensive product notifications of change in product design, constituents etc – indicating the speed in which electronic cigarette products are evolving in the EU. An indicative example of submissions and notifications in some European Member States is reported in Table 1: the extremely high numbers are a clear indication of the complexity of the issue, due to the need to evaluate so many different products, the majority of which were related to the notification of new electronic cigarette refills, although the system still contains some obsolete products that are no longer marketed in the EU.

While the EU-CEG data are helpful for monitoring the market and signal hazards related to harmful ingredients in e-liquids, and other factoes, some limitations are present, mainly related to the need for independent assessors to check the large body of data submitted by manufacturers.

Table 1: Notifications in EU-MS (EU-CEG data Sep 2020).

Country	Files submitted (total, including updates)	Unique products country (total)	Unique products country (active 09/2020)
AT	240352	78098	70098
BE	172268	34837	18671
BG	195915	40439	32986
CY	161399	37058	30585
CZ	234138	49790	42942
DE	583252	200721	190327
DK	45293	12258	6528
EE	228568	43390	34778
ES	230383	52417	45093
FI	86230	22496	8901
FR	235248	56304	41415
UK	380752	76651	61703
GR	183810	37841	29405
HR	161850	33381	27919
HU	69274	16734	9370
IE	300581	60576	52199
IT	220413	55143	46180
LT	193097	42177	34462
LU	57469	15320	10290
LV	66549	16428	6377
MT	132025	31013	25710
NL	247555	49264	39034
PL	107849	24262	14561
PT	81054	20879	13819
RO	137480	31847	26019
SE	142975	30624	18897
SI	149601	30522	22667

SK	186416	38943	32535

Except for nicotine, only ingredients shall be used in the nicotine-containing liquid that do not pose a risk to human health in heated or unheated form. Several additives are prohibited, like vitamins or other additives that create the impression that a tobacco product has a health benefit or presents reduced health risks, caffeine or taurine or other additives and stimulant compounds that are associated with energy and vitality, additives having colouring properties for emissions, additives that facilitate inhalation or nicotine uptake, and additives that have CMR properties in unburnt form (TPD, Article 7).

Nicotine-containing liquids are only allowed to be placed on the market, if the nicotine concentration does not exceed 20 mg/ml. Electronic cigarettes shall deliver the nicotine doses at consistent levels under normal conditions of use. In order to limit the risks associated with nicotine, maximum sizes for refill containers, tanks and cartridges are set. Nicotine-containing liquid is only placed on the market in dedicated refill containers not exceeding a volume of 10 ml, in disposable electronic cigarettes or in single use cartridges, the cartridges or tanks do not exceed a volume of 2 ml. Electronic cigarettes should deliver nicotine doses at consistent levels to avoid the risk of accidental consumption of high doses.

Electronic cigarettes and refill containers need to be child- and tamperproof, including by means of childproof labelling, fastenings and opening mechanisms. Products need to be equipped with an information leaflet and warnings.

6.4 Chemical ingredients in e-liquids

The SCHEER focusses this Opinion on the most frequent chemicals originally used in e-liquids and others that may be generated by chemical reactions through heating of the e-liquid and/or the device itself and to which users of electronic cigarettes may be exposed to through the inhaled aerosol. The Opinion makes use of information from competent authorities in the Netherlands and Greece, who have compiled lists of most common ingredients of e-liquids (see tables in Annex 2). Similar information sets are available to all regulators for their respective countries. The SCHEER examined information pertaining to i) nicotine, ii) carriers (e.g. glycerol and propylene glycol) considered of high importance and present with high frequency at high levels and iii) ingredients present in more than 10% of products tested with a median amount > 1 mg or present in less than 10 % of products tested but with a median amount of > 10 mg (see table 2).

Table 2: Most frequently used ingredients in e-liquids other than nicotine according to the criteria described above as reported to national competent authorities of the Netherlands and Greece

Ingredient name		**Most frequently used (%)**	**Recipe quantity Median (mg)**	**Concentration Median (mg/mL)**
Glycerol	NL GR	94.1	4968 5000	506
Propylene Glycol	NL GR	85.8	4152 4174	429.6
Vanillin (F)	NL GR	35.2	7 8	0.89
Ethyl maltol (F)	NL GR	32.0	5.9 10	1

Ethyl Butyrate (F)	NL GR	28.4	3.6 3.2	0.34
Ethyl Acetate	NL GR	23.2	1.1 1.5	0.17
Ethanol (F)	NL GR	23.1	31 26	2.8
Maltol (F)	NL GR	22.8	1.3 2	0.22
Ethyl Vanillin (F)	NL GR	19.4	6.8 8.7	0.88
Furaneol (F)	NL GR	19.3	2 2.5	0.27
Methyl cyclopentenolone	NL GR	18.3	2	
Cis-3-hexenol (F)	NL GR	17.8	1.5	
Isoamyl Acetate (F)	NL GR	16.3	2.3	
Ethyl 2-Methyl Butyrate (F)	NL GR	16.0	2.2	
Acetic Acid	NL GR	15.7	1.2 1.2	0.13
Triacetin (F)	NL GR	14.4	5.6	
Benzyl Alcohol (F)	NL GR	14.2	3.3 4.6	0.5
Menthol (F)	NL GR	12.1	18	
Hexyl Acetate (F)	NL GR	10.3	1	
Sucralose (F)	NL GR	8.3	11	

Data based on information from the Netherlands (NL) supported by data from Greece (GR). More information, e.g. on maximum values are given in Annex 2
(F) indicates those chemicals used as flavourings

A survey conducted in 2017 and related to ~20,000 e-liquids marketed in the Netherlands, classified 19,266 e-liquids into the 16 main categories of the e-liquid flavour wheel, and among 16,300 e-liquids (85%) for which sufficient information were available, identified 245 unique flavour descriptions (Havermans *et al.*, 2021). The categories containing the highest number of e-liquids were fruit (34%) and tobacco (16%), the latter preferred by dual users (using electronic cigarettes as well as traditional cigarettes). Various miscellaneous flavours such as "sandwich", "buttermilk" and "lavender" were also identified, whereas the unflavoured e-liquids were a minority (n=266).

Nicotine concentrations varied ranging from 0 to 20 mg/mL. The percentage of e-liquids with high nicotine concentrations (18 mg/mL) was highest within the unflavoured category (40%). The reason for this is hypothetically attributed by the Authors to the fact that unflavoured e-liquids are often used as a 'nicotine booster' by consumers in order to add nicotine to hand-made e-liquid mixes (Havermans *et al.*, 2021). This was confirmed by

another recent paper reporting that the top flavour categories in an analysis of 277 refill fluids were "fruity", "minty/mentholic", "floral", "caramellic", and "spicy" (Omaiye *et al.,* 2019). Among the analysed e-liquids (of which 170 contained nicotine), 85% had total flavour concentrations >1 mg/ml, and 37% were >10 mg/ml (1% by weight). Of the 170 e-liquids containing nicotine, 56% had a total flavor chemical/nicotine ratio >2.

For the same set and each flavour category identified in the Dutch survey, flavourings present in more than 10% of the products were identified: of the 219 unique ingredients present in more than 100 e-liquids, 213 were flavourings. The mean number of flavourings per e-liquid was found to be was 10±15. The most frequently used flavourings were vanillin (present in 35% of all liquids), ethyl maltol (32%) and ethyl butyrate (28%) (Krüsemann *et al.*, 2021).

Analytical methodology for qualitative and/or quantitative determination of the constituents from e-liquid and aerosol of e-cigarettes are differentiated as presented in Annex 1 (tables A1.1 to A1.3.).

6.5 Assessment of Health Risks

In order to evaluate the health risks related to the use of electronic cigarettes, the SCHEER followed different lines of evidence. The SCHEER is of the opinion that it is mainly the chemicals present in the aerosol that are responsible for possible health effects for users of electronic cigarettes. Relevant compounds in the aerosol have been identified. They may have their origin in the e-liquid, but they may also emit from the electronic device during use. They are considered and assessed by the SCHEER independently from their origin. For the risk assessment, their hazard profile is described. The exposure to those compounds is assessed using measured data as well as assumptions based on electronic cigarette use protocols and consumer behaviour. The SCHEER also considered data on health impacts on users of electronic cigarettes from epidemiological studies or clinical trials.

Further potential health effects associated with the use of electronic cigarettes are poisoning from ingestion of liquid nicotine, particularly by young children, as well as injuries due to burns and explosions. It has been noted, however, that the EU injury database (IDB) does not (yet) include the relatively new product "electronic cigarette": collecting information related to case report on injuries due to burns and explosions of the electronic cigarette devices in the official IDB would be beneficial.

6.5.1 Consumer behaviour related to exposure assessment

In order to assess the quantities of chemicals to which consumers are exposed when using electronic cigarettes, specific information on consumer behaviour is needed, like the frequency of use, number of puffs, puff duration, puff volume and puff interval. The SCHEER compiled available information on prevalence rates, electronic cigarette use behaviour and on electronic cigarette use protocols to estimate exposure to different chemicals for electronic cigarette users. Exposure can be measured or it can be calculated on the basis of exposure scenarios modelling typical consumer behaviour.

Frequency of use of electronic cigarettes

The frequency of use of electronic cigarettes is increasingly rising, particularly in the USA and Europe, with prevalence rates of regular and/or current use among adults ranging between 0.9% and 1.8%, respectively (Levy *et al.*, 2017, Brown *et al.*, 2014; Laverty *et al.*, 2018). Corresponding rates of ever use of electronic cigarettes is notably higher in the aforementioned regions, with prevalence rates ranging as high as 7.7% to 11.8% in the USA and Europe, respectively (Levy *et al.*, 2017, Laverty *et al.*, 2018).

Analyses of the "Special Eurobarometer 458" (May 2017) reported that in 2017 an estimated 63 million Europeans aged 15 or older had ever used electronic cigarettes (95% CI, 59.9 million-66.2 million), and 7.6 million (95% CI, 6.5 million-8.9 million) were regular electronic cigarette users. In 2017 across the then 28 European Member States, men were more likely than women to have ever tried electronic cigarettes (Adjusted Odds Ratio 1.25, 95%CI: 1.15 to 1.60). Younger people were also more likely to have ever tried electronic cigarettes (p for trend across age groups <0.001) as were those with more years in education. Both former (aOR7.49, 95%C.I. 6.51 to 8.61) and current tobacco smokers (aOR 22.88, 95%C.I: 20.16 to 25.97) were more likely to have ever tried electronic cigarettes than never-smokers.

Although regression analyses were not available for the 2020 Eurobarometer data, there was wide variation among EU Member States in the proportions of ever users of electronic cigarettes in 2020 as noted by the Eurobarometer report.In seven countries, at least two in ten respondents have at least tried e-cigarettes once or twice: Ireland (29%), Estonia (25%), France and the United Kingdom (both 22%), Luxembourg and Latvia (both 21%) and Belgium (20%). At the other end of the spectrum, less than one in ten report the same in Poland (6%), Malta, Portugal and Romania (all 7%) and Hungary (9%). Overall, one in seven (14%) have at least tried e-cigarettes in the EU and the UK: Notably the Eurobarometer report also identified that the younger the respondents, the more likely they are to have at least tried e-cigarettes or heated tobacco products. A quarter of young people (aged 15-24) have at least tried electronic cigarettes, compared with 8% of the oldest respondents (aged 55 or over).

Use in young adults, children and adolescents

The 2015 National Youth Tobacco Survey (NYTS) in the US reported that 27.1% of middle and high school students ever used electronic cigarettes[7]. Rates of ever use were similar in the 2016 survey, ranging from 17.5% among 8th grade students to 29.0% among 10th graders, and 33.8% among high school seniors (Schulenberg *et al.*, 2017). The most recent youth rates reported from the PATH survey (Wave 1 in 2013–2014) indicate much lower rates of ever use, with only 10.7 percent of youth ages 12 to 17 reporting ever using an electronic cigarette even once or twice (Backinger, 2017). Conversely, rates in the 2015 YRBS are substantially higher, with 44.9 percent of high school students reporting ever using "electronic aerosol products" (Kann *et al.*, 2016). The proportion of youth who reported ever using electronic cigarettes varies substantially across surveys. With respect to use in the past 30 days, the 2016 NYTS reported that 4.3 percent of middle school students and 11.3 percent of high school students reported any electronic cigarette use in the past 30 days (Jamal *et al.*, 2017). Data presented shows the percentage of high school and middle school students who have ever used electronic cigarettes, 2011 to 2016, in NYTS. MTF rates for 2016 are similar, with 6.2 percent of 8th graders, 11.0 percent of 10th graders, and 12.5 percent of 12th grade students reporting electronic cigarette use in the past 30 days (Schulenberg *et al.*, 2017). Again, youth use rates reported in the PATH Wave 1 survey in 2013–2014 are the lowest, with only 3.1 percent of youth age 12 to 17 reporting current use (Backinger, 2017), while rates among high school students in the 2015 YRBS are again the highest, at 24.1 percent (Kann *et al.*, 2016).

Electronic cigarette use behaviour

Patterns of electronic cigarette use, such as puff topography and number of puffs per day, are important to understand the real-life exposure to the aerosol from electronic cigarettes. Two reviews on electronic cigarette use behaviour were selected (DeVito and Krishnan-Sarin, 2018; Evans and Hoffman, 2014). The recent (2018) review of DeVito and Krishnan-Sarin concluded that electronic cigarette users tend to take longer puffs and have longer use bouts than combustible cigarette users (DeVito and Krishnan-Sarin, 2018). All other factors held constant, longer puff duration increases nicotine delivery from electronic

[7] https://www.ncbi.nlm.nih.gov/books/NBK507192/

cigarettes. Importantly, the validity of nicotine delivery measures does not appear to be undermined by the presence of a topography-measuring device on the electronic cigarette, although it may affect user's subjective experience. The four studies (Strasser *et al.*, 2016; Behar, *et al.*, 2015; Norton *et al.*, 2014; Farsalinos *et al.*, 2015a) reviewed in DeVito and Krishnan-Sarin, 2018 are summarised in table A3.1 in Annex 3. Average puff number is diverse, as sessions are defined in different ways. Average puff duration ranges from 2.1 to 3.5 seconds, average inter-puff interval from 11.2 to 29.6 seconds, and average puff volume from 51 to 118.2 ml (only two studies). However, it has to be noted, that there is diversity in test subjects, test products, and test methods.

The older (2014) review of Evans and Hoffmann concluded that, compared with traditional cigarettes, electronic cigarette average puff duration was significantly longer, and electronic cigarette use required stronger suction (Evans and Hoffman, 2014); it needs to be noted that none of the studies was performed with standardized, validated topography equipment. The four studies (Etter and Bullen, 2011; Hua *et al.*, 2013; Farsalinos *et al.,* 2013; Trtchounian *et al.*, 2010) reviewed in Evans and Hoffman, 2014 are also summarised in table A3.1 in Annex 3. Only number of puffs, and puff duration, no puff volume and puff interval were studied. The average puff duration was reported in two studies (for more details see Annex 3) and is slightly longer than those reported in the recent review described above. The average number of puffs widely differs, as some are per session, and some per day.

In supplementary table A3.2 in Annex 3, the SCHEER summarises findings from recent, non-review studies published in 2018-2019. Eleven relevant studies on human electronic cigarette topography were found (McAdam *et al.*, 2019; St Helen *et al.,* 2018; Spindle *et al.*, 2018; Vansickel *et al.*, 2018; Kosmider *et al*., 2020; Robinson *et al.*, 2018; Lee *et al.*, 2019; Lee *et al.*, 2018a; Kosmider *et al.,* 2018b; Guerrero-Cignarella *et al.*, 2018; Farsalinos *et al.,* 2018; Dawkins *et al.*, 2018).

Average puff number is diverse, as sessions are defined in different ways. Average puff duration ranges from 1.8 to 5.9 seconds, average inter-puff interval from 22 to 38 seconds (only two studies), and average puff volume from 48 to 134 ml. However, it needs to be noted that there is diversity in test subjects, test products, and test methods.

In conclusion, electronic cigarette users tend to take longer puffs and have longer use bouts than combustible cigarette users. Average puff duration ranges from 1.8-5.9 seconds, average inter-puff interval 11-38, average puff volume 48-134 ml. Note that there is diversity in test subjects, test products, and test methods.

The weight of evidence for electronic cigarette use behaviour is judged to be moderate to strong. The highest uncertainty is related to differences between individuals and types of devices.

6.5.2 Exposure assessment

A large number of devices and liquids are available on the market and new ones are frequently added. Besides this, there is also large variation in individual exposures due to the variability in concentrations in the inhaled aerosol, the duration of exposure, the frequency of exposure events (electronic cigarette use sessions) and the frequency of inhalation during sessions of electronic cigarette use. This is a great challenge for the exposure assessment for users of electronic cigarettes and for those exposed to exhaled air from these users (second-hand exposed persons). Below aerosol concentrations are evaluated as originating from simulation of electronic cigarette use by an emission-generating machine and as measured in aerosol from electronic cigarette users. It needs to be noted that different protocols are used to create emissions, resulting in a wide range of data. It would be advisable to develop standardised protocols to make emission levels more

comparable. In addition, second-hand exposure is evaluated as measured in exhaled breath.

6.5.2.1 Aerosol characteristics

Electronic-cigarette aerosol is composed of droplets of e-liquids, which contain mainly propylene glycol, glycerol, nicotine, water, flavourings (if added to e-liquid), and also small amounts of by-products of thermal decomposition of some of these constituents (Sosnowski, 2018, Goniewicz *et al.*, 2014b; Jensen *et al.*, 2015). Emitted (inhaled) aerosol is highly concentrated and contains mainly submicrometric-size particles. Electronic cigarette aerosol is composed of droplets of e-liquids (Sosnowski, 2018). These droplets are surrounded by air and a mixture of aerosols. The major e-liquid components have a high boiling point (propylene glycol: 180°C and glycerol: 300°C), hence a low volatility. The equilibrium saturated vapor pressure of PG at room temperature is below 17 Pa (0.13 mmHg) and of glycerol even less: 0.13 Pa (0.001 mmHg). Accordingly, the concentration of these aerosols around droplets is low as compared to typical concentrations of water vapor, which is characterized by the equilibrium pressure of ~2,350 Pa (17.6 mmHg; Maloney, 2008).

Higher power setting results in a shift towards larger particle sizes resulting in more mass being available to form primary particles. As power is increased more e-liquid will aerosolise and be available (Lerner *et al.*, 2015).

Based on laboratory simulation, a 10-puff session would result in 2.5–72.5 mg e-liquid inhaled, with 37–69% of aerosol being < 4 μm in size (highly respirable). For e-liquid containing 20 mg/mL nicotine, this would be an intake of 0.08–1.45 mg nicotine. Data on total puff volume and nicotine intake can contribute to the development of a standard protocol for laboratory testing of electronic cigarette products (Behar *et al.*, 2015).

For establishing a standard laboratory protocol for the generation of aerosols from electronic cigarettes, the topography data are needed to understand baseline characteristics pertaining to electronic cigarette use, taking into account the following variables: (1) a topographically adaptable device for different device types; (2) quantification of the flows required for the activation of each brand; (3) the various behaviors of users; (4) variations between mark topographies (5) electronic cigarette topography parameters (volume and duration of down). Due to these challenges and the rapid evolution of electronic cigarette design and performance, it may be useful to consider creating more standard laboratory protocols for electronic cigarette testing. Factors to consider when creating test protocols are performance differences for different electronic cigarette styles (Trtchounian *et al*., 2010; Williams *et al.*, 2015; Williams and Talbot, 2011).

Validation of an appropriate protocol and methods by developing one or more standardized puffing protocols for electronic cigarettes, different from the standard puffing protocol for traditional cigarettes, involves the development and validation of methods to produce aerosols and analysis the following parameters:

- target constituents present in electronic cigarettes,
- average puffing conditions observed between users,
- development and validation of a standardized method for measuring particle size,
- distribution and respiratory deposition of electronic cigarette aerosols,
- development of analytical methods for testing chemicals in electronic cigarette liquids and aerosols, with emphasis on the screening and identification of potentially toxic compounds, including the study of the effects of power and temperature and other characteristics of the device that generates such compounds, using exposure conditions and animal models that are relevant to real-life inhalation exposure in humans. (Recommendation 6-2 of the Food and Drug Administration and other US federal research sponsors and / or device manufacturers. It is noted by the SCHEER

that EU policy restrictions exist on animal safety testing on chemicals (e.g. Regulation (EC) No 1223/2009).

The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) method 81[8] recommends 3.0 sec puff duration and 55 mL puff volume. For a standardized puff, 100 mL glass syringe, a 60 mL puff was conducted over a 3-second period with 20 mL preceding the puff to establish steady flow and 20 mL following puff to clear aerosol from the tubing for a total volume of 100 mL and dilution factor of 1.67x. After 10x dilution, the diluted aerosol was injected into a sampling bag pre-filled with 2.7 L of HEPA filtered air (Floyd *et al.*, 2018).

Electronic cigarette use induces relatively high concentrations of ultrafine particles (<100 nm), the exposure level of ultrafine particles of the mainstream aerosol can reach up to 4x 10^9 particles/cm³ (Fuoco *et al.,* 2019) or 1x10^9 particles/cm^3 (Ingebrethsen *et al.*, 2012). The PM_1 mass concentration fluctuated between 15 and 120x10³ g/cm³ and the PM_1 number concentration varied from 90 to 580x 10³ particles/ cm³. When the aerosol is released in a room (35 m³) the particles have a rather short lifetime of 10–20 s. The mean ambient air total particle concentration is 8.0x 10³ ± 3.05x 10³ particles/cm^3, whereas that emitted from the electronic cigarette using the different liquids is of the order of 10^6 to 10^7 particles/cm^3 (Lampos *et al.,* 2019).

Electronic cigarette aerosols normally exhibit a bimodal particle size distribution: nanoparticles (11–25 nm count median diameter) and submicron particles (96–175 nm count median diameter). Each mode has comparable number concentrations (107–108 particles/cm^3) (Margham *et al.*, 2016; Mikheev *et al.,* 2016).

Also, the particle size distribution (PSD) indicated a trimodal aerosol with two modes in the measurement range at 40 and 200 nm and one mode in the Aerodynamic Particle Sizer (APS) measurement range at ~1000 nm (Schripp *et al.,* 2013).

Electronic cigarette particles generated from different components have different size. For example, propylene glycol-based e-liquids (count median diameter (CMD) = 145±8 nm and mass median diameter [MMD] = 3.06±0.17µm) were smaller than those generated from vegetable glycerin-based e-liquids (CMD = 182±9 nm and MMD = 3.37±0.21 µm). Puff volume also impacted aerosol particle size: CMD and MMD were 154±11 nm and 3.50±0.27µm, 163±6 nm and 3.35±0.24 µm, and 146±12 nm and 2.95±0.14 µm, respectively, for 35, 90, and 170 ml puffs. Estimated electronic cigarette particle mass deposition fractions in tracheobronchial and bronchoalveolar regions were 0.504-0.541 and 0.073-0.306, respectively (Son *et al.*, 2020*)*.

Particles analysed in the Scanning Electron Microscopy (SEM) ranged in size from about 1 to 20 µm. To determine if metal nanoparticles (100 nm) were present in aerosol, samples were examined by transmission electron microscopy (TEM) and Energy Dispersive X-Ray Spectroscopy (EDS). Tin, chromium and nickel, silicate beads, and nanoparticles were found in cartomizer aerosol, in some cases probably greater than a conventional cigarette (Williams *et al.*, 2013).

Volume-weighted median droplet diameters (d_{50}) from a variety of electronic cigarette devices were typically less than 500 nm by Laser Diffraction (LD) and less than 300 nm for electrical mobility (EM), slightly larger than equivalent tobacco smoke measurements of approximately 210 nm (Cabot *et al., 2014*).

[8] CORESTA (2015) No. 81—Routine Analytical Machine for E-Cigarette Aerosol Generation and Collection—Definitions and Standard Conditions.

Estimation of the health risk specifically associated with the inhaled nanoparticles from electronic cigarettes is currently impossible due to the lack of data. Two clear observations are reported: nanoparticles are present in the aerosol and some of them contain metals. But it is not clear which fraction of the observed particles of electronic cigarettes are solid, insoluble nanoparticles, since these particles are considered (partly independent on their composition) to bear an additional health risk. Due to the scarce data, nanoparticles are not taken into account in the final risk assessment of electronic cigarette use.

Weight of evidence
Strong to moderate evidence is found concerning the increased exposure to particles due to electronic cigarette use, during which the number of particles reaches levels of 107–108 particles/ cm^3 and higher. Still insufficient information is available on the particle size and size distribution. An ultra-fine particles fraction has been identified, containing also micro-meter sized particles. Due to the lack of characterisation data of particles generated by electronic cigarette use, it is not possible to weigh the evidence concerning the nature of these different fractions. No clear data can be found on whether the particles fractions detected are liquid or solid and whether these particles contain other contaminants (e.g. metals). Due to the scarce data, nanoparticles are not taken into account in the final risk assessment of electronic cigarette use, included in this SCHEER Opinion.

6.5.2.2 Exposure to aerosols, qualitative description

Electronic cigarette users

The compounds identified in the aerosols inhaled by users of electronic cigarettes originate from the liquids used or directly from the electronic cigarette device or indirectly from chemical reactions. The most frequently detected compounds found can be organised as follows (US-NAS, 2018; Zhang *et al.*, 2018; Klager *et al.*, 2017). It is noted that, in view of the rapidly changing nature of electronic devices used, some exposure data may not apply any more or may only be valid in specific countries.

1. **Originating <u>from e-liquids</u>:** nicotine, solvent carriers (propylene glycol, ethylene glycol and glycerol), tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), phenolic compounds, flavourings as well as tobacco alkaloids. TSNAs and tobacco alkaloids are related to impurities in the nicotine added to the liquids. VOCs detected include toluene, phenols, xylenes, ethyl acetate, ethanol, methanol, pyridine, acetylpyrazine, 2,3,5-trimethylpyrazine, octamethylcyclo-tetrasiloxane, benzene, ethylbenzene, styrene (US-NAS, 2018). With regard to flavours: table 6 shows common flavours used in e-liquids. The total number of flavours was already reported to be more than 7000 in 2014 (Zhu *et al.*, 2014). Many flavours are alcohols or aldehydes (Tierney *et al.*, 2016). Klager *et al.* (2017) found that diacetyl and acetoin were the most prevalent of the flavouring chemicals in electronic cigarette aerosols being found in more than 60% of samples. In another study, 159 sweet-flavoured liquids from 36 American and European manufacturers resulted in diacetyl and/or acetylpropionyl being found in over 70% of sampled liquids and their aerosols (Farsalinos *et al.*, 2015a). It is relevant to note for the risk assessment that specific carrier liquids or additives may only be present in a small fraction of the e-liquids available on the market. Examples are diethylene glycol, benzene, toluene or TSNAs (Visser *et al.,* 2015).

2. **<u>Formed by chemical reaction in the heating element</u>:** aldehydes, free radicals and reactive oxygen species, furans. Aldehydes include predominantly acetaldehyde and formaldehyde. Other aldehydes may be measured such as acrolein (propenal), propionaldehyde (propanal), (methyl)benzaldehyde, isobutyraldehyde and others. The aerosol of electronic cigarettes is generated when the electronic liquid comes in contact with a coil heated to a temperature of roughly 100–250 °C within a chamber, which is thought to cause pyrolysis of the e-liquid and could also lead to

decomposition of other liquid ingredients (Rowell and Tarran, 2015). It has, for instance, been reported that ester hydrolysis of triacetin forming acetic acid occurs during aerosolization. The acetic acid, which is an ingredient itself, acts as a catalyst in the degradation of propylene glycol and glycerol, used as carriers, increasing the formation of formaldehyde hemiacetals, acrolein, and acetaldehyde (Vreeke *et al.*, 2018). Another example is offered by sugar-derived furans in electronic cigarette aerosols (Soussy *et al.*, 2016): sucralose, a sweetener authorised in the European Union as E 955, decomposed and dechlorinated with formation of possibly harmful chlorinated compounds when heated to temperatures higher than 120 °C (BfR, 2019).

The heating power determines the degree of thermal degradation of solvent carriers to carbonyls (Geiss *et al.*, 2016) as well as the mass of aerosol produced. Glycerol has been shown to produce acrolein, formaldehyde and acetaldehyde due to thermal decomposition (pyrolysis) in temperature-dependent amounts (Paine *et al.*, 2007) with, for instance, small amounts of acrolein being formed in some ionic environments at 350 °C, and all three aldehydes being formed at 600 °C. A steep increase in the generated carbonyls was observed when applying a battery-output of at least 15 W corresponding to 200–250 °C on the heating coil (Geiss *et al.*, 2016; Farsalinos and Gillman, 2018, see table 4). Oxidants and reactive oxygen species (OX/ROS) have been found in the electronic cigarette aerosols. OX/ROS could react with other chemicals in the electronic cigarette aerosol because they are highly reactive, causing alterations its chemical composition (Rowell and Tarran, 2015). McNeill *et al.* (2018) discuss the phenomenon of 'dry puff' when the e-liquid is overheated which creates an aversive taste. Such conditions lead to a much higher emission of aldehydes. Electronic cigarette users will, however, avoid using electronic cigarettes under these conditions.

3. **Mostly originating from the device:** metals. Metals reported in aerosols are aluminium, antimony, arsenic, boron, cadmium, chromium, copper, iron, lanthanum, lead, nickel, potassium, silver, tin, titanium, zinc (US-NAS,2018).

The levels of nicotine, tobacco-specific nitrosamines (TSNAs), aldehydes, metals, volatile organic compounds (VOCs), flavours, and tobacco alkaloids in electronic cigarette aerosols vary greatly (Cheng, 2014), depending on several factors, including the e-liquid contents, puffing rate, type of device, and the battery voltage or heating power (Kim, 2016 US-NAS, 2018).

Second-hand exposure

Harmful components are partially exhaled by users of electronic cigarettes. Because electronic cigarettes are only active when users take a puff, electronic cigarettes do not produce aerosol when no puff is being taken. Therefore, electronic cigarettes do not emit harmful compounds when no puff is being taken, in contrast to tobacco cigarettes. Nevertheless, non-users may be exposed to exhaled air following a puff.

In a recent study, the TackSHS Survey (Amalia *et al.*, 2021), country-specific weekly prevalence (%) and duration (minutes/day) of electronic cigarette second-hand aerosol (SHA) exposure in selected indoor settings was investigated in 12 European countries. Overall, 16.0% (4.3-29.6%) of electronic cigarette non-users were exposed to SHA in any indoor setting at least weekly. The median duration of SHA exposure among those who were exposed was 43 minutes/day, range 0 – 120 minutes/day.

Hess *et al.* (2016) and Abidin *et al.* (2017) systematically reviewed 16 and 4 studies, respectively, on the composition of indoor air analysed for components of exhaled air from electronic cigarette users and compared it with background levels. The exhaled air contained elevated levels of particulate matter, nicotine, glycerol, propylene glycol, formaldehyde and acetaldehyde, VOCs and metals. Cotinine was elevated in saliva, urine

and serum. Other studies reviewed by US-NAS (2018) confirm these findings. In one of the studies reviewed, Schober *et al.* (2014) reported an increase of PAHs over the control level in indoor air, established one day before electronic cigarette use. No other reports were found on the generation of PAHs in inhaled or exhaled aerosols except a recent publication that detected very low levels in indoor air, slightly elevated over background (Drooge *et al.*, 2019).

Several studies examined the composition of residues from exhaled aerosol on surfaces in various settings. The residues were found to contain mainly nicotine, plus other alkaloids and TSNAs (Bush and Goniewicz, 2015; Khachatoorian *et al*., 2019; Marcham *et al.,* 2019)

6.5.2.3 Quantification of aerosol concentrations

Electronic cigarette users
The aerosol composition is frequently quantified by simulating the use of electronic cigarettes under controlled conditions in emission-generating machines. Experimental variables are the puff volume, puff flow rate, puff frequency, the type and temperature of the e-cigarette device, and the voltage of the battery. The most controlled studies are discussed below. It is noted that, in view of the rapidly changing nature of electronic devices used some exposure data may no longer apply or may only be valid in specific countries.

Visser *et al.* (2014 and 2015) used an emission-generating machine in order to sample the aerosol of different types of e-liquid and first and second-generation electronic cigarettes in a reproducible manner. Exposure results are summarised in table 3.

Table 3: Measured concentrations in aerosol of electronic cigarettes (Visser *et al.,* 2014 and 2015). For the calculation of the median, all samples were included (also samples for which the measured concentration was below the detection limit; n=12 for the nitrosamines, n=17 for the other values). LOQ stands for 'limit of quantification'. Puff volume is 70 ml. Puff duration is 4 seconds. Puff interval is 20 seconds.

	number >LOQ	**range** min	max	**Median**	**unit**
carrier liquid and nicotine					
nicotine	14	0.001	0.142	0.051	mg/puff
propylene glycol	16	< 0.05	6.8	2.8	mg/puff
glycerol	17	< 0.02	5.0	2.7	mg/puff
di-ethylene glycol	2	< 0.6	18.0	< 0.6	µg/puff
tri-ethylene glycol	2	< 1.6	93.0	< 1.6	µg/puff
aldehydes					
formaldehyde	11	<0.2	33	0.2	µg/puff
acetaldehyde	1	<2	4.7	<2	µg/puff
acrolein	2	<0.2	3.3	<0.2	µg/puff
diacetyl	2	<10	16	<10	µg/puff
Nitrosamines[1]					
NNN	1	< 0.6	269	< 0.6	pg/puff
NAT	6	< 0.6	85	0.3	pg/puff
NAB	2	< 0.6	10	< 0.6	pg/puff
NNK	9	< 0.6	122	4.0	pg/puff
Metals					
vanadium	3	< 0.05	0.11	< 0.05	ng/puff
chromium	16	< 0.05	9.3	6.7	ng/puff

manganese	7	< 0.05	0.47	< 0.05	ng/puff
Cobalt	7	< 0.05	0.58	< 0.05	ng/puff
Nickel	7	< 0.1	6.4	< 0.1	ng/puff
copper	17	0.38	24	2.1	ng/puff
Zinc	17	2.7	67	17	ng/puff
arsenic	0	< 0.05	< 0.05	< 0.05	ng/puff
molybdenum	4	< 0.05	1.3	< 0.05	ng/puff
cadmium	10	< 0.01	0.10	0.01	ng/puff
Tin	17	0.72	86	1.1	ng/puff
Lead	17	0.16	2.1	0.59	ng/puff
uranium	0	< 0.01	< 0.01	< 0.01	ng/puff

[1] NNN = N'-nitrosonornicotine, NAT = N'-nitrosoanatabine, NAB= N'-nitrosoanabasine, NNK =4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

Full data are available on www.rivm.nl/bibliotheek/rapporten/2015-0144_data.xlsx. Carrier liquids and nicotine were almost completely aerosolised, and their concentrations in the aerosol are therefore determined nearly entirely by the power output of the aerosoliser and the behaviour of the user. Dry puff conditions were avoided. However, it was shown that short-chain aldehydes and ketones present in the aerosol do not originate from the e-liquid but are formed during aerosolisation. It was argued that propylene glycol and glycerol may partially decompose when heated. The concentrations of those substances in the aerosol varied greatly. Two apparently identical aerosolisers made by the same manufacturer and filled with the same e-liquid yielded aerosol formaldehyde concentrations that differed by a factor of more than twenty-five.

Studies reporting on specific chemical groups in aerosols quantitatively will be discussed below.

Nicotine

The constancy of nicotine levels in successive production batches is a criterion of quality, but research showed that there is little relationship between nicotine concentration in e-liquids and nicotine concentration in the resulting aerosol, because the composition of the aerosol also depends on the characteristics of the electronic cigarette (temperature, coil, power, ventilation (Goniewicz *et al.*, 2014a; Peace *et al.*, 2016).

US-NAS (2018) also concluded, based on an extensive review of nicotine exposure, that there is conclusive evidence that exposure to nicotine from electronic cigarettes is highly variable and depends on product characteristics and that there is substantial evidence that nicotine intake from electronic cigarette devices among experienced adult electronic cigarette users can be comparable to that from combustible tobacco cigarettes.

Glycerol and glycols

Besides the research of Visser *et al.* (2014, 2015), specific studies on quantification of glycerol and glycols in aerosols were not available.

Carbonyls

The following table (based on Geiss *et al.*, 2016, Farsalinos and Gillman, 2018, and US-NAS, 2018) summarizes studies using an emission-generating machine, specifically designed to measure aldehydes.

