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Scientific Committee on Health, Environmental and Emerging Risks
SCHEER

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Preliminary Opinion on electronic cigarettes

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The SCHEER adopted this Opinion by written procedure on 23 September 2020.

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 concludes that on health effects

a) for users of electronic cigarettes

1. the overall weight of evidence for risks of local irritative damage to the respiratory tract is i) **moderate** for heavy users of electronic cigarette due to the cumulative exposure to polyols, aldehydes and nicotine, and ii) **not to be excluded** for average and light users. 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 **strong**.
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 poisoning and injuries due to burns and explosion, is **strong**. However, the incidence is low.
5. the overall weight of evidence for risks of other long-term adverse health effects, such as pulmonary disease, CNS and reprotoxic effects, plausible based on the hazard identification and limited human evidence, cannot be established due to **lack of consistent data**.
6. to date, there is **no specific data** that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure (but may enhance attractiveness).

b) for second-hand exposed persons

1. the overall weight of evidence is **moderate** for risks of local irritative damage to the respiratory tract.
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 **strong** 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.

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-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, 23 September 2020

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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.

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http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

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1. SUMMARY

The European 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 help the Commission in assessing the potential need for legislative amendments under the Directive or other regulatory/enforcement measures. 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 of 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. Exposure of electronic cigarette users is considered 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. However, consistency of the data

is judged to be low and the weight of evidence for second-hand exposure assessment is judged to be weak to moderate.

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 is no harmonised classification to clearly identify their hazards, 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, 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 whether children and adolescents have higher risk than adults when regularly second-hand exposed 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 (14.6%) in the EU. Among the general adult and young populations 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 factor 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.

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 76% 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 strong 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.

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. During this timeframe, experimentation with the use of electronic cigarettes for smoking cessation increased (3.7% to 9.7%), while on the contrary the use of pharmacotherapy (14.6% to 11.1%) and smoking cessation services (7.5% to 5.0%) declined across the EU. 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 experimentation with electronic cigarettes 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 for tobacco products (Article 20(6)). The regulation of flavours, local advertising and age limits are left to Member States.

[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

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 the five levels strong, moderate, weak, uncertain or not possible. 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 proportion. They are affecting nicotine delivery, appeal, and ease of product use influencing the individual preferences that may play a role in use patterns.

There are currently four generations of electronic cigarettes in the EU market, but this evolves in a very rapid way and other products, already marketed in the USA, are expected to come soon. It is noted that products as well as liquids used differ between EU and the US, with US allowing higher nicotine concentrations with respect to the limit of 20 mg/ml nicotine set by 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 most common ingredients of e-liquids from competent authorities compilation. 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 is 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 origin: 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 to which 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 with frequent addition of new ones. 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 smoking habits; 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 the characterisation of smoking protocols[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 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 cigarette use by the SCHEER.

Individuals may be second-hand exposed 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 those reported for exposure of electronic cigarette users. Data on second-hand exposure are however scarce, reported in different units and related to highly different exposure scenarios, device designs, topography, and liquid compositions. The consistency of the data therefore is judged to be low. The weight of evidence for second-hand exposure assessment is judged to be weak to 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 the cardiovascular system appear particularly relevant for the SCHEER conclusions on the use of electronic cigarettes. However, besides these, 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 harmonised classification to clearly identify their hazards, and

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

the toxicological profile has not been fully investigated, e.g. for many of them the toxicity following inhalation is unknown, nor whether 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, since 2016, the World Health Organization (WHO)[6] 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".

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 cough 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 it's 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 related risk is low.

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.

According to the literature, the level of evidence regarding the cardiovascular effects of nicotine contained in cigarettes and the related pathophysiological mechanisms is considered from moderate to strong, and it can be assumed that similar mechanisms exist regarding the exposure to nicotine from electronic cigarettes use.

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, 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 whether

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

children and adolescents have higher risk than adults when regularly second-hand exposed 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, and 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 affected by a high degree of uncertainty. As a consequence risks could not be properly assessed based on 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 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 was based on aerosol analysis data obtained from pre-defined exposure scenarios for daily users and on exhaled air, for second-hand exposure. 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 lines of evidence described in 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), and taking into account the moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes, 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:
    - These substances are all identified as irritants.
    - 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.
    - The model studies revealed low margins of exposure (MoEs) for irritative effects for individual chemicals and these will be even lower in an additive approach.
    - The alveolar concentrations of nicotine calculated are higher than or comparable to effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour.
    - 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, while the risk was considered not to be excluded or uncertain for average and light users.