Table 4: Experimental studies determining carbonyl compounds in electronic cigarette aerosols

Reference	Methodology for carbonyl trapping/analysis	Type of electronic cigarette(s)	Liquid(s) used	carbonyl emissions
Uchiyama *et al.*, 2013	Emission-generating machine (puff volume: 55 ml, puff duration: 2 seconds, puff interval: 30 seconds), direct trapping in DNPH, HPLC and GC/MS	Second-generation electronic cigarettes, 10 brands, variable voltage	Not specified	Formaldehyde up to 79000 ng/puff acetaldehyde up to 52000 ng/puff acrolein up to 9900 ng/puff aceton up to 6400 ng/puff glyoxal up to 29000 ng/puff methylglyoxal up to 33000 ng/puff
Klager *et al.*, 2017	Emission-generating machine (puff volume: 48-80 ml, puff duration: 2 seconds, puff interval: 60 seconds), direct trapping on DNPH-sorbent, HPLC	26 first-generation electronic cigarettes	Not reported	formaldehyde: up to 99.4 µg/l aerosol acetaldehyde: 0.022-20.4 µg/l aerosol croton aldehyde: up to 82.9 µg/l aerosol No correlation with flavourings
Ogunwale *et al.*, 2017	Emission-generating machine (puff volume: 91 ml, puff duration: 4 seconds, puff interval: 30 seconds, trapping in coated silicon microreactors, GC-MS	4 electronic cigarette products, second generation, variable voltage		formaldehyde: 18-7400 ng/puff acetaldehyde: 15-6310 ng/puff acrolein: 2-580 ng/puff acetone 129—1250 ng/puff
Sleiman *et al.*, 2016	Emission-generating machine (puff volume: 50 ml, puff duration: 3.0 seconds, puff interval: 20 seconds), direct trapping on DNPH-sorbent, HPLC	Two types of electronic cigarette, variable voltage	Propylene glycol and glycerol; ethanol, propylene oxide and acetol also present	formaldehyde: up to 90000 ng/puff acetaldehyde: up to 50000 ng/puff acrolein: up to 30000 ng/puff
Geiss *et al.*, 2016	Emission-generating machine (puff volume: 50 ml, puff duration: 5 seconds, puff interval: 30 seconds), direct trapping on DNPH-sorbent, HPLC	Third-generation electronic cigarette with variable voltage/wattage (5 W, 10 W, 15 W, 20 W, 25 W tested). Heating element with 1.6-Ω resistance, 2,200-mAh battery	Glycerol (50%), PG (40%), water, fragrance, nicotine	formaldehyde: 24 (at 5W–1,559 (at 20 W) ng/puff acetaldehyde: 13–348 ng/puff acrolein: not detected - 2.5 ng/puff
Gillman *et al.*, 2016	Emission-generating machine (puff volume: 55 ml, puff duration: 4 seconds, puff interval: 30 seconds, direct trapping on DNPH-sorbent, HPLC	Different generations of electronic cigarettes, 5 types, variable voltage	Propylene glycol (48%) and glycerol (48%)	formaldehyde: 50-51000 ng/puff acetaldehyde: 30–40700 ng/puff acrolein: < 20-5500 ng/puff
Laugesen, 2015	Emission-generating machine (puff volume: 70	First-generation electronic cigarette		formaldehyde: 0.48-2.5 µg/l

Reference	Methodology for carbonyl trapping/analysis	Type of electronic cigarette(s)	Liquid(s) used	carbonyl emissions
	ml, puff duration 3 seconds, puff interval: 10 seconds, direct trapping in DNPH-solution, HPLC			aerosol acetaldehyde: 0.58-1.52 µg/l aerosol acrolein: 0.4-2.1 µg/l aerosol
Farsalinos *et al.*, 2015c	Emission-generating machine (puff volume: 60 ml, puff duration 4 seconds, puff interval: 30 seconds, direct trapping in DNPH-solution, HPLC	New generation rebuildable tank electronic cigarette,	Glycerol (45%) propylene glycol (45%), water (8%)	formaldehyde: up to 1100 ug/puff acetaldehyde: up to 450 ug/puff acrolein: up to 100 ug/puff Much higher levels at dry puff conditions
Tayyarah and Long, 2014	Emission-generating machine (puff volume: 55 ml, puff duration 2 seconds, puff interval 30 seconds), smoke/aerosol collected in two DNPH-containing impingers, HPLC	Two disposable and three rechargeable electronic cigarettes; no detailed information on electronic cigarette properties available	(1) Glycerol/PG (20/70%), water, nicotine, fragrance; (2) Glycerol (80%), water, nicotine, fragrances	Expressed as total carbonyls: <900 ng/puff acetaldehyde: up to 320 ng/puff acrolein: up to 190 ng/puff propionaldehyde: up to 110 ng/puff Formaldehyde: not detected
Bekki *et al.*, 2014	Emission-generating machine (puff volume: 55 ml, puff duration: 2 seconds, puff interval: 30 seconds, 10 puffs), direct trapping on cartridges (hydroquinone and DNPH), HPLC	13 Japanese electronic cigarette brands; no detailed information on electronic cigarette properties available	No detailed information available	formaldehyde: 660–3,400 ng/puff acetaldehyde: 20–2,600 ng/puff acrolein: 110–2,000 ng/puff (at 20 W) propionaldehyde: 40–1,500 ng/puff
Goniewicz *et al.*, 2014b	Emission-generating machine (puff volume: 70 ml, puff duration: 1.8 seconds, puff interval: 10 seconds, 15 puffs), sorbent trapping, HPLC	12 electronic cigarette brands, first-generation; no detailed information on electronic cigarette properties available	No detailed information available	formaldehyde: 21–374 ng/puff acetaldehyde: 13–91 ng/puff acrolein: 4.6–201 ng/puff (at 20 W)
Hutzler *et al.*, 2014	Emission-generating machine (puff volume: 55 ml, puff duration: 3 seconds, puff interval: 30 seconds, puffing until no aerosols observable), collected in two DNPH-containing impingers, HPLC	First-generation electronic cigarette; no detailed information on electronic cigarette properties available	Prefilled cartridges; no detailed information available	formaldehyde: ~5000 ng/puff acetaldehyde: ~8000 ng/puff acrolein: 3500 ng/puff

DL = detectable level; DNPH = 2,4-dinitrophenylhydrazine; HPLC = high-performance liquid chromatography; PG = propylene glycol.

Farsalinos and Gillman (2018) point to the fact that the majority of exposure studies do not control for the generation of dry puffs, particularly in studies using variable power devices, which could result in testing conditions and reported carbonyl levels that have no clinical relevance or context. The diversity of puffing regimes and reported units make comparison

difficult as well the distinction between realistic exposure conditions and dry puff conditions, characterized by low levels of liquid, limited liquid supply, high power and/or long puff duration. Studies with controlled realistic conditions are rare.

VOCs
Goniewicz *et al.* (2014b) measured 11 VOCs in aerosol generated from 12 brands of electronic cigarettes (see table 4). Toluene and *m*- and *p*-xylene were found in almost all examined electronic cigarettes: toluene levels ranged from 0.2 mg to 6.3 mg per one electronic cigarette (150 puffs). Xylene levels equalled background.

TSNAs
Farsalinos *et al.* (2015b) analysed TSNAs (for chemical names: see Table 3), using a second-generation device and three commercial e-liquids. No TSNAs were detected in the aerosol. Goniewicz *et al.* (2014b) measured NNN at 0.8-4.3 ng/150 puffs and NNK at 1.1-28.3 ng/150 puffs in aerosols from 9 out of 12 brands of electronic cigarettes.

Flavourings
Farsalinos *et al.* (2015a) evaluated sweet-flavoured electronic cigarette liquids and their aerosols for the presence of diacetyl (DA) and acetyl propionyl (AP). DA and AP were found in 74.2% of the 159 samples. Typical mean daily exposures via aerosol from an emission-generating machine (puff volume 55 ml, puff duration 4 seconds, puff interval 30 seconds). were reported to be 56 µg/day (interquartile range 26–278 µg/day) for DA and 91 µg/day (interquartile range 20–432 µg/day) for AP. When 24 electronic cigarette flavours in 4 brands were tested in an emission-generating machine (2 electronic cigarettes within 30 seconds, puff interval 60 seconds, puff volume 45-80 ml) the maximum aerosol concentrations for the most prevalent flavours diacetyl (62%) and acetoin (65%) were 3.69 and 23.8 ug/m^3, respectively (Klager *et al.*, 2017).

Metals
Goniewics *et al.* (2014b) analysed the aerosols generated by an emission-generating test machine for 12 metals and identified and quantified cadmium (0.01 to 0.22 µg per 150 puffs), nickel (0.11 to 0.29 µg per 150 puffs), and lead (0.03 to 0.57 µg per 150 puffs) without data on speciation. Farsalinos *et al.* (2015d) also reported on another study in which, in addition, a range of other metals were quantified, but the type of electronic cigarette was qualified as outdated. Mikheev *et al.* (2016) detected metals in electronic cigarette emissions (As, Cr, Ni, Cu, Sb, Sn, Zn), again without data on speciation. The amounts in most cases varied by several orders of magnitude. The authors explained the large variations in metal levels by electronic cigarette manufacturing inconsistencies and variation in the duration of e-liquid exposure to the high temperature, because the e-liquid delivery rate to the heated wire may not be well controlled in commercial electronic cigarettes.

A review regarding experimental simulation of electronic cigarette use has been published, reporting the detection of an array of metals in electronic cigarette aerosols, ranging from potentially toxic heavy metals like Ni, Cd, Cr, Mn, Pb, As, B, Sn, Ba, Al, Zr, Ti, Ag, Li, Ca, K, Zn, Fe, Na, Mg, and Cu (Williams *et al*., 2017). The levels were highly variable, also due to the fact that the approach used for mimicking the electronic cigarette use for electronic cigarette aerosols varied in different studies in terms of number, frequency and duration of puffs (Beauval *et al.*, 2017; Goniewicz *et al.*, 2014b. and sampling methods). In addition, the sampling methods and the detection techiques for metals were also different (Williams *et al.*, 2013; Palazzolo *et al*., 2016). Most of the studies showed the presence of Ni, Cr, Pb, Sn, Al, Cd, and Cu (Dunbar *et al*., 2018). Relatively small levels of other metals like As, Fe, and Zn were reported (Mikheev *et al.*, 2016; Olmedo *et al.*, 2018). The presence of Ni in electronic cigarette aerosol was reported in nine studies, and its levels varied between 5 and 7.33 ng/10 puffs (Goniewicz *et al.*, 2014b), while Cr was reported in six studies with levels ranging from 7 to < 200 ng/10 puffs in two studies (Olmedo *et al.*, 2018). Pb with levels ranging from 2 to 38 ng/10 puffs was reported in six studies (Olmedo *et al.*, 2018).

Likewise, Al was reported in about five studies in concentrations ranging from 266 to 394 ng/10 puffs (Williams *et al.*, 2013; Schober *et al.*, 2014; Goniewicz, 2014b; Cooper *et al.,*2016); Brown *et al.*, 2014). Cd was reported in four studies with levels ranging from 0.66 to 14.6 ng/10 puffs and Sn was reported in six studies with a concentration ranging from 36 to < 6000 ng/10 puffs (Margham *et al.*, 2016). Cu was observed in eight studies (Bernhard *et al.,*2005) with levels ranging from 11 to 2247 ng/10 puffs in two studies (Palazzolo *et al.,*2016; Lerner *et al.*, 2015b). Similarly, Mn was reported in four studies at a concentration of 2 to 35 ng/10 puffs in two studies (Mikheev *et al.,* 2016; Olmedo *et al.*, 2018). A more recent systematic review (Zhao *et al.*, 2020) confirmed the high variation shown in the results of 12 studies.

Conclusions on exposure associated to electronic cigarette use

The relevant compounds for the RA in electronic cigarette aerosols are mainly the solvent carriers (glycols and glycerol), nicotine, flavourings (if added to e-liquid), nitrosamines (TSNAs), by-products of thermal decomposition of some of these constituents, notably carbonyls, and metals originating from the device.

The risk assessment will be based on the aerosol concentrations found in the Visser *et al.* study (2014 and 2015). The following table 5 compares the concentrations found in this study with, for comparison, maximum concentrations reported elsewhere. All values are converted to a mass/volume unit. It is relevant to note for the risk assessment that some substances, e.g. TSNAs, are only present in a small fraction of the e-liquids available on the market. (Visser *et al*., 2015).

Table 5: Reported maximum concentrations of compounds in aerosols from electronic cigarettes

Compound	Maximum median aerosol concentration Visser *et al.*, 2014 and 2015 (µg/l)	Maximum aerosol concentration other studies[2](µg/l)		
		Margham, 2016	Olmedo, 2018	Halstead *et al.*, 2020
nicotine	2000	581.8		
propylene glycol	97000	12890		
glycerol	71000	28.709		
formaldehyde	470	2.218		
acetaldehyde	70	1.927		
acrolein	50	1.272		
diacetyl	220	0.0343		
acetoin	nm[1]	nm		
NNN[3]	0.0038	0.00098		
NAT[3]	0.0012	0.000236		
NAB[3]	0.0001	nm		
NNK[3]	0.0017	0.00018		
V	0.133	nm	nm	nm

Cr	0.0067	0.00725	0.0295	nm
Mn	0.0083	nm	0.00142	nm
Co	0.091	nm	nm	0.03
Ni	0.343	0.0112	0.112	nm
Cu	0.133	0.0343	nm	nm
Zn	0.0014	0.224	nm	0.02
Cd	1.22	nm	nm	0.015
Sn	0.03	nm	nm	0.05
Pb	nm	<0.00909	0.0275	nm
As	nm	0.00345	0.00104	nm

[1] nm= not measured [2] Other studies than Visser *et al.,*in this section 6.5.2.3.
[3] NNN = N'-nitrosonornicotine, NAT = N'-nitrosoanatabine, NAB= N'-nitrosoanabasine, NNK =4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

The higher carbonyl levels in several studies most probably are generated under dry puff conditions and can be considered unusable for the risk assessment. Also, the levels of formaldehyde, acetaldehyde and acetone measured in aerosols depend on the nicotine level; it is greater when the puffing regimen is associated with the 6 mg/mL nicotine liquid compared with the 24 mg/mL nicotine liquid (Kosmider *et al.,* 2018a).

In spite of the high overall variability of results, caused by unstandardized experimental settings and expressed by the large ranges reported, the quality and the consistency of the data selected is judged to be medium to high. Exposure of electronic cigarette users is considered to be sufficiently characterised for risk assessment.

The weight of evidence for external exposure assessment for users of electronic cigarettes is judged to be moderate to strong based on the medium to high quality and consistency of the data selected. The highest uncertainty is related to the proper distinction of realistic versus dry puff conditions and the corresponding carbonyl concentrations.

Second-hand exposure
Visser *et al.* (2019) collected the exhaled breath of 17 volunteers while they were using electronic cigarettes and measured the levels of contaminants. Three electronic cigarette/e-liquid combinations were used. Subjects took a specified number of puffs and exhaled onto a trapping device immediately after each puff via a mouthpiece. Samples of control breath (without using the electronic cigarette) were obtained from each subject at the start of the experiment. Analysis of exhaled aerosol are summarised in table 6, providing the information on second-hand exposure. The maximum levels will be used in specific exposure scenarios for the risk assessment in section 6.5.5.3. See that section for the conversion to room concentrations.

Table 6: Chemical analysis of exhaled aerosol (Visser *et al.*, 2019). The columns with ranges and medians list average amounts recovered in the first exhaled breath after inhaling a puff. LOQ stands for 'limit of quantification'.

	n	**range** min	max	**Median**		**unit**
carrier liquid and nicotine **nicotine**	17	<LOQ	2140	108		ng
propylene glycol	17	< LOQ	127	<LOQ		µg
glycerol	17	<LOQ	<LOQ	<LOQ		µg

Aldehydes						
formaldehyde	4	<LOQ	<LOQ	<LOQ		ng
acetaldehyde	4	<LOQ	<LOQ	<LOQ		ng
acrolein	4	<LOQ	<LOQ	<LOQ		ng
Nitrosamines[1]						
NNN	9	< LOQ	111	29		pg
NAT	9	< LOQ	40	14		pg
NAB	9	< LOQ	8	2		pg
NNK	9	< LOQ	71	15		pg
NDMA equivalent	9	<LOQ	77	28		pg
total TSNAs						
Metals						
copper	3	<LOQ	2.92	<LOQ		ng
all other metals	3	<LOQ	<LOQ	<LOQ		ng

[1] For chemical names pf TSNAs: see Table 3. NDMA = N- nitrosodimethylamine

Martin *et al.* (2019) also found a strong reduction in nicotine content using an emission-generating machine: the amount of nicotine per puff for an active user was reduced with a factor of 5000 at a distance of 1 m from the active user to 5 ng (Martin *et al.,* 2019). Schober *et al.* (2014) measured levels of potential electronic cigarette pollutants in a ventilated room of 45 m^3 while per session three volunteers consumed electronic cigarettes with and without nicotine for two hours. During the consumption of electronic cigarettes, substantial amounts of 1,2-propylene glycol (mean 199.2 µg/m^3, glycerol (mean 72.2 µg/m^3) and nicotine (mean 2.2 µg/m^3) were found in the gas-phase with control levels all below 0.04 µg/m^3, as well as elevated concentrations of PM2.5 (mean 197 µg/m^3 versus 8 µg/m^3 for control, maximum 514 µg/m^3). The concentration of putative carcinogenic PAH in indoor air increased by 20% to 147 ng/m^3, and aluminum showed a 2.4-fold increase with no increases for other metals. These increases may be questioned since control environmental measurements were performed on a separate day.

Analysis for propylene glycol, glycerol and nicotine in chamber studies revealed peak levels of 2164, 136 and 0.6 µg/m^3, respectively (Geiss *et al.*, 2016). Liu *et al.* (2017) measured room concentrations of 34 chemicals after electronic cigarette use by 37 healthy volunteers. The cumulative four-hour room air levels of the chemicals measured above the LOQ were relatively small and mainly concerned nicotine (up to 2.83 µg/m^3), propylene glycol (up to 317 µg/m^3), and glycerol (up to 242 µg/m^3). Schober *et al*. (2019) measured particles and VOCs in seven passenger cars during continuous electronic cigarette use. Five of the seven tested cars showed a strong increase in the PM2.5 concentration to 75-490 µg/m^3. The concentration of propylene glycol increased in five cars interiorst to 50–762 µg/m^3. In four vehicles, the nicotine concentration increased to 4–10 µg/m^3. Carbonyl concentrations were not elevated above background.

Conclusions on second-hand exposure

The compounds identified in exhaled air of electronic cigarette users include particulate matter, nicotine, glycerol, propylene glycol, formaldehyde and acetaldehyde, VOCs, metals and, in rare cases, PAH. The reported concentrations are orders of magnitude lower for all these substances than those reported for exposure of electronic cigarette users. This is understandable given the high dilution rates: if we assume a volume of 1 L for 10 puffs than the dilution factor will be 50,000 for a room of 50 m^3.

Data on second-hand exposure are reported in different units and related to highly different exposure scenarios, device designs, topography, and liquid compositions. The consistency of the data selected is judged to be medium.

The weight of evidence for second-hand exposure assessment is judged to be moderate. The highest uncertainty is related to the comparison of concentrations in indoor air due to the highly different exposure scenarios.

6.5.3 Hazard identification of most relevant compounds

Beside nicotine and its derivates, chemicals which are also used as additives in the traditional cigarette and other tobacco products are among the most used ingredients in e-liquids. Some of them are included in the list of priority substances identified by the SCENIHR in its Opinion Tobacco Additives 1 (2016), used by the Commission to adopt the Commission Implementing Decision (EU) 2016/787 laying down a priority list of additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations, identifying 15 priority chemicals. As discussed in Section 6.5.2, the e-liquid components nicotine, solvent carriers (propylene glycol, ethylene glycol and glycerol), tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), phenolic compounds, flavourings, and tobacco alkaloids can be found back in the aerosols of electronic cigarettes. In addition, the aerosols contain pyrolysis products of the liquids (i.e., aldehydes, free radicals and reactive oxygen species, furans) and metals, originating from the heated device.

These ingredients can be toxic, affecting different target organs and with different mechanisms involved. In addition, reactions between ingredients can also occur, leading to the formation of other chemicals, such as aldehydes (Conklin *et al.,* 2018; Farsalinos *et al*., 2018; 2016; Vreeke *et al.*, 2018) (see previous section on Exposure).

For most of the listed ingredients of e-liquids and the components of aerosols, there is no harmonised classification to clearly identify their hazard, and the toxicological profile has not been fully investigated, e.g. for many of them the toxicity following inhalation is unknown, and it is equally uncertain whether they form degradation products in the conditions of use.

Nicotine and nitrosamines
For electronic cigarette refill vials to be placed onto the market under the TPD, electronic cigarettes must deliver nicotine doses at consistent levels under normal conditions of use (Art20;3f); must not contain nicotine in excess of 20 mg/ml (Art20;3b). A pre-post TPD assessment of the most popular brands (n=255) across 9 European Member States indicated that more than half of the top selling products in the European market (57.6% pre vs. 52.5% post assessment) were shown to have a discrepancy in nicotine concentration wider than ±10% of the amount labelled on the product – indicating the importance of quality control during production (Girvalaki *et al.,* 2018; 2019).

Nicotine is a parasympathomimetic alkaloid and has an effect on the heart rate and blood pressure, the stimulating effect prevailing at low doses. Furthermore, it acts on the gastrointestinal tract and the central nervous system. The dose and the route and duration of administration determine whether there will be a stimulating effect or an inhibition of circulation. At toxic doses, central stimulation is followed by inhibition, e.g. central inhibition of respiration.

With respect to intoxication of humans, estimates range from 60 mg from self-testing up to more recent estimates of 0.5–1 g of ingested nicotine, corresponding to an oral LD50 of 6.5–13 mg/kg (Mayer, 2014).

According to the harmonised classification and labelling approved by the European Union, nicotine is fatal if swallowed, is fatal in contact with skin, is fatal if inhaled and is toxic to aquatic life with long lasting effects. Additionally, the classification provided by companies

to ECHA in REACH registrations identifies that this substance causes serious eye damage and causes skin irritation.

The nicotine used in e-liquids is extracted from tobacco, and the purity of the extracted nicotine can vary depending upon manufacturer and grade. Nicotine extracts may contain natural impurities such as other tobacco alkaloids, but also degradation products like nicotine-N-oxides, cotinine, nornicotine, anatabine, myosmine, anabasine, and β-nicotyrine (Flora *et al.*, 2016).

While nicotine is not considered a human carcinogen, several tobacco-specific nitrosamines (TSNA) derived from nicotine and other tobacco alkaloids are carcinogenic in laboratory animals. Numerous studies in rodents and primates, both in vitro and in vivo, demonstrate that nitrosamine ketone (NNK), its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and N-Nitrosonornicotine (NNN) are extensively metabolized and form electrophilic intermediates that form covalent adducts with DNA and hemoglobin (IARC, 2004). Although no adequate studies of the relationship between exposure to NNN and human cancer have been reported, there is sufficient evidence that NNN causes cancer in experimental animals. Exposure to NNN affects the liver and it is reasonably anticipated to be a human carcinogen. NNK and NNAL are potent systemic lung carcinogens in rats. Tumors of the nasal cavity, liver, and pancreas are also observed in NNK- or NNAL-treated rats. NNK and NNAL are suspected to cause cancer in humans.

Carbonyl compounds

Relevant oxidation products related to the use of electronic cigarettes are formaldehyde, acetaldehyde and acrolein. Formaldehyde is of high chemical reactivity, causing local irritation or corrosion at exposed epithelia, acute and chronic toxicity and has genotoxic properties. At concentrations above 0.1 ppm in air, formaldehyde can irritate the eyes and mucous membranes in humans. There is also convincing evidence for skin sensitisation by the active substance. Formaldehyde interacts with protein, DNA and RNA in vitro. Formation of DNA-protein links is thought to lead to clastogenic effects. In long-term experiments with rats exposed by inhalation, formaldehyde caused tumours in the epithelium of the nasal mucosa. Eczema and changes in lung function have been observed at 0.6 to 1.9 ppm in humans (ATSDR, 2010; ECHA, 2017). The occupational exposure limits recommended by the SCOEL are 0.3 ppm (0.37 mg/m^3) for long term and 0.6 ppm (0.74 mg/m^3) for short-term exposure. National values for occupational exposure limits vary from 2 ppm to 0.12 ppm (ECHA, 2019).

Acetaldehyde is irritant to skin, eyes, mucous membranes, and respiratory tract. Symptoms of exposure include nausea, vomiting, and headache but also drowsiness, delirium, hallucinations. The perception threshold for acetaldehyde in air is in the range between 0.07 and 0.25 ppm. In rats, after chronic inhalation exposure, acetaldehyde leads to adenocarcinoma of the olfactory epithelium (750 ml/m^3) and squamous cell carcinoma of the respiratory epithelium of the nasal mucosa (1500 ml/m^3) and, in hamsters, to tumors of the nose and larynx. Acetaldehyde is genotoxic in vitro and in vivo. SCE, DNA adducts, DNA crosslinks and mutations in mammalian cells without metabolic activation are observed in vitro. Acetaldehyde has also been shown to be clastogenic in vivo. In mice, acetaldehyde induces micronuclei in the bone marrow, so systemic availability can be assumed. The occupational exposure limit in Germany is set at 50 ppm (91 mg/m^3) (MAK, 2008).

Inhaled acrolein is highly toxic. It is irritating to the upper respiratory tract even at low concentrations. Its odour threshold is 0.16 ppm. In subchronic and chronic inhalation studies on various species, irrespective of the concentration, irritative effects on the respiratory tract, predominantly on the nose, up to hyper- and metaplastic changes on the nasal epithelium occur. Direct contact with liquid acrolein causes rapid and severe eye and skin irritation or burns. In experiments with volunteers, acrolein is irritating to the eyes at 0.15 ml/m^3. Acrolein reacts with DNA bases in vitro to form cyclic adducts. Cyclophosphamide, from which acrolein and other alkylating metabolites are formed, causes

in vivo DNA adducts. In vitro, acrolein has a direct genotoxic effect in various test systems. Mutations were caused in Drosophila both in germ cells and in somatic cells. Two in vivo studies on mutagenicity and cytogenetics in rats were negative. Carcinogenicity studies with dermal, inhalation and oral administration to hamsters, rats and mice showed no evidence of a carcinogenic effect. Acrolein is also thought to be involved in the development of bladder tumors (MAK, 1997). For acrolein a European occupational exposure limit has been set at 0.02 ppm (0.05 mg/m^3) in Commission Directive (EU) 2017/164.

Carriers
Glycerol or propylene glycol are used as aerosolising agents (or as carriers); sometimes they are also considered flavourings, but they are not expected to impart a noticeable flavour. For the toxicological features of glycerol and propylene glycol see also the SCENIHR Opinion on Tobacco additives 1 (2016) and the RAC Opinion on propane-1,2-diol (2016[9]).

Flavourings
Flavouring agents are frequently used as components of e-liquids (table 2) and are present in the aerosol as well. Most of them are listed as generally recognized as safe (GRAS) by the FDA and approved by EFSA as food additives showing low toxicity after oral uptake. However, as said, their toxicity after inhalation, the major route of exposure for electronic cigarette users, is largely untested. When analysing chemicals in e-liquids and their aerosols as well as their potential hazards, , several e-liquids contained flavours with known allergenic properties (Hutzler *et al.*, 2014). Most importantly, other can cause airway resistance (Pisinger and Dossing, 2014) and respiratory irritation (Tierney *et al.*, 2016).

Menthol is a multifunctional additive. It is an effective anaesthetic, antitussive agent that may increase the sensation of airflow and inhibit respiratory rate (SCENIHR, 2016), thereby allowing increased lung exposure to nicotine and other e-liquid ingredients.

For the toxicological features of the most frequently used flavours (Vanillin, Ethyl maltol, Ethyl Butyrate), as well as for Maltol and Menthol, it is possible to refer to SCENIHR Opinion Tobacco additives 1 (2016).

It has been shown, that beside product type and battery output voltage also certain flavours significantly affected toxicity and alter inflammatory response of electronic cigarette aerosols in human bronchial epithelial cells in vitro (Leigh *et al.,* 2016; Lerner *et al.,* 2015a) Decreased cell viability and increased oxidative stress levels were observed at 24 hrs after primary human bronchial epithelial cells were exposed to aerosol from 200 puffs at the air-liquid interface (Scheffler *et al*., 2015).

The chemical reactivity of the flavouring compounds used in electronic cigarettes has not been extensively investigated. It has been reported that the aerosolization of flavoured e-liquid generates toxic aldehydes. It is not clear whether aldehydes derive from flavourings or most likely from aerosolising agents in e-liquid such as propylene glycol and glycerol (Vreeke *et al.*, 2018) The generation of aldehydes has been associated to oxidative stress (Lerner *et al.*, 2015b; Muthumalage *et al.*, 2018) and inflammatory responses (Gerloff *et al.*, 2017; Leigh *et al.*, 2016).

Metals
In addition, several metals have been identified in the aerosol, which mainly were released from the material of the electronic cigarette. The highest values have been reported for Chromium, Copper, Zinc, Tin and Lead, for which the toxicological profile is described in the following paragraphs. Data have been obtained by previous evaluations conducted by International Agencies.

[9] https://echa.europa.eu/documents/10162/c02bcec3-641b-6770-a361-99776015680e

Chromium
In nature the three main forms are Cr (0), Cr (III) and Cr (VI). The bioavailability of Cr (III) is very low while Cr (VI) can pass through the cell membrane, but generally when in contact with tissues is reduced to Cr (III), although not completely. Information on the form in which Cr is present in aerosol generated by electronic cigarette use are not available.

Oral absorption for Cr (III) is between 0.13 and 2.8% and is influenced by the water solubility of the compounds, while Cr (VI) is absorbed between 1 and 6.9%.

In general, Cr (III) salts have low oral toxicity. Discordant results are reported for the effects on reproduction and developmental toxicity probably due to the experimental protocols. Based on the available data, Cr (III) is not considered carcinogenic in animal models. The most relevant NOAELs are 506 and 286 mg Cr (III) / kg bw per day respectively from a sub-chronic and long-term rat toxicity study after oral administration.

Based on available dose-response data in humans and animals, the most sensitive noncancer effects of chromium (VI) compounds are respiratory (nasal and lung irritation, altered pulmonary function), gastrointestinal (irritation, ulceration and non-neoplastic lesions of the stomach and small intestine), which appear to be portal-of-entry effects for inhalation and oral exposure, respectively. In addition, haematological and reproductive are also observed (ATSDR, 2012).

Effects on the male reproductive system of rodents after acute and medium-term exposures and also effects on development (embryotoxicity and increase of fetal malformations) due to exposure during gestation were also highlighted. Cr (VI) compounds are genotoxic in vitro, but the results of in vivo studies after oral exposure are controversial. However, it is clearly genotoxic after ip administration indicating that the reducing capacity of the gastrointestinal tract can affect its genotoxicity in vivo. Cr (VI) if inhaled (as demonstrated for professional exposures) can induce tumours. With regard to current knowledge, it cannot be excluded that data available on animals on a possible carcinogenic activity following ingestion are also not relevant for humans. A "virtual safety dose" (VSD) of 0.0002 µg / kg bw / d has been identified, recommended by ECHA and also adopted by SCHER's Opinion on the presence of Cr (VI) in toys (SCHER, 2015). There are no indications of carcinogenic effects following skin absorption.

Due to the extremely high boiling point of chromium, inhalation exposure can occur in the form of particle-bound chromium or chromium dissolved in droplets and effects depend on the inhaled Cr salt. As an example, occupational exposure to chromium (VI) trioxide has been reported to result in marked damage to the nasal mucosa and perforation of the nasal septum, whereas exposure to insoluble (VI) compounds results in damage to the lower respiratory tract. Nasal irritation and mucosal atrophy and decrease in pulmonary function occurred at occupational exposure levels ≥0.002 mg chromium (VI)/m^3 as chromium trioxide mist (ATSDR, 2012).

Exposure at both occupational levels, but also to low levels of chromium as found in consumer products, could result in sensitization or a reaction in sensitized individuals. Chromium (VI) sensitization typically presents as allergic contact dermatitis resulting from dermal exposures in sensitized individuals, although respiratory effects of sensitization (asthma) may also occur.

Copper
Humans can be exposed to Copper (Cu) via drinking water, the diet or the environment, also inhaling air or dust containing the metal, it has been reported that copper may enter the lungs of workers exposed to copper dust or fumes. Since Copper is an essential trace element (ETE) its absorption is strictly and efficiently regulated in order to maintain the amount of copper in the body fairly constant, it is therefore variable depending on the need as a protective measure. Copper is highly toxic if protective mechanisms are bypassed (i.v.,

i.p. dosing). Copper is excreted via both faeces and urine. The toxicity of copper vs dose is depicted by a clear 'U' curve, with relevant effects caused by both deprivation (below the levels considered as necessary for the physiological functioning of the organism) and excess. Copper deficiency causes more and far severe adverse health effects than copper toxicity.

Long-term exposure to copper dust can irritate nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea; oral exposure to high results in nausea, vomiting, stomach cramps, or diarrhea. However, the available data on the toxicity of inhaled copper are very scant and were considered inadequate for the derivation of reference values by different agencies (ATSDR, 2004).

The repeated dose toxicity data is mainly based on copper sulphate taken via the oral route but read across for other compounds. No relevant animal data are available after inhalation and dermal exposure. After repeated oral dosing, liver, forestomach and kidneys are target organs of toxicity in rats. There is some indication in animals that daily ingestion of dietary copper causes tolerance to high doses. An external NOAEL=16.3 mg Cu/kg/day was derived from a feeding study in rats, as reported on the ECHA web site[10].

Copper (sulphate) has been negative in bacterial mutagenicity tests but has caused chromosome aberrations in mammalian cells in vitro, at high concentrations and in vivo after an i.p. administration but no genotoxicity was evidenced after oral administration. The assumed mechanism(s) of genotoxicity are generation of reactive oxygen species and/or inhibition of DNA-repair enzymes. It can be concluded that copper (sulphate) is not mutagenic. Copper is not classified as a human carcinogen because there are no adequate human or animal cancer studies, but seems that carcinogenicity is not a concern for copper.

Zinc

Zinc (Zn) is an essential element needed for the functioning of many physiological processes: nearly 200 zinc-containing enzymes have been identified, including many dehydrogenases, aldolases, peptidases, polymerases, and phosphatases.

Absorption of ingested zinc is highly variable (10–90%) and is mainly affected by the homeostatic mechanisms to maintain the Zn levels almost constant in the organism working at the gastrointestinal absorption and excretion, the latter occurring mainly (75%) via the faeces, and only to a smaller extent via urine and sweat. The biological half-time of retained zinc in humans is of the order of 1 year.

Zinc is characterised by a low acute toxicity, depending on the form the organism is exposed to; acute toxicity arises from the ingestion of excessive amounts of zinc salts, either accidentally or deliberately as an emetic or dietary supplement. Acute toxic effects of inhaled zinc have been reported in industrial workers exposed to zinc fumes; the symptoms include pulmonary distress, fever, chills, and gastroenteritis.

A high-zinc diet has been shown to induce hypocalcaemia and bone resorption in rats. In humans, manifest copper deficiency is the major consequence of the chronic ingestion of zinc. In 1982, JECFA proposed a provisional maximum tolerable daily intake (PMTDI) of 1.0 mg/kg of body weight. The USEPA reported a TDI of 0.3 mg/kg of body weight.

10 https://echa.europa.eu/it/copper-voluntary-risk-assessment-reports?diss=true&search_criteria_ecnumber=231-159-6&search_criteria_casnumber=7440-50-8&search_criteria_name=copper

The effects of inhalation exposure to zinc and zinc compounds occur within the respiratory tract, although with some variability in the degree of effects depending on the inhaled compound. Following inhalation of zinc oxide, and to a lesser extent zinc metal and many other zinc compounds (in the range 77–600 mg zinc/m^3), the most commonly reported effect is reversible and known as "metal fume fever", characterized by chest pain, cough, dyspnoea, reduced lung volumes, nausea, chills, malaise, and leucocytosis (ATSDR, 2005a).

Tin

Both tin and inorganic tin compounds are generally poorly absorbed (< 5%) from the gastrointestinal tract. Absorbed tin is rapidly excreted primarily via the kidneys and only to a smaller extent via the bile.

Tin and inorganic tin compounds are characterised by a low acute toxicity: at very high doses of inorganic tin compounds (of the order of the LD_{50}) affect the central nervous system, producing effects such as ataxia, muscular weakness and central nervous system depression. In humans, concentrations of 150 mg/kg in canned beverages or 250 mg/kg in other canned foods may produce acute manifestations of gastric irritation in certain individuals.

The only observed effect in long-term studies in rats treated orally with tin was a slight increase in the relative spleen weight at the mid and high doses, but no histopathological changes were observed. The NOAEL in this study was the lowest dose that is 20 mg/kg of body weight per day. There are no data to indicate any adverse effects in humans associated with chronic exposure to tin (JECFA, 2006).

JECFA confirmed in 2006 the PTWI of 14 mg/kg of body weight established from a TDI of 2 mg/kg of body weight on the basis of the gastrointestinal irritancy, the threshold for which is about 200 mg/kg in food.

Humans chronically exposed to inorganic tin (e.g., stannic oxide dust or fumes) through inhalation in occupational setting manifest a benign form of pneumoconiosis known as stannosis, which involves mainly the lower respiratory system. Some cases of fatal acute intoxication were also reported. Limited inhalation data from intermediate-duration studies in animals indicate that organotins can produce lung alterations, irritation of the respiratory airways, skin, and eyes, and liver and kidney effects, but the data base was not robust enough to derive any reference value (ATSDR, 2005b).

Lead

Absorption of Lead (Pb) in the gastrointestinal tract depends on the chemical-physical properties of the ingested material and the age of the exposed individuals. The extent of absorption is on average 15-20% in adults and higher in children: 40-50% (RIVM, 2008).

Skin absorption is generally considered to be much lower, estimated between 0 and 0.3%. Once absorbed, lead is transported in the blood and distributed to soft tissues, such as the liver and kidneys, and to the bones where it can accumulate with age. The average life of Pb in blood and bones are 30 days and 10 to 30 years respectively.

The most relevant information on exposure and related health effects comes from the measurement of lead in the blood (B-Pb); determinable levels in bones and teeth give indications of past exposures. Due to its persistence in the body, chronic toxicity is the crucial point for assessing the potential risk of Pb for health. Studies on animal models (rodents and non-human primates) have shown that chronic exposure to low lead levels cause: neurotoxicity, especially developmental learning deficits, cardiovascular problems with raised blood pressure and nephrotoxicity. Consequently, these three endpoints are considered as the potential adverse critical effects to be taken into account for the risk assessment.

For lead, a massive amount of data can be derived from epidemiological studies that can rely on internal dose metrics (B-Pb), which reflect Pb body burden, irrespective of the route of exposure. The primary systemic toxic effects of Pb are the same regardless of the route of entry into the body.

In humans, the central nervous system is the main target of Pb toxicity in the developmental age. In fact, in children a high level in Pb blood has been inversely associated with a reduced IQ and reduced cognitive functions up to at least 7 years of age. In adults, an association between increased systolic blood pressure and chronic kidney disease and relatively low levels of B-Pb has been established.

Genotoxicity data indicate that Pb may have an indirect weak genotoxic potential, involving the formation of reactive oxygen species and interference with DNA repair processes at non-cytotoxic concentrations. The IARC has classified inorganic Pb as a probable carcinogen for humans (Group 2A), but in rodents, the tumors show up only at extremely high doses of treatment.

Neurotoxicity in children and cardiovascular and nephrotoxic effects in adults are therefore the critical effects to be considered for risk assessment.

BMDL01 were calculated for adults relating to the effects on blood pressure and on the kidney using the values of blood circulating Pb (B-Pb) equal to 36 and 15 µg/L, corresponding to an external exposure of of 1.50 µg/kg bw per day and 0.63 µg/kg bw per day, respectively, calculated by usign toxicokinetic models. Similarly for children, a BMDL01 (i.e. a dose corresponding to an additional risk of 1% for neurological impairment) of 12 µg / L (B-Pb) equal to an external dose of 0.50 µg/kg bw per day was derived (EFSA, 2010).

Plasticizers

Very recently, diethyl phthalate (DEP) and diethylhexyl phthalate (DEHP), known as plasticizers, have been identified in e-liquids. DEP is used as solvent or plasticizer in the packaging of flavours, cosmetics, detergent industry, while DEHP is used as plasticizer in polyvinyl chloride (PVC) products. They are found in e-liquid packaging or during production processes, and even their concentration are below phthalate exposure limits (Diethyl phthalate and diethylhexyl phthalate were detected in concentration ranges of 0.01–1745.20 mg/L (47.6% detection frequency) and 0.06–81.89 mg/L (79.1% detection frequency) in the replacement liquids), they are possibly carcinogenic to humans (Oh *et al.*, 2015).

Also, dibutyl phthalate (DPB) and dibutyl sebacate, known as plasticizers, too, have been tentatively identified by GC-QTOF-MS, at different part of electronic cigarettes involving plastics, for example at inner end cap or packaging cap (https://www.waters.com/webassets/cms/library/docs/2017asms_lai_electronic cigarettes.pdf. However, it is noted that phthalates have not been detected in aerosols.

Weight of evidence

Information on toxicity of nicotine and tobacco-specific nitrosamines, carbonyl compounds and metals has been collected from international bodies and organisations. Therefore, this information is considered to provide strong evidence. For chemicals, for which there is little information on toxic properties, mainly flavourings, the evidence is considered to be moderate or weak.

Table 7: Toxicity and adverse health effects associated to compounds present in electronic cigarettes e-liquids/aerosol (subject to inhalation)

Health effects	IRRITANT	IRRITANT	CNS	CVD	Genotoxicity/	Other

Compounds	(skin and eye membranes)	(respiratory tract[1]/GIT mucosa[2])	(neuro-toxicity)	(heart-rate and blood pressure)	Carcinogenicity (nasal cavity, liver, lung)	(repro-toxicity[1]/ brain develop-ment[2])
Carriers (*) (Propylene glycol, glycerol)	X	X[1], X[2]				
Nicotine	X	X[1]	X	X		
Nitrosamines TSNA: *(NNK, NAT, NNAL,NNN)*					X	
Carbonyl compounds (VOC): *Formaldehyde* *Acetaldehyde* *Acrolein*	X X X	X[1] X[1] X[1]	X		X X	
Flavourings (**)	X					
Metals: *Chromium VI* *Copper* *Zinc* *Tin* *Lead*		X[1], X[2] X[1] X[2]	X X X	X	X	X[1] X[2]

(*) – irritant effects to skin and eye have been notified to ECHA but data is scarce for the respiratory tract and GIT,

(**) Flavourings cover a wide variety of compounds, in its majority considered as GRAS (Generally Recognized As Safe) and allowed to be used as food additives; notwithstanding, GRAS status is not sufficient proof of safety as tobacco additive because the component is inhaled not ingested and combustion products may be toxic. Some are classified under CLP as irritants to skin and/or serious eye damage.

6.5.4 Human evidence for health impacts of electronic cigarettes

The health impacts of electronic cigarette use are still difficult to establish due to the lack of long-term data from epidemiological studies or clinical trials. However, since 2016, the World Health Organization (WHO)[11] has already noted that, while electronic cigarettes might be "less harmful" than conventional cigarettes, electronic cigarettes still *"are harmful to health and are not safe"*. Therefore, WHO suggested to "*deter electronic cigarette promotion to non-smokers and young people; prohibit unproven health claims about electronic cigarettes; prevent/Bar/Ban involvement of the tobacco industry in the marketing and promoting of e- cigarettes"*. Although, electronic cigarettes are relatively new in terms of exposure to humans, and more research is needed over a longer period of time, there is large scientific body of studies suggesting that electronic cigarettes' use can pose various health risks to the user; e.g., acute or chronic cardiovascular disease (CVD) problems, can irritate the lungs, as well as induce other symptoms, like cough, chest pain, nausea, vomiting, or diarrhea, and sometimes fatigue, fever, or even weight loss (Thirión-Romero *et al.*, 2019). In this section, a brief summary of studies regarding health impacts of electronic cigarettes on human is presented.

[11] https://www.who.int/fctc/cop/cop7/FCTC_COP_7_11_EN.pdf

Cardiovascular diseases

The most consistent evidence regarding the effect of electronic cigarettes on human health concerns cardiovascular diseases. In November 2019, the *European Heart Network (EHN)* published a position document regarding the cardiovascular consequences of electronic cigarette's use[12]. The EHN concluded that there is mixed evidence for the effects of electronic cigarettes on the cardiovascular system from short-term exposure. In particular, it was noted that "*while some studies have found a higher risk compared to smoking combustible tobacco cigarettes, short-term electronic cigarette use is likely less harmful to the cardiovascular system than smoking conventional cigarettes*", whereas the long-term effects on the cardiovascular system are still unknown due to the lack of robust data. However, the authors underlined that, despite the fact that there is "no evidence", this should not be interpreted as no effect, and findings from recent studies suggest that use may pose a higher risk than so far assumed. It is clear the need for longitudinal studies to elucidate long-term effects of electronic cigarette use on the cardiovascular system and whether electronic cigarette use is less hazardous to cardiovascular health than conventional cigarette smoking in the longer term. In addition, the United States Food and Drug Administration (FDA) has also highlighted the adverse health impacts of electronic cigarette use (Chen, 2013). The detrimental acute effects of electronic cigarette use on cardio-metabolic features include adverse vascular and cardiac impacts (including effects on blood pressure and heart rate) (Qasim *et al.*, 2017). Based on the evidence available to date, the individual and interactive effects of flavour and additives used in electronic cigarettes collectively detrimentally impact CVD health, including the propagation of increased heart rate and increased diastolic blood pressure, posing users at elevated subsequent risk for manifesting CVD. The underlying pathophysiological mechanisms remain to be elucidated, however, it has been hypothesized that via sympathetic nervous stimulation, as well as endothelial cell dysfunction and oxidative stress (Higashi *et al.*, 2009), (atomized) nicotine impacts vasculature (Zhang *et al.*, 2018) and arterial stiffness (Vlachopoulos *et al.*, 2016) similarly to conventional tobacco smoking, ultimately inducing hypertension, a well-established CVD risk factor. While due to lag time effects, robust evidence remains limited to date, it is hypothesized that these risks are anticipated to be highest among the most susceptible populations, including children and adolescents. Specifically, the detrimental health impacts of electronic cigarette use on cardio-metabolic features, including effects on blood pressure and heart rate (Qasim *et al.*, 2017) are hypothesized to result via the effects of atomized nicotine on the sympathetic nervous system, inducing cardiac arrhythmias and elevated blood pressure, as well as adverse long-term adverse impacts on vasculature (Zhang *et al.*, 2018) similar to those of conventional tobacco smoking, such as arterial stiffness (Vlachopoulos *et al.*, 2016). Furthermore, electronic cigarette use is also associated with key underlying pathophysiological mechanisms implicated in CVD onset and progression, including endothelial cell dysfunction and oxidative stress (Higashi *et al.*, 2009,) similar to that of tobacco smoking, including rapid surges in the number of circulating endothelial progenitor cells (Antoniewicz *et al.*, 2016), ultimately inducing vascular injury.

Nicotine remains a very important toxin present in electronic cigarette. Most of the cardiovascular effects demonstrated in humans are consistent with the known sympathomimetic effects of nicotine. Acute exposure to (high amounts) of inhaled nicotine may cause dizziness, nausea, or vomiting. Following (acute) exposure to the electronic cigarette with nicotine, there was a significant shift in cardiac sympathovagal balance towards sympathetic predominance. The decrease in the high-frequency component and the increases in the low-frequency component and the low-frequency to high-frequency ratio were significantly greater following exposure to nicotine-containing electronic cigarettes use. The acute sympathomimetic effect of nicotine containing electronic cigarette can

[12] http://www.ehnheart.org/images/EHN_e-cigarettes_final_final.pdf

possibly be associated with increased cardiac risk populations with and without known cardiac disease.

Recent findings demonstrate that volatile liquids containing nicotine may induce adverse cardiovascular effects attributed to its toxic impact on myocardial cells. E-liquids of electronic cigarettes containing nicotine have an alkaline pH, which is expected to influence nicotine absorption, since a larger proportion of nicotine is in its unionized ("free-base" nicotine) form, which is more easily and rapidly absorbed through biological membranes (Stepanov and Fujioka, 2015, DeVito and Krishnan-Sarin, 2018). Even so, electronic cigarette users exposed to 11 mg/mL of nicotine content in e-liquids had increased cardiac output and heart rate (Farsalinos *et al.*, 2014). Regular electronic cigarette use with nicotine containing liquid is associated with a shift towards sympathetic predominance in heart rate and associated variability (Franzen *et al.*, 2018), as well as vascular calcification and impaired vascular function (Babic *et al.*, 2019), leading to prolonged elevated systolic blood pressure (Franzen *et al.*, 2018).

Table 8 summarizes the major cardiovascular effects of nicotine contained in cigarettes and pathophysiological mechanisms (Benowitz *et al.,* 2016). According to the literature, the level of evidence regarding the underlined mechanisms is considered from moderate to strong. It could be assumed that similar mechanisms exist regarding electronic cigarettes use (Benowitz *et al.,* 2016).

Table 8: Cardiovascular effects of nicotine
• Haemodynamics effects (increased heart rate, blood pressure, myocardial contractility) • Endothelial dysfunction • Lipid abnormalities (lower HDL-cholesterol, higher triglycerides) • Insulin resistance • Ventricular arrhythmogenesis • Trial arrhythmogenesis • Remodelling, fibrosis • Heart failure

More recently, the European Association of Preventive Cardiology (EAPC) in a recent position paper highlighted the rapid evolution of the electronic cigarette and the increasing use among adolescents and young individuals. In addition, it is reported that although the long-term direct cardiovascular effects remain largely unknown, the existing evidence suggests that the e-cigarette should not be regarded as a cardiovascular safe product.

Lung diseases

Short-term use of an electronic cigarette has acute effects on airways physiology and respiratory symptoms in COPD smokers, asthmatic smokers, "healthy" smokers and healthy never-smokers. Evidence arising from both experimental and observational studies, support that electronic cigarette use may induce pulmonary toxicity, which is anticipated to emerge as a major public health concern (Chun *et al.*, 2017, Jankowski *et al.*, 2017). Specifically, studies in both animal models and human populations demonstrate that acute electronic cigarette use triggers oxidative stress and increased airflow resistance (Vardavas *et al.*, 2012), either by increased mucin secretion via altered neutrophil related pathways (Reidel *et al.*, 2018) and/or by damage of epithelial airway cells which lead to persistent inflammation and secretion of mediators (namely defensins and matrix metalloproteinases) inducing lung tissue destruction (Chen *et al.*, 2019).

In another recent cross-sectional study of inflammasome protein release in human BAL fluid to compare various smoking categories that include exclusive electronic cigarette use, it

was observed that macrophage counts among electronic cigarette users were intermediate between smokers and never-smokers, suggesting that electronic cigarette use can affect innate immunity. Conclusively, studies up to now suggest that high nicotine levels delivered via aerosol increase the risk for nicotine poisoning and may cause airway inflammation, whereas other ingredients of electronic cigarettes, such as flavourings and triglycerides (Muthumalage *et al*., 2020), may contribute to considerable electronic-cigarette-vaping-lung-injury (EVALI).

Diminished pulmonary function is hence anticipated, particularly among susceptible populations. In fact, electronic cigarette use in adolescents has been associated with the presence of asthma (Clapp and Jaspers, 2017). In another, large-scale study of a total of 45,128 students, use of electronic cigarettes in the past 30 days, was associated with several respiratory symptoms (i.e., cough or phlegm). In a recent study among women of childbearing age, it was observed that compared to nonsmokers, current electronic cigarette users, without a history of cigarette smoking, were associated with 74% higher odds of having asthma. Moreover, the likelihood of having COPD was almost 3-times higher for electronic cigarette users with a history of cigarette smoking. In addition, dual users had 5-times higher likelihood of COPD as compared to non-smokers.

Furthermore, studies in cell lines of human epithelial lung and fibroblast cell lines revealed that the aforementioned cell lines are sensitive to electronic cigarette exposure, inducing production of ROS and pro-inflammatory cytokines, apoptosis, and necrosis (Chen *et al.*, 2019), all hallmarks for tumor growth and development. The effects of long-term use particularly in relation to lung cancer remain to be determined in larg-scale, prospective epidemiological investigations (Chun *et al.*, 2017, Murthy, 2017).

Acute effects

Acute effects of electronic cigarette use have been reported in some cases. Palamidas *et al.,* studied the short-term effects of nicotine electronic cigarettes use in healthy volunteers, asthmatics and COPD patients. Short-term use was associated: a) with increased heart rate in all subjects except in the COPD group, b) decreased oxygen saturation in "healthy" and COPD smokers, c) increased airway resistance (Raw) in asthmatic smokers, "healthy" smokers, and healthy never-smokers and d) decreased specific airway conductance (sGaw) in healthy subjects. Moreover, short-term use of nicotine-free electronic cigarettes increased Raw and decreased sGaw among healthy never-smokers (Palamidas *et al.*, 2017). Acute mouth / throat irritation, and cough are reported by a sub-group of electronic cigarette users, but these effects are not attributed to the nicotine content (Palamidas *et al*., 2017). It could be suggested that these effects are caused by hyperventilation, which is associated with long puffing time (Polosa *et al.*, 2011).

Other health effects

There are also some indications about electronic cigarette use and other health problems. In a recent systematic review conducted among 18 investigations, the carcinogenic potential of electronic cigarettes and the occurrence of head and neck cancers was revealed, albeit with a low level of evidence. Moreover, within this context, findings from several investigations reviewed corroborated that electronic cigarette use induces DNA damage via increased oxidative stress, with most profound effects being associated with flavoured e-liquid use (Flach *et al.*, 2019). It is apparent that as the long-term health effects of electronic cigarettes remain for the most part unknown to date, further investigations regarding their impacts upon both pulmonary and other health systems are urgently needed (Klein *et al.*, 2019).

Few studies have reviewed actual use of electronic cigarettes in pregnant women. In particular, in a survey conducted in 316 pregnant women from a University of Maryland prenatal clinic, 13% of participants reported prior or current use of electronic cigarettes,

and 0.6% reported current daily use (Mark *et al.*, 2015). When analysing by various potential confounders, authors found that those who had ever used electronic cigarettes (ever-users) were slightly older and more likely to identify as white when compared to never-users, whereas no health effects were reported. In another study Ashford *et al,* (2016) administered a survey to 194 current or former female tobacco users (101 whom were pregnant) at a University of Kentucky. Of the pregnant participants, 22.7% were current electronic cigarette users and 37.6% were former users; again, no health effects were reported. Moreover, in a report commissioned by Public Health England, a lack of evidence was reported on the prevalence of using electronic cigarettes in pregnancy in England, the effects of using electronic cigarettes on smoking during pregnancy and following childbirth, as well as on the effects of using electronic cigarettes on maternal health or pregnancy outcomes.

Yuan *et al.* (2015) reviewed clinical and preclinical data concerning sensitivity of the adolescent brain to nicotine. They reported that nicotine exposure in adolescence and the subsequent aberrant activation of nAChRs can lead to persisting changes in neuronal signalling which may have potentially severe consequences for teen addiction, cognition, and emotional regulation. Sailer *et al.* studied the impact of nicotine replacement therapies (NRT) and electronic nicotine delivery systems (ENDS) on fatal brain development. In case of NRT, it was concluded that NRT during pregnancy cannot be considered as a safe alternative to conventional tobacco smoking. Currently, no studies assessing ENDS safety during pregnancy are available, but there are some studies in vitro and on animal models with positive results. ENDS were linked to impaired placental trophoblast function, diminished alveolar cell proliferation and postnatal lung growth (Sailer *et al.,* 2019).

A recent epidemiological study by Pham *et al.* (2020) explored the association between electronic cigarette use and adverse mental health status. The cross-sectional analysis was conducted in Canada using data from the 2015 and 2016 (n=53,050). The association between electronic cigarette use and mental health was found to be modified by smoking status and gender in most of the epidemiological models. The effect was somewhat more pronounced in non-smoking electronic cigarettes users, and in female electronic cigarette users, who tended to have higher odds of adverse mental health than male users. The study relied on respondent self-report, and the cross-sectional nature and thus does not allow us to clarify the direction of this association. Therefore, authors concluded that electronic cigarettes as a possible risk factor for mental health and the potentially harmful effects of second-hand aerosols should be clarified using future longitudinal studies.

The oral cavity is the initial point of contact of electronic cigarette smoke and the first affected system in humans. Oral health depends on an intricate balance in the interactions between oral bacteria and the human immune system. Emerging evidence from subjects with periodontitis as well as periodontaly healthy subjects demonstrates that electronic cigarette use is associated with a compositional and functional shift in the oral microbiome, with an increase in opportunistic pathogens and virulence traits. Dysbiosis of oral microbial communities underlies the etiology of periodontitis, caries, and oral cancer.

Electronic cigarette nicotine poisonings

Another potential health effect associated with the use of electronic cigarettes is poisoning from ingestion of e-liquid containing nicotine, particularly by young children (European Commission, 2016). Within the context of electronic cigarettes, the concern lies within the high concentration of liquid nicotine contained within devices, which at high doses can substantiate the risk of severe toxicity that may result in neurological and neuromuscular harm, respiratory failure and even death (Bassett *et al.*, 2014; Dinakar and O'Connor, 2016; Eggleston *et al.*, 2016). A number of case reports and reports from poison centres have documented incidents of unintentional exposure to e-liquids, including among young children(Chang and Rostron, 2019; Eggleston *et al.*, 2016; Maessen *et al.*, 2020; CI Vardavas *et al.*, 2017) and in rare cases resulting in fatality (Eggleston *et al.*, 2016). Notably, among the 148 cases of acute intoxication due to exposures to e-cigarettes

reported to the Czech Toxicological Information Centre over a 7-year period (2012-2018), more than 60% were in the group of children below 12 years (Obertova *et al*., 2020). The main route of exposure was ingestion of e-liquid contained in cartridges or refillable tanks, which were not characterized by a childproof fastening and opening mechanism.
Among those above the age of 10 years, nicotine intoxication from e-liquids has primarily occurred by way of a suicide attempt, rather than unintentional ingestion (Maessen *et al.*, 2020; Park and Min, 2018). The level of nicotine that may produce acute toxicity has been estimated by the European Chemical Agency's Committee for Risk Assessment to be 5 mg per kg bodyweight (RAC, 2015). The most frequently reported symptoms of nicotine intoxication include vomiting, tachycardia, headache. In addition to ingestion, route of exposure can also be via ocular, dermal, or inhalation. In a study evaluating nicotine poisonings (n=277) reported to poison centres in eight European Union (EU) Member States (Austria, Hungary, Ireland, Lithuania, Netherlands, Portugal, Sweden and Slovenia) from 2012-2015, the most frequent symptoms reported were vomiting, nausea and dizziness, similar results are reported for the US (Chang and Rostron, 2019; Chatham-Stephens *et al.*, 2014; Vardavas *et al.*, 2017b). The majority of cases were unintentional (71.3%), related to refillable electronic cigarettes (87.3%), with exposures primarily via ingestion (54.%), followed by 28.6% inhalation, 9% ocular and 7.9% dermal (Vardavas *et al.*, 2017b). While respiratory exposure was more frequent among paediatric patients, ocular exposure was more frequent among adults (Vardavas *et al.*, 2017b). These parallel findings from the UK, in which 36.4% of the exposure incidents (2007-2013) were for children ages 4 and younger (Thomas *et al*., 2014) and from the US indicating that 50% of cases were among children (Chatham-Stephens *et al*., 2014). Medical outcomes were minor in effect (53.8%) or no effect at all (39.4%), with 6.3% moderate effects, and 1 case of a major clinical outcome. No deaths were reported. While presenting symptoms at the poisoning centres are characteristic of nicotine, they may potentially also be attributable to other ingredients in electronic cigarette liquids, namely flavours, which contain substances identified as respiratory irritants (see also 6.5.3 and table 7) (Girvalaki *et al.*, 2018; Vardavas *et al.*, 2017a).

In order to mitigate the potential risks of electronic cigarette poisonings, the EU Tobacco Products Directive (TPD) 2014/40/EU (European Parliament and the Council of the European Union, 2014), along with Commission Implementing Decisions EU 2016/586 (2016) (Commission Implementing Decision (EU) 2016/586 of 14 April 2016 on technical standards for the refill mechanism of electronic cigarettes (notified under document C(2016) 2093), n.d.) and EU 2015/2183 (2015)(Commission Implementing Decision (EU) 2015/2183 of 24 November 2015 establishing a common format for the notification of electronic cigarettes and refill containers (notified under document C(2015) 8087), n.d.), sets forth standards for electronic cigarette product safety, packaging, and reporting. Specifically, EU TPD Article 20 stipulates a maximum limit for e-liquid refill volumes (≤10 mL) and nicotine content of the vial (≤20 mg/mL), as well as requires the existence of child-resistant fastening and a tamper-proof system. A study evaluating compliance with the EU TPD parameters before and after its implementation, among the most commonly used electronic cigarette refill products in nine European countries found that there was general compliance for child-resistant packaging and the product's nicotine content and volume after TPD implementation *(*Girvalaki *et al*., 2019).

Health effects related to second-hand exposure to aerosol from electronic cigarettes

Particularly in relation to cardiovascular and other health effects of passive smoking secondary to electronic cigarettes use, it has been documented that the complete blood counts of otherwise naïve passive smokers are not affected by such exposures (Flouris *et al.*, 2013). Additionally, despite high levels of carbonyl emissions as reported in several studies above, limited impacts on cardiovascular and/or other health outcomes have been documented (Farsalinos and Gillman, 2018). However, a limited number of studies were done that mimick real-life situations (Ballbe *et al.*, 2014, Flouris *et al.*, 2013), examine the

impacts of passive smoking due to electronic cigarettes currently exists (Shearston *et al.*, 2019) andprimarily evaluate the effects upon airborne nicotine levels, serum cotinine, lung function, complete blood counts and inflammatory marker levels (Shearston *et al.*, 2019). Of these, only one study evaluates the effects of regular passive smoking exposure due to electronic cigarettes within the home, and this study demonstrats increased levels of ambient air nicotine and biomarkers of nicotine (Ballbe *et al.*, 2014). However, when studying second-hand exposure at home (or work), other source of nicotine contamination within the home (or workplace) should be taken into account, as e-cigarette users are often former smokers.

Although the database on the long-term consequences of second-hand exposure to electronic cigarettes on human health is not reach, it is well established that passive smoking detrimentally impacts cardiovascular health, with recent meta-analyses revealing that such exposure increases CVD risk by 23% (Lv *et al.*, 2015), including ischemic and coronary heart disease risk by 25-30% (He *et al.*, 1999, Dunbar *et al.*, 2013, Law *et al.*, 1997). It is hypothesized that passive smoking CVD risk in a non-linear dose-effect relationship, detrimentally impacts the health event even at low exposure levels (Argacha *et al.*, 2018), as a result of nicotinic stimuli on both the sympathetic system and vascular oxidative stress (Barnoya and Glantz, 2005, Whincup *et al.*, 2004). Surprisingly, particularly in relation to cardiovascular and other health effects of passive smoking secondary to electronic cigarettes, the authors found that the complete blood counts of otherwise naïve passive smokers are not affected by such exposures (Flouris *et al.*, 2013). Additionally, despite high levels of carbonyl emissions as reported in several studies above, limited impacts on cardiovascular and/or other health outcomes have been documented (Farsalinos and Gillman, 2018). However, it is noteworthy that to date data on the long-term consequences of passive smoking of electronic cigarettes on human health are lacking (Hiemstra and Bals, 2016).

Indoor electronic cigarette use can lead to deposition of aerosol components on surfaces. A recent review Díez-Izquierdo *et al.* (2018) analysed the reported concentration of nicotine, nitrosamines and/or cotinine as components of third-hand smoke (THS) in indoor dust. The reported THS concentrations could be linked to harmful effects on cells, in animal models, and in people including children. However, the authors concluded that only speculations can be made on the long-term effects of these exposures (Díez-Izquierdo *et al.*, 2018).

Health effects of electronic cigarette use on young adultss, children and adolescents

With regard to the health effects of electronic cigarette use in children and adolescents, these are associated with the particular ingredients of electronic cigarettes liquids most often preferred by this population group. Specifically, as aforementioned, apart from nicotine, e-liquids have an array of flavours, strengths, and types; particularly with regard to added flavours, a recent systematic review of 66 investigations revealed that consumers prefer flavoured electronic cigarettes. Preferences varied by age, gender, and smoking history, with several flavours being perceived as having diminished risk of harm from electronic cigarettes use (Zare *et al.*, 2018). It is noteworthy that adolescents (Zare *et al.*, 2018) (along with young adults (Harrell *et al.*, 2017a, Harrell *et al.*, 2017b) were most likely to initiate use with flavoured types, while young adults were observed to prefer menthol and/or other sweet flavours (Zare *et al.*, 2018). As such, use of flavoured volatile liquids may pose a gateway for electronic cigarettes use, which may be later escalated to nicotine use, particularly among vulnerable populations such as children and adolescents (Harrell *et al.*, 2017a, Harrell *et al.*, 2017b). Most guilefully, though, those with the sweetest taste (namely strawberry and/or cinnamon) and most likely to be readily adopted by younger populations as they are erroneously presumed to be less harmful (Pepper *et al.*, 2016), were found to be of highest toxicity (Leigh *et al.*, 2016, Pisinger and Dossing, 2014, Bahl *et al.*, 2012). Specifically, liquid flavours were found to be highly cytotoxic to human

embryonic and mouse neural stem cells, as well as human pulmonary fibroblasts, inducing alterations in gene expression (Pisinger and Dossing, 2014, Bahl *et al.*, 2012). However, the long-term effects of such exposure on health, particularly during pivotal developmental periods (namely pregnancy and childhood), remain to be elucidated (De Long *et al.*, 2014) and are not predictable based on currently available data (Tierney *et al.*, 2016). Hence, these adverse health effects are upheld to be highest among susceptible populations, such as children and adolescents, who based on market date most frequently utilize electronic cigarettes containing potentially harmful chemicals, such as sweet flavours and additives.

In addition, with regard to the respective effects of passive smoking secondary to electronic cigarettes use, there exists a complete paucity of evidence regarding the acute and long-term effects of passive smoking secondary to electronic cigarettes on cardiovascular and other health outcomes in children and adolescents. Therefore, further research investigations are urgently mandated for evaluating the effects of passive smoking induced by electronic cigarettes use in susceptible populations, particularly such as children and adolescents who may be regularly exposed within their home environments.

Electronic cigarettes and injuries due to burns and explosions

As additional health effects, electronic cigarette use can be the cause of injuries due to burns and explosions. Reports of spontaneous explosions and/or fires of electronic cigarettes have been reported, and cases are predominantly attributed to the malfunction of lithium-ion batteries – a risk that can be substantially mitigated through appropriate legislative action. Electric, thermal or mechanical damage to lithium-ion batteries (via persistent over-charging, over-heating or crushing, respectively) can result in the erosion of integral safety features (Nicoll *et al*., 2016). Such damage can trigger a hazardous short circuit, initiating a ''thermal runaway'' reaction whereby internal battery overheating causes a battery fire or explosion, and subsequent burn and blast injuries. Injury mechanisms associated in explosions related to the use of electronic cigarettes, include thermal burns with flames, blasts lesions secondary to the explosion, chemical burns caused by the leakage of corrosive lithium ion compounds following explosion, Nicoll *et al*., 2016) and thermal burns without flames (overheating) (Serror *et al*., 2018). These mechanisms may be single or associated. Electronic cigarette explosion injuries can be classified as direct and indirect injuries (Patterson *et al*., 2017). Direct injuries result directly from the explosion of the device. These mainly include localized hand injuries, face injuries (head and neck), waist/groin injuries, as well as inhalation injuries from using the device. Hand injuries, including severe burns, loss of digits or high-pressure injection of e-liquids, (Foran *et al*., 2017) occur when the electronic cigarette device explodes while being held by the victim or while being kept in their pocket (and the hand is used to extinguish the fire) Serror *et al*., 2018, Patterson *et al*., 2017). Face injuries occur when the electronic cigarette is being held up to the face for inhalation. These can include ocular and oral/maxillofacial trauma due to thermal, chemical and blunt force injuries. Ocular injuries may cause significant and permanent visual impairment due to injuries to the cornea, conjunctiva and anterior segment and permanent fovea damage and visual loss due to choroidal rupture following an explosion (Khairudin *et al*., 2016). The directionality of blasts toward the upper and posterior oral cavity and palate may cause fractures, burns, lacerations, dental injuries (including dental avulsion and fractures), as well as cranial injuries (Archambeau *et al*., 2016). Inhalation injuries include upper airway injuries and irritation resulting from direct flash or explosion of the electronic cigarette device (Archambeau *et al*., 2016; Patterson *et al.,* 2017). Waist/groin injuries occur when the electronic cigarette device is stored in the victim's pants' pocket and ignites the victim's clothing, resulting in deep burns in the pelvic area. The majority of burns occur when the device explodes while stored in the users pocket, making the groin and genital area the most commonly affected area of the body in reported cases (Serror *et al*., 2018; Toy *et al*., 2017; Brownson *et al*., 2016; Hassan *et al*., 2016; Arnaout *et al*., 2017). Indirect electronic cigarette explosion injuries occur as a consequence of fire when the device ignites and causes a house or car fire, causing subsequent flame burn injuries and inhalation injuries (Patterson *et al*., 2017). The pattern and severity of electronic cigarette related injuries depend on the status of the device

(charging, in- use, stored) and its positioning relative to the user. Severe injuries are more likely when the electronic cigarette device is in the victim's mouth, in very close proximity to their face, or in a pocket (U.S. Fire Administration, 2017). Additionally, explosion generates a relatively concentrated area of direct thermal injury, creating an entryway into the skin for toxic chemicals and introducing chemical burns. The quantity of toxic chemicals that are subsequently introduced into the lesions varies, and the amounts that would cause permanent toxic injury is unknown (Kite *et al*., 2016).

Safety Gate notification for electronic cigarette and related products from 2012 to 2020

By searching for the key-word 'electronic cigarette' on the Rapid Alert System for dangerous non-food products (now called Safety Gate, once known as RAPEX), which is the EU rapid alert system notifying Member States about risks to the health and safety of consumers (excluding pharmaceutical and medical devices), 54 entries were found. They come from 14 different MS, indicating that the potential risk is spread all over Europe. Considering the country of origin of the notified products, excluding a few 'unkown', almost 50% was from China, 1 fwas from the United States and the rest was from EU MS.

Only 10 entries refer to risk due to 'Electrical appliances and equipments', related to electronic cigarette charger, battery, and adapter. The nature of risk was classified as

- Electric shock (n=7) due the following defect: The insulation is not sufficient, and a user may come into contact with live parts and receive an electric shock.
- Electric shock/fire (n=2) due the following defect: The electrical insulation is inadequate: beside the electric shock, generation of fire is also considered possible.
- Burn/fire/injuries (n=1) due the following defect: An external short circuit can occur in the battery, leading to an internal temperature and pressure increase. The battery and the device it is used for can consequently explode, releasing shrapnel and or/leading to a fire

The products did not comply with the requirements of the Low Voltage Directive and the relevant European standard EN 60335 EN 60960 and EN 62133-2 and their withdrawal from the market was established, in some cases paralleled to a recall of the products from end users.

The remaining entries are classified as risks coming from 'chemical products' and generally refer to e-liquid content. In two cases, the product was considered not compliant due to the lack of a child-proof fastening and opening mechanism, independently from the content and for that reason they were withdrawn from the market. However, the lack of a child-proof fastening and opening mechanism was also described for other products, for which the e-liquid composition was also not compliant.

All the other cases (n= 42) did not comply with the requirements of the TPD. The risk was linked to different causes, listed below:

1) An excessive amount of nicotine: values ranged from 23.5 up to very high ones (100-150 and 250 mg/ml were the highest values). The content was declared in the label. The products did not comply with the requirements of the TPD.
2) Nicotine content was wrongly declared in the label (e.g. labelled as <20mg/ml, while actually containing >20 mg/ml). Beside the TPD, the products did not comply with the Regulation on the classification, labelling and packaging of substances and mixtures (CLP).
3) The presence of nicotine was not reported on the labelling, although the liquid contained nicotine. The products did not comply with the TPD or the CLP.
4) The product contains an excessive volume of liquid, which contains nicotine.
5) The product lacks the adequate labelling and warnings. The product does not comply with the CLP Regulation.
6) In two cases, the products were considered to be misleading for consumers since they can be mistaken for foodstuff. Indeed, one of them refers to a drink both in

respect of packaging and in terms of organoleptic characteristics, i.e. intense aroma of cocoa, while a second one has a label depicting fruits. So besides being not compliant with the CLP, the products did not comply with the requirements of Directive 87/357/EEC on products which, appearing to be other than they are, endanger the health or safety of consumers.

Overall, the risk was associated mainly to nicotine content, especially if the user, due to inadequate safety label bearing risk-related indications, has no information about the safe and correct use of the product, e.g. how to properly dilute the product and avoid the dangers incurred when the product comes into contact with the skin or if it is ingested.

Conclusions for poisoning and injuries due to burns and explosion

For both poisoning and injuries due to burns and explosion, the evidence for the intrinsic capability to cause health problems is strong, but the incidence is quite low: only few case reports are available, the collection of injury events has not yet foreseen by the EU IDB, and the notifications to the Rapid Alert System for dangerous non-food products not compliant with the ralted regulations are limited. Therefore, the related risk is low.

Conclusion and weight of evidence consideration

There is a moderate, but growing level of evidence from human data suggesting that electronic cigarette use has harmful health effects, especially but not limited to the cardiovascular system. However, more studies, in particular on long-term health effects, are needed. For acute health effects, only one valuable clinical study was identified. Pulmonary changes such as increased airway resistance and decreased airway conductance were observed in healthy volunteers. If assessed in cohort studies, there is a low incidence of acute effects of electronic cigarette use are mouth/throat irritation and coughing, whcich are reported by a sub-group of users and seem not to be related to the nicotine content. The weight-of-evidence is moderate for local irritative damage to the respiratory tract of electronic cigarette users.

In addition, with regard to the respective effects of second-hand exposure of children and adolescents secondary to electronic cigarettes use, the weight of evidence cannot be established as there exists a complete paucity of evidence regarding the acute and long-term effects on cardiovascular and other health outcomes in this group. Therefore, further research investigations are urgently mandated for evaluating the effects induced by electronic cigarettes use in susceptible populations, particularly such as children and adolescents who may be regularly exposed within their home environments.

6.5.5 Risk assessment

In this section, the results of exposure assessments will be compared to the results of dose-response analyses, such as PoDs and human limit values, for substances in the aerosol of electronic cigarettes.

Given the numerous substances potentially present in aerosol from electronic cigarettes, the SCHEER selected those it considered to be priorities for the risk assessment (Section 6.5.5.1). The preferred approach for the risk assessment is being explained in Section 6.5.5.2. Risk assessments are presented based on simulations and measured concentrations for electronic cigarette users.

6.5.5.1 Prioritisation for risk assessment

Prioritisation was performed based on the concentrations measured in aerosol (section 6.5.2.3, table 5) and the hazards and human health impacts identified (section 6.5.3 and 6.5.4). In addition, a comparison is made to the list of compounds recommended to be measured in aerosol of electronic cigarettes according to the tobacco and electronic

cigarette industry dominated CEN for the purpose of regulatory submission under the TPD (CEN, 2018) and to the list of the European Association for the Co-ordination of Consumer Representation in Standardisation (ANEC, 2019). The CEN-list includes nicotine, in situ formed formaldehyde, acrolein, acetaldehyde and the hardware related metals cadmium, chromium, iron, lead, mercury, nickel, titanium and aluminium. ANEC (2019) addressed substances in e-liquids (solvents, contaminants and flavours) as well as substances formed (degradation products) or released (from materials) during electronic cigarette use. Priority was given to substances frequently found in screened literature, substances with highest measured concentrations and substances with identified (low) thresholds.

It is noted that the composition of the aerosols as measured only match with the lists of top ingredients in liquids as presented in Annex 2 (present in > 10% liquids) for nicotine, carrier liquids, ethyl acetate and ethanol. The latter two compounds were not quantified. Other ingredients on the list, present in liquid in concentrations > 1 mg/ml and detected in aerosols, were: acetoin, diacetyl, and acetylpropionyl. None of the other listed ingredients were quantified in aerosols. Comparing the list of table 5 with the CEN-list and the ANEC-list it can be concluded that table 5 is the most comprehensive list. However, it is noted that CEN additionally lists iron, mercury, titanium and aluminium.

The focus of the risk assessment will be on the organic substances in Table 5. Table 5 also shows typical maximum concentrations for these substances.

6.5.5.2 Dose metrics in the risk assessment of electronic cigarettes

In risk assessment, the hazard information preferably needs to show an exposure regimen close to that of the exposure scenario under investigation. The dose metric to be used depends on the mode of action of the chemical, its toxicokinetics and the dynamics of the chemical in the aerosol and could be the concentration in the aerosol in different regions of the respiratory tract, the inhaled dose per time interval, the absorbed dose per time interval, or a cumulative dose over partial or total lifetime. In a review on toxicokinetics and dynamics of use of electronic cigarettes, Bos *et al.* (2021) applied this concept to the electronic cigarette. The daily exposure to aerosol from an electronic cigarette is a compilation of multiple peak exposures with irregular time intervals. An increase in the dose is achieved by an increase in puffing frequency and duration whereas, at the same time, the exposure concentration will not or hardly change. Bos *et al.,* performed simulations in which the exposure scenario was compared with that for the general population (continuous exposure of 24 hours per day), starting from the same total inhaled daily dose. It was shown that peak air concentrations during a puff can be easily two orders of magnitude higher than the inhaled concentration of the general population, be it with regular non-exposures between sessions.