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

- The overall weight of evidence for rosk of long-term systemic effects on the cardiovascular system is **strong**. The lines of evidence are the following:
    - Heart rate and blood pressure effects were identified as hazards for nicotine (and lead).
    - 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.
    - Based on human evidence, there is a moderate and growing evidence for harmful health effects for electronic cigarette users, especially, for cardiovascular disease.
    - The alveolar concentrations of nicotine calculated in the model studies are higher than effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour.

- The overall weight of evidence for risk for 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 lines of evidence are the following:
    - Nitrosamines, formaldehyde and acetaldehyde have been identified as genotoxic and carcinogenic.
    - The human evidence is very limited and does not allow a conclusion.
    - In the model calculations, exposure to the nitrosamines increased the calculated risk of tumour development in the respiratory tract, especially, in heavy users. It is assumed that this risk may increase due to cumulative exposure to these chemicals.
    - The formaldehyde-induced damage to the respiratory epithelium can be a precursor to tumour formation and 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.

- The weight of evidence for 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.

- The overall weight of evidence for risk for other long-term adverse health effects, such as pulmonary disease and CNS- and reprotoxic effects, plausible based on the hazard identification and limited human evidence, cannot be established due to **lack of consistent data**.

- To date, there is **no specific data** that specific flavourings used in the EU pose health risks for electronic cigarette users following repeated exposure (but may enhance attractiveness). The concentrations of aldehyde 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.

Overall assessment for second-hand exposed persons

Based on the lines of evidence described in 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), and taking into account the weak to moderate weight of evidence for the second-hand exposure assessment, 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:
  - This irritation is mainly due to exposure to glycols. Glycols are identified as irritants.
  - The model studies revealed low MoEs for irritative effects from propylene glycol.
  - MoEs for nicotine do not point at a risk for respiratory irritation.
  - Exposure of second-hand exposed persons to glycerol or aldehydes is negligible or orders of magnitude lower than for electronic cigarette users.

- The overall weight of evidence for risk for systemic cardiovascular effects in second-hand exposed persons due to exposure to nicotine is **weak to moderate**. The lines of evidence are the following:
  - Heart rate and blood pressure effects were identified as hazards for nicotine.
  - In the model calculations, the MoEs for cardiovascular effects are low.
  - 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.

- The overall weight of evidence for a carcinogenic risk due to cumulative exposure to TSNAs is **weak to moderate**. The lines of evidence are the following:
  - Nitrosamines have been identified as genotoxic and carcinogenic.
  - The MoEs calculated for the carcinogenic risk from TSNAs are low.
  - Human evidence is lacking.

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 "Special Eurobarometer 458" from May 2017, 15% of the respondents have at least tried electronic cigarettes and 2% use them regularly. Among young people (15-24 years), ever use is higher than average (25%), 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 also was substantially higher than reported ever use among other age groups. 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 more recent review on the prevalence of electronic cigarette use among the general adult and young populations 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. 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 topic are dealing with data from the US. Products on the US market may differ considerably with 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 harm perceptions and increase willingness to try and initiate use of electronic cigarettes. Adolescents consider flavour the most important factor 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. Flavoured electronic cigarettes are used at electronic cigarette initiation by the majority of youth. 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 "Special Eurobarometer 458" reports that the most popular flavour of electronic cigarette is fruit flavour (47%), followed by tobacco flavour (36%), menthol or mint (22%) and candy flavour (18%). Alcohol flavoured electronic cigarettes are the least popular, favoured by only 2% of respondents. Tobacco-flavoured electronic cigarettes are much more popular among those aged 55 or more (66%) vs those aged between 15 and 24 (19%), whereas younger respondents are much more likely to prefer fruit-flavoured electronic cigarettes (72%, compared with 17% of the oldest cohort) and somewhat more likely to prefer candy-flavoured electronic cigarettes (22%, compared with 11%). 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 increase product liking, 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 76% 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.