From this, it was concluded by Bos *et al.* (2021) that direct risks could not be assessed based on health-based guidance values (HBGVs), as also noted by USDHHS (2016). Since there are no HBGVs for smoking or using electronic cigarettes and existing HBGVs are not applicable to the electronic cigarette use scenario, it was advised to perform a risk assessment in which chemical-specific information that is relevant for the scenario (i.e., intensity, duration, and frequency) is taken into account. Because the available hazard information, often based on animal experiments, will mostly be obtained with an exposure regimen that will also significantly differ from the electronic cigarette use scenario, a direct comparison of exposure and hazard characteristics will generally not be possible. Farsalinos and Gillman (2018) also point out that reporting carbonyl emissions as mg/m^3 could be relevant to environmental emissions (second-hand exposure) but is problematic when assessing exposure to users due to the intermittent nature of electronic cigarette use.

As a pragmatic alternative, the Margin of Exposure (MoE) approach may be applied. A MoE is the ratio of a reference point (the Point of Departure or PoD), taking into account *in vitro* or *in vivo* experiments and corresponding to an exposure that causes a low but measurable

response, and the exposure estimate in humans (EFSA, 2005). This approach offers the possibility to take the specific exposure characteristics into account. The minimal value required for the MoE to come to a conclusion of no or low concern depends on the hazard information available and on the exposure characteristics and thus will be different for different scenarios. In general, only interspecies and inter-individual differences in susceptibility need to be taken into account in the evaluation of the MoE if no adverse effects are observed at the PoD. Typically, a MOE of minimally a factor of 100 is then considered to be required for non-carcinogenic effects. If the exposure scenario from which the PoD is derived significantly differs from the human exposure scenario under consideration, these differences need to be bridged by taking them into account in the evaluation of whether a MoE is sufficient to reach a conclusion of low concern.

6.5.5.3 Risk assessment based on modelled topography of electronic cigarette consumption and second-hand exposure scenarios

Assessment for electronic cigarette users

Because of the extremely variable individual differences in the levels of exposure to ingredients in liquids and aerosol Visser *et al.* (2014 and 2015) performed a risk assessment based upon three pre-defined exposure scenarios for daily users. They used the aerosol analysis data for two out of the 12-17 e-liquid samples shown in Section 6.5.2, Table 3 and the calculations explained in the previous section. The risk assessment was done for all substances in table 3 except metals. Fragrances were also not included in this analysis. The use topography information used for this assessment was derived from scientific literature and was supplemented with market survey data on the frequency and nature of electronic cigarette use. The following three exposure scenarios were defined:

1. Light user: 15 inhalations per day, 1 puff per 4 minutes, with a total daily use duration of sixty minutes.
2. Average user: 60 inhalations per day, 1 puff per 2 minutes, with a total daily use duration of 120 minutes.
3. Heavy user: 500 inhalations per day, 2 puffs per minute with a total daily use duration of 240 minutes.

Given the use topography discussion in section 6.5.1, it can be concluded that the heavy use scenario seems realistic, but this may not be a worst case scenario with regard to the average puff volumes of 70 ml (can run up to 118 ml) which determines the dose inhaled. On the other hand, the number of puffs per day, determining the exposure duration, seems very high.

For local effects on the respiratory tract, the MoE was based on the estimated maximum median alveolar concentration calculated from the puff dose, the volume per puff (70 ml), a low absorption rate (30%) and the dilution rate in the lungs. With respect to the latter: the aerosol concentration in the respiratory tract will be lowered since, together with the puff, air will also be inhaled. For systemic effects, the MoE was based on the calculated total absorbed daily dose. On the hazard side, a suitable animal experiment was chosen to derive the PoD.

It was concluded for the e-liquid samples considered that:

- Exposure to the polyols brings a high risk of irritative damage to the respiratory tract in heavy users of electronic cigarettes (MoEs 0.27 – 16, no MoE for diethylene-glycol) and that this risk cannot be excluded in light and average users (MoEs 0.6-36). It was considered likely that the mechanism by which the various polyols damage the respiratory epithelium is the same in all cases and therefore that cumulative effects are likely. The possibility of heavy users experiencing systemic effects (reduced lymphocyte count) as a result of exposure to propylene glycol

cannot be excluded (MoEs 6.7-30). There was no risk for systemic effects from polyols for other scenarios for use of electronic cigarettes.

- Exposure to nicotine may induce effects on the respiratory tract since the alveolar concentrations calculated are higher than (effects likely) or comparable to (effects cannot be excluded) effect concentrations in human volunteer studies with nicotine, showing coughing and constriction of the airways. Systemic effects on the cardiovascular system are considered possible since the absorbed doses are higher than effect levels in human volunteer studies with nicotine, showing changes in heart-beat and systolic blood pressure. There may be a risk for adverse effects on the foetus for heavy users since the absorbed doses calculated were slightly lower than effect concentrations in a study with monkeys. Nicotine dependence and addiction will be discussed in Section 6.6.
- Exposure to the tobacco-specific nitrosamines (e.g. NNK) will increase the risk of tumour development in the respiratory tract in heavy users (MoEs 24-766); in light and average users, the additional tumour risk may vary between negligible (typical MoE 1685) and increased (typical MoE 54) depending on the type of liquid.
- With regard to aldehydes: formaldehyde, acrolein and diacetyl were present in concentrations sufficient for potential damage to the respiratory tract for heavy users (MoEs 0.11-34), while the risk was considered not to be excluded (MoEs 0.24 – 0.9) or uncertain for average and light users (MoEs 5 -75). It was noted that formaldehyde-induced damage to the respiratory epithelium can be a precursor to tumour formation and that in a few cases, the formaldehyde concentrations were sufficient to create a risk of tumour development in the respiratory tract, maybe exacerbated by the presence of acetaldehyde, acrolein and diacetyl. No definite conclusion was drawn. Other systemic risks were considered low for these substances.

Cumulative assessment groups can be identified for irritative effects on the respiratory tract and for carcinogenicity. In an additive approach, the total exposure to polyols, aldehydes and nicotine will lead to a very low MoE and adverse effects on the respiratory tract will be very likely. The evidence for irritative effects can be considered strong. Carcinogenic effects can be expected to occur due to exposures to nitrosamines and formaldehyde. The assessment above already takes into account additive effects from the nitrosamines involved if present. The carcinogenic effect from formaldehyde, if it occurs at all, proceeds via a different mechanism of action than carcinogenicity from nitrosamines. Additivity (i.e. cumulative effects of different chemicals) is not warranted here.

Assessment for second-hand exposure

Visser *et al.* (2016 and 2019) evaluated two specific second-hand exposure scenarios. The first scenario concerns a daily car trip of one hour in a small unventilated car of 2 m^3 with two electronic cigarette users (puffing frequency 0.5 per minute, 1 hour of use). The exposed person is a child, sitting in the same car. This exposure scenario approximates the highest levels of exposure that may be expected in everyday situations. The second scenario concerns a daily exposure of four hours in an office-sized space (30 m^3) with one electronic cigarette user (puffing frequency 2 per minute, 4 h of use). Based on the exposure levels of Table 6 the concentrations for the assessment of local effects and the systemic dose were calculated for propylene glycol, nicotine, TSNAs and copper. The air concentration (final concentration (mg/m3) reached at the end of the use period) and internal systemic exposure (expressed as mg/kg bw), were used. For each chemical, the exposure concentrations were calculated from the highest amounts exhaled by the volunteers (see Table 6), taking into account ventilation, pulmonary retention (0% for local effects, 50% for systemic effects), and the fact that exhalation of the chemical may not have been complete in the first exhalation but may continue with subsequent exhalations.. Using 50% retention for systemic effects can be considered a worst-case default value in view of the much higher alveolar retention of, for instance, nicotine. This leads to an overestimation of the bystander exposure.

The estimated air concentrations for the individual chemicals were compared with human limit values with respect to chronic exposure for the general population. Air concentrations of chemicals below their (WHO Air Quality Guideline) limit value are considered not to result in adverse health effects. In cases where appropriate human health-based limit values were lacking, the risk assessment was based on a Margin of Exposure (MOE) approach.

It was concluded (by Visser *et al.*, 2016 and 2019) that:

- The risk for local effects on the respiratory tract of propylene glycol cannot be excluded for scenario 1 (MoEs 17-18) and is low for scenario 2 (MoE 74-81). There is no risk for systemic effects (MoEs 535-1475).
- Glycerol was not detected in exhaled air and therefore the risk for second-hand exposed persons is considered low.
- Local effects from nicotine exposure are not expected (MoEs 170-750. In a worst case approach, the MoE for systemic cardiovascular effects is 2.1 for scenario 1: adverse systemic effects are expected. For scenario 2, systemic cardiovascular effects cannot be excluded either (MoE 6).
- Aldehydes are not detected in exhaled air allowing the conclusion that there is no risk for adverse effects for second-hand exposed persons.
- For TSNAs MoEs are 521 and 2297 for scenario 1 and 2, respectively. A carcinogenic risk cannot be excluded for scenario 1 and is uncertain for scenario 2.

6.5.5.4 Other risk assessments

Assessment for electronic cigarette users

Several reviews are available that predominantly compare exposure levels of substances in aerosol from electronic cigarettes with health-based guidance values (e.g., Farsalinos *et al.*, 2015d; Zulkifli *et al.*, 2016; McNeill *et al.*, 2018; US-NAS, 2018). As argued in Section 2.1, such values are based on more continuous exposure scenarios that are completely different from electronic cigarette exposure scenarios that are characterised by multiple peak exposures with irregular time intervals of zero or background exposure only. Therefore, such risk assessment are not applicable for the purpose of this Opinion, unless they show that the puff concentrations measured are below these standards and therefore clearly point at the absence of any risk with a wide margin. This is the case for the review by Farsalinos *et al.* (2015d) in which metal levels in aerosol, found in two studies, were compared to 3 different health based guidance values: the Permissible Daily Exposure (PDE) from inhalational medications, defined by the United States Pharmacopeia, the Minimal Risk Level (MRL), defined by the US Agency for Toxic Substances and Disease Registry (ATSDR), and the Recommended Exposure Limit (REL), defined by the US National Institute of Occupational Safety and Health (NIOSH). In spite of the assumption of a very high puff frequency of 1200/day to estimate daily exposure, none of the levels detected were above these limits except for a 10% increase for cadmium above the PDE for one of the 13 products investigated. This study was re-evaluated by Zulkifli *et al.* (2016) who calculated hazard quotients based on a comparison of the metal concentrations measured with reference concentrations and cancer slope factors/minimal risk levels from US-EPA/ ATSDR. In this assessment, hazard quotients higher than 1 were not only found for cadmium (28.5) but also for nickel (1.6), aluminium (9.4) and titanium (2.4). Lifetime cancer risks for cadmium, chromium, lead and nickel were all below 1.10^{-6}. Note that these quotients are based on the assumption of continuous exposure and therefore likely to be overestimated. In another approach Farsalinos and Rodu (2018) determined the liquid consumption that would exceed permissible daily exposures (PDEs) defined for inhalation medications for metals. They calculated that for almost all metals, except nickel, unrealistically high levels of liquid need to be consumed in order for total daily exposure to exceed established limits.

In a recent review Stephens *et al.* (2018) calculated an aggregated lifetime cancer risk for different first- and second-generation electronic cigarettes based on concentration-weighted

inhalation potencies and concentrations of IARC-classified carcinogenic substances in undiluted aerosol. Exposure data came from the published literature. The daily use volume was estimated at 30 l/day. The substances were: acetaldehyde, formaldehyde, NNN, NNK, cadmium, lead and nickel. Although the absolute unit risk estimates used may not be applicable to this specific exposure scenario, the relative contribution to the aggregate cancer potency suggest that the carcinogenic risk was determined mainly by carbonyls and, if present, cadmium, but is highly variable. Nitrosamines appeared to be minor contributors. Scungio *et al.* (2018) also evaluated the overall carcinogenic risk of substances condensed on particulate matter from electronic cigarettes. The excess lifetime cancer risk (ELCR) was estimated based on inhalation slope factors of IARC Group 1 pollutants, their mass concentration condensed on the aerosol particles, the measured doses of deposited particles and electronic cigarette use characteristics. The pollutants were arsenic, cadmium, nickel, NNN and NNK. The ELCR values for mainstream aerosol with and without nicotine were found to be below 10^{-5}. It is noted that slope factors were used for continuous exposure over a lifetime, but that the ELCR was averaged for the number of years of using electronic cigarettes to better match the actual exposure scenario.

Hahn *et al.* (2014) assessed the risk of measured constituents of electronic cigarettes by a MoE estimation based on the use levels found (see section 1.1) and toxicological PoDs. However, this assessment was exclusively based on oral data and therefore the SCHEER considers the conclusions not applicable to electronic cigarette exposure scenarios.

Risk assessments for fragrances were not found. The SCHEER agrees with McNeill *et al.* (2018) in concluding that 'to date, there is no clear evidence that specific flavourings pose health risks but there are suggestions that inhalation of some could be a source of preventable risks'. However, as noted earlier, inhalation toxicology data are scarce for flavourings that are mainly being assessed for oral exposure through food.

Tierney *et al.* (2016) analysed flavour chemicals in two brands of electronic cigarettes. Many of the products contained the same flavour chemicals (vanillin and ethyl vanillin, maltol and ethyl maltol, benzaldehyde and benzyl alcohol, and ethyl butyrate and ethyl acetate), a significant number of which (6/24) were aldehydes, recognised toxicologically to be 'primary irritants' of the mucosa of the respiratory tract. Based on a rough comparison with the occupational exposure limits for vanillin and benzaldehyde, it was concluded that aerosol exposure may be close to or even exceed these limits. It was also shown (Erythropel *et al.*, 2019) that reactions occur between flavouring and solvent components such as propylene glycol, resulting in compounds, e.g. aldehyde–propylene glycol acetals, having toxicological properties that differ from either the flavourings or solvent components with hitherto unknown consequences for the risk assessment.

Assessment for second-hand exposure

Hess *et al.* (2016) reviewed 16 studies, with varying designs and of different quality, investigating potential adverse health effects of passive exposure to electronic cigarette aerosols. The conclusion of this qualitative meta risk assessment was that the majority of studies concluded that passive exposure to electronic cigarette aerosol may pose a health risk to second-hand exposed persons. Only four studies were negative, but these studies were reported to have been undertaken by tobacco employees or funded by the National Vapers Club. None of the studies looked at potential long-term impacts from exposure to electronic cigarette aerosol.

Liu *et al.* (2017) measured room concentrations of 34 chemicals after e-cigarette use by 37 healthy volunteers. The cumulative four-hour room air levels of the chemicals measured above the LOQ were relatively small and mainly concerned nicotine, propylene glycol and glycerol. Cumulative 4-h. levels of nicotine, PG and glycerol measured were several-fold below the time-weighted average limits used in workplace exposure evaluation.

Scungio *et al.* (2018) evaluated the excess lifetime carcinogenic risk (ELCR) of substances on particulate matter in second-hand aerosol from electronic cigarettes and found about two orders of magnitude of difference between ELCR associated to mainstream aerosol (that were below 1.10^{-5}) and second-hand aerosol.

6.5.5.5 Risk estimates from epidemiology

In a Cochrane systematic review of epidemiological studies into adverse events with a follow-up of 6-24 months, three random clinical trials (RCT) and nine cohort studies were found eligible for further analysis. The quality of the evidence was judged to be weak (GRADE-system: further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate). No studies reported serious adverse effects considered related to electronic cigarette use. One RCT provided data on the proportion of participants experiencing any adverse events with a relative risk of 0.99 (electronic cigarette versus nicotine patch, n=456) and 0.97 (electronic cigarette versus placebo, n=298). Cohort studies found mouth and throat irritation, dissipating over time, to be the most frequently reported adverse effect in electronic cigarette users (Hartmann-Boyce *et al.*, 2016; update of Hajek, 2014). In a further update, including in total 50 studies, none of the included studies (short- to mid-term, up to two years) detected serious adverse events considered possibly related to EC use. The most commonly reported adverse eIects were throat/mouth irritation, headache, cough, and nausea, which tended to dissipate over time (Hartmann-Boyce *et al*., 2020).

6.5.5.6 Conclusions

On risks for electronic cigarette users

Based on the exposure assessment (Section 6.5.2) the hazard identification (Section 6.5.3), the human health impacts (Section 6.5.4) and the risk assessment (Section 6.5.5), the SCHEER concludes for exposure of electronic cigarette users that:

- The overall weight of evidence is moderate for risk of local irritative damage to the respiratory tract of electronic cigarette users due to the cumulative exposure to polyols, aldehydes and nicotine. The lines of evidence are the following:
    - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.
    - These substances are all identified as irritants (strong weight of evidence)
    - In cohort studies, mouth and throat irritation, dissipating over time, was the most frequently reported adverse effect in electronic cigarette users. The overall reported incidence was low (moderate weight of evidence).
    - The model studies revealed low MoEs for irritative effects for individual chemicals and these will be even lower in an additive approach. It is noted that nicotine salts are less irritating. With regard to the risk calculation on aldehydes: formaldehyde, acrolein and diacetyl were present in concentrations sufficient for potential damage to the respiratory tract for heavy users (moderate weight of evidence).
    - The alveolar concentrations of nicotine calculated in the model studies are higher than or comparable to effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour (moderate weight of evidence).

- The overall weight of evidence for risk of long-term systemic effects on the cardiovascular system is moderate. The lines of evidence are the following:
    - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.

  - There is strong evidence regarding the cardiovascular effects of nicotine – based on increase of heart rate, hypertension and vascular calcification (strong weight of evidence).
  - The level of evidence regarding the cardiovascular effects of nicotine contained in electronic cigarettes and the related pathophysiological mechanisms is considered to range from moderate to strong.
  - The absorbed doses of nicotine calculated in the model studies are higher than effect levels in studies with human volunteers exposed repeatedly to nicotine vapour showing cardiovascular effects (moderate weight of evidence).
  - Based on human evidence, there is moderate weight of evidence for cardiovascular effects triggered by nicotine, however, the weight of evidence related to long-term effects is weak due to lack of longitudinal studies and taking into account the possible substance mixture effects in e-cigarettes (e-liquids/aerosols.

- The overall weight of evidence for risk of respiratory tract carcinogenicity due to long-term, cumulative exposure to nitrosamines and due to exposure to acetaldehyde and formaldehyde is weak to moderate. The lines of evidence are the following:
  - Moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes.
  - Nitrosamines, formaldehyde and acetaldehyde have been identified as genotoxic and carcinogenic (strong weight of evidence).
  - In the model calculations. exposure to the nitrosamines increased the calculated risk of tumour development in the respiratory tract, especially in heavy users. If TSNA is present in the e-liquids, it is assumed that this risk will increase due to cumulative exposure to these chemicals (moderate weight of evidence).
  - The formaldehyde-induced damage to the respiratory epithelium is a precursor to tumour formation and may be exacerbated by the presence of acetaldehyde, acrolein and diacetyl (weak weight of evidence).
  - The human evidence is very limited and does not allow a conclusion (weak weight of evidence).

- The weight of evidence for risk of adverse effects from the metals in aerosols, specifically carcinogenicity, is weak. This conclusion is mainly based on the comparison between measured exposure levels in aerosols and health-based guidance values (weak weight of evidence).

- The overall weight of evidence for risks of other long-term adverse health effects. such as pulmonary disease, CNS and reprotoxic effects, based on the hazard identification and human evidence, is weak, and further consistent data are needed.

- The overall carcinogenic risk of substances condensed on particulate matter from electronic cigarettes was found to be below 10^{-5}.

- To date, there is no consistent data that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure.

- The concentrations of aldehydes resulting from flavourings are considered too low to add substantially to the already apparent cumulative risk to the respiratory tract from the aldehydes generated in the electronic cigarette and from polyols and nicotine. The weight of evidence is weak due to the absence of inhalation toxicological data and specific risk assessments.

- The overall weight of evidence for poisoning and injuries due to burns and explosion is strong. However, the incidence is low. Therefore, the risk is expected to be low.

On risks for second-hand exposure

Based on the exposure assessment (Section 6.5.2), the hazard identification (Section 6.5.3), the hazard assessment (Section 6.5.4) and the risk assessment (Section 6.5.5), the SCHEER concludes that:

- The overall weight of evidence is moderate for risk of local irritative damage to the respiratory tract. The lines of evidence are the following:
  - Moderate weight of evidence for second-hand exposure
  - This irritation is mainly due to exposure to glycols. Glycols are identified as irritants (strong weight of evidence).
  - The model studies revealed low MoEs for irritative effects from propylene glycol (moderate weight of evidence).
  - The assessment of second-hand nicotine exposure does not point at a risk for respiratory irritation (moderate weight of evidence).
  - Second-hand exposure of bystanders to glycerol, propylene glycol or aldehydes is negligible or orders of magnitude lower than for electronic cigarette users (moderate weight of evidence).

- The overall weight of evidence for risk of systemic cardiovascular effects in second-hand exposed persons due to exposure to nicotine is weak to moderate. The lines of evidence are the following:
  - Moderate weight of evidence for second-hand exposure.
  - Heart rate and blood pressure effects were identified as hazards for nicotine (strong weight of evidence).
  - In the model calculations, the worst case MoEs for cardiovascular effects are low (moderate weight of evidence).
  - There exists a complete paucity of human evidence regarding the acute and long-term effects on cardiovascular and other health outcomes in children and adolescents (weak weight of evidence).

- The overall weight of evidence for a carcinogenic risk due to cumulative exposure to TSNAs and to substances on particulate matter is weak to moderate. The lines of evidence are the following:
  - Moderate weight of evidence for second-hand exposure.
  - Nitrosamines have been identified as genotoxic and carcinogenic (strong weight of evidence).
  - The MoEs calculated for the carcinogenic risk from TSNAs are low (moderate weight of evidence). If TSNA is present in the e-liquids. it is assumed that this risk will increase due to cumulative second-hand exposure to these chemicals.
  - The excess lifetime carcinogenic risk of substances on particulate matter in second-hand aerosol from electronic cigarettes was found to be below 10^{-7} (moderate weight of evidence).Human evidence is lacking (weak weight of evidence).

Further research is needed on whether children and adolescents are at greater risk than adults of being adversely affected by regular second-hand exposure to electronic cigarettes within their home environments.

6.6 Role in the initiation of smoking (particularly focusing on young people)

In this section, electronic cigarette awareness, initiation, perception and reasons for use will be discussed, with a focus on adolescents as a vulnerable group. In total, seven reviews were found in the period 2016-2019 that covered this topic. It needs to be noted that most of the included studies were carried out in the US. The SCHEER is aware, that US data do not necessarily reflect the exact situation in the EU, but trends coming from the US frequently also impact European markets. For the EU, information from the Eurobarometer was considered and comparison to the US was provided as far as possible.

Electronic cigarettes are rapidly becoming a new trend among adolescents and the number of users increased from 7.2% in 2012, to 11.6% in 2014 to 14.6% in 2017 in the EU. According to the 2020 Eurobarometer, 14% of the respondents have at least tried electronic cigarettes and 2% use them regularly. Indeed, 25% of young people (aged 15-24) have at least tried e-cigarettes, compared with 8% of the oldest respondents (aged 55 or over). Notably, among the 15-24-year-olds who were ever users of electronic cigarettes, 16.9% transitioned to regular users, however, the rate of transition between experimentation and regular use was higher in other age groups.

A recent review on the prevalence of electronic cigarette use among the general adult in Europe concluded that the prevalence of current electronic cigarette use ranged from 0.2% to 27%, ever-use ranged from 5.5% to 56.6% and daily use ranged from 1% to 2.9%. It also showed a higher prevalence of electronic cigarette use among males, adolescents and young adults, smokers of conventional cigarettes, and former smokers (Kapan, *et al.,* 2020).

A 2019 review describes the motivations for electronic cigarette use amongst young adults aged 18-25 and compares the reasons for using electronic cigarette of people who currently or formerly used tobacco products to those who had never smoked tobacco prior electronic cigarette use (Kinouani, *et al.,* 2020). Independently of smoking status, curiosity was the most frequently reported reason for initiating the use of electronic cigarettes in young adults. Reasons for continuing to use electronic cigarettes were various. The continued use of electronic cigarettes could be either a means to replicate smoking habits, or a way for a different and personalized use of nicotine by inhalation. Overall, reasons for using electronic cigarettes in young adults are varied and are not limited to stopping smoking.

Similar conclusions can be drawn from a 2018 review of reasons for electronic cigarette use as reported by electronic cigarette users, cigarette smokers, dual users, and non-users, among both adults and youth. Adults' perceptions and reasons for electronic cigarette use are often related to smoking cessation, while youth like the novelty of the product (Romijnders, *et al.,* 2018). Young non-users perceived the electronic cigarette as a cool and fashionable product that mimics the smoking routine and is rather safe to use. In general, perceived benefits included avoidance of smoking restrictions, the product being cool and fashionable, having health benefits, lower costs compared to cigarettes, positive experiences (mimics smoking routine, enjoyable taste, throat hit, weight control, increases concentration), safety of use, smoking cessation or reduction purposes, social acceptability, and perceived benefits for second-hand exposed persons.[13]

[13] Expected benefits among one or more of the groups include the product having an enjoyable taste, being healthier than cigarettes, improving breathing, increasing concentration, satisfying nicotine need, availability of variety of flavours, and controlling weight. Experienced benefits among one or more of the groups include the possibility to avoid smoking restrictions by dual use of tobacco products and electronic cigarettes, curiosity and novelty, perceived health benefits (regained sense of smell and taste, improved breathing, decreased coughing, improved dental health, increased athletic performance, increased alertness, aid to concentration, reduces stress), product appeal, also as compared to cigarettes (pleasure of product use, taste of flavours, throat hit, convenience of product, possibility to alter technical specifications, lower costs compared to cigarettes, easily accessible, discrete in use (no lingering smell, able to hide use), practical in use (no lighter, no ashtray, one puff, and able to

In the EU, among the 2020 Eurobarometer respondents, 4% of 15-24-year olds were current electronic cigarette users, 3% reported to have used to use it but stopped and 18% of 15-24 year olds had tried it at least once or twice. Among all respondents, the most frequently mentioned reason (54%) for taking up electronic cigarettes was to stop or reduce tobacco consumption. Other reasons included electronic cigarettes being perceived as less harmful (34%), lower cost (23%), liking the flavours (18%), and the ability to use them in places where smoking is not allowed (17%). When comparing these results with those of the previous survey in 2017, the most notable changes are a decrease in the proportion of users saying they started using e-cigarettes to stop or reduce tobacco smoking (-4 percentage points) and significant increases in the shares of those who mention that they liked the flavours of e-cigarettes (+8 pp), that they believed that vaping was less harmful than using tobacco (+6 pp) and that their friends used e-cigarettes (+5 pp).

The youngest respondents are the least likely to say that they started using e-cigarettes to stop or reduce their tobacco consumption (33% compared with 58-64% among other age groups), but the most likely to mention that they believed that vaping was less harmful than using tobacco (45% compared with 34-37%), that they liked the flavours of e-cigarettes (36% compared with 11-24%), that their friends used e-cigarettes (35% compared with 9-16%) or that e-cigarettes were cool or attractive (13% compared with 5-8%).

Regarding product type, pod devices have especially become a more socially acceptable alternative to combustible cigarettes among adolescents and young adults. and have become popular among this age group as a result of (1) sleek designs. (2) user-friendly functions. (3) less aversive smoking experiences. (4) desirable flavours and (5) the ability to be used discreetly in places where smoking is forbidden (Fadus *et al.,*2019). One of these products is currently the most popular retail electronic cigarette brand in the USA accounting for 76% of the retail electronic cigarette market at the end of 2018 (Fadus *et al.,* 2019). It would be interesting to collect such data from the EU as well. Unlike the US, where there is no upper limit on nicotine levels in e-liquids, the EU TPD prescribes that nicotine levels in e-liquids should not exceed 20 mg/ml. It is important to note that the upper limit of 20 mg/ml nicotine can be compensated for by technological modifications in the device. yielding similar nicotine emissions levels as the American version that used high nicotine levels in the liquid (see below in the section on nicotine) (Mallock *et al.*, 2020).

Regarding flavours, a 2019 review found consistent evidence that flavours attract both youth and adults to use electronic cigarettes (Meernik *et al.,* 2019). Flavours decrease harm perceptions and increase willingness to try and initiate use of electronic cigarettes. Among adults, electronic cigarette flavours increase product appeal and are a primary reason for many adults to use the product. In the sections below, specific flavour, preferences are discussed.

Addictiveness and attractiveness related to ingredients

In this section, data from eight reviews that covered electronic cigarette flavours and/or nicotine, from the period 2016-2019 will be discussed.

Flavours

E-liquids are available in many flavours not found in traditional tobacco products, a commonly-cited reason for electronic cigarette use (reviewed in Goldenson *et al.,* 2019).

store the device)), smoking cessation purposes (alternative for smoking cigarettes, avoidance of withdrawal of nicotine, cut back cigarettes, use as smoking cessation aid, deal with cravings. Finally, the social environment is important (fitting in, pressure of social environment, recommended by friends or family, role models use e-cigarettes).

Most e-liquid brands are available in a variety of youth-appealing flavours, ranging from fruits, desserts, candy, and soda to traditional tobacco (reviewed in Walley *et al.,* 2019). The number of available e-liquid flavours exceeded 7500 in 2014 and is still increasing (in Krüsemann *et al.,* 2019). Forty-three main flavour categories have been found in literature, eg, tobacco, menthol, mint, fruit, bakery/dessert, alcohol, nuts, spice, candy, coffee/tea, beverages, chocolate, sweet flavours, vanilla, and unflavoured (Krüsemann *et al.,* 2019).

A review on flavour preferences showed that sweet preference in children and adolescents was higher than in adults (Hoffman *et al.,* 2016). Examples of preferred food-related tastes and odours for young people included cherry, candy, strawberry, orange, apple and cinnamon (Hoffman *et al.,* 2016). All of these flavours are used for e-liquids (Hoffman *et al.,* 2016). Tobacco products in flavours preferred by young people may impact tobacco use and initiation, while flavours preferred by adults may impact product switching or dual use (Hoffman *et al.,* 2016).

Flavoured electronic cigarettes are used at electronic cigarette initiation by the majority of youth (Goldenson *et al.,* 2019). These flavours enhance the appeal of electronic cigarettes by creating sensory perceptions of sweetness and coolness and masking the aversive taste of nicotine (Goldenson *et al.,* 2019). Use of flavoured electronic cigarettes is higher among youth and young adults (vs. older adults) and among non-smokers (vs. combustible cigarette smokers) (Goldenson *et al.,* 2019). Overall, consumers preferred flavoured electronic cigarettes, and such preference varied with age groups and smoking status (Zare *et al.,* 2018).

Adolescents consider flavour the most important electronic cigarette attribute in trying electronic cigarettes and were more likely to initiate using through flavoured electronic cigarettes (reviewed in Zare *et al.,* 2018). Young adults overall preferred sweet, menthol, and cherry flavours, while non-smokers in particular preferred coffee and menthol flavours (Zare *et al.,* 2018). Adults in general also preferred sweet flavours (though smokers like tobacco flavour the most) and disliked flavours that elicit bitterness or harshness (Zare *et al.,* 2018).

The above-mentioned pod device with a large US-market share is a brand of electronic cigarette that has recently received significant media attention because of its rapid uptake by adolescents (Walley *et al.,* 2019). The appealing flavourings available (e.g., mango, fruit medley, menthol) can mask unwanted tastes and smells, and are often cited as a reason for experimentation among young users (reviewed in Fadus *et al.,* 2019).

Several flavours (candy and fruit flavours) were associated with decreased harm perception, while tobacco flavour was associated with increased harm perception (Zare *et al.,* 2018) among adult and youth electronic cigarette users, adult and youth cigarette smokers, and non-users (reviewed in Romijnders *et al.,* 2018). If non-users were not to perceive fruit- and candy-flavoured e-liquids as harmless, they might be less inclined to initiate electronic cigarette use (Romijnders *et al.,* 2018). Moreover, manufacturing labels are not always comprehensive with regard to e-liquid constituents and therefore might not alert the consumer to the potential for harmful effects (Sood *et al.,* 2018).

Overall, thousands of e-liquid flavours are available in tobacco and other flavours. Flavours are an important part of e-liquid appeal, and most consumers prefer flavoured e-liquids. Non-tobacco, sweet flavours are preferred by youth and non-smokers, and non-tobacco flavours are associated with decreased risk perception of electronic cigarettes. In the current EU-TPD, the use of all flavours is allowed, as long as they "do not pose a risk to human health in heated or unheated form" (TPD Article 20.3). The responsibility for adopting rules on flavours remains with the Member States. Currently, unlike tobacco and roll-your-own tobacco, where products with a strong smell or taste other than tobacco are banned because of their attractiveness for young people, there are currently no provisions regarding the attractiveness of electronic cigarette taste and smell. In the EU, according to

the 2020 Eurobarometer, a relative majority are in favour of banning flavours in electronic cigarettes (47% in favour vs. 35% against). The share of respondents in favour of banning flavours in e-cigarettes has increased by seven percentage points since this question was last asked in the 2017 Eurobarometer. Interestingly, the older the respondents, the more likely they are to be in favour of banning flavours in e-cigarettes (41% of those aged 15-24, compared with 49% of those aged 55 or more) maybe because these groups are interested in using flavoured electronic cigarettes. Another option might be the regulate flavours that are specifically attractive to young people.

The 2020 Eurobarometer reports that the most popular flavour of electronic cigarette is fruit flavour (48%), followed by tobacco flavour (36%), menthol or mint (30%) and chocolate or candy flavour (20%). Alcohol-flavoured electronic cigarettes are the least popular, favoured by only 4% of respondents. The older the e-cigarette users, the more likely they were to prefer tobacco-flavoured e-cigarettes: 56% of those aged 55 or more give this answer, compared with 22% of those aged between 15 and 24. The reverse is true for fruit-flavoured e-cigarettes: three quarters of those aged 15-24 mention this flavour, compared with 18% of the oldest cohort. The youngest users are also the most likely to mention menthol or mint flavour (46%, compared with 25-27% among older users) and candy flavours (30%, compared with 10-23%).

The older the electronic cigarette users, the more likely they are to prefer tobacco-flavoured e-cigarettes: 56% of those aged 55 or more give this answer, compared with 22% of those aged between 15 and 24. The reverse is true for fruit-flavoured e-cigarettes: three quarters of those aged 15-24 mention this flavour, compared with 18% of the oldest cohort. The youngest users are also the most likely to mention menthol or mint flavour (46%, compared with 25-27% among older users) and candy flavours (30%, compared with 10-23%).

According to the EHN, the fact that people, and particularly young people, who have never smoked are increasingly taking up electronic cigarette use deserves much attention as they are at substantial risk of becoming regular cigarette smokers. Moreover, it was recommended (1) that flavours should be prohibited, mainly because they are likely to attract children and young people (2) the same regulations as for conventional cigarettes should be set for electronic cigarettes (i.e., regarding marketing, advertising, labelling and packaging, buying restrictions, age limits and the use of electronic cigarettes in public places, which should be prohibited).

Nicotine

Nicotine-containing e-liquids have a stimulating effect on the reward system within the brain, which is implicated in the development of addiction (in Krüsemann *et al.,* 2018)). Whereas flavours are added to increase product liking, addictive substances such as nicotine play a role in motivation and influence the reward system through mechanisms of learning and wanting (in Krüsemann *et al.,* 2018). Specific to youth, nicotine addiction and dependence leading to lifelong tobacco use is a major concern when considering electronic cigarette use (Walley *et al.,* 2019). Nicotine addiction is an adaption to nicotine exposure over time, and thus the high concentrations of nicotine in electronic cigarettes are of major concern.

Consumer preference for nicotine strength and types depends on smoking status, electronic cigarette use history, and gender (Zare *et al.,* 2018). Non-smokers and inexperienced electronic cigarette users tended to prefer no nicotine or low nicotine electronic cigarettes while smokers and experienced electronic cigarette users preferred medium and high nicotine electronic cigarettes (Zare *et al.,* 2018). Weak evidence exists regarding a positive interaction between menthol flavour and nicotine strength (Zare *et al.,* 2018).

Typical nicotine absorption from a conventional cigarette is 1 mg (range 0.3–2 mg), with blood nicotine levels ranging from an average of 15 to 30 ng/mL (Walley, *et al.,* 2019). Studies of electronic cigarette use have revealed that, depending on duration of use and user puffing topography, serum levels of nicotine can be as high with electronic cigarette use as with use of a conventional cigarette (Walley *et al.,* 2019).

In one study, the urinary cotinine concentrations (a biomarker for nicotine exposure) among adolescents using the above-mentioned pod device with a large US market share was even higher than the urinary cotinine concentrations of those who smoked conventional cigarettes (Walley *et al.,* 2019). A recent study (2019) from Imperial Tobacco found that for electronic cigarettes with nicotine salts (lactate) the rate of nicotine absorption into the bloodstream was as rapid as that for conventional cigarette. The use of nicotine salts in electronic cigarettes enables cigarette-like pulmonary delivery of nicotine that reduces the desire to smoke (O'Connell *et al.,* 2019).

The above-mentioned pod device with a large market share is a brand of electronic cigarette that has recently received significant media attention because of its rapid uptake by adolescents. The popular pod device utilizes protonated nicotine, which the company claims to provide a more satisfying experience to the user by reducing aversive experiences of taste, smell, and throat irritation (Fadus *et al.,* 2019). In addition to PG and glycerol, the pod is advertised to contain benzoic acid (a naturally occurring acid found in the tobacco plant) and nicotine (Walley *et al.,* 2019). As of August 2018, it advertises pods with two nicotine concentrations of 5% (59 mg/mL) and 3% (35 mg/mL). Each pod is marketed as equivalent to ~1 pack of cigarettes (ie, 200 puffs).

As explained above, the EU TPD upper limit of 20 mg/ml does not mean that users will be exposed to lower levels of nicotine, as they can puff more intensely and adapt their device settings.

In conclusion, nicotine is an addictive substance and its levels range widely in e-liquids. Consumer preference for nicotine strength and types depends on smoking status, electronic cigarette use history, and gender. Serum levels of nicotine can be as high with electronic cigarette use as with use of a conventional cigarette. Traditional e-liquids use free-base nicotine. Use of nicotine salts reduces throat irritation and enables high peak levels of nicotine, similar to those of a tobacco cigarette. Note that according to the EU-TPD, the nicotine level in the liquid may not exceed 20 mg/ml (TPD Article 20.3). Additionally, liquids that do not contain nicotine are not covered by the TPD. However, such liquids are still on the market; e-liquids without nicotine are regulated via other laws (although in some EU Member States, e-liquids without nicotine are regulated in the same way as nicotine-containing e-liquids, and covered by the Tobacco Law), and nicotine levels exceeding 20 mg/ml have also been signalled, even in physical shops. It is also interesting to note that a modified version of the popular pod device with a large market share is now available on the EU market, with technological adjustments to the wick (Mallock *et al.,* 2020). This product type compensates for the lower nicotine levels in the liquid, and the increased aerosolization results in nicotine delivery per puff approximately equal to the American original using high nicotine levels in the liquid. This suggests similar addictiveness potential of the enhanced European version and the original American product.

Role as a gateway product or renormalisation of traditional tobacco smoking

One of the four core purposes of this scientific Opinion is to assist the Commission in assessing the most recent scientific and technical information on electronic cigarettes with regard to their role as a gateway to smoking and with respect to the initiation of smoking particularly focusing on young people. Within this context, there are two hypotheses that need to be tested, the *gateway hypothesis* (in which the use of electronic cigarettes lead never-tobacco users to begin using other tobacco products) (Bunnell *et al.,* 2014; Kandel

and Kandel, 2014) and the *renormalisation hypothesis* (in which the public acceptance of electronic cigarette use may lead to a renomalisation of tobacco use (Fairchild *et al.,* 2014)). Indeed, with adult and adolescent smoking rates decreasing due to tobacco control efforts, there remains concern if the expansion of electronic cigarettes may hinder tobacco control efforts and impact smoking rates as adolescents and young adults who were likely to never use any form of nicotine products start experimenting with electronic cigarettes and other forms of nicotine delivery.

Experimentation with tobacco products among non-tobacco using youth that experiment with electronic cigarettes (gateway)

To be able to attribute causality between an exposure and an outcome, a causal study design is necessary. One such study design that could potentially shed light on the potential impact of electronic cigarette experimentation on subsequent tobacco use is a prospective cohort study design. To this extent, a recent systematic review and meta-analysis of cohort studies that assessed initial use of electronic cigarettes and subsequent cigarette smoking has been published and included nine individual cohort studies among youth – all of which are based in the US (Soneji *et al.*, 2017). This meta-analysis included 17 389 adolescents and young adults, aged between 14 and 30 years at baseline, and 56.0% were female. The pooled probabilities of cigarette smoking initiation were 30.4% for baseline ever electronic cigarette users and 7.9% for baseline never-electronic cigarette users. The pooled probabilities of past 30-day cigarette smoking at follow-up were 21.5% for baseline past 30-day electronic cigarette users and 4.6% for baseline non-past 30-day electronic cigarette users. Adjusting for known demographic, psychosocial, and behavioural risk factors for cigarette smoking, the pooled odds ratio for subsequent cigarette smoking initiation was 3.62 (95% CI, 2.42-5.41) for ever vs never-electronic cigarette users, and the pooled odds ratio for past 30-day cigarette smoking at follow-up was 4.28 (95% CI, 2.52-7.27) for past 30-day electronic cigarette vs non-past 30-day electronic cigarette users at baseline. It is important to note that a moderate level of heterogeneity was identified, as the studies followed differed in their survey methods, sample sizes, age groups and follow up. It is important to note, however, that the exposures and outcome in all cases were clearly defined. An earlier systematic review (Chatterjee *et al.,* 2016) also found similar results using data from four longitudinal studies that were subsequently also included in the meta analysis of Soneji *et al.* (2017).

Additional evidence was assessed through a systematic review by Glasser *et al.*, covering 26 heterogenous studies of longitudinal design that included both adolescents and young adults and assessed electronic cigarette use at baseline and cigarette smoking at follow-up. Results suggest that, among never-smokers, electronic cigarette use is associated with future (6 months to 2.5 years) cigarette experimentation; findings which may be limited by small sample size, measurement of experimental use and potentially confounding variables (Glasser *et al.*, 2019). In this systematic review, three studies were located within European Member States (2 in the UK, one in NL). One in Scotland noted that ever-electronic cigarette users at baseline had a higher odds compared to never-electronic cigarette users of transitioning to cigarette smoking one year later in adjusted analyses (aOR = 6.64, 95%C.I = 3.60-12.26) (Best *et al.,* 2017). The other in England noted that ever smoking a cigarette at follow up was predicted by baseline ever use of electronic cigarettes (aOR 4.06, 95% C.I: 2.94-5.60) (Conner *et al.*, 2017). Similarly, although not included in the above systematic review, East *et al.* (2018), identified that the odds of smoking initiation in ever users of electronic cigarettes were (OR=12.31, 95% CI: 5.06–29.94) (Adjusted OR=10.57, 95% CI: 3.33–33.50).

A systematic review and meta-analysis of studies in the UK by Aladeokin *et al.* (2019), which included eight studies (involving 73076 adolescents), from the UK, of which the above three were included in the meta-analysis and identified that the odds of smoking initiation for non-smoking adolescents who used electronic cigarettes was 3.86

(95%C.I:2.18-6.82). The only other EU study identified by the above review was in the Netherlands. Within this cohort study adolescents who ever used an electronic cigarette with nicotine at baseline were at 11.90 higher odds of having smoked a conventional cigarette 6 months later than those who never used an electronic cigarette with nicotine (95% CI 3.36–42.11) -albeit with the limitation of a small sample size as indicated by wide confidence intervals (Treur *et al.*, 2018).

Other systematic reviews and meta-analyses of population studies have also assessed the role of electronic cigarette experimentation on subsequent tobacco use but are either compiled of only studies of cross sectional design (which can infer associations but not causal associations) or studies that predominantly are of cross sectional design. Zhong *et al.*performed a systematic review and meta-analysis of six studies with 91,051 participants, including 1452 with ever electronic cigarettes use, and identified that never-smoking adolescents and young adults, who used electronic cigarettes, have more than 2 times increased odds of intention to cigarette smoking (OR = 2.21, 95% CI: 1.86-2.61), compared to those who never used, with low evidence of between-study heterogeneity (p = 0.28, I^2 = 20.1%). Among never-smoking adolescents and young adults, electronic cigarettes use was associated with increased smoking intention (Zhong *et al.*, 2016).

On the antipode, however a number of studies indicate that exposure to electronic cigarette use may not be directly related to smoking uptake among youth. A time trend analyses on national representative data on electronic cigarette and tobacco use in the US by Levy *et al.* (2019) noted a decline in past 30-day smoking prevalence between 2014-2017, which coincides with the timeframe of electronic cigarette proliferation in the US, however the authors noted that while there has been a decrease in smoking rates during the past years in the US, this could also be attributable to the influence of other tobacco control interventions. Another review of studies -a tobacco industry manuscript- of the gateway effect examining how extensively studies (n=15) accounted for confounders associated with smoking initiation in youths noted that the reported studies may not have addressed for all confounders of smoking initiation (Lee *et al.,* 2018b).

Notably the studies used in the above meta-analyses and reviews are predominantly from the US and other non European Union countries, many of which have a very different regulatory environment, different population perspectives of electronic cigarettes and substantially different prevalence of both tobacco and electronic cigarette use, all of which combined or individually may impact substantially the direction and the slope of the association between experimentation with electronic cigarettes and subsequent use of other tobacco products. Even among those studies performed in Europe, the majority are from the UK. However, it has to be noted, that the UK has taken some policy approaches different to the rest of the EU.

The 2018 US National Academies of Science, Engineering and Medicine (NASEM) report concluded that there is "*strong evidence of plausibility and specificity of a possible causal effect of electronic cigarette use on smoking*". However, it is important to note that the current literature covers a period during which electronic cigarette products on the market did not contain nicotine salts and before the prolific expansion of such products in the US: this can impact the oucome of future studies. Research performed in the US indicate that such products may significantly contribute to overall nicotine product use among youth (Vallone *et al.*, 2019).

Experimentation with electronic cigarettes among non-smoking adults and youth in the EU

There is limited national or regional evidence using population based cross-sectional or cohort studies, with the Eurobarometer being one of the key, albeit cross-sectional, datasets available. Evidence in these datasets indicate an increase in the prevalence of electronic cigarette use and transition from experimentation to regular use, however the

Eurobarometer surveys by design cannot attribute causality nor have they assessed transitions from electronic cigarette use to tobacco product use. Furthermore, the Eurobarometer and other such population-based studies may not always adjust for potential confounding factors or common underlining risk factors.

A recent systmematic review and meta-analysis was identified and included fifteen cohort studies (Zhang *et al*., 2021). The pooled results suggested that ever e-cigarette users were more likely to initiate smoking than non-e-cigarette users (AOR=2.91; 95% CI: 2.61–3.23; I2 =61.0%; 15 trials, n=68943), however publication bias was noted. An additional meta-analysis of 11 studies showed a similar significant longitudinal association between vaping and smoking [aOR) = 2.93, 95% confidence interval (CI) = 2.22, 3.87], with the authors also noting potential issues of publication bias and issues of potential confounding (Chan *et al.*, 2020).

Previous secondary data set analyses using the 2012, 2014 and 2017 Eurobarometer datasets had indicated that ever use of an electronic cigarette in the EU Member States increased from 7.2% (95% CI 6.7 - 7.7) in 2012, to 11.6% (95% CI 10.9 - 12.3) in 2014 to 14.6% (95% CI 13.9–15.3) in 2017. Across the whole of the EU 1.8% of the adult population (95% CI 1.5 to 2.1) were current regular electronic cigarette users in 2017, compared with 1.5% (1.2–1.8) in 2014 (Filippidis *et al.*, 2017; Laverty *et al.*, 2018). In 2014, across the EU MS having ever used electronic cigarettes was 5.75 times more likely among 18-24-year olds compared to those >55 years of age, with aORs found to decrease with the increase in the respondents age after controlling for potential confounding factors. Among those who had ever used electronic cigarettes, participants aged 15–24 years were less likely to be regular user than those aged ≥55 years (16.9% vs. 38.1%). After adjusting for age and smoking status both ever use (OR = 1.46, 1.37 to 1.55) and current regular use of electronic cigarettes were more common in 2017 than 2014 (OR = 1.32, 1.11 to 1.55).

In 2017, it is important to note that 25% of 15-24-year olds had reported ever trying electronic cigarettes, a substantially higher rate than experimentation in other age categories. This difference in experimentation was 8.23 times higher in the 15-24-year old group when compared to those 55 and older, but was also substantially higher than reported ever use among other age groups (p for trend across age groups < 0.001). Notably, among the 15-24-year olds who were ever-users of electronic cigarettes, 16.9% transitioned to regular users, however, the rate of transition between experimentation and regular use was higher in other age groups. (Laverty *et al.*, 2018). Data from the Eurobarometer report also show that the younger the respondents, the more likely they are to have at least tried e-cigarettes. For instance, 25% of young people (aged 15-24) have at least tried e-cigarettes, compared with 8% of the oldest respondents (aged 55 or over). Moreover, the youngest (aged 15-24) among those who have never used e-cigarettes or have only tried them once or twice are slightly more likely to find them appealing compared with the oldest among these respondents (11% compared with 5%). Logistic regression analyses of the 2020 Eurobarometer data were not available at the time of writing of the report, nor was information on the type of device being used (older vs. newer pod-type generation electronic cigarettes).

Denormalization of cigarette smoking is a successful strategy to reduce cigarette smoking as smokers who perceived societal disapproval of smoking are more likely to intend to quit smoking, and subsequently quit smoking (Hammond, 2006). Thus, renormalization of cigarette smoking could lead to a resurgence of cigarette smoking (Choi, 2017). To this extent, there is a possibility that the use of design, manufacture, or marketing strategies that are implemented for electronic cigarettes and are prohibited or extensively regulated for cigarettes, such as flavours, advertising strategies, and packaging, may be used to attract the youth market to electronic cigarettes. Using data from the 2014 Eurobarometer for tobacco survey across the EU MS, among ever-dual product users (ever-cigarette and ever-electronic cigarette users), respondents who identified price; packaging; flavour;

brand; amount of nicotine; or design as important factors for the choice of cigarettes were more likely to identify the same factor as important for their choice of electronic cigarettes. Indeed those aged 15–24 were more likely than older respondents to cite external packaging [adjusted prevalence ratio (aPR = 2.06, 95% CI 1.00–4.23)] and design features (aPR = 1.99, 1.20–3.29) as important reasons for their choice of electronic cigarettes, (Laverty *et al.*, 2016).

At the EU Member State level, a cross-sectional survey of 6902 German students recruited in six German states noted that in that population, 38.8% of the students were exposed to electronic cigarette advertisements; ever use of electronic cigarettes was 21.7% and of combustible cigarettes was 21.8% (Hansen *et al.*, 2018), through which the authors noted that exposure to electronic cigarette marketing actions might increase the susceptibility to use of tobacco products directly, due to similarity in product shape and marketing themes for combustible cigarette and electronic cigarette products.

Overall, the SCHEER is of the opinion that there is moderate evidence that electronic cigarettes are a gateway to smoking/for young people. There is also strong evidence that nicotine in e-liquids is implicated in the development of addiction and that flavours have a relevant contribution for attractiveness of use of electronic cigarette and initiation.

6.7 Role of electronic cigarettes in the cessation of traditional tobacco smoking and dual use

Smoking cessation has additionally been recognised as an essential component of the WHO's MPOWER package for tobacco control and the WHO Framework Convention for Tobacco Control (FCTC) (WHO, 2008). WHO has selected a 30% reduction in tobacco use as one of 25 goals to be achieved by 2025, and the WHO Regional Office for Europe has professed their ultimate goal as having a European region free of tobacco use (WHO, 2015).

Due to the large health benefits of smoking cessation for both the individual and public health overall, it is essential to implement strategies to assist smokers in quitting. Using the Eurobarometer datasets, research has indicated that in the EU and among current and former smokers, those who had ever attempted to quit without assistance increased from 70.3% in 2012 to 74.8% in 2017 to 76% in 2020. During this timeframe, ever use of electronic cigarettes for smoking cessation increased (3.7% to 9.7% to 11%), The use of pharmacotherapy (14.6% to 11.1% to 13%) and smoking cessation services (7.5% to 5.0% to 6%) did not show clear trends (Eurobarometer, 2020) (Filippidis *et al.*, 2019). Notably, the differences in cessation methods across European Member States were associated with the existence of comprehensive national smoking cessation policies. Recent data on quitting activity, including attempts to quit and intention to quit, and use of cessation assistance among a cohort of smokers from eight European countries, indicated that ever use of an electronic cigarette as a smoking cessation device in the last quit attempt differed substantially across different European Member States, ranging from 5% in Spain to 51.6% in England – highlighting the differences across the EU (Hummel *et al.*, 2018).

In light of the above population experimentation with electronic cigarettes, it is important to assess through reviews of existing evidence, cohort studies and randomised control trials to assess the weight of evidence available. To this extent, a previous Cochrane Review (Hartmann-Boyce, 2016) included 24 studies (three RCTs, two of which were eligible for meta-analysis, and 21 cohort studies)- up to 2015, in which the authors noted that there is evidence from two trials that electronic cigarettes help smokers to stop smoking in the long term compared with placebo electronic cigarettes. However, the small number of trials, low event rates and wide confidence intervals around the estimates mean that our confidence in the result is rated 'low' by GRADE standards. Malas *et al.* (2016) identified 62 relevant references appraised in accordance with the GRADE system, in which the quality of the

evidence in support of electronic cigarettes' effectiveness in helping smokers quit was assessed as very low to low, and the evidence on smoking reduction was assessed as very weak to moderate.

In 2019, a new RCT was published (Hajek *et al.*, 2019). In this study, motivated smokers attempting to quit and who were not current users of either product were randomised to either electronic cigarettes or nicotine replacement therapy (NRT) for 52 weeks (n=886). At 1 year, the abstinence rate was 17.7% in the electronic cigarette group and 8% in the NRT group. Notably, participants who did not achieve abstinence and used electronic cigarettes showed a significant reduction in their exhaled carbon monoxide, suggesting decreased tobacco consumption. The study concluded that use of electronic cigarettes was more effective than use of NRT for smoking cessation in the trial when both were accompanied by behavioural support.

In 2019 another RCT was published (conducted in 2016–2017 in New Zealand), comparing electronic cigarettes with and without nicotine as an adjunct to NRT in the form of a nicotine patch (Walker *et al.*, 2020). The study randomized smokers motivated to quit. In this study, smokers using nicotine-containing electronic cigarettes were more likely to have biochemically verified, continuous cigarette abstinence at 6-month follow-up than those randomized to patch plus nicotine-free electronic cigarettes or to nicotine patch alone (7%, 4%, and 2%, respectively).

Taking the above RCTs into account and the information available through systematic reviews that have synthesized the observational literature on the impact of electronic cigarette use the most recent 2020 Surgeon general's report on Smoking Cessation (Surgeon General 2020) concluded that "*The evidence is inadequate to infer that e-cigarettes, in general, increase smoking cessation*". Moreover, the report also concluded that *"the evidence is suggestive but not sufficient to infer that the use of e-cigarettes containing nicotine is associated with increased smoking cessation compared with the use of e-cigarettes not containing nicotine, and the evidence is suggestive but not sufficient to infer that more frequent use of e-cigarettes is associated with increased smoking cessation compared with less frequent use of e-cigarettes."*

The above Cochrane review was updated in October 2020 (Hartman-Boyce, 2020) including 50 completed studies, representing 12,430 participants, of which 26 are RCTs. The authors identified four studies that compared e-cigarette use with NRT and identified that quit rates were higher in people randomized to nicotine EC than in those randomized to NRT (risk ratio (RR) 1.69: 1.25 to 2.27; I2 = 0%; 3 studies, 1498 participants). However, these results are limited by imprecision, and are based on only three studies, with the results primarily influenced (70.6%) by the sample size of the aforementioned Hajek *et al*. study with the newly added study that was limited to preoperative veterans, with a small sample size (Lee, 2018c) that contributed 2.2% of the weight.

In addition to the above, the 2020 Cochrane review identified that quit rates were higher in people randomized to nicotine EC than to placebo EC (RR 1.71, 95% CI 1.00 to 2.92; I2 = 0%; 3 studies, 802 participants. The change to the 2016 Cochrane review is via the addition of one more RCT (Lucchiari *et al*., 2020), which however has wide CI that overlap the null (1.18, 95%C.I: 0-57 – 2.46). We must note however that these studies may have included earlier forms of e-cigarettes and may not have represented the nicotine delivery capable of electronic cigarettes now on the market in the EU.

A very recent systematic review and meta-analysis, assessing more recent data published between 2015-2020, suggested that electronic cigarettes may be superior to NRT or placebo on smoking cessation (RR=1.55; 95% CI: 1.00–2.40;I2=57.6%; low certainty; 5 trials, n=4025). This meta-analysis also included data on nine cohort studies for which the pooled results suggested that e-cigarettes were not associated with smoking cessation (AOR=1.16; 95% CI: 0.88–1.54; I2=69.0%; 9 trials, n=22220). Subgroup analysis on the

frequency of e-cigarette use suggested that intensive electronic cigarette use was more effective in achieving cessation than non-use (AOR= 2.03; 95% CI: 1.35–3.05; I2=37.8%; 4 trials, n=1144) (Zhang *et al.,* 2021).

There is a lack of robust longitudinal data on the effect of electronic cigarettes on smoking cessation. Until such research is available, electronic cigarettes should only be considered to support smoking cessation for a limited time and under supervision. Furthermore, it should also be noted that the conclusions of some of the RCTs and reviews are only based on quitting at six months and do not take into account what proportion of smokers may relapse into smoking or dual use of e-cigarettes and cigarettes after the initial six months. However, as the majority of RCT assessed within the literature possibly referred to devices of earlier design, further research is needed to assess the impact of newer e-cigarette products on population based smoking cessation, using large population-based cohort data, with sufficient follow up time to assess potential relapse.

7. CONSIDERATION OF THE RESPONSES RECEIVED DURING THE PUBLIC CONSULTATION

A public consultation on this Opinion was open on the website of the Scientific Committees from 23 September to 26 October 2020.

Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

128 organisations and a number of individuals participated in the public consultation, providing input to different parts of the Opinion, resulting in 691 contributions collected in a table "Results of the public consultation on SCHEER's preliminary opinion SCHEER's preliminary Opinion on electronic cigarettes."

Frequently occurring comments were answered in a "Table of frequent comments" and included issues regarding the lack of comparison with tobacco smoking, the literature search and selection, the risk assessment methodology, the estimation of the risk of second-hand exposure, the delivery of nicotine by e-cigarettes, the lack of recent data on e-cigarette use, and the conclusions on the gateway effect, attractiveness and cessation.

In many cases, the Opinion was adapted based on these and other, less frequent, comments, and a selection of the additional literature was suggested. A major change in the conclusions was the change of the WoE for the gateway effect from 'strong' to 'moderate' and a change of the WoE for the risk of second-hand exposure from 'weak to moderate' to 'moderate'.

8. MINORITY OPINIONS

None.

9. REFERENCES

Abidin, N.Z., Abidin, E.Z., Zulkifli, A., Karuppiah, K., Ismael, S.N.S. and Nordin, A.S.A. (2017). Electronic cigarettes and indoor air quality: a review of studies using human volunteers. Reviews on Environmental Health. 32(3), 235–44. doi:10.1515/reveh-2016-0059.

Aladeokin, A., Haighton, C. (2019). Is adolescent e-cigarette use associated with smoking in the United Kingdom?: A systematic review with meta-analysis. Tobacco Prevention & Cessation. 5, 15. doi:10.18332/tpc/108553.

Amalia, B., Liu, X., Lugo, A., Fu, M., Odone, A., van den Brandt, P., Semple, S., Clancy, L., Soriano, J.B. Fernández, E., Gallus, S. and the TackSHS Project Investigators (2021). Exposure to secondhand aerosol of electronic cigarettes in indoor settings in 12 European countries: data from the TackSHS Survey. Tob Control 30(1), 49-56. http://dx.doi.org/10.1136/tobaccocontrol-2019-055376.

ANEC (2019). European Association for the Co-ordination of Consumer Representation in Standardisation. E-cigarettes and e-liquids - Limits for chemicals Basis for discussion. ANEC-PT-2019-CEG-005.

Antoniewicz, L., Bosson, J.A., Kuhl, J., Abdel-Halim, S.M., Kiessling, A., Mobarrez, F., Lundback, M. (2016). Electronic cigarettes increase endothelial progenitor cells in the blood of healthy volunteers. Atherosclerosis, 255, 179-185. doi: 10.1016/j.atherosclerosis.2016.09.064.

Archambeau, B.A., Young, S., Lee, C., Pennington, T., Vanderbeek, C., Miulli, D., Neeki, M. (2016). E-cigarette blast injury: complex facial fractures and pneumocephalus. Western Journal of emergency medicine, 17(6), 805. https://doi.org/10.5811/westjem.2016.7.31354.

Argacha, J. F., Bourdrel, T., Van De Borne, P. (2018). Ecology of the cardiovascular system: A focus on air-related environmental factors. Trends Cardiovasc Med, 28(2), 112–26. https://doi.org/10.1016/j.tcm.2017.07.013.

Arnaout, A., Dewi, F., Nguyen, D. (2017). Re: Burn injuries from exploding electronic cigarette batteries: An emerging public health hazard. Journal of Plastic, Reconstructive & Aesthetic Surgery, 70(7), 981–2. doi:10.1016/j.bjps.2017.02.021.

Ashford, K., Wiggins, A., Butler, K., Ickes, M., Rayens, M. K., Hahn, E. (2016). e-Cigarette Use and Perceived Harm Among Women of Childbearing Age Who Reported Tobacco Use During the Past Year. Nursing research, 65(5), 408–14. https://doi.org/10.1097/NNR.0000000000000176.

ATSDR (2004). Public Health Service, Agency for Toxic Substances and Disease Registry, Toxicological profile for Copper. https://www.atsdr.cdc.gov/toxprofiles/tp132.pdf

ATSDR (2005a). Public Health Service, Agency for Toxic Substances and Disease Registry, Toxicological profile for Zinc. https://www.atsdr.cdc.gov/ToxProfiles/tp60.pdf

ATSDR (2005b). Public Health Service, Agency for Toxic Substances and Disease Registry, Toxicological profile for Tin and Tin compounds. https://www.atsdr.cdc.gov/ToxProfiles/tp55.pdf

ATSDR (2010). Public Health Service, Agency for Toxic Substances and Disease Registry, Adendum to the toxicological profile for formaldehyde.

ATSDR (2012). Public Health Service, Agency for Toxic Substances and Disease Registry, Toxicological profile for Chromium. https://www.atsdr.cdc.gov/toxprofiles/tp7.pdf

Babic, M., Schuchardt, M., Tolle, M., Van Der Giet, M. (2019). In times of tobacco-free nicotine consumption: The influence of nicotine on vascular calcification. Eur J Clin Invest, 49(4), e13077. https://doi.org/10.1111/eci.13077.

Backinger, C.L. (2017). Youth use of electronic cigarettes. https://cdn.ymaws.com/www.srnt.org/resource/resmgr/conferences/2017_annual_meeting/SRNT_2017_Abstract_Book__Web.pdf

Bahl, V., Lin, S., Xu, N., Davis, B., Wang, Y. H. & Talbot, P. (2012). Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models. Reprod Toxicol, 34(4), 529–37.

Ballbe, M., Martinez-Sanchez, J.M., Sureda, X., Fu, M., Perez-Ortuno, R., Pascual, J.A., Salto, E., Fernandez, E. (2014). Cigarettes vs. e-cigarettes: Passive exposure at home measured by means of airborne marker and biomarkers. Environ Res, 135, 76-80. https://doi.org/10.1016/j.envres.2014.09.005.

Barnoya, J. and Glantz S.A. (2005). Cardiovascular effects of secondhand smoke: nearly as large as smoking. Circulation 111(20), 2684-2698. https://doi.org/10.1161/CIRCULATIONAHA.104.492215.

Bassett, R.A., Osterhoudt, K., Brabazon, T. (2014). Nicotine poisoning in an infant. The New England journal of medicine, 370(23), 2249–50. https://doi.org/10.1056/NEJMc1403843.

Beauval, N., Antherieu, S., Soyez, M., Gengler, N., Grova, N., Howsam, M., Hardy, E.M., Fischer, M., Appenzeller, B.M.R., Goossens, J.F., Allorge, D., Garçon, G., Lo-Guidice, J.M., Garat, A. (2017). Chemical evaluation of electronic cigarettes: multicomponent analysis of liquid refills and their corresponding aerosols. J Anal Toxicol 41, 670–8. https://doi.org/10.1093/jat/bkx054.

Behar, R.Z., Hua, M., Talbot, P. (2015). Puffing topography and nicotine intake of electronic cigarette users. PLoS One, 10(2), e0117222. doi:10.1371/journal.pone.0117222.

Behar, R.Z., Luo, W., McWhirter, K.J. *et al.* (2018). Analytical and toxicological evaluation of flavor chemicals in electronic cigarette refill fluids. Sci Rep 8, 8288. https://doi.org/10.1038/s41598-018-25575-6.

Bekki, K., Uchiyama, S., Ohta, K., Inaba, Y., Nakagome, H., and Kunugita, N. (2014). Carbonyl compounds generated from electronic cigarettes. International Journal of Environmental Research and Public Health 11, 11192–11200. doi: 10.3390/ijerph111111192.

Benowitz, N.L., & Burbank, A.D. (2016). Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. Trends in cardiovascular medicine, 26(6), 515–23. https://doi.org/10.1016/j.tcm.2016.03.001.

Bernhard, D., Rossmann, A., Wick, G. (2005). Metals in cigarette smoke. IUBMB life, 57(12), 805–9. https://doi.org/10.1080/15216540500459667

Bertholon, J.F., Becquemin, M.H., Annesi-Maesano, I. and Dautzenberg, B. (2013). Electronic Cigarettes: A Short Review. Respiration, 86 (5), 433–8. doi:10.1159/000353253.

Best, C., Haseen, F., Currie, D., Ozakinci, G., MacKintosh, A.M., Stead, M., Eadie, D., MacGregor, A., Pearce, J., Amos, A., Frank, J., Haw, S. (2017). Relationship between trying

an electronic cigarette and subsequent cigarette experimentation in Scottish adolescents: a cohort study. Tobacco control, 27(4), 373–8. Advance online publication. https://doi.org/10.1136/tobaccocontrol-2017-053691.

Bos, P.M.J., Soeteman-Hernández, L.G. and Talhout, R. (2021). Risk assessment of components in tobacco smoke and e-cigarette aerosols: a pragmatic choice of dose metrics.

Breland, A., Soule, E., Lopez, A., Ramôa, C., El-Hellani, A., Eissenberg, T. (2017). Electronic cigarettes: what are they and what do they do? Ann N Y Acad Sci. 1394(1), 5–30. doi: 10.1111/nyas.12977.

Brown, C.J., Cheng, J.M. (2014). Electronic cigarettes: product characterization and design considerations. Tob Control 23(Suppl 2), ii4–ii10. doi: 10.1136/tobaccocontrol-2013-051476.

Brown, J., West, R., Beard, E., Michie, S., Shahab, L., McNeill, A. (2014). Prevalence and characteristics of e-cigarette users in Great Britain: Findings from a general population survey of smokers. Addictive behaviors, 39(6), 1120–5. https://doi.org/10.1016/j.addbeh.2014.03.009.

Brownson, E.G., Thompson, C.M., Goldsberry, S., Chong, H.J., Friedrich, J.B., Pham, T. N., Gibran, N.S. (2016). Explosion Injuries from E-Cigarettes. New England Journal of Medicine, 375(14), 1400–2. doi:10.1056/nejmc1608478.

Bunnell, R.E., Agaku, I.T., Arrazola, R.A., Apelberg, B.J., Caraballo, R.S., Corey, C.C., Coleman, B.N., Dube, S.R., King, B.A. (2014). Intentions to smoke cigarettes among never-smoking US middle and high school electronic cigarette users: National Youth Tobacco Survey, 2011–2013. Nicotine Tob Res 17(2),228–235. doi:10.1093/ntr/ntu166.

Bush, D., Goniewicz, M.L. (2015). A pilot study on nicotine residues in houses of electronic cigarette users, tobacco smokers, and non-users of nicotine-containing products. The International journal on drug policy, 26(6), 609–11. https://doi.org/10.1016/j.drugpo.2015.03.003.

Cabot, R., Yurteri, C.Ü., Mcaughey, J. (2014). Droplet size measurement of e-cigarette aerosol, CORESTA Congress, Quebec, Smoke Science/Product Technology Groups, ST 40.

CEN (2018). Electronic cigarettes and e-liquids - Constituents to be measured in the aerosol of vaping products. European Committeee for Standardization, Brussels, Belgium. Technical Report, CEN/TR 17236:2018 E.

Chakma, J.K., Dhaliwal R.S., Mehrotra R. Indian Council of Medical Research (2019). White Paper on Electronic Nicotine Delivery System. The Indian journal of medical research, 149(5), 574–83. https://doi.org/10.4103/ijmr.IJMR_957_19.

Chan, G.C.K, Stjepanović, D., Lim, C., Sun, T., Shanmuga Anandan, A., Connor, J.P., Gartner, C., Hall, W.D., Leung, J. (2020). Gateway or common liability? A systematic review and meta-analysis of studies of adolescent e-cigarette use and future smoking initiation. Addiction. 2020 Sep 4. DOI: 10.1111/add.15246.

Chang, J.T. and Rostron, B.L. (2019). Electronic nicotine delivery system (ENDS) liquid nicotine exposure in young children presenting to US emergency departments, 2018. Injury epidemiology 6. England, 43. DOI: 10.1186/s40621-019-0219-6.

Chatham-Stephens, K., Law, R., Taylor, E., Melstrom, P., Bunnell, R., Wang, B., Apelberg, B., Schier, J.G. (2014). Notes from the field: calls to poison centers for exposures to

electronic cigarettes-United States, September 2010-February 2014. MMWR. Morbidity and mortality weekly report 63(13), 292–3. DOI: mm6313a4 [pii].

Chatterjee, K., Alzghoul, B. Innabi A. and Meena N. (2016). Is vaping a gateway to smoking: a review of the longitudinal studies. Int J Adolesc Med Health 30(3). DOI: 10.1515/ijamh-2016-0033.

Chen, I.L. (2013). FDA summary of adverse events on electronic cigarettes. Nicotine Tob Res 15(2), 615-6. doi: 10.1093/ntr/nts145.

Chen, I.L., Todd, I.,Fairclough, L.C. (2019). Immunological and pathological effects of electronic cigarettes. Basic Clin Pharmacol Toxicol, 125(3), 237-52. doi: 10.1111/bcpt.13225.

Cheng, T. (2014). Chemical evaluation of electronic cigarettes. Tob Control, 23 (Supplement 2), ii11–ii17. doi:10.1136/tobaccocontrol-2013-051482.

Choi, K., Grana, R., Bernat, D. (2017). Electronic Nicotine Delivery Systems and Acceptability of Adult Cigarette Smoking Among Florida Youth: Renormalization of Smoking? J Adolesc Health. 60(5), 592–8. doi:10.1016/j.jadohealth.2016.12.001.

Chun, L.F., Moazed, F., Calfee, C.S., Matthay, M.A., Gotts, J.E. (2017). Pulmonary toxicity of e-cigarettes. Am J Physiol Lung Cell Mol Physiol, 313(2), L193-L206. doi: 10.1152/ajplung.00071.2017.

Clapp, P.W. and Jaspers I. (2017). Electronic Cigarettes: Their Constituents and Potential Links to Asthma. Curr Allergy Asthma Rep 17(11), 79. doi: 10.1007/s11882-017-0747-5.

Commission Implementing Decision (EU) 2015/2183 of 24 November 2015 establishing a common format for the notification of electronic cigarettes and refill containers (notified under document C(2015) 8087) (n.d.). Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32015D2183&qid=1524052550933 (accessed 6 December 2017).

Commission Implementing Decision (EU) 2016/586 of 14 April 2016 on technical standards for the refill mechanism of electronic cigarettes (notified under document C(2016) 2093) (n.d.). Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=uriserv:OJ.L_.2016.101.01.0015.01.ENG (accessed 6 December 2017).

Conklin, D.J., Ogunwale, M.A., Chen, Y., Theis, W.S., Nantz, M.H., Fu, X.A., Chen, L.C., Riggs, D.W., Lorkiewicz, P., Bhatnagar, A., Srivastava, S. (2018). Electronic cigarette-generated aldehydes: The contribution of e-liquid components to their formation and the use of urinary aldehyde metabolites as biomarkers of exposure. Aerosol Sci Technol. 52(11), 1219–32. doi: 10.1080/02786826.2018.1500013.

Conner, M., Grogan, S., Simms-Ellis, R., Flett, K., Sykes-Muskett, B., Cowap, L., Lawton, R., Armitage, C.J., Meads, D., Torgerson, C., West, R., Siddiqi, K. (2017). Do electronic cigarettes increase cigarette smoking in UK adolescents? Evidence from a 12-month prospective study. Tobacco control, 27(4), 365–72. Advance online publication. https://doi.org/10.1136/tobaccocontrol-2016-053539.

Cooper, M., Harrell, M.B., Perry, C.L. (2016). Comparing young adults to older adults in e-cigarette perceptions and motivations for use: implications for health communication. Health Educ Res 31(4), 429– 38. doi: 10.1093/her/cyw030.

Das Bundesinstitut für Risikobewertung (BfR) (2019). Harmful compounds might be formed when foods containing the sweetener Sucralose are heated BfR opinion No 012/2019 of 9 April 2019 https://www.bfr.bund.de/cm/349/harmful-compounds-might-be-formed-when-foods-containing-the-sweetener-sucralose-are-heated.pdf

Dawkins, L., Cox, S., Goniewicz, M., McRobbie, H., Kimber, C., Doig, M., Kosmider, L. (2018). 'Real-world' compensatory behaviour with low nicotine concentration e-liquid: subjective effects and nicotine, acrolein and formaldehyde exposure. Addiction, 113(10), 1874-82. doi:10.1111/add.14271.

De Long, N.E., Barra, N.G., Hardy, D.B., Holloway, A.C. (2014). Is It Safe to use smoking cessation therapeutics during pregnancy? Expert Opin Drug Saf, 13, 1721-31.

DeVito, E.E., Krishnan-Sarin, S. (2018). E-cigarettes: Impact of E-Liquid Components and Device Characteristics on Nicotine Exposure. Curr Neuropharmacol, 16(4), 438-59. doi:10.2174/1570159x15666171016164430.

Díez-Izquierdo, A., Cassanello-Peñarroya, P., Lidón-Moyano, C., *et al.* (2018). Update on thirdhand smoke: A comprehensive systematic review. Environmental Research 167, 341–371. doi: 10.1016/j.envres.2018.07.020.

Dinakar, C. and O'Connor, G.T. (2016). The Health Effects of Electronic Cigarettes. The New England journal of medicine 375(14), 1372–81. doi: 10.1056/NEJMra1502466.

Drooge, B.L. van, Marco, E., Perez, N. and Grimalt, J.O. (2019). Influence of electronic cigarette vaping on the composition of indoor organic pollutants, particles, and exhaled breath of bystanders. Environmental Science and Pollution Research 26, 4654–66. doi.org/10.1007/s11356-018-3975-x.

Dunbar, A., Gotsis, W., Frishman, W. (2013). Second-hand tobacco smoke and cardiovascular disease risk: an epidemiological review. Cardiol Rev, 21, 94-100.

Dunbar, Z.R., Das, A., O'Connor, R.J., Goniewicz, M.J., Wei, B., Travers, M.J. (2018). Brief report: lead levels in selected electronic cigarettes from Canada and the United States. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph15010154.

East, K, Hitchman, S.C., Bakolis, I., Williams, S., Cheeseman, H., Arnott, D., McNeill, A. (2018). The Association between Smoking and Electronic Cigarette Use in a Cohort of Young People. J Adolesc Health. 62(5), 539-47. doi:10.1016/j.jadohealth.2017.11.301.

ECHA (2017). Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Evaluation of active substances. Assessment Report Formaldehyde.

ECHA (2019). Worker exposure to formaldehyde and formaldehyde releasers https://echa.europa.eu/documents/10162/13641/investigationreport_formaldehyde_workers-exposure_final_en.pdf/ac457a0c-378d-4eae-c602-c7cd59abc4c5.

EFSA (European Food Safety Authority) (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. (Request No EFSA-Q-2004-020), adopted on 18 October, 2005. The EFSA Journal, 282, 1-31.

EFSA (European Food Safety Authority) (2010). Scientific opinion on lead in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). The EFSA Journal, 8(4), 1570.

Eggleston, W., Nacca, N., Stork, C.M., Marraffa, J.M. (2016). Pediatric death after unintentional exposure to liquid nicotine for an electronic cigarette. Clinical Toxicology. 54(9), 1-2 doi: 10.1080/15563650.2016.1207081.

Erythropel, H.C., Jabba, S.V., DeWinter, T.M., Mendizabal, M., Anastas, P.T., Jordt, S.E., Zimmerman, J.B. (2019). Formation of flavorant-propylene Glycol Adducts With Novel Toxicological Properties in Chemically Unstable E-Cigarette Liquids. Nicotine Tobacco Research 21(9), 1248-58. doi:10.1093/ntr/nty192.

Etter, J.F., Bullen, C. (2011). Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. Addiction, 106(11), 2017-28. doi:10.1111/j.1360-0443.2011.03505.x.

European Commission, Report from the Commission to the European Parliament and the Council on the potential risks to public health associated with the use of refillable electronic cigarettes. COM/2016/0269 final.

European Parliament and the Council of the European Union (2014). Directive 2014/40/EU. Official Journal of the European Union 2014(April), 1–38.

Evans, S.E., Hoffman, A.C. (2014). Electronic cigarettes: abuse liability, topography and subjective effects. Tob Control, 23(2), ii23-29. doi:10.1136/tobaccocontrol-2013-051489.

Fadus, M.C., Smith, T.T., Squeglia, L.M. (2019). The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. Drug Alcohol Depend, 201, 85-93. doi:10.1016/j.drugalcdep.2019.04.011.

Fairchild, A.L., Bayer, R., Colgrove, J. (2014). The renormalization of smoking? E-cigarettes and the tobacco "endgame". N Engl J Med 370(4), 293–5. doi:10.1056/NEJMp1313940.

Famele, M., Ferranti, C., Abenavoli, C., Palleschi, L., Mancinelli, R. and Draisci, R. (2014). The Chemical Components of Electronic Cigarette Cartridges and Refill Fluids: Review of Analytical Methods. Nicotine and Tobacco Research, 17 (3), 271–9. doi:10.1093/ntr/ntu197.

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2013). Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: implications for research protocol standards definition and for public health authorities' regulation. Int J Environ Res Public Health, 10(6), 2500-14. doi:10.3390/ijerph10062500

Farsalinos, K. E., Spyrou, A., Tsimopoulou, K., Stefopoulos, C., Romagna, G., Voudris, V. (2014). Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. Sci Rep, 4, 4133.

Farsalinos, K.E., Kistler, K.A., Gillman, G. and Voudris, V. (2015a). Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Nicotine and Tobacco Research, 17, 168–74. doi:10.1093/ntr/ntu176.

Farsalinos, K.E., Gillman, G., Poulas, K. and Voudris, V. (2015b). Tobacco-specific nitrosamines in electronic cigarettes: comparison between liquid and aerosol levels. International Journal of Environmental Research and Public Health, 12, 9046-53. doi:10.3390/ijerph120809046.

Farsalinos, K. E., Voudris, V., Poulas, K. (2015c). E-cigarettes generate high levels of aldehydes only in "dry puff" conditions. Addiction 110: 1352–6. doi: 10.1111/add.12942.

Farsalinos, K.E., Voudris, V. and Poulas, K. (2015d). Are metals emitted from electronic cigarettes a reason for health concern? A risk-assessment analysis of currently available literature. Int J Environ Res Public Health, 12: pp. 5215-32. doi: 10.3390/ijerph120505215.

Farsalinos, K.E., Spyrou, A., Stefopoulos, C., Tsimopoulou, K., Kourkoveli, P., Tsiapras, D., Voudris, V. (2015e). Corrigendum: Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naive users (smokers). Sci Rep, 5, 13506. doi:10.1038/srep13506.

Farsalinos, K. E., Poulas, K., Voudris, V., Le Houezec, J. (2016). Electronic cigarette use in the European Union: analysis of a representative sample of 27 460 Europeans from 28 countries. Addiction 111(11), 2032-40. doi: 10.1111/add.13506.

Farsalinos, K.E. and Gillmann, G. (2018). Carbonyl emissions in E-cigarette aerosol: a systematic review and methodological considerations. Frontiers in Physiology, 8, 1119. doi: 10.3389/fphys.2017.01119.

Farsalinos, K.E. and Rodu, B. (2018). Metal emissions from e-cigarettes: a risk assessment analysis of a recently-published study. Inhalation Toxicology, 30, 321-6. doi: 10.1080/08958378.2018.1523262.

Farsalinos, K.E., Poulas, K., Voudris, V. (2018). Changes in Puffing Topography and Nicotine Consumption Depending on the Power Setting of Electronic Cigarettes. Nicotine Tob Res, 20(8), 993-997. doi:10.1093/ntr/ntx219.

Filippidis, F.T., Laverty, A.A., Gerovasili, V., Vardavas, C.I. (2017). Two-year trends and predictors of e-cigarette use in 27 European Union Member states. Tob Control. 26(1), 98–104. doi:10.1136/tobaccocontrol-2015-052771.

Filippidis, F.T., Laverty, A.A., Mons, U., Jimenez-Ruiz, C., Vardavas, C.I. (2019). Changes in smoking cessation assistance in the European Union between 2012 and 2017: pharmacotherapy versus counselling versus e-cigarettes. Tob Control. 28(1), 95–100. doi:10.1136/tobaccocontrol-2017-054117.

Flach, S., Maniam, P., Manickavasagam, J. (2019). E-cigarettes and head and neck cancers: A systematic review of the current literature. Clin Otolaryngol, 44, 749-56. doi: 10.1111/coa.13384.

Flora. J.W., Wilkinson. C.T., Sink. K.M., McKinney. D.L. and Miller. J.H. (2016). Nicotine-related impurities in e-cigarette cartridges and refill e-liquids." Journal of Liquid Chromatography & Related Technologies. 39 (17-18). 821–9. doi: 10.1080/10826076.2016.1266500.

Flouris, A.D., Chorti, M.S., Poulianiti, K.P., Jamurtas, A.Z., Kostikas, K., Tzatzarakis, M.N., Hayes W.A., Tsatsakis, A.M. and Koutedakis, Y. (2013). Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. Inhalation Toxicology, 25(2), 91-101.

Floyd, E.L., Queimado, L., Wang, J., Regens, J.L., Johnson, D.L. (2018). Electronic cigarette power affects count concentration and particle size distribution of vaping aerosol. PLoS ONE 13(12), e0210147. https://doi.org/10.1371/journal.pone.021014.

Foran, I., Oak, N.R., Meunier, M.J. (2017). High-Pressure Injection Injury Caused by Electronic Cigarette Explosion. JBJS Case Connector, 7(2), e36. doi:10.2106/jbjs.cc.16.00177.

Franzen, K.F., Willig, J., Cayo Talavera, S., Meusel, M., Sayk, F., Reppel, M., Dalhoff, K., Mortensen, K.,Droemann, D. (2018). E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: A randomized, double-blinded pilot study. Vasc Med, 23(5), 419-25. doi: 10.1177/1358863X18779694.

Fuoco, F., Buonanno, G., Stabile, L., Vigo, P. (2014). Influential parameters on particle concentration and size distribution in the mainstream of e-cigarettes. Environ. Pollut. 184, 523–9. doi: 10.1016/j.envpol.2013.10.01.

Geiss, O., Bianchi, I., Barrero-Moreno, J. (2016).Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapour. International Journal of Hygiene and Environmental Health, V. 219(3), 268-277 http://dx.doi.org/10.1016/j.ijheh.2016.01.004.

Gerloff, J.; Sundar, I.K., Freter, R., Sekera, E.R., Friedman, A.E., Robinson, R., Pagano, T., Rahman, I. (2017). Inflammatory Response and Barrier Dysfunction by Different e-Cigarette Flavoring Chemicals Identified by Gas Chromatography-Mass Spectrometry in e-Liquids and e-Vapors on Human Lung Epithelial Cells and Fibroblasts. Appl. In Vitro Toxicol. 3, 28–40. doi: 10.1089/aivt.2016.0030.

Gillman, I.G., Kistler, K.A., Stewart, E.W., Paolantonio, A.R. (2016). Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. Regulatory Toxicology and Pharmacology 75, 58–65. doi: 10.1016/j.yrtph.2015.12.019.

Girvalaki, C., Tzatzarakis, M., Kyriakos, C.N., Vardavas, A.I, Stivaktakis, P.D., Kavvalakis, M., Tsatsakis A., Vardavas C. (2018). Composition and chemical health hazards of the most common electronic cigarette liquids in nine European countries. Inhalation toxicology 30(9–10). England, 361–9. doi: 10.1080/08958378.2018.1527879.

Girvalaki, C., Vardavas, A., Tzatzarakis, M., Kyriakos, C.N., Nikitara, K., Tsatsakis, A.M., Vardavas, C.I. (2019). Compliance of e-cigarette refill liquids with regulations on labelling, packaging and technical design characteristics in nine European Member states. Tob Control. 29(5), 531-6. doi: 10.1136/tobaccocontrol-2019-055061.

Glasser, A.M., Collins, L., Pearson, J.L., Abudayyeh, H., Niaura, R.S., Abrams, D.B., Villanti A.C. (2017). Overview of Electronic Nicotine Delivery Systems: A Systematic Review. Am J Prev Med.52(2), e33-e66. doi: 10.1016/j.amepre.2016.10.036.

Glasser, A., Abudayyeh, H., Cantrell J. and Niaura R. (2019). Patterns of E-Cigarette Use Among Youth and Young Adults: Review of the Impact of E-Cigarettes on Cigarette Smoking. Nicotine Tob Res 21(10), 1320-30. doi: 10.1093/ntr/nty103.

Goldenson, N.I., Leventhal, A.M., Simpson, K.A., Barrington-Trimis, J.L. (2019). A Review of the Use and Appeal of Flavored Electronic Cigarettes. Curr Addict Rep, 6(2), 98-113. doi:10.1007/s40429-019-00244-4.

Goniewicz, M.L., Hajek, P., McRobbie, H. (2014a). Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. Addiction 109, 500–7. https:// doi.org/10.1111/add.12410.

Goniewicz, M.L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Peyton III, J. and Benowitz, N. (2014b). Levels of selected carcinogens and toxicants in aerosol from electronic cigarettes. Tobacco Control, 23(2), 133–9. doi: 10.1136/tobaccocontrol-2012-050859.

Guerrero-Cignarella, A., Luna Diaz, L.V., Balestrini, K., Holt, G., Mirsaeidi, M., Calderon-Candelario, R., Campos, M.A. (2018). Differences in vaping topography in relation to

adherence to exclusive electronic cigarette use in veterans. PLoS One, 13(4), e0195896. doi:10.1371/journal.pone.0195896.

Hahn, J., Monakhova, Y.B., Hengen, J., Kohl-Himmelseher, M., Schüssler, J., Hahn, H., Kuballa, T. and Lachenmeier, D.W. (2014). Electronic cigarettes: overview of chemical composition and exposure estimation. Tobacco Induced Diseases, 12, 23. doi:10.1186/s12971-014-0023-6.

Hajek, P., Etter, J.F., Benowitz, N., Eissenberg, T. and McRobbie, H. (2014). Electronic cigarettes: review of use, content, safety, effects on smokers and potential for harm and benefit. Addiction, 109 (11), 1801–10. doi:10.1111/add.12659.

Hajek, P., Phillips-Waller, A., Przulj, D., Pesola, F., Myers Smith, K., Bisal, N., Li, J., Parrott, S., Sasieni, P., Dawkins, L., Ross, L., Goniewicz, M., Wu, Q., McRobbie, H.J. (2019). A Randomized Trial of E-Cigarettes versus Nicotine-Replacement Therapy. The New England journal of medicine, 380(7), 629–37. https://doi.org/10.1056/NEJMoa1808779

Halstead, M., Gray, N., Gonzalez-Jimenez, N., Fresquez, M., Valentin-Blasini, L., Watson, C., & Pappas, R. S. (2020). Analysis of Toxic Metals in Electronic Cigarette Aerosols Using a Novel Trap Design. Journal of analytical toxicology, 44(2), 149–55. https://doi.org/10.1093/jat/bkz078.

Hammond, D., Fong, G.T., Zanna, M.P., Thrasher, J.F., Borland, R. (2006). Tobacco denormalization and industry beliefs among smokers from four countries. American journal of preventive medicine, 31(3), 225–32. https://doi.org/10.1016/j.amepre.2006.04.004.

Hansen, J., Hanewinkel, R., Morgenstern, M. (2018). Electronic cigarette marketing and smoking behaviour in adolescence: a cross-sectional study. ERJ Open Res. 4(4), 00155-2018. doi:10.1183/23120541.00155-2018.

Harrell, M.B., Loukas, A., Jackson, C.D., Marti, C.N., Perry, C.L. (2017a). Flavored Tobacco Product Use among Youth and Young Adults: What if Flavors Didn't Exist? Tobacco regulatory science, 3(2), 168–73. https://doi.org/10.18001/TRS.3.2.4.

Harrell, M.B., Weaver, S.R., Loukas, A., Creamer, M., Marti, C.N., Jackson, C.D., Heath, J.W., Nayak, P., Perry, C.L., Pechacek, T.F., Eriksen, M.P. (2017b). Flavored e-cigarette use: Characterizing youth, young adult, and adult users. Preventive medicine reports, 5, 33–40. https://doi.org/10.1016/j.pmedr.2016.11.001.

Hartmann-Boyce, J., McRobbie, H., Bullen, C., Begh, R., Stead, L.F. and Hajek, P. (2016). Electronic cigarettes for smoking cessation (Review). Cochrane Database of Systematic Reviews.9, CD010216. doi: 10.1002/14651858.CD010216.pub3.

Hartman-Boyce, J., McRobbie, H., Lindson, N., Bullen, C., Begh, R. Theodoulou, A., Notley, C., Rigotti, N.A., Turner, T., Butler, A.R., Fanshawe, T.R., Hajek, P. (2020). Electronic cigarettes for smoking cessation (Review). Cochrane Database of Systematic Reviews. 10, CD010216. doi: 10.1002/14651858.CD010216.pub4.

Hassan, S., Anwar, M.U., Muthayya, P., Jivan, S. (2016). Burn injuries from exploding electronic cigarette batteries: An emerging public health hazard. Journal of Plastic, Reconstructive & Aesthetic Surgery, 69(12), 1716–8. doi:10.1016/j.bjps.2016.09.014

Havermans, A., Krüsemann, E., Pennings, J., de Graaf, K., Boesveldt, S., Talhout, R. (2021). Nearly 20 000 e-liquids and 250 unique flavour descriptions: an overview of the Dutch market based on information from manufacturers. Tob Control30, 57-62.doi:10.1136/tobaccocontrol-2019-055303.

He, J., Vupputuri, S., Allen, K., Prerost, M.R., Hughes, J., Whelton, P.K. (1999). Passive smoking and the risk of coronary heart disease--a meta-analysis of epidemiologic studies. The New England journal of medicine, 340(12), 920–6. https://doi.org/10.1056/NEJM199903253401204.

Hess, I.M.R., Lachireddy, K., Capon, A. (2016). A systematic review of the health risks from passive exposure to electronic cigarette aerosol. Public Health Res Practice 26, e2621617 doi: http://dx.doi.org/10.17061/phrp2621617.

Hiemstra, P.S. and Bals R. (2016). Basic science of electronic cigarettes: assessment in cell culture and in vivo models. Respir Res 17(1), 127. https://doi.org/10.1186/s12931-016-0447-z.

Higashi, Y., Noma, K., Yoshizumi, M., Kihara, Y. (2009). Endothelial function and oxidative stress in cardiovascular diseases. Circulation journal: official journal of the Japanese Circulation Society, 73(3), 411–8. .

Hoffman, A.C., Salgado, R.V., Dresler, C., Faller, R.W., Bartlett, C. (2016). Flavour preferences in youth versus adults: a review. Tob Control, 25(Suppl 2), ii32-ii39. doi:10.1136/tobaccocontrol-2016-053192.

Hua, M., Yip, H., Talbot, P. (2013). Mining data on usage of electronic nicotine delivery systems (ENDS) from YouTube videos. Tob Control, 22(2), 103-6. doi:10.1136/tobaccocontrol-2011-050226.

Hummel, K., Nagelhout, G.E., Fong, G.T., Vardavas, C.I., Papadakis, S., Herbeć, A., Mons, U., van den Putte, B., Borland, R., Fernández, E., de Vries, H., McNeill, A., Gravely, S., Przewoźniak, K., Kovacs, P., Trofor, A.C., Willemsen, M.C. (2018). Quitting activity and use of cessation assistance reported by smokers in eight European countries: Findings from the EUREST-PLUS ITC Europe Surveys. Tobacco induced diseases, 16(Suppl 2), A6. https://doi.org/10.18332/tid/98912

Hutzler, C., Paschke, M., Kruschinkski, S., Henkler, F., Hahn, J., Luch, A. (2014). Chemical hazards present in liquids and vapors of electronic cigarettes. Archives of Toxicology, 88, 1295-1308. doi: 10.1007/s00204-014-1294-7.

IARC (2004). Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 83 Tobacco Smoke and Involuntary Smoking.

Ingebrethsen, B., Cole, S.K., Alderman, S.L. (2012). Electronic cigarette aerosol particle size distribution measurements. Inhalation Toxicology. 24 (14), 976-84. DOI: 10.3109/08958378.2012.744781

Jamal, A., Gentzke, A., Hu, S.S. Cullen, K.A., Apelberg, B.J., Homa, D.M., King, B.A. (2017). Tobacco use among middle and high school students – United States. 2011–2016. MMWR Morb Mortal Wkly Rep. 66, 597–603. doi: 10.15585/mmwr.mm6623a1.

Jankowski, M., Brożek, G., Lawson, J., Skoczyński, S., Zejda, J.E. (2017). E-smoking: Emerging public health problem? International journal of occupational medicine and environmental health, 30(3), 329–44. https://doi.org/10.13075/ijomeh.1896.01046.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2006). Safety evaluation of certain contaminants in food. Inorganic Tin WHO FOOD ADDITIVES SERIES: 55 pages 318-350 World Health Organization, Geneva, 2006 https://apps.who.int/food-additives-contaminants-jecfa- database/chemical.aspx?chemID=515

Jensen, R.P., Luo, Pankow, J.F., Strongin, R.M., Peyton, D.H. (2015). Hidden formaldehyde in E-cigarette aerosols. New England Journal of Medicine 372, 392-4. doi: 10.1056/NEJMc1413069.

Kandel, D.B., Kandel, E.R. (2014). A molecular basis for nicotine as a gateway drug. N Engl J Med. 371(21), 2038-9. doi:10.1056/NEJMc1411785.

Kann, L., McManus, T., Harris, W.A., Shanklin, S.L., Flint, K.H., Hawkins, J., Queen, B., Lowry, R., Olsen, E.O., Chyen, D., Whittle, L., Thornton, J., Lim, C., Yamakawa, Y., Brener, N., Zaza, S. (2016). Youth Risk Behavior Surveillance - United States, 2015. Morbidity and mortality weekly report. Surveillance summaries (Washington, D.C. 2002), 65(6), 1–174. https://doi.org/10.15585/mmwr.ss6506a1.

Kapan, A., Stefanac, S., Sandner, I., Haider, S., Grabovac, I., Dorner, T.E. (2020). Use of Electronic Cigarettes in European Populations: A Narrative Review. International journal of environmental research and public health, 17(6), 1971. https://doi.org/10.3390/ijerph17061971.

Khachatoorian, C., Jacob, P., 3rd, Sen, A., Zhu, Y., Benowitz, N.L., Talbot, P. (2019). Identification and quantification of electronic cigarette exhaled aerosol residue chemicals in field sites. Environmental research, 170, 351–8. https://doi.org/10.1016/j.envres.2018.12.027.

Khairudin, M.N., Zahidin, A.Z.M., Bastion, M.L.C. (2016). Front to back ocular injury from a vaping-related explosion. Case Reports, 2016, bcr2016214964. doi: 10.1136/bcr-2016-214964.

Kim K-H. (2016). Mass change tracking approach as collection guidelines for aerosol and vapor samples released during e-cigarette smoking. Analyt Methods 8, 2305–11.

Kinouani, S., Leflot, C., Vanderkam, P., Auriacombe, M., Langlois, E., Tzourio, C. (2020). Motivations for using electronic cigarettes in young adults: A systematic review. Substance abuse, 41(3), 315–22. https://doi.org/10.1080/08897077.2019.1671937.

Kite, A.C., Le, B.Q., Cumpston, K.L., Hieger, M.A., Feldman, M.J., Pozez, A.L. (2016). Blast Injuries Caused by Vape Devices. Annals of Plastic Surgery, 77(6), 620-2. doi:10.1097/sap.0000000000000875.

Klager, S., Vallarino, J., MacNaughton, P., Christiani, D.C., Lu, Q. and Allen, J.G. (2017). Flavoring chemicals and aldehydes in E-cigarette emissions. Environmental Science and Technology 51, 10806−13. doi: 10.1021/acs.est.7b02205.

Klein, M.D., Sokol, N.A., Stroud, L.R. (2019). Electronic Cigarettes: Common Questions and Answers. Am Fam Physician, 100(4), 227-35.

Kosmider, L., Kimber, C., Kurek, J., Corcoran, O., Dawkins, L. (2018a). Compensatory Puffing with Lower Nicotine Concentration E-liquids Increases Carbonyl Exposure in E-cigarette Aerosols, Nic Tob Res 20 (8), 998-1003. https://doi.org/10.1093/ntr/ntx162.

Kosmider, L., Jackson, A., Leigh, N., O'Connor, R., Goniewicz, M.L. (2018b). Circadian Puffing Behavior and Topography among E-cigarette Users. Tob Regul Sci, 4(5), 41-9. doi:10.18001/TRS.4.5.4.

Kosmider, L., Cox, S., Zaciera, M., Kurek, J., Goniewicz, M.L., McRobbie, H., Kimber, C. Dawkins, L. (2020). Daily exposure to formaldehyde and acetaldehyde and potential health risk associated with use of high and low nicotine e-liquid concentrations. Sci Rep 10, 6546. https://doi.org/10.1038/s41598-020-63292-1

Krüsemann, E., Boesveldt, S., de Graaf, K., Talhout, R. (2019). An E-Liquid Flavor Wheel: A Shared Vocabulary Based on Systematically Reviewing E-Liquid Flavor Classifications in Literature. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco, 21(10), 1310–9. https://doi.org/10.1093/ntr/nty101.

Krüsemann, E., Havermans, A., Pennings, J., de Graaf, K., Boesveldt, S., Talhout, R. (2021). Comprehensive overview of common e-liquid ingredients and how they can be used to predict an e-liquid's flavour category. Tobacco control, 30(2), 185–91. https://doi.org/10.1136/tobaccocontrol-2019-055447.

Lampos, S., Kostenidou, E., Farsalinos, K., Zagoriti, Z., Ntoukas, A., Dalamarinis, K., Savranakis, P., Lagoumintzis, G., Poulas, K. (2019). Real-Time Assessment of E-Cigarettes and Conventional Cigarettes Emissions: Aerosol Size Distributions, Mass and Number Concentrations. Toxics, 7(3), 45. https://doi.org/10.3390/toxics7030045.

Laugesen, M. (2015). Nicotine and toxicant yield ratings of electronic cigarette brands in New Zealand. New Zealand Medical Journal 128, 77–82.

Laverty, A.A., Vardavas, C.I., Filippidis, F.T. (2016). Design and marketing features influencing choice of e-cigarettes and tobacco in the EU. Eur J Public Health. 26(5), 838–41. doi:10.1093/eurpub/ckw109.

Laverty, A.A., Filippidis, F.T., Vardavas, C.I. (2018). Patterns, trends and determinants of e-cigarette use in 28 European Union Member States 2014-2017. Prev Med. 116, 13-8. doi:10.1016/j.ypmed.2018.08.028.

Law, M.R., Morris, J.K., Wald, N.J. (1997). Environmental tobacco smoke exposure and ischaemic heart disease: an evaluation of the evidence. BMJ (Clinical research ed.), 315(7114), 973–80. https://doi.org/10.1136/bmj.315.7114.973.

Lee, Y.O., Nonnemaker, J.M., Bradfield, B., Hensel, E.C., Robinson, R.J. (2018a). Examining Daily Electronic Cigarette Puff Topography Among Established and Nonestablished Cigarette Smokers in their Natural Environment. Nicotine Tob Res, 20(10), 1283-8. doi:10.1093/ntr/ntx222.

Lee, P.N., Coombs K.J., and. Afolalu E.F. (2018b). Considerations related to vaping as a possible gateway into cigarette smoking: an analytical review. F1000Res 7, 1915. https://doi.org/10.12688/f1000research.16928.3.

Lee, S.M., Tenney, R., Wallace, A.W, Arjomandi, M. (2018c). E-cigarettes versus nicotine patches for perioperative smoking cessation: a pilot randomized trial. Peer J 6(9), e5609. doi: 10.7717/peerj.5609.

Lee, Y.O., Morgan-Lopez, A.A., Nonnemaker, J.M., Pepper, J.K., Hensel, E.C., Robinson, R.J. (2019). Latent Class Analysis of E-cigarette Use Sessions in Their Natural Environments. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco, 21(10), 1408–13. https://doi.org/10.1093/ntr/nty164

Leigh, N.J., Lawton, R.I., Hershberger, P.A., Goniewicz, M.L. (2016). Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). Tob. Control. 25, ii81–ii87. 10.1136/tobaccocontrol-2016-053205.

Lerner, C.A., Sundar, I.K., Yao, H., Gerloff, J., Ossip, D.J., McIntosh, S. *et al.* (2015a). Vapors Produced by Electronic Cigarettes and E-Juices with Flavorings Induce Toxicity, Oxidative Stress, and Inflammatory Response in Lung Epithelial Cells and in Mouse Lung. PLoS ONE 10(2), e0116732. https://doi.org/10.1371/journal.pone.0116732.

Lerner, C.A., Sundar, I.K., Watson, R.M., Elder, A., Jones, R., Done, D., Kurtzman, R., Ossip, D.J., Robinson, R., McIntosh, S., Rahman, R. (2015b). Environmental health hazards of e-cigarettes and their components: oxidants and copper in e-cigarette aerosols. Environ Pollut 198, 100–7. https://doi.org/10.1016/j.envpol.2014.12.033 25

Levy, D.T., Yuan, Z., Li, Y. (2017). The Prevalence and Characteristics of E-Cigarette Users in the U.S. International journal of environmental research and public health, 14(10), 1200. https://doi.org/10.3390/ijerph14101200.

Levy, D.T., Warner, K.E., Cummings, K.M., Hammond, D., Kuo, C., Fong, G. T., Thrasher, J.F. Goniewicz, M.L. Borland, R. (2019). Examining the relationship of vaping to smoking initiation among US youth and young adults: a reality check. Tob Control 28(6), 629-35. doi: 10.1136/tobaccocontrol-2018-054446.

Liu, J., Liang, Q., Oldham, M.J., Rostami, A.A., Wagner, K.A., Gillman, I.G., Patel, P., Savioz, R., Sarkar, M. (2017). Determination of selected chemical levels in room air and on surfaces after the use of cartridge- and tank-based E-vapor products or conventional cigarettes. Int. J. Environ. Res. Public Health 14, 969. https://doi.org/10.3390/ijerph14090969.

Lucchiari, C., Masiero, M., Mazzocco, K., Veronesi, G., Maisonneuve, P., Jemos, C., Omodeo Salè, E., Spina, S., Bertolotti, R., Pravettoni, G. (2020). Benefits of e-cigarettes in smoking reduction and in pulmonary health among chronic smokers undergoing a lung cancer screening program at 6 months, Addictive Behaviors 103, 106222, https://doi.org/10.1016/j.addbeh.2019.106222.

Lv, X., Sun, J., Bi, Y., Xu, M., Lu, J., Zhao, L., Xu, Y. (2015). Risk of all-cause mortality and cardiovascular disease associated with secondhand smoke exposure: a systematic review and meta-analysis. International journal of cardiology, 199, 106–15. https://doi.org/10.1016/j.ijcard.2015.07.011.

Maessen, G.C., Wijnhoven, A.M., Neijzen, R.L., Paulus, M.C., van Heel, D., Bomers, B., Boersma, L.E., Konya, B., van der Heyden, M. (2020). Nicotine intoxication by e-cigarette liquids: a study of case reports and pathophysiology. Clinical Toxicology 58(1). 1–8. https://doi.org/10.1080/15563650.2019.1636994.

MAK (1997) 2-Propenal (Acrolein), MAK Value Documentation.

MAK (2008) Acetaldehyd. MAK Value Documentation.

Malas, M., van der Tempel, J., Schwartz, R., Minichiello, A., Lightfoot, C., Noormohamed, A., Andrews, J., Zawertailo, L., Ferrence, R. (2016). Electronic Cigarettes for Smoking Cessation: A Systematic Review. Nicotine Tob Res. 18(10), 1926-36. doi: 10.1093/ntr/ntw119.

Mallock, N., Trieu, H.L., Macziol, M. *et al.* (2020). Trendy e-cigarettes enter Europe: chemical characterization of JUUL pods and its aerosols. Arch Toxicol 94, 1985–94. https://doi.org/10.1007/s00204-020-02716-3.

Maloney, J.O. (2008). Perry‘s Chemical Engineers’ Handbook. New York, NY: McGraw Hill.

Marcham, C.L., Floyd, E.L., Wood, B.L., Arnold, S., Johnson, D.L. (2019). E-cigarette nicotine deposition and persistence on glass and cotton surfaces, Journal of Occupational and Environmental Hygiene, 16(5), 349-54, doi: 10.1080/15459624.2019.1581366.

Margham, J., McAdam, K., Forster, M., Liu, C., Wright, C., Mariner, D. and Proctor, C. (2016). Chemical composition of aerosol from an E cigarette: a quantitative comparison with cigarette smoke. Chemical Research in Toxicology, 29(10), 1662-78. https://doi.org/10.1021/acs.chemrestox.6b00188.

Mark, K.S., Farquhar, B., Chisolm, M.S. Coleman-Cowger, V.H., & Terplan, M. (2015). Knowledge, Attitudes, and Practice of Electronic Cigarette Use Among Pregnant Women. Journal of addiction medicine, 9(4), 266–72. https://doi.org/10.1097/ADM.0000000000000128.

Martin, D., Penin-Ibanez, M., Gonzalez Gonzalvez, A., Santos-Delgado, M.J., Gonzalez Urena, M. (2019). On the passive exposure to nicotine from traditional cigarettes versus e-cigarettes. International Journal of Public Health Research 7(1), 11-7. http://www.openscienceonline.com/journal/ijphr.

Mayer B (2014). How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Arch Toxicol 88, 5–7. doi: 10.1007/s00204-013-1127-0

McAdam, K., Warrington, A., Hughes, A., Adams, D., Margham, J., Vas, C., Proctor, C. (2019). Use of social media to establish vapers puffing behaviour: Findings and implications for laboratory evaluation of e-cigarette emissions. Regul Toxicol Pharmacol, 107, 104423. doi:10.1016/j.yrtph.2019.104423.

McNeill, A., Brose, L.S., Calder, R., Bauld, L. and Robson, D. (2018). Evidence review of e-cigarettes and heated tobacco products. Public Health England, London, UK.

Meernik, C., Baker, H.M., Kowitt, S.D., Ranney L.M, Goldstein A.O. (2019). Impact of non-menthol flavours in e-cigarettes on perceptions and use: an updated systematic review. BMJ Open 9(10), e031598. https://doi.org/10.1136/bmjopen-2019-031598.

Mikheev, V.B., Brinkman, M.C., Granville, C.A., Gordon, S.M., Clark, P.I. (2016). Real-time measurement of electronic cigarette aerosol size distribution and metals content analysis. Nicotine & Tobacco Research 18, 1895–1902. https://doi.org/10.1093/ntr/ntw128.

Murthy, V.H. (2017). E-Cigarette Use among Youth and Young Adults: A Major Public Health Concern. JAMA Pediatr 171(3), 209-10. doi: 10.1001/jamapediatrics.2016.4662.

Muthumalage T., Prinz M., Ansah K.O., Gerloff J., Sundar I.K., Rahman I. (2018). Inflammatory and Oxidative Responses Induced by Exposure to Commonly Used e-Cigarette Flavoring Chemicals and Flavored e-Liquids without Nicotine. Front. Physiol. 8, https://doi.org/10.3389/fphys.2017.01130.

Muthumalage, T., Lucas, J.H., Wang, Q., Lamb, T., McGraw, M.D., Rahman, I. (2020). Pulmonary Toxicity and Inflammatory Response of Vape Cartridges Containing Medium-Chain Triglycerides Oil and Vitamin E Acetate: Implications in the Pathogenesis of EVALI. Toxics . 8, 46. https://doi.org/10.3390/toxics8030046.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2018. Public health consequences of e-cigarettes. Washington, DC: The National Academies Press. doi: https://doi.org/10.17226/24952.

Nicoll, K.J., Rose, A.M., Khan, M.A.A., Quaba, O., Lowrie, A.G. (2016). Thigh burns from exploding e-cigarette lithium ion batteries: First case series. Burns, 42(4), e42–e46. doi:10.1016/j.burns.2016.03.027.

Norton, K.J., June, K.M., O'Connor, R.J. (2014). Initial puffing behaviors and subjective responses differ between an electronic nicotine delivery system and traditional cigarettes. Tob Induc Dis, 12(1), 17. doi:10.1186/1617-9625-12-17.

Obertova, N., Navratil, T., Zak, I., Zakharov, S. (2020). Acute exposures to e-cigarettes and heat-not-burn products reported to the Czech Toxicological Information Centre over a 7-year period (2012-2018). Basic & clinical pharmacology & toxicology, 127(1), 39–46. https://doi.org/10.1111/bcpt.13393.

O'Connell, G., Pritchard, J.D., Prue, C., Thompson, J., Verron, T., Graff, D., & Walele, T. (2019). A randomised, open-label, cross-over clinical study to evaluate the pharmacokinetic profiles of cigarettes and e-cigarettes with nicotine salt formulations in US adult smokers. Intern Emerg Med. 14(6), 853-61. doi:10.1007/s11739-019-02025-3.

Ogunwale, M.A., Li, M., Ramakrishnam Raju, M.V., Chen, Y., Nantz, M.H., Conklin, D.J., Xiao-An Fu (2017). Aldehyde detection in electronic cigarette aerosols. ACS Omega. 2, 1207–1214. doi: 10.1021/acsomega.6b00489.

Oh, J.A., Shin, H.S. (2015). Identification and Quantification of Several Contaminated Compounds in Replacement Liquids of Electronic Cigarettes by Gas Chromatography-Mass Spectrometry. Journal of chromatographic science, 53(6), 841–8. https://doi.org/10.1093/chromsci/bmu146.

Olmedo, P., Goessler, W., Tanda, S., Grau-Perez, M., Jarmul, S., Aherrera, A., Chen, R., Hilpert, M., Cohen, J.E., Navas-Acien, A., Rule, A.M. (2018). Metal Concentrations in e-Cigarette Liquid and Aerosol Samples: The Contribution of Metallic Coils. Environmental health perspectives, 126(2), 027010. https://doi.org/10.1289/EHP2175.

Omaiye, E.E., McWhirter, K.J., Luo, W. *et al.* (2019). High concentrations of flavor chemicals are present in electronic cigarette refill fluids. Sci Rep 9, 2468. https://doi.org/10.1038/s41598-019-39550-2.

Orellana-Barrios, M.A., Payne, D., Mulkey, Z. and Nugent, K. (2015). Electronic cigarettes-a narrative review for clinicians. The American Journal of Medicine, 128(7), 674–81. doi:10.1016/j.amjmed.2015.01.033.

Paine, J.B., Pithawalla, Y.B., Naworal, J.D., Thomas Jr., C.E. (2007) .Carbohydrate pyrolysis mechanisms from isotopic labeling: Part 1: the pyrolysis of glycerin: discovery of competing fragmentation mechanisms affording acetaldehyde and formaldehyde and the implications for carbohydrate pyrolysis, J. Anal. Appl. Pyrolysis, 80(2), 297-311. https://doi.org/10.1016/j.jaap.2007.03.007.

Palamidas, A., Tsikrika, S., Katsaounou, P.A., Vakali, S., Gennimata, S., Kaltsakas, G., Gratziou, C., Koulouris, N. (2017). Acute effects of short term use of e-cigarettes on airways physiology and respiratory symptoms in smokers with and without airways obstructive diseases and in healthy non smokers. Tobacco Prevention & Cessation, 3(March), 5. https://doi.org/10.18332/tpc/67799

Palazzolo, D.L., Crow, A.P., Nelson, J.M., Johnson, R.A. (2016). Trace metals derived from electronic cigarette (ECIG) generated aerosol: potential problem of ECIG devices that contain nickel. Front Physiol. https://doi.org/10.3389/fphys.2016.00663

Park, E.J. and Min, Y.G. (2018). The Emerging Method of Suicide by Electronic Cigarette Liquid: a Case Report. J Korean Med Sci. 33(11), e52doi: 10.3346/jkms.2018.33.e52.

Patterson, S.B., Beckett, A.R., Lintner, A., Leahey, C., Greer, A., Brevard, S.B., Kahn, S.A. (2017). A Novel Classification System for Injuries After Electronic Cigarette Explosions. Journal of Burn Care & Research, 38(1), e95–e100. doi:10.1097/bcr.0000000000000471.

Peace, M.R., Baird, T.R., Smith, N., Wolf, C.E., Poklis, J.L. and Poklis, A. (2016). Concentration of nicotine and glycols in 27 electronic cigarette formulations. Journal of Analytical Toxicology, 40(6), 403–7. doi: 10.1093/jat/bkw037.

Pepper, J.K., Ribisl, K.M., Brewer, N.T. (2016). Adolescents' interest in trying flavoured e-cigarettes. Tobacco control, 25(Suppl 2), ii62–ii66. https://doi.org/10.1136/tobaccocontrol-2016-053174.

Pham, T., Williams, J.V.A., Bhattarai, A., Dores, A.K., Isherwood, L.J., Patten, S.B. (2020). Electronic cigarette use and mental health: A Canadian population-based study. Journal of affective disorders, 260, 646–52. https://doi.org/10.1016/j.jad.2019.09.026

Pisinger, C. and Dossing M. (2014). A systematic review of health effects of electronic cigarettes. Prev Med 69: 248-60. doi: 10.1016/j.ypmed.2014.10.009.

Polosa, R., Caponnetto, P., Morjaria, J.B., *et al.* (2011). Effect of an electronic nicotine delivery device (e-Cigarette) on smoking reduction and cessation: a prospective 6-month pilot study. BMC Public Health 11(1), 786. doi: 10.1186/1471-2458-11-786.

Price, L.R., Martinez, J. (2020). Cardiovascular, carcinogenic and reproductive effects of nicotine exposure: A narrative review of the scientific literature. F1000Research, 8, 1586. https://doi.org/10.12688/f1000research.20062.2.

Qasim, H., Karim, Z.A., Rivera, J.O., Khasawneh, F.T. Alshbool, F.Z. (2017). Impact of Electronic Cigarettes on the Cardiovascular System. J Am Heart Assoc, 6(9), e006353. doi: 10.1161/JAHA.117.006353.

RAC, The European Chemicals Agency's Committee for Risk Assessment. Opinion proposing harmonised classification and labelling at EU level of Nicotine (ISO). Adopted 10 September 2015.

Reidel, B., Radicioni, G., Clapp, P.W., Ford, A.A., Abdelwahab, S., Rebuli, M.E., Haridass, P., Alexis, N.E., Jaspers, I., Kesimer, M. (2018). E-Cigarette Use Causes a Unique Innate Immune Response in the Lung, Involving Increased Neutrophilic Activation and Altered Mucin Secretion. Am J Respir Crit Care Med, 197(4), 492-501. doi: 10.1164/rccm.201708-1590OC.

REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL on the potential risks to public health associated with the use of refillable electronic cigarettes.COM/2016/0269 final.

RIVM (National Institute for Public Health and the Environment) (2008). Report 320003001/2008 on 'Chemicals in Toys A general methodology for assessment of chemical safety of toys with a focus on elements'.

Robinson, R.J., Hensel, E.C., Al-Olayan, A.A., Nonnemaker, J.M., Lee, Y.O. (2018). Effect of e-liquid flavor on electronic cigarette topography and consumption behavior in a 2-week natural environment switching study. PLoS One, 13(5), e0196640. doi:10.1371/journal.pone.0196640.

Romijnders, K., van Osch, L., de Vries, H., Talhout, R. (2018). Perceptions and Reasons Regarding E-Cigarette Use among Users and Non-Users: A Narrative Literature Review. Int J Environ Res Public Health, 15(6). doi:10.3390/ijerph15061190.

Rowell, T.R. and Tarran, R. (2015). Will Chronic E-Cigarette Use Cause Lung Disease? American Journal of Physiology. Lung Cellular and Molecular Physiology, 309 (12), L1398–L1409. doi:10.1152/ajplung.00272.2015.

Sailer, S., Sebastiani, G., Andreu-Férnández, V., *et al.* (2019). Impact of Nicotine Replacement and Electronic Nicotine Delivery Systems on Fetal Brain Development. International Journal of Environmental Research and Public Health 16(24), 5113. doi: 10.3390/ijerph16245113.

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Additives used in tobacco products, 25 January 2016.

SCHEER 2018, Memorandum on weight of evidence and uncertainties. doi:10.2875/386011.

SCHER (Scientific Committee on Health and Environmental Risks), Final Opinion on Chromium VI in toys, 22 January 2015.

Schober, W., Szendrei, K., Matzen, W., Osiander-Fuchs, H., Heitmann, D., Schettgen, T., Jörres, R.A. and Fromme, H. (2014). Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. International Journal of Hygiene and Environmental Health, 217(6), 628-37. doi: 10.1016/j.ijheh.2013.11.003.

Schober, W. Fembacher, L., Frenzen, A., Fromme, H. (2019). Passive exposure to pollutants from conventional cigarettes and new electronic smoking devices (IQOS, e-cigarette) in passenger cars, International Journal of Hygiene and Environmental Health 222, 486-93.
https://doi.org/10.1016/j.ijheh.2019.01.003.

Schripp, T., Markewitz, D., Uhde, E. and Salthammer, T. (2013). Does e-cigarette consumption cause passive vaping? Indoor Air, 23(1), 25-31. doi: 10.1111/j.1600-0668.2012.00792.x.

Schulenberg, J.E., Johnston, L.D., O'Malley, P.M., Bachman, J.G., Miech, R., Patrick, M.E. (2017). Monitoring the Future national survey results on drug use, 1975-2016: Volume II: College students & adults ages 19-55. 2017.

Scungio, M., Stabile, L. and Buonanno, G. (2018). Measurements of electronic cigarette-generated particles for the evaluation of lung cancer risk of active and passive users. Journal of Aerosol Science 115, 1-11. https://doi.org/10.1016/j.jaerosci.2017.10.006.

Serror, K., Chaouat, M., Legrand, M. M., Depret, F., Haddad, J., Malca, N., Boccara, D. (2018). Burns caused by electronic vaping devices (e-cigarettes): A new classification proposal based on mechanisms. Burns, 44(3), 544-8. doi:10.1016/j.burns.2017.09.005

Shearston, J., Lee, L., Eazor, J., Meherally, S., Park, S.H., Vilcassim, M.R., Weitzman, M.Gordon, T. (2019). Effects of exposure to direct and secondhand hookah and e-cigarette aerosols on ambient air quality and cardiopulmonary health in adults and children: protocol for a panel study. BMJ Open, 9, e029490. doi: 10.1136/bmjopen-2019-029490.

Scheffler, S., Dieken, H., Krischenowski, O., Aufderheide, M. Cytotoxic evaluation of e-liquid aerosol using different lung-derived cell models (2015). Int. J. Environ. Res. Public Health, 12, 12466-74.
Sleiman, M., Logue, J. M., Montesinos, V.N., Russell, M.L., Litter, M.I., Gundel, L.A., Destaillats, H. Hugo Destaillats (2016). Emissions from electronic cigarettes: key parametersaffecting the release of harmful chemicals. Environmental Science and Technology 50, 9644–51. doi: 10.1021/acs.est.6b01741.

Son, Y., Mainelis, G., Delnevo, C., Wackowski, O. A., Schwander, S., & Meng, Q. (2020). Investigating E-Cigarette Particle Emissions and Human Airway Depositions under Various E-Cigarette-Use Conditions. Chemical research in toxicology, 33(2), 343–52. https://doi.org/10.1021/acs.chemrestox.9b00243.

Soneji, S., Barrington-Trimis, J L., Wills, T.A., Leventhal, A. M., Unger, J.B., Gibson, L.A., Yang, J., Primack, B.A., Andrews, J.A., Miech, R.A., Spindle, T.R., Dick, D.M., Eissenberg, T., Hornik, R.C., Dang, R., Sargent, J.D. (2017). Association Between Initial Use of e-Cigarettes and Subsequent Cigarette Smoking Among Adolescents and Young Adults: A Systematic Review and Meta-analysis. JAMA pediatrics, 171(8), 788–97. https://doi.org/10.1001/jamapediatrics.2017.1488.

Sood, A.K., Kesic, M.J., Hernandez, M.L. (2018). Electronic cigarettes: One size does not fit all. J Allergy Clin Immunol, 141(6), 1973-82. doi:10.1016/j.jaci.2018.02.029.

Sosnowski, T.R. and Odziomek, M. (2018). Particle size dynamics: toward a better understanding of electronic cigarette aerosol interactions with the respiratory system. Frontiers in Physiology, 9, 853. doi:10.3389/fphys.2018.00853.

Soussy, S., EL-Hellani, A., Baalbaki, R., Salman, R., Shihadeh, A., Saliba, N.A. (2016). Detection of 5-hydroxymethylfurfural and furfural in the aerosol of electronic cigarettes. Tob. Control. 25 (Suppl 2), ii88–ii93. https://doi.org/10.1136/tobaccocontrol-2016-053220.

Special Eurobarometer 458. (2017). Attitudes of Europeans towards tobacco and electronic cigarettes. doi:10.2875/245123.

Special Eurobarometer 506. (2020). Attitudes of Europeans towards tobacco and electronic cigarettes. doi:10.2875/490366.

Spindle, T.R., Talih, S., Hiler, M.M., Karaoghlanian, N., Halquist, M.S., Breland, A.B., Eissenberg, T. (2018). Effects of electronic cigarette liquid solvents propylene glycol and vegetable glycerin on user nicotine delivery, heart rate, subjective effects, and puff topography. Drug Alcohol Depend, 188, 193-9. doi:10.1016/j.drugalcdep.2018.03.042.

St Helen, G., Shahid, M., Chu, S., Benowitz, N.L. (2018). Impact of e-liquid flavors on e-cigarette vaping behavior. Drug Alcohol Depend, 189, 42-8. doi:10.1016/j.drugalcdep.2018.04.032.

Stepanov, I., Fujioka, N. (2015). Bringing attention to e-cigarette pH as an important element for research and regulation. Tobacco control, 24(4), 413–4. https://doi.org/10.1136/tobaccocontrol-2014-051540.

Stephens, W.E. (2018). Comparing the cancer potencies of emissions from aerosolised nicotine products including e-cigarettes with those of tobacco smoke. Tob Control 27, 10–17. doi:10.1136/tobaccocontrol-2017-053808.

Strasser, A.A., Souprountchouk, V., Kaufmann, A., Blazekovic, S., Leone, F., Benowitz, N. L., Schnoll, R.A. (2016). Nicotine Replacement, Topography, and Smoking Phenotypes of E-cigarettes. Tob Regul Sci, 2(4), 352-62. doi:10.18001/TRS.2.4.7.

Strongin, M.R. (2019). Annu Rev Anal Chem (Palo Alto Calif). 12(1), 23–39. doi:10.1146/annurevanchem-061318-115329.

Surgeon General 2020. U.S. Department of Health and Human Services. Smoking Cessation. A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and

Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Tayyarah, R., Long, G.A. (2014). Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. Regulatory Toxicology and Pharmacology 70,704–10. https://doi.org/10.1016/j.yrtph.2014.10.010

Thirión-Romero, I., Pérez-Padilla, R., Zabert, G., Barrientos-Gutiérrez, I. (2019). Respiratory impact of electronic cigarettes and "low-risk" tobacco. Rev Invest Clin. 71(1), 17–27. https://doi.org/10.24875/RIC.18002616.

Thomas, E., Spears, R.A., Alldridge, G., *et al.* (2014). E-cigarette liquid refills-a safe beverage? Analysis of enquiries to the UK National Poisons Information Service from 2007 to 2013. In: Clinical Toxicology, 52, 338–9. DOI: 10.3109/15563650.2014.906213.

Tierney, P.A., Karpinski, C.D., Brown, J.E., Luo, W., Pankow, J.F. (2016). Flavour chemicals in electronic cigarette fluids. Tobacco control, 25(e1), e10–e15. https://doi.org/10.1136/tobaccocontrol-2014-052175.

Toy, J., Dong, F., Lee, C., Zappa, D., Le, T., Archambeau, B., Neeki, M.M. (2017). Alarming increase in electronic nicotine delivery systems-related burn injuries: A serious unregulated public health issue. The American Journal of Emergency Medicine, 35(11), 1781–2. doi:10.1016/j.ajem.2017.05.029.

Treur, J.L., Rozema, A.D., Mathijssen, J.J.P., van Oers, H., Vink, J.M. (2018). E-cigarette and waterpipe use in two adolescent cohorts: cross-sectional and longitudinal associations with conventional cigarette smoking. Eur J Epidemiol. 33(3), 323–34. doi:10.1007/s10654-017-0345-9.

Trtchounian, A., Williams, M., Talbot, P. (2010). Conventional and electronic cigarettes (e-cigarettes) have different smoking characteristics. Nicotine Tob Res, 12(9), 905-12. doi:10.1093/ntr/ntq114.

U.S. Fire Administration. July 2017. Electronic Cigarette Fires and Explosions in the United States 2009 – 2016. Available at: https://www.usfa.fema.gov/downloads/pdf/publications/electronic_cigarettes.pdf

Uchiyama, S., Ohta, K., Inaba, Y., Kunugita, N. (2013). Determination of carbonyl compounds generated from the E-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. Analytical Science. 29, 1219–22. https://doi.org/10.2116/analsci.29.1219.

USDHHS (2016). E-Cigarette use among youth and young adults. A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Vallone, D.M., Bennett, M., Xiao, H., Pitzer, L., Hair, E.C. (2019). Prevalence and correlates of JUUL use among a national sample of youth and young adults. Tobacco control, 28(6), 603–9. https://doi.org/10.1136/tobaccocontrol-2018-054693.

Vansickel, A.R., Edmiston, J.S., Liang, Q., Duhon, C., Connell, C., Bennett, D., Sarkar, M. (2018). Characterization of puff topography of a prototype electronic cigarette in adult exclusive cigarette smokers and adult exclusive electronic cigarette users. Regul Toxicol Pharmacol, 98, 250-6. doi:10.1016/j.yrtph.2018.07.019.

Vardavas, C.I., Anagnostopoulos, N., Kougias, M., Evangelopoulou, V., Connolly, G.N. Behrakis, P.K. (2012). Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. Chest, 141(6), 1400-6. doi: 10.1378/chest.11-2443.

Vardavas, C., Girvalaki, C., Vardavas, A., Papadakis, S., Tzatzarakis, M., Behrakis, P., Tsatsakis, A. (2017a). Respiratory irritants in e-cigarette refill liquids across nine European countries: a threat to respiratory health? The European respiratory journal, 50(6), 1701698. https://doi.org/10.1183/13993003.01698-2017.

Vardavas, C.I., Girvalaki, C., Filippidis, F.T., Oder, M., Kastanje, R., de Vries, I., Scholtens, L., Annas, A., Plackova, S., Turk, R., Gruzdyte, L., Rato, F., Genser, D., Schiel, H., Balázs, A., Donohoe, E., Vardavas, A.I., Tzatzarakis, M. N., Tsatsakis, A.M., Behrakis, P.K. (2017b). Characteristics and outcomes of e-cigarette exposure incidents reported to 10 European Poison Centers: a retrospective data analysis. Tobacco induced diseases, 15,(1), 36. https://doi.org/10.1186/s12971-017-0141-z

Visser, W., Geraets, L., Klerx, W., Hernandez, L., Stephens, E., Croes, E., Schwillens, P., Cremers, H., Bos, P., Talhout, R. (2014). De gezondheidsrisico's van het gebruik van e-sigaretten. National Institute for Public Health and the Environment, Bilthoven, the Netherlands, RIVM report 2014-0143 (in Dutch), Available from: http://www.rivm.nl/bibliotheek/rapporten/2014-0143.pdf

Visser, W., Geraets, L., Klerx, W., Hernandez, L., Stephens, E., Croes, E., Schwillens, P., Cremers, H., Bos, P., Talhout, R. (2015). The health risks of using e-cigarettes. National Institute for Public Health and the Environment, Bilthoven, the Netherlands, RIVM rapport 2015-0144, Available from: http://www.rivm.nl/bibliotheek/rapporten/2015-0144.pdf

Visser, W., Geraets, L., Bos, P., Ramlal, R., Fokkens, P., Klerx, W., Cremers, H., Schwillens, P. and Talhout, R. (2016). De gezondheidsrisico's van e-sigaretten voor omstanders [The health risks of electronic cigarette use to bystanders]. National Institute for Public Health and the Environment, Bilthoven, the Netherlands, RIVM rapport 2016-0036 (in Dutch), Technical Appendix in English), Available from: http://www.rivm.nl/bibliotheek/rapporten/2016-0036.pdf

Visser, W.F., Klerx, W.N., Cremers, H.W.J.M., Ramlal, R., Schwillens, P.L. and Talhout, R. (2019). The health risks of electronic cigarette use to bystanders. International Journal of Environmental Research and Public Health 16, 1525. doi.org/10.3390/ijerph16091525

Vlachopoulos, C., Ioakeimidis, N., Abdelrasoul, M., Terentes-Printzios, D., Georgakopoulos, C., Pietri, P., Stefanadis, C., Tousoulis, D. (2016). Electronic Cigarette Smoking Increases Aortic Stiffness and Blood Pressure in Young Smokers. Journal of the American College of Cardiology, 67(23), 2802–3. https://doi.org/10.1016/j.jacc.2016.03.569.

Vreeke, S., Peyton, D.H., Strongin, R.M. (2018). Triacetin Enhances Levels of Acrolein, Formaldehyde Hemiacetals, and Acetaldehyde in Electronic Cigarette Aerosols. ACS omega, 3(7), 7165–70. https://doi.org/10.1021/acsomega.8b00842.

Walker, N., Parag, V., Verbiest, M., Laking, G., Laugesen, M., Bullen, C. (2020). Nicotine patches used in combination with e-cigarettes (with and without nicotine) for smoking cessation: a pragmatic, randomised trial. Lancet Respir Med. 8(1), 54-64. doi: 10.1016/S2213-2600(19)30269-3.

Walley, S.C., Wilson, K.M., Winickoff, J.P., Groner, J. (2019). A Public Health Crisis: Electronic Cigarettes, Vape, and JUUL. Pediatrics, 143(6). doi:10.1542/peds.2018-2741.

Wang, G., Liu, W., Song, W. (2019). Toxicity assessment of electronic cigarettes. Inhalation toxicology, 31(7), 259–73. https://doi.org/10.1080/08958378.2019.1671558.

Whincup, P.H., Gilg, J.A., Emberson, J.R., Jarvis, M.J., Feyerabend, C., Bryant, A., Walker, M., Cook, D.G. (2004). Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. BMJ (Clinical research ed.), 329(7459), 200–5. https://doi.org/10.1136/bmj.38146.427188.55.

WHO (2008). World Health Organization. WHO report on the global tobacco epidemic: The MPOWER package. Geneva, 2008.

WHO (2015). World Health Organization - Regional Office for Europe. Roadmap of actions to strengthen implementation of the WHO Framework Convention on Tobacco Control in the European Region 2015–2025: making tobacco a thing of the past.

WHO (2016). Electronic nicotine delivery systems and electronic non-nicotine delivery systems (ENDS/ENNDS). World Health Organisation, Conference of the Parties to the WHO Framework Convention on Tobacco Control Seventh session Delhi, India, 7–12 November 2016, FCTC/COP/7/11.

Williams, M., Talbot, P. (2011). Variability among electronic cigarettes in the pressure drop, airflow rate, and aerosol production. Nicotine Tob Res 13(12), 1276–83. https://doi.org/10.1093/ntr/ntr164.

Williams M, Talbot P. (2019). Design Features in Multiple Generations of Electronic Cigarette Atomizers. Int J Environ Res Public Health, 16(16), 2904. doi: 10.3390/ijerph16162904. PMID: 31416115; PMCID: PMC6720609.

Williams, M., Villarreal, A., Bozhilov, K., Lin, S., Talbot, P. (2013). Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PloS one, 8(3), e57987. https://doi.org/10.1371/journal.pone.0057987.

Williams, M., Ghai, S., Talbot, P. (2015). Disposable electronic cigarettes and electronic hookahs: evaluation of performance. Nicotine Tob Res 17(2), 201–8. https://doi.org/10.1093/ntr/ntu118.

Williams, M., Bozhilov, K., Ghai, S., Talbot, P. (2017). Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. PloS one, 12(4), e0175430. https://doi.org/10.1371/journal.pone.0175430.

Yuan, M., Cross, S.J., Loughlin, S.E., *et al.* (2015). Nicotine and the adolescent brain. The Journal of Physiology 593(16), 3397–3412. doi: 10.1113/JP270492.

Zare, S., Nemati, M., & Zheng, Y. (2018). A systematic review of consumer preference for e-cigarette attributes: Flavor, nicotine strength, and type. PLoS One, 13(3), e0194145. doi:10.1371/journal.pone.0194145.

Zhang, G., Wang, Z., Zhang, K., Hou, R., Xing, C., Yu, Q. and Liu, E. (2018). Safety Assessment of Electronic Cigarettes and Their Relationship with Cardiovascular Disease. International Journal of Environmental Research and Public Health 15, 75. doi:10.3390/ijerph15010075.

Zhang, Y., Bu, F., Dong, F., Wang, J.H., Zhu, S.J., Zhang, X.W., Robinson, N., Liu, J.P. (2021). The effect of e-cigarettes on smoking cessation and cigarette smoking initiation: An evidence-based rapid review and meta-analysis. Tobacco induced diseases, 19, 04. https://doi.org/10.18332/tid/131624.

Zhao, D., Aravindakshan, A., Hilpert, M., Olmedo, P., Rule, A.M., Navas-Acien, A. and Aherrera, A. (2020). Metal/Metalloid Levels in Electronic Cigarette Liquids, Aerosols, and Human Biosamples: A Systematic Review Environmental Health Perspectives 128(3), 036001-1/13. https://doi.org/10.1289/EHP5686.

Zhong, J., Cao, S., Gong, W., Fei F. and Wang M. (2016). Electronic Cigarettes Use and Intention to Cigarette Smoking among Never-Smoking Adolescents and Young Adults: A Meta-Analysis. Int J Environ Res Public Health 13(5), 465. doi:10.3390/ijerph13050465.

Zhu, S.-H., Sun, J.Y., Bonnevie, E., Cummins, S.E., Gamst, A., Yin, L. and Zhu, M.L. (2014). Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. TobaccoControl, 23: pp.iii3–iii9. doi: 10.1136/tobaccocontrol-2014-051670.

Zulkifli, A., Abidin, E.Z., Abidin, N.Z., Nordin, A.S.A., Praveena, S.M., Ismail, S.N.S., Rasdi, I., Karuppiah, K. and Rahman, A.A. (2016). Electronic cigarettes: a systematic review of available studies on health risk assessment. Reviews in Environmental Health, 33(1), 43-52. doi 10.1515/reveh-2015-0075.

10. LIST OF ABBREVIATIONS

AB	anabasine
AT	anatabine
BN	β-nicotyrine
BTEX	acronym for benzene, toluene ethylbenzene, and xylenes
CE	collision energy
CT	cotinine
DP	declustering potential
e-cig, e-cigarette	electronic cigarette
MRM	multiple reaction monitoring
MS	myosmine
NC	nicotine
NN	nornicotine
NO	nicotine-N'-oxides
PAHs	polycyclic aromatic hydrocarbons
TSNA	tobacco-specific nitrosamines
VOC	volatile organic compound
GC/FID	gas chromatography coupled with flame ionization detector
GC/MS	gas chromatography coupled with mass spectrometry
GC/NPD	gas chromatography coupled with nitrogen-phosphorus detector
GC/TSD	gas chromatography coupled with thermionic specific detector
HPLC/DAD	high-performance liquid chromatography coupled with diode array detector
HPLC/UV	high-performance liquid chromatography coupled with ultraviolet/visible spectroscopic detector;
HS GC/MS	head space gas chromatography coupled with mass spectrometry
ICP/MS	inductively coupled plasma coupled with mass spectrometry
ICP/OES	inductively coupled plasma coupled with optical emissions spectroscopy;
Ion trap	Ion trap mass analyzer
LC/MS/MS	liquid chromatography coupled with tandem mass spectrometry
LC/TOF	liquid chromatography coupled with time-of-flight mass spectrometry
NMR	nuclear magnetic resonance
SIFTMS	selected ion flow tube and mass spectrograph
TSNAs	tobacco-specific nitrosamines
UHPLC/DAD	ultra high-performance liquid chromatography coupled with diode array detector
VOCs	volatile organic compounds
EMA	electrical mobility analyzer
ESI/MS	electro-spray ionization mass spectrometry
GC/FID	gas chromatography coupled with flame ionization detector
GC/MS	gas chromatography coupled with mass spectrometry
GC/NPD	gas chromatography coupled with nitrogen-phosphorus detector
GCTSD	gas chromatography coupled with thermionic specific detector
HPLC/DAD	high-performance liquid chromatography coupled with diode array detector
HPLC/UV	high-performance liquid chromatography coupled with ultraviolet/visible spectroscopic detector
HS GC/MS	head space gas chromatography coupled with mass spectrometry
MS-EI	electron impact mass spectrometry
MSMS	tandem mass spectrometry; NMR, nuclear magnetic resonance
PAHs	polycyclic aromatic hydrocarbons
SIFTMS	selected ion flow tube and mass spectrograph
NNK	nitrosamine ketone
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNN	N-Nitrosonornicotine

SMPS	scanning mobility particle sizer
SMPS-CPC	scanning mobility particle sizer and condensation particle counter
ST	spectral transmission method
WPS	wide range particle spectrometer

ANNEX 1: ANALYTICAL METHODS

Analytical methodology for qualitative and/or quantitative determination of the constituents from e-liquid and aerosol of e-cigarettes are differentiated as is presented in the tables A1.1 to A1.3.

The analytical methods depend on the chemical compound's matrixes, as follows:

- **Nicotine** in e-liquids using gas chromatography with flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) [*1*], and HPLC methods, where the nicotine in e-liquids is analyzed with validation parameters (LOD, LOQ, linearity, accuracy, precision) [*2*-5].

- **Glycols** could be analysed by using gas chromatography equipped with flame ionization detector or gas chromatography/mass spectrometry (GC/MS), whereas carbonyl and other volatile organic compounds determinations have been performed by HPLC/DAD and GC/MS, respectively [3, 43].

- **Propylene glycol** was found to be present in all liquids, because it was used as the solvent for nicotine and flavours. The gas chromatography with mass spectrometry (GC-MS) has been used for the qualitative determination of this ingredient and nicotine, too. The method was adequate for nicotine but poorer for the remaining e-liquid ingredients, mainly flavors [6].

- **Heavy metals** have been performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). Currently, there are several published methods to measure [7-10].

- **Tobacco-specific impurities**, generated from nicotine used for e-liquid production, extracted from tobacco, as: minor alkaloids like nornicotine, anatabine, anabasine, myosmine, cotinine, nicotine-N'-oxides (cis and trans isomers), β-nicotyrine and β-nornicotyrine and are thought to arise by bacterial activity or oxidation during tobacco processing [11]. Nicotine and cotinine in tobacco are largely present as the levorotary (S)-isomers (only 0.1 - 0.6 % of total nicotine content is (R)-nicotine) whereas anabasine, anatabine and nornicotine in tobacco exist as mixture of enantiomers.

- **Degradation products of nicotine** can also occur during the manufacturing processes of e-liquids and high amounts of nicotine-related substances as: formaldehyde, acetaldehyde or acrolein may be generated [12,13]. In particular, formaldehyde classified as carcinogenic to humans, has been described in several studies, at varying levels depending on the experimental conditions. The vaping conditions seem to strongly affect carbonyl generation.

The specific analytical methods for these compounds both for electronic cigarette-liquids and for electronic cigarette aerosols are presented in tables A1.1 to A1.3.

Table A.1.1: Analytical methods for the main compounds in e-liquids and aerosols

Literature	Nicotine	TSNAs	Aldehydes	Metals	VOCs	Phenols	PAHs	Drugs	Alkaloids
6	LC/MS/MS								
11	UHPLC/DAD, GC/FID, GC/MS								
14	GC/TSD								
15		UPLC/MS	HPLC/DAD	ICP/MS	GC/MS				
17		LC/MS/MS							
18	LC/MS/MS/trap								
19			HS GC/MS						
20	LC/TOF								
21			HPLC/UV		GC/MS				
22	NMR								
23				ICP/OES					
24	GC/FID, GC/MS								
25	GC/NPD	GC/MS	HPLC/UV		HS GC/MS		GC/MS		
26			HPLC/UV						
3	HPLC/DAD								HPLC/DAD
27			HPLC/UV						
28; 42	HPLC/UV, GC/MS	LC/MS/MS							HS GC/MS or MSMS
29	HPLC/UV	LC/MS/MS							HPLC/UV, GC/MS
30		LC/MS/MS	SIFTMS	ICP/MS	SIFTMS	SIFTMS	GC/MS		
3								HPLC/DAD or MSMS	

Table A.1.2: Published Analytical Methods for analytes in e-liquids and aerosols [31]

Analytes or classes of analytes	Matrices	Analytical techniques	References
Nicotine	Refill liquid	GC/FID	32
		HPLC/DAD	33
	Cartridge[a]	GC/FID	34
		HPLC-UV	35
	Cartridge, aerosol	GC-TSD	36
Nicotine and	Cartridge	HSGC-MS	28

Analytes or classes of analytes	Matrices	Analytical techniques	References
nicotine-related compounds	Cartridge[a], refill liquid, aerosol	HPLC/DAD	3
Tobacco-specific nitrosamines	Cartridge[a]	LC-MS/MS	30; 28
	Refill liquid	LC-MS/MS	32 ; 19
Diethylene glycol	Cartridge[a]	GC/MS (1H-NMR[b])	28
Propylene glycol	Refill liquid	GC/FID (GC/MS[b])	3
Glycerin	Refill liquid	GC/FID (enzymatic analysis[b])	32
VOCs	Refill liquid	GC/MS	32
Carbonyl compounds and other VOCs	Cartridge	HS-SPME GC-MS	30
Carbonyl compounds	Refill liquid	HS-SPME GC-MS[c]	19
	Aerosol	HPLC/DAD[c]	37-39
Heavy metals	Cartridge[a]	ICP-MS	30
	Aerosol	ICP-MS	37-39
		ICP-OES	40; 42

[a]It requires extraction procedures with organic solvent.
[b]Confirmatory method.
[c]Derivatization step previously.

Table A.1.3: Compounds and matrixes for analyses [43, 44]

Electronic cigarette liquid	VOCs Acetaldehyde propionaldehyde	HS-GC-MS
	Nicotine, anatabine, myosmine, beta-nicotyrine	HPLC-DAD
	Nicotine Nicotine from flavorings Menthol, benzyl alcohol, vanillin	GC-MS, GC-FID
	Carbonyls Acetaldehyde, formaldehyde, acrolein	SPME- GC-MS
	PAH TSNA NNN, NNK, NAB, NAT	GC-MS LC-MS-MS
	PAH NAP, ANT, FLR, PYR, BAA, CHY, BAP, BBF, BFK, DBA,. FLT	GC-MS
	Heavy metals	Sn. Cu. Ni
Electronic cigarette aerosols. aerosol. smoke	VOCs Acetaldehyde propionaldehyde	HS-GC-MS
	Acetaldehyde. formaldehyde. acroleine. glyoxal	HPLC-UV. HPLC-PDA
	Acetaldehyde, formaldehyde, methyl 1,3-butadiene	TD-GC-MS
	Acetaldehyde, formaldehyde, acrolein	HS-GC-MS
	Acetaldehyde, formaldehyde, acrolein, acetone	HPLC
	Formaldehyde, malonaldehyde, acrolein, glyoxal	SPE-GC-MPD, SPME-GC
	Carbonyls	GC-FID, LDI-FTI CRMS, GC-MS, HPLC-UV
	Formaldehyde, malonaldehyde, acrolein, glyoxal	SPE-GC-NPD
	Nicotine, anatabine, myosmine, beta-nicotyrine	HPLC-DAD
	TSNA NNN, NNK, NAB, NAT	GC-MS GC-FID, LDI-FTI CRMS, GC-MS, HPLC-UV
	Volatile, flavouring agents Polypropilene glycol, glycerol	
	PAH NAP, ANT, FLR, PYR, BAA, CHY, BAP. BBF. BFK.	GC-MS

	DBA. FLT	
	Heavy metals. Sn. Cu Ni, Si, Al	SEM/EDS, ICP-OES

References:

1. Flora, J. W., Wilkinson, C. T., Sink, K. M., McKinney, D. L. and Miller, J. H. (2016). Nicotine-related impurities in e-cigarette cartridges and refill e-liquids," Journal of Liquid Chromatography & Related Technologies, 39 (17-18), 821–9. doi: 10.1080/10826076.2016.1266500.

2. Vinit, V., Gholap, V.V., Kosmider, L. and Halquist, M.S. (2018). A Standardized Approach to Quantitative Analysis of Nicotine in e-Liquids Based on Peak Purity Criteria Using High-Performance Liquid Chromatography. Journal of Analytical Methods in Chemistry, Article ID 1720375, 11 pages, https://doi.org/10.1155/2018/1720375.

3. Trehy, M. L., Ye, W. Hadwiger, M.E. *et al.* (2011). Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. Journal of Liquid Chromatography & Related Technologies, 34(14), 1442–58. https://doi.org/10.1080/10826076.2011.572213.

4. Cheng, T. (2014). Chemical evaluation of electronic cigarettes. Tobacco Control , 23, ii11–ii17. doi:10.1136/tobaccocontrol-2013-051482.

5. Liu, X. Joza, P., and Rickert, B. (2017). Analysis of nicotine and nicotine-related compounds in electronic cigarette liquids and aerosols by liquid chromatography-tandem mass spectrometry," Beiträge zur Tabakforschung International/Contributions to Tobacco Research, 27(7), 154–67, DOI: 10.1515/cttr-2017-0016.

6. Aszyk, J., Kubica, P., Kot-Wasik, A., Namieśnik, J., Wasik, A. (2017). Comprehensive determination of flavouring additives and nicotine in e-cigarette refill solutions. Part I: Liquid chromatography-tandem mass spectrometry analysis. Journal of chromatography. A, 1519, 45–54. https://doi.org/10.1016/j.chroma.2017.08.056.

7. Ohashi, Shintaro. (2018). Development and Validation of an ICP-MS Method for Simultaneous Determination of Selected Metals in Electronic Cigarette Aerosol. Beiträge zur Tabakforschung International/Contributions to Tobacco Research. 28. 2-13. 10.2478/cttr-2018-0002. .

8. Mikheev, V.B., Brinkman, M.C., Granville, C.A., Gordon, S.M and Clark, P.I (2016). Real-Time Measurement of Electronic Cigarette Aerosol Size Distribution and Metals Content Analysis; Nicotine Tob. Res. 18, 1895–1902. DOI: 10.1093/ntr/ntw128

9. Palazzolo, D.L., Crow, A.P., Nelson, J.M. and Johnson, R.A. (2017). Trace Metals Derived from Electronic Cigarette (ECIG) Generted Aerosol: Potential Problem of ECIG Devices That Contain Nickel. Front. Physiol. 7 article 663. DOI: 10.3389/fphys.2016.00663

10. Goniewicz, M.L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob, P., 3rd, & Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tobacco control, 23(2), 133–9. https://doi.org/10.1136/tobaccocontrol-2012-050859.

11. Etter, J.F., Zather, E., and Svensson, S. (2013). Analysis of refill liquids for electronic cigarettes. Addiction 108(9), 1671–9. doi: 10.1111/add.12235.

12. Farsalinos, K.E., Voudris, V., and Poulas, K. (2015). E-cigarettes generate high levels of aldehydes only in 'dry puff' conditions. Addiction 110(8), 1352–6. doi: 10.1111/add.12942.

13. Farsalinos, K.E., Kistler, K.A., Gillman, G., and Voudris, V. (2015). Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Nicotine Tob. Res. 17(2), 168–74; doi: 10.1093/ntr/ntu176.

14. Flora, J.W., Meruva, N., Huang, C.B., Wilkinson, C.T., Ballentine, R., Smith, D.C., Werley, M.S., McKinney, W.J. (2016). Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols. Regulatory toxicology and pharmacology74, 1–11. https://doi.org/10.1016/j.yrtph.2015.11.009.

15. Goniewicz, M.L., Hajek, P., McRobbie, H. (2014). Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. Addiction 109(3), 500–7. https://doi.org/10.1111/add.12410.

16. Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob, P., 3rd, & Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tobacco control, 23(2), 133–9. https://doi.org/10.1136/tobaccocontrol-2012-050859.

17. Davis, B., Dang, M., Kim, J., Talbot, P. (2015). Nicotine concentrations in electronic cigarette refill and do-it-yourself fluids. Nicotine Tob Res., 17(2), 134–41. https://doi.org/10.1093/ntr/ntu080.

18. Kim, H.J., Shin, H.S. (2013). Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography-tandem mass spectrometry. Journal of chromatography. A, 1291, 48–55. https://doi.org/10.1016/j.chroma.2013.03.035.

19. Aszyk, J., Kubica, P., Kot-Wasik, A., Namieśnik, J., & Wasik, A. (2017). Comprehensive determination of flavouring additives and nicotine in e-cigarette refill solutions. Part I: Liquid chromatography-tandem mass spectrometry analysis. Journal of chromatography. A, 1519, 45–54. https://doi.org/10.1016/j.chroma.2017.08.056.

20. Lim, H.H., Shin, H.S. (2013). Measurement of aldehydes in replacement liquids of electronic cigarettes by headspace gas chromatography-mass spectrometry. B Kor Chem Soc. 34(9).2691–2696.

21. Kirschner, R., Gerona, R., Jacobitz, K. (2013).Nicotine content of liquid for electronic cigarettes. Clin Toxicol 2013, 51, 684.

22. Schripp, T., Markewitz, D., Uhde, E., Salthammer, T. (2013). Does e-cigarette consumption cause passive vaping? Indoor Air 2013; 23, 25–31. https://doi.org/10.1111/j.1600-0668.2012.00792.x.

23. Uryupin, A.B., Peregudov, A.S., Kochetkov, K.A., *et al.* (2013). Qualitative and quantitative compositions of fluids for electronic cigarettes. Pharm Chem J 46, 687–92. https://doi.org/10.1007/s11094-013-0871-z.

24. Williams, M., Villarreal, A., Bozhilov, K., Lin, S., & Talbot, P. (2013). Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PloS one, 8(3), e57987. https://doi.org/10.1371/journal.pone.0057987.

25. Cheah, N.P., Chong, N.W., Tan, J., Morsed, F.A., & Yee, S.K. (2014). Electronic nicotine delivery systems: regulatory and safety challenges: Singapore perspective. Tob control, 23(2), 119–25. https://doi.org/10.1136/tobaccocontrol-2012-050483.

26. McAuley, T.R., Hopke, P.K., Zhao, J., & Babaian, S. (2012). Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. Inhalation toxicology, 24(12), 850–7. https://doi.org/10.3109/08958378.2012.724728.

27. Ohta, K., Uchiyama, S., Inaba, Y., Nakagom, H., and Kunugita, N. (2011). Determination of carbonyl compounds generated from the electronic cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. Bunseki Kagaku 60, 791−7.

28. Uchiyama, S., Ohta, K., Inaba, Y., and Kunugita, N. (2013). Determination of carbonyl compounds generated from the E-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. Anal. Sci. 29, 1219−22;

29. Westenberger, B.J. Evaluation of e-cigarettes. Washington, DC: U.S. Food and Drug Administration, 2009 http://www.fda.gov/downloads/drugs/scienceresearch/ucm173250.pdf

30. Farsalinos, K., Gene Gillman, I., Hecht, S., Polosa, R., Thornburg, J. (2017). Analytical Assessment of e-Cigarettes. From Contents to Chemical and Particle Exposure Profiles. 1st Edition. Elsevier. 2017

31. Laugesen, M. (2008). Safety Report on the Ruyan E-cigarette Cartridge and Inhaled Aerosol. Christchurch: Health New Zealand Ltd., 2008. http://www.healthnz.co.nz/RuyanCartridgeReport30-Oct-08.pdf

32. Famele, M., Ferranti, C., Abenavoli, C., Palleschi, L., Mancinelli, R., Draisci, R. (2015). The chemical components of electronic cigarette cartridges and refill fluids: review of analytical methods. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco, 17(3), 271–9. https://doi.org/10.1093/ntr/ntu197.

33. Schober, W., Szendrea, K., Matzea, W., *et al.* (2014). Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. Int J Hyg Environ Health. 217, 628–37. doi: 10.1016/j.ijheh.2013.11.003.

34. Davis, B., Dang, M., Kim, J., Talbot, P. (2014). Nicotine concentrations in electronic cigarette refill and do-it-yourself fluids. Nicotine Tob Res. 17(2), 134-41. doi: 10.1093/ntr/ntu080.

35. Cheah, N.P., Chong, N.W., Tan, J., Morsed, F.A., Yee, S.K. (2014). Electronic nicotine delivery systems: regulatory and safety challenges: Singapore perspective. Tobacco control, 23(2), 119–25. https://doi.org/10.1136/tobaccocontrol-2012-050483.

36. Meruva, N.K., Benvenuti, M.E., Cleland, G.E., Burgess, J.A. (2016). Simultaneous Determination of Nicotine and Related Impurities in E-Liquids and E-Cigarettes Using UPLC-UV-MS. Waters, UK: 2016.

37. Goniewicz, M.L., Hajek, P., McRobbie, H. (2014). Nicotine content of electronic cigarettes, its release in aerosol and its consistency across batches: regulatory implications. Addiction 109, 500–7. doi: 10.1111/add.12410.

38. Goniewicz, M.L., Gupta, R., Lee, Y.H., Reinhardt, S., Kim, S., Kim, B., *et al.* (2015). Nicotine levels n electronic cigarette refill solutions: a comparative analysis of products from the US, Korea, and Poland. The International Journal on Drug Policy 26(6), 583–8. DOI: 10.1016/j.drugpo.2015.01.020.

39. Goniewicz, M.L., Hajek, P., McRobbie, H. (2014). Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. Addiction 109(3), 500–7. https://doi.org/10.1111/add.12410.

40. Goniewicz, M.L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob, P., 3rd, Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tobacco control, 23(2), 133–9. https://doi.org/10.1136/tobaccocontrol-2012-050859.

41. Pappas, R.S., Gray, N., Gonzalez-Jimenez, N., Fresquez, M., Watson, C.H. (2016). Triple Quad-CP-MS measurement of toxic metals in mainstream cigarette smoke from spectrum research cigarettes. Journal of Analytical Toxicology, 40, 43–48.

42. Beauval, N., Howsam, M., Antherieu, S., Allorge, D., Soyez, M., Garçon, G., *et al*. (2016). Trace elements in e-liquids - Development and validation of an ICP-MS method for the analysis of electronic cigarette refills. Regulatory Toxicology and Pharmacology, 79, 144–8.

43. Lisko, J.G., Tran, H., Stanfill, S.B., Blount, B.C., Watson, C.H. (2015). Chemical composition and valuation of nicotine, tobacco alkaloids, PH, and selected flavors in e-cigarette cartridges and refill solutions. Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and tobacco, 17, 1270–8.

44. El-Hellani, A., El-Hage, R., Baalbaki, R., Salman, R., Talih, S., Shihadeh, A., *et al*. (2015). Free-ase and protonated nicotine in electronic cigarette liquids and aerosols. Chemical Research in toxicology, 28, 1532–7.

1
2
3
4
5

ANNEX 2: INGREDIENTS IN E-LIQUIDS

Table A2.1: Ingredients determined in e-liquids in the Netherlands

Ingredient name	%age present	1st Qu. amount (mg)	Median amount (mg)	3rd Qu. amount (mg)
Glycerol	94.1	477	4968	7000
Nicotine	88.4	3	32	120
Propylene Glycol	85.8	271	4152	5571
Water	45.0	50	223	630
Vanillin	35.2	0.47	7	34
Ethyl maltol	32.0	0.5	5.9	27
Ethyl Butyrate	28.4	0.36	3.6	14
Ethyl Acetate	23.2	0.24	1.1	6.9
Ethanol	23.1	1.5	31	115
Maltol	22.8	0.17	1.3	9.6
Ethyl Vanillin	19.4	0.3	6.8	31
Furaneol	19.3	0.39	2	9.9
Methyl cyclopentenolone	18.3	0.15	2	14
gamma-Decalactone	18.2	0.12	0.49	4
Cis-3-hexenol	17.8	0.37	1.5	7.7
Isoamyl Acetate	16.3	0.31	2.3	15
Ethyl 2-Methyl Butyrate	16.0	0.18	2.2	11
Acetic Acid	15.7	0.14	1.2	6.1
Butyric Acid	15.0	0.22	0.84	5.7
Linalool	14.5	0.16	0.9	3.2
Triacetin	14.4	0.4	5.6	24
Benzyl Alcohol	14.2	0.68	3.3	18
Ethyl Hexanoate	13.6	0.11	0.54	4.8

Benzaldehyde	12.4	0.1	0.33	5.9
Menthol	12.1	2.5	18	71
Isoamyl Isovalerate	11.5	0.2	0.77	7.2
delta-Decalactone	11.2	0.13	0.34	2
Hexanoic Acid	11.1	0.12	0.42	2.1
Ethyl Propionate	10.9	0.1	0.55	3.9
gamma-Undecalactone	10.9	0.15	0.42	5.8
Hexyl Acetate	10.3	0.15	1	4.3
2-Methyl Butyric Acid	9.8	0.18	1.6	7.1
Piperonal	9.6	0.15	0.47	6
gamma-Nonalactone	9.5	0.2	0.74	2.9
Ethyl Isovalerate	9.5	0.17	0.54	6.3
4-(4-Hydroxyphenyl)-2-butanone	9.4	0.21	1.4	8
Methyl Cinnamate	9.4	0.13	0.47	4.1
Benzyl Acetate	9.2	0.1	0.85	3.6
Cis-3-hexenyl Acetate	9.2	0.15	0.8	3
Anisaldehyde	9.0	0.04	0.24	1.5
delta-Dodecalactone	8.7	0.077	0.29	2.1
Sucralose	8.3	2.3	11	23
Limonene	7.9	0.27	3.3	15
Beta-Ionone	7.5	0.1	0.36	1
Acetoin	7.5	0.09	1	6.1
gamma-Octalactone	7.3	0.1	0.4	2.1
Anisyl Alcohol	7.0	0.1	0.58	1.7
Isoamyl Butyrate	6.8	0.15	0.95	6
Lemon oil	6.3	0.13	1.2	12
Guaiacol	6.1	0.07	0.22	0.67
Eugenol	6.0	0.1	1.2	11
2-Acetylpyrazine	6.0	0.22	1.5	6.8
Dihydrocoumarin	5.9	0.15	0.74	2.7

2.3.5-Trimethylpyrazine	5.7	0.066	2	16
Citral	5.6	0.1	0.9	5.3
Alpha-Ionone	5.6	0.12	0.6	2
Allyl Hexanoate	5.5	0.11	1	3.6
4-Methyl-5-Thiazole Ethanol	5.5	0.03	0.3	1.8
beta-Damascone	5.5	0.1	0.51	4.9
alpha-Terpineol	5.5	0.1	0.69	3.1
gamma-Hexalactone	5.1	0.14	0.53	1.2
Dimethyl Sulfide	5.0	0.06	0.13	1
Isobutyl Acetate	4.9	0.1	1.1	10
Isoamyl Alcohol	4.5	0.1	0.52	1.6
beta-Damascenone	4.4	0.03	0.18	1
Octanoic Acid	4.4	0.16	0.2	3.6
Propionic Acid	4.3	0.1	0.61	5
2-Phenylethanol	4.2	0.041	0.13	1
Triethyl Citrate	4.1	0.45	4.6	26
Geraniol	4.1	0.1	0.33	1.9
Lime oil	4.0	1	3.3	18
Butyl Butyryl Lactate	3.9	0.12	1	6
trans-2-Hexenal	3.9	0.13	1	5.5
Cinnamaldehyde	3.8	0.12	2	11
Methyl Anthranilate	3.7	0.1	0.77	5.9
Orange oil	3.7	0.12	1	2.1
Hexanal	3.6	0.02	0.29	2
Ethyl Lactate	3.6	0.1	0.41	2.1
n-Hexanol	3.6	0.14	0.61	4.3
Geranyl acetate	3.5	0.1	0.45	8.1
Lactic Acid	3.4	1	3.2	25
Linalyl Acetate	3.4	0.07	0.3	1.8
Cis-3-Hexenyl	3.3	0.1	0.24	3.6

Butyrate				
Ethyl Acetoacetate	3.3	0.2	1	9.1
Benzyl Benzoate	3.1	0.17	1.1	7.5
Citric Acid	3.1	0.02	0.21	0.9
2.3-Pentanedione	3.1	0.27	2	7
Eucalyptol	3.0	0.58	3	12
gamma-Dodecalactone	3.0	0.12	1.5	3
Furfural	3.0	0.05	0.34	5.9
Menthone	2.9	0.2	5.4	24
2.3.5.6-Tetramethylpyrazine	2.9	0.02	0.47	13
Butyl Butyrate	2.8	0.1	0.25	2.4
5-Methyl Furfural	2.7	0.02	0.69	2.8
Methyl-alpha-ionone	2.6	0.23	0.72	4.5
Methylthio Methyl Pyrazine	2.4	0.035	0.06	0.14
Propenyl Guaethol	2.4	0.14	0.59	1
Ethyl methyl phenylglycidate	2.4	0.1	1	1.8
Caramel	2.4	0.13	1	2.9
Butyl Acetate	2.3	0.075	1.1	5.8
Furfuryl Alcohol	2.3	0.1	1	4.8
Menthyl acetate	2.3	0.076	1.2	14
Anethole	2.3	1	9.8	26
Ethyl Octanoate	2.3	0.05	0.22	2
2-Methylbutyl acetate	2.2	0.05	0.06	0.33
trans-Anethole	2.2	1.3	9.6	35
2.6-Dimethyl-5-heptenal	2.1	0.18	0.6	3.9
alpha-Pinene	2.1	0.8	3.4	8.8
beta-Pinene	2.1	0.35	3.2	6.5

2.3-Dimethylpyrazine	2.1	0.27	2	19
Cedrol	2.1	24	36	61
Acetaldehyde	2.0	0.2	1.3	6.6
Ethyl Heptanoate	2.0	0.1	0.66	12
2-Acetyl Pyridine	2.0	0.08	1.2	9.4
Decanoic Acid	1.9	0.1	0.2	2
1.4-Dimethoxybenzene	1.9	0.01	0.023	0.18
Amyl acetate	1.9	0.21	1	2.3
Citronellol	1.9	0.056	0.23	2
Myrcene	1.9	0.17	3	12
alpha-Damascone	1.8	0.06	6.5	8.6
trans-2-Hexenol	1.8	0.12	3	7.2
beta-Caryophyllene	1.8	0.05	0.42	4.9
alpha-Methylbenzyl acetate	1.8	0.18	0.53	2.2
Isovaleraldehyde	1.8	0.04	0.19	2.4
Peppermint Oil	1.8	1	2.4	22
Hexyl Butyrate	1.7	0.084	0.1	2.2
Veratraldehyde	1.7	0.52	3	5.4
Ethyl Decanoate	1.6	0.04	0.2	0.81
Thio Menthone	1.6	0.018	0.04	0.13
Fenugreek	1.6	0.1	0.39	1
Neryl Acetate	1.6	0.034	0.18	4.7
Strawberry Extract	1.6	0.1	0.2	9.9
2.5-Dimethylpyrazine	1.5	0.028	0.24	1.3
Cocoa Extract	1.5	1	4.5	11
Ethyl menthane carboxamide	1.5	1.1	4.2	19
Citronellyl Acetate	1.5	0.023	0.13	1.3
Ethyl Cinnamate	1.5	0.05	0.13	1.4
Ethyl Nonanoate	1.5	0.3	1	12

Isoamyl Phenyl Acetate	1.5	0.19	1	2.4
Blood Orange Oil	1.5	0.11	1.3	11
Methyl Thiobutyrate	1.5	0.04	0.1	0.34
Carob	1.5	0.06	0.12	3
Carvone	1.5	0.34	3.6	22
2-Propanol	1.4	0.1	6	207
Benzyl Butyrate	1.4	0.068	0,45	6,1
Isobutyl Alcohol	1.4	0.023	0.08	0,29
Ethyl 2-Phenyl Acetate	1.4	0.025	0.14	0,56
4,5-Dimethyl-3-Hydroxy-2,5-Dihydrofuran-2-One	1.4	0.1	1	3,1
Vanillin Propylene Glycol Acetal	1.3	0.1	0.2	1,3
Dimethyl Anthranilate	1.3	0.1	0.2	1
trans-2-Hexenoic acid	1.3	0.07	0.28	0,96
2-Isopropyl-N,2,3-trimethylbutyramide	1.3	0.46	31	351
Bucchu Leaf Oil	1.3	0.08	0.17	1
Cornmint Oil	1.3	1	6.8	70
Sugar	1.3	1	1	18
Cassia oil	1.3	0.1	0.45	6,.2
n-Butanol	1.3	0.12	1	1
Decanal	1.2	0.02	0.05	0.3
Nerol	1.2	0.02	0.08	0.46
Methyl Salicylate	1.2	0.1	1	1.7
2-Acetyl Furan	1.2	0.03	0.08	0.36
Peru Balsam	1.2	0.06	0.14	0.25
Sodium Benzoate	1.2	0.04	0.06	0.16
Sodium Citrate	1.2	0.04	0.06	0.16
Potassium Sorbate	1.1	0.04	0.06	0.16

5-methyl-2-Phenyl-2-Hexenal	1.1	0.2	0.4	7.9
Amyl Butyrate	1.1	0.18	1	21
n-Octanal	1.1	0.02	0.1	0.91
Oleic Acid	1.1	0.1	0.51	10
Acetal	1.1	0.07	0.41	1
Spearmint oil	1.1	0.15	1	13
2-3-Hexanedione	1.1	1.3	2.8	4
4-(4-methoxyphenyl)butan-2-one	1.1	0.1	0.2	5.1
1-Pentanol	1.0	0.4	1.3	11

1
2

Table A.2.2: Most frequently determined ingredients in e-liquids in Greece

	Recipe quantity (mg)					Concentration (mg/ml)				
Name	**1stQu.**	**Median**	**Mean**	**3rdQu.**	**Max.**	**1stQu.**	**Median**	**Mean**	**3rdQu.**	**Max.**
Propylene glycol	1086	4174	3593	5112	442185	170.2	429.6	375	515.3	44218.5
Nicotine	10.59	30.3	65.91	117	9470	1.08	3.435	7.163	12	947
Glycerol	756	5000	14760	6265	8510000	100	506	1492	630	8510000
Vanillin	1	8	27.57	30	2100	0.1	0.8878	2.8576	3.09	210
Water	32.72	157.86	367.47	559	4331	3.391	16.39	37.925	58.882	433.1
Ethyl maltol	0.98	9.99	27.23	27.14	1734.8	0.1	1	2.705	2.787	173.48
Ethyl butyrate	0.526	3.164	13.361	12.96	885.76	0.05616	0.33616	1.33052	1.308	44.1
Ethyl alcohol	3.372	26	101.703	102.27	3060.19	0.36456	2.8	10.35433	10.36	233.196
Maltol	0.34	2	13.64	9	5142.23	0.0376	0.218	1.3988	0.9	514.223
Ethyl acetate	0.228	1.5	9.861	6.786	2000	0.023	0.166	0.9756	0.6847	200
Furaneol	0.3889	2.4833	12.6772	11.5475	2000	0.04128	0.26755	1.25596	1.152	200
Ethyl vanillin	1	8.71	28.39	31.25	1900	0.1	0.8837	2.8249	3.2	190
Isoamyl acetate	0.25	1.97	13.93	11.29	557.41	0.0278	0.2	1.4801	1.13	72.52
cis-3-Hexen-1-ol	0.24	1.64	7.47	7	442.88	0.02592	0.16965	0.73883	0.664	20.4
γ-Decalactone	0.1272	0.75	3.6199	3	165	0.014	0.077	0.367	0.3	16.5
Benzyl alcohol	0.477	4.552	19.882	18.583	3709	0.054	0.5	2.026	2	370.9
Ethyl 2-methylbutyrate	0.4	2.24	15.99	10.63	2250	0.045	0.2316	1.5503	1.0685	225
Acetic acid	0.28	1.22	6.848	5.425	885.76	0.02865	0.12897	0.64998	0.55289	20
Butyric acid	0.1415	0.9263	5.394	3.79	200	0.016	0.1	0.537	0.386	20
Linalool	0.1415	0.5215	4.8911	2.39	450	0.011	0.0533	0.4849	0.2614	45

ANNEX 3: OVERVIEW PUFFING PARAMETERS AND TESTING CONDITIONS

Table A3.1: Overview of puffing parameters and testing conditions in studies reviewed in (DeVito and Krishnan-Sarin, 2018) and (Evans and Hoffman, 2014). The references have been included in the main reference list, section 9.

average								
Puff number	**Puff duration (s)**	**Inter-puff interval (s)**	**Puff volume (ml)**	**Time of session**	**Test subject**	**Test product**	**Test methods**	**ref**
13.2 (SD = 9.46)	2.06 (SE = 0.7)	11.2 (SD = 5.2)	n.a.	165.6 seconds (SD = 89.5)	28 cigarette smokers	5 electronic cigarettes brands, 18mg/ml	Analysis video-recording *ad libitum* sessions on day 10	(Strasser *et al.*, 2016)
32±8	2.65±0.98	17.9±7.5	51±21	n.a.	20 experienced electronic cigarette users	2 types: 16mg/ml (Blu Cigs) and 18mg/ml (V2 Cigs)	Cress-micro flowmeter, 10-minute sessions	(Behar, Hua, & Talbot, 2015)
8.7 +- 1.6	3.0 +- 0.8	29.6 +- 11.7	118.2 +- 13.3	n.a.	18 cigarette smokers	'cigarette-like', 11 mg/ml (Vapor Corp)	CReSS device	(Norton, June, & O'Connor, 2014)
~90 vapers, ~85 smokers	3.5 ± 0.2 s in vapers, 2.3 ± 0.2 s in smokers	n.a.	n.a.	n.a.	Vapers (n=24) Smokers (n=23)	new-generation electronic cigarette device 18 mg/ml nicotine	electronic cigarette device stored puff number and duration. ad libitum session	(K. E. Farsalinos *et al.*, 2015)
120/day	n.a.	n.a.	n.a.	n.a.	3587 participants, 70% former tobacco smokers .	Av. 18 mg/mL nicotine	online survey	(Etter & Bullen, 2011)
n.a.	electronic cigarette users range 1.9–8.3 s, average 4.3 ±1.5 traditional cigarettes 2.4	n.a.	n.a.	n.a.	Electronic cigarette and traditional cigarette users		videos analysis of ad libitum puff and exhalation duration	(Hua, Yip, & Talbot, 2013)

	±0.8.							
electronic cigarette user 43	electronic cigarette user 4.2±0.7, inhalation 1.3±0.4 traditional cigarette smokers using electronic cigarettes, duration 2.4±0.5 s and inhalation 2.0±0.4 s				45 experienced electronic cigarette users and 35 traditional cigarette smokers (naïve to electronic cigarettes)	second-generation electronic cigarette device	randomised cross-over design in which users were video-recorded	(K. E. Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013)

177±15 to 313±115 to exhaust the cartridge.					traditional cigarette and electronic cigarette users	two electronic cigarette; one had a reservoir of e-liquid that was three times smaller than the other	specially designed topography equipment. Differences were observed in vacuum required and aerosol density between brands	(Trtchounian. Williams. & Talbot. 2010)

Legend:
Cigarette smokers (N=28) were randomized to one of 5 electronic cigarette brand/types (all of which contained 18mg/ml nicotine e-liquid) for 9 days of take-home use (Strasser *et al.*. 2016) reviewed in (DeVito & Krishnan-Sarin. 2018). Video-recordings showed that topography differed between smoking and using electronic cigarettes, with electronic cigarette sessions having longer puffs (20% longer) and shorter interpuff intervals (25 sec vs. 11sec). There were no effects of brand on topography.
A topography study with a Cress-micro flowmeter with two popular electronic cigarette types found substantial individual differences in puffing topography, but on average more puffs (32 (8)) and longer puffs (2.65 (0.98) seconds) for electronic cigarettes relative to typical combustible cigarette topography with more puffs and longer puffs for Blue vs. V2, and no significant difference in puff topography between electronic cigarette only users and dual users of electronic cigarettes and combustible cigarettes. Together, these findings suggest that electronic cigarette users adjust topography to compensate for lower efficiency devices, to achieve sufficient nicotine levels (Behar *et al.*, 2015) reviewed in (DeVito & Krishnan-Sarin, 2018).

Cigarette smokers with no past-month use of electronic cigarettes self-administered own brand cigarettes or electronic cigarettes and found reduced craving in response to own brand cigarettes but not electronic cigarettes (Norton *et al.*, 2014) reviewed in (DeVito & Krishnan-Sarin, 2018). Puff volume (118. 2(13.3) vs 67.5 (6.3) ml) and puff velocity (52.0(4.7) vs 36.1(1.8) ml/s)) and inter-puff interval (29.6(11.7) vs 21.3(6.2); not significant) for electronic cigarettes relative to own brand combustible cigarette were increased. (Norton *et al.*, 2014). Puff duration (3.0 (0.8) electronic cigarette vs 3.0(1.0) cigarette) was equivalent across both. Puff count (13.2(1.1) vs 8.7(1.6)) was higher for the cigarette

During an ad libitum session, experienced and naïve groups did not differ in the number of puffs they self-administered, but experienced users took longer puffs on average (3.5 vs. 2.3 seconds) (K. E. Farsalinos *et al.*, 2015) reviewed in (DeVito & Krishnan-Sarin, 2018).

Etter and Bullen (online survey, 3587 participants, 70% former tobacco smokers) found that daily use of electronic cigarettes was 120 puffs per day (five refills per day; averaging 24 puffs per refill and 18 mg/mL) **ref**.

Hua *et al*. (videos analysis of ad libitum puff and exhalation duration for individuals using electronic cigarettes and traditional cigarettes) observed that electronic cigarette users showed a large variation in puff duration (range 1.9–8.3 s), with average puff duration significantly longer (4.3 s, SD ±1.5) than puff duration for the traditional cigarettes (2.4 s, SD ±0.8). The values for average duration of exhalation did not differ significantly between electronic cigarette users (1.7 s, SD 1.1) and traditional cigarette smokers (1.6 s, SD 0.7).

Farsalinos using a second-generation electronic cigarette device studied 45 experienced electronic cigarette users and 35 traditional cigarette smokers (naïve to electronic cigarettes) in a randomised cross-over design in which users were video-recorded. electronic cigarette user puff duration (4.2±0.7 s), inhalation (1.3±0.4 s) and puff number (43 puffs) were different from traditional cigarette smokers using electronic cigarettes, who had shorter puff durations (2.4±0.5 s) and longer inhalation (2.0±0.4 s).

Trtchounian *et al.* conducted two studies that examined the smoking characteristics of traditional cigarettes and electronic cigarettes using specially designed topography equipment. Differences were observed in vacuum required and aerosol density between brands. Total puffs ranged from 177±15 to 313±115 to exhaust the cartridge. Interestingly, the two electronic cigarette produced almost the same average number of puffs even though one had a reservoir of e-liquid that was three times smaller than the other, indicating that puff number is influenced by factors in addition to reservoir size.

Table A3.2: Overview of puffing parameters and testing conditions found in recent studies (2018-2019)

average								
Puff number	**Puff duration (s)**	**Inter-puff interval (s)**	**Puff volume (ml)**	**Time of session**	**Test subject**	**Test product**	**Test methods**	**ref**
	3 s on average 5.6 95th percentile						Analysis of large database of public-domain videos; near natural settings	(McAdam *et al.*, 2019); British American Tobacco
Average strawberry, 73+/-35; tobacco, 69+/-46 usual e-liquid 106+/-67	strawberry 3.2+/-1.3 tobacco 2.8+/-1.1 usual e-liquid 4.3+/-1.6					strawberry vs tobacco flavour (18mg/mL), and their usual brand e-liquid (3-18mg/mL).	3-day inpatient crossover study; 90-minute videotaped ad libitum session	(St Helen, Shahid, Chu, & Benowitz, 2018)
Prescribed 10	4.3-5.9 Shorter puffs	Prescribed 30	97-134 Smaller		Thirty experienced electronic	differient liquid propylene glycol:glycerol	nicotine- abstinent for at least 12 hours, two	(Spindle *et al.*, 2018)

	for higher glycerol levels		puffs for higher glycerol levels		cigarette users	ratio; device power (7.3W) and liquid nicotine concentration (18mg/ml) constant	electronic cigaretteIG-use bouts (10 puffs, 30 s interpuff interval)	
	CS cigarette 1.7+/-0.4s CS electronic cig 2.3+/-0.8 electronic cigarette 3.0+/-1.3		CS cigarette 44.1+/-10.5ml CS electronic cig 47.9+/-18.2 electronic cigarette 53.4+/-19.2		13 adult exclusive cigarette smokers (CS) and 10 adult electronic cig users (electronic cigarette)	prototype electronic cigarette, 2% nicotine	ad lib conditions in a clinic 7-hr use session. using SODIM Smoking Puff Analyzer Mobile Device (SPA/M). CS also smoked a single cigarette	(Vansickel *et al.*, 2018); Altria
	mean 2.2 for tobacco, 1.9 for menthol and 2.4 for berry				34 experienced ENDS users	tobacco flavor for one week, and either berry or menthol flavor for one week	natural environment observational study; RIT wPUMTM monitor to record date, time and puff topography	(Robinson, Hensel, Al-Olayan, Nonnemaker, & Lee, 2018)
	Established 3.3 vs. 1.8 nonestablished	38.1 vs. 21.7	110.3 vs. 54.7.	566.3 vs. 279.7 more sessions per day 5.3 vs. 3.5	20 young adult (18-25) established cigarette smokers and nonestablished cigarette smokers.	Disposable electronic cigarettes	wireless hand-held monitoring device in users' everyday lives over 1 week. Online surveys	(Lee, Nonnemaker, Bradfield, Hensel, & Robinson, 2018)
class 1: 14.7 class 2 16.7	Session class 1 2.0 Session class 2 4.4		Session class 1 59.9 Session class 2 290.9		34 current second-generation e-cigarette users		wireless portable use monitor (wPUMTM) continuously over 2 weeks in their everyday live	(Lee, Morgan-Lopez, *et al.*, 2018)

156.2+/-10.3, clustered in 10.2+/-7.9 puffs per puffing session	3.0+/-1.2 sec		73.4+/-51.5 ml		24 adult regular electronic cigarette users		personal electronic cigarettes ad-lib over the course of 24 hours. calibrated CReSS pocket topography monitors	(Kosmider, Jackson, Leigh, O'Connor, & Goniewicz, 2018b)
RP success group 139.4 ± 138.0; Failure group 114.6 ± 94.0 MP success group 218.0 ± 173.3; Failure group: 159.9 ± 76.7	RP 5.7 ± 1.4 and 3.7 ± 1.5 MP 6.1 ± 1.3 and 4.4 ± 1.9				25 active TC smokers were asked to replace TC with electronic cigarette		Observational non-blinded study wirh replacement and maintenance phase. Vaping information downloaded from the electronic cigarette device	(Guerrero-Cignarella *et al.*, 2018)
10 W 46 [16] 6 W (57 [20]	10 W 3.8 [0.8] 6 W 4.6 [1.0]					Experienced adult vapers (n = 21)	Own liquids; atomizer and battery provided by researcher Two 30-minute sessions, device power set at 6 W and 10 W.	(K. Farsalinos, Poulas, & Voudris, 2018)
272-338	3.61-4.46	26.23-37.32			Twenty experienced electronic cigarette users		Counterbalanced, repeated measures with four conditions differing in nicotine level and yes/no adjustable power. Ad libitum using.	(Dawkins *et al.*, 2018)

Legend:
A British American Tobacco study analysed a large database of public-domain videos to establish electronic cigarette puffing behaviour in near natural settings. A 3 s puff duration, as used in the recently published ISO puffing standard ISO 20,768:2018, appears appropriate for average behaviours. A puff duration of around 5.6 s appears to represent 95th percentile puffing behaviours amongst vapers, and could be considered for a more intense puffing regime.(McAdam *et al.*, 2019)
A 3-day inpatient crossover study addressed differences in puffing behaviour for strawberry vs tobacco flavour (18mg/mL), and their usual brand e-liquid (3-18mg/mL). Relatively small differences in puff topography were found in puff topography for the different flavours.(St Helen *et al.*, 2018)
Thirty experienced electronic cigarette users, nicotine- abstinent for at least 12 hours, completed test sessions differing only by liquid propylene glycol:glycerol ratio; while device power (7.3W) and liquid nicotine concentration (18mg/ml) remained constant. When 100% propylene glycol based liquids were used, participants took shorter and smaller puffs but obtained significantly more nicotine relative to the glycerol-based conditions, resulting in higher total nicotine exposure. However, the experience was significantly less "pleasant" and "satisfying" relative to the other liquids. (Spindle *et al.*, 2018)
An Altria study evaluated whether a SODIM Smoking Puff Analyzer Mobile Device (SPA/M) was useful to measure puff topography during use of a prototype electronic cigarette in exclusive cigarette smokers (CS) and electronic cig users (electronic cigarette) under ad lib conditions in a clinic. When compared to a single use of their own

brand cigarettes, CS took longer puffs with similar puff volume from the electronic cigarette prototype. The puff duration, flow rate and peak flow were significantly lower ($p<0.05$) with the electronic cigs compared to cigarettes.(Vansickel *et al.*, 2018)

A natural environment observational study was conducted on experienced ENDS users to measure the effect of e-liquid flavor on topography and consumption behavior. The RIT wPUMTM monitor was used to record to record the date and time and puff topography for every puff taken by N = 34 participants over the course of two weeks. Results provide strong evidence that flavor affects the topography behaviors of mean puff flow rate and mean puff volume, and there is insufficient evidence to support an influence of flavor on mean puff duration and mean puff interval.(Robinson *et al.*, 2018)

Electronic cigarette topographies of established cigarette smokers and nonestablished cigarette smokers were compared using a . wireless hand-held monitoring device in users' everyday lives over 1 week. Young adult (aged 18-25) participants (N = 20) used disposable electronic cigarettes with the monitor as they normally would and responded to online surveys. Established cigarette smokers had larger first puff volume (130.9 mL vs. 56.0 mL, $p < .05$) and larger puff volume per session (1509.3 mL vs. 651.7 mL, $p < .05$) compared with nonestablished smokers. At marginal significance, they had longer sessions (566.3 s vs. 279.7 s, $p = .06$) and used electronic cigarettes more sessions per day (5.3 s vs. 3.5 s, $p = .14$). Established cigarette smokers also used electronic cigarettes for longer puff durations (3.3 s vs. 1.8 s, $p < .01$) and had larger puff volume (110.3 mL vs. 54.7 mL, $p < .05$) compared with nonestablished smokers. At marginal significance, they had longer puff interval (38.1 s vs. 21.7 s, $p = .05$).(Lee, Nonnemaker, *et al.*, 2018)

Puff topography data were collected using a wireless portable use monitor (wPUMTM) continuously over 2 weeks among N = 34 current second-generation e-cigarette users in their everyday lives. Multilevel latent profile analysis resulted in two session classes and three person types. Session class 1 was characterized by 14.7 puffs per session (PPS), low puff volume (59.9 ml), flow rate (28.7 ml/sec), and puff duration (202.7 sec x 100). Session class 2 was characterized by 16.7 PPS with a high puff volume (290.9 ml), flow rate (71.5 ml/sec), and puff duration (441.1 sec x 100). Person class 1 had almost exclusively "light" class 1 sessions (98.0%), whereas person class 2 had a majority of "heavy" class 2 sessions (60.7%) and person class 3 had a majority of "light" class 1 sessions (75.3%) but some "heavy" class 2 sessions (24.7%).(Lee, Morgan-Lopez, *et al.*, 2018)

Puffing behavior and topography were examined using calibrated CReSS pocket topography monitors over 24 hours among regular electronic cigarette users. Twenty-four adult electronic cigarette users (15 male) vaped their personal electronic cigarettes ad-lib over the course of 24 hours. Over 24 hours participants took on average 156.2+/-10.3 puffs, clustered in 10.2+/-7.9 puffs per puffing session with an average puff interval of 15.4+/-22.0 sec. A single puff lasted on average 3.0+/-1.2 sec, had a volume of 73.4+/-51.5 ml, and was taken with the average flow rate of 24.7+/-10.2 ml/sec.(Kosmider *et al.*, 2018b)

In an observational non-blinded study, active cigarette smokers were asked to replace cigarettes with electronic cigarettes over 4 weeks (replacement phase, RP) followed by exclusive electronic cigarette use for an additional 12 weeks (maintenance phase, MP). From 25 subjects that followed the protocol, sixteen succeeded in completing the RP and 8 the MP (32%). Success subjects showed significantly longer puff duration (seconds per vape) and total overall aerosol exposure (number of vapes x average vape duration or vape-seconds) in both study phases. Furthermore, subjects in the success group continued to increase the number of vapes, device voltage and wattage significantly as they transitioned into the MP.(Guerrero-Cignarella *et al.*, 2018)

Changes in puffing topography of experienced electronic cigarette users (vapers) were evaluated when changing power settings in electronic cigarette battery devices. Participants used their own liquids and an atomizer and battery provided by the researchers. Puff number and puff duration were lower at 10 W (46 [16] puffs and 3.8 [0.8] s) compared with 6 W (57 [20] puffs and 4.6 [1.0] s). Liquid and nicotine consumption was higher at 10 W (373 [176] mg and 4.2 [2.4] mg, respectively) compared with 6 W (308 [165] mg and 3.5 [2.3] mg, respectively).(K. Farsalinos *et al.*, 2018)

The effects were compared of (i) high versus low nicotine concentration e-liquid, (ii) fixed versus adjustable power and (iii) the interaction between the two on: (a) behaviour, (b) subjective effects, (c) nicotine intake and (d) exposure to acrolein and formaldehyde in everyday setting when using electronic cigarettes. Twenty experienced electronic cigarette users vaped ad libitum over 4 weeks (1 week per condition).Use of a lower nicotine concentration e-liquid may be associated with compensatory behaviour (e.g. higher number and duration of puffs) and increases in negative affect, urge to vape and formaldehyde exposure.(Dawkins *et al.*, 2018).

ANNEX 4: LITERATURE – SEARCH TERMS USED

Literature search on electronic cigarettes

The Scientific Committee on health, environmental and emerging risks, has received from the Commission a request for a scientific opinion on electronic cigarettes: https://ec.europa.eu/health/sites/health/files/scientific_committees/scheer/docs/scheer_q_013.pdf

In order to ensure that all relevant scientific information is available to the Scientific Committee for its assessment, we would like to ask you to carry-out a literature search.

The terms used in the searches should be:

- Smoking
- nicotine
- nicotine addiction
- nicotine concentration in e-cigarette
- heated tobacco
- Electronic Nicotine Delivery Systems
- evaporation-products
- Vaping
- ingredient
- liquid
- impurities
- addiction
- flavour
- additives
- Propyleneglycol
- Glycerine
- intoxikation
- dehabituation
- behaviour
- passive smoking
- steam density
- concentration of ingredients
- content
- effect
- health effect
- analytic
- technic and design
- risk
- risk assessment
- exposure assessment
- mixture toxicity

AND

e-cigarette **OR** electronic cigarette

The types of documents:

- peer reviewed articles
- journal entries
- book chapters
- government and non-government funded publications.

The terms should be searched in: Title, abstract, key word fields.

The period covered: no restriction