Health effects of electronic cigarette use are mainly due to nicotine, but are also associated with the particular flavour ingredients (including menthol) which are perceived as having diminished risk of harm from electronic cigarettes use, which are most often preferred by this population group and can contribute to attractiveness and addictiveness.

Overall, the SCHEER is of the opinion that there is **strong** 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 also **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 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. During this timeframe, experimentation with the use of electronic cigarettes for smoking cessation increased (3.7% to 9.7%), while on the contrary the use of pharmacotherapy (14.6% to 11.1%) and smoking cessation services (7.5% to 5.0%) declined across the EU. 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 experimentation with electronic cigarettes 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.

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 use for the Opinion firstly review articles published between 01.01.2015 and April 2019. If necessary, the primary sources were also used, as well as further articles of importance published after April 2019. 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 on 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, emmission) 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) simulate tobacco cigarettes by 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 consumption of nicotine-containing aerosol via a mouth piece, 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: disposable and refillable versions in different designs and there is a rapid development of the devices and their contents. 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 shapes and forms, electronic cigarettes are devices used to inhale a liquid that may contain nicotine and/or other chemicals and consist of a lithium battery, pressure sensor, control circuit board, and in some cases a light emitting diode. Electronic cigarettes were originally developed in China in 2003 to mimic conventional cigarettes and smoking via concomitant motor and sensory stimulation, including hand-to-mouth movement and visible "smoke" production (Cobb *et al.*, 2011).

This Opinion is restricted to the terms of references given by the European Commission. It covers electronic cigarette products complying with the TPD. Electronic cigarettes not containing nicotine are 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, 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, 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 induces the production of the characteristic aerosol associated with electronic cigarette use (Wang *et al.*, 2019). In addition, temperature driven chemical reactions occur and result in formation of degradation products (Visser *et al.*, 2014 and 2015; see also table 3).

The early devices looked like a conventional cigarette, often including a small light on the tip that lit when the user puffed. These early systems were generally inefficient at delivering nicotine, in part because the particle sizes of the aerosol were too large to penetrate deep into the lungs (Glantz *et al.*, 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 (Breland *et al.*, 2017).

There are currently four generations of electronic cigarettes (Glasser *et al.*, 2017; Farsalinos *et al.*, 2014; Strongin, 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 get 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).

It should be noted, that the electronic cigarette brand with the largest US market share (~75% as of 2019 and growing notable for their popularity among teens) is an electronic cigarette that uses changeable, nicotine salt-based liquid cartridges and temperature regulation to produce an aerosol as an alternative to traditional cigarettes. 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. It is like first-generation devices in that it does 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.

This electronic cigarette brand started entering the EU market in Q2 of 2018 and since Q1 of 2019 it is available in almost all European Member states. Although the trend needs to be monitored, in the EU the nicotine content has to be lower in line with the TPD restrictions as compared to that in the USA.

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 regulation on 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 include 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 and taking into account, inter alia, any addictive effect; 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 no more marketed in EU.

While the EU-CEG data are helpful for monitoring the market and signal hazards related to e.g. harmful ingredients in e-liquids, some limitations are present, mainly related to the need of checking by independent assessors the big 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

Country	Files submitted (total, including updates)	Unique products country (total)	Unique products country (active 09/2020)
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, where the nicotine concentration does not exceed 20 mg/ml. This concentration allows for a delivery of nicotine that is considered to be comparable to the permitted dose of nicotine derived from a standard cigarette during the time needed to smoke such a cigarette. 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, which have compiled lists of most common ingredients of e-liquids (see tables in Annex 2). Information indicate that the ingredients used in the Netherlands and in Greece are representative for the EU market in general. The SCHEER considered 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 and their Classification according to **CLP** (CE) n. 1272/2008 as reported to national competent authorities of the Netherlands and Greece

Ingredient name		**Most frequently used (%)**	**Recipe quantity Median (mg)**	**Concentration Median (mg/mL)**	**CLP**
Glycerol	NL GR	94.1	4968 5000	506	None
Propylene Glycol	NL GR	85.8	4152 4174	429.6	H302, H315, H319
Vanillin (F)	NL GR	35.2	7 8	0.89	H302, H315, H319
Ethyl maltol (F)	NL GR	32.0	5.9 10	1	H302
Ethyl Butyrate (F)	NL GR	28.4	3.6 3.2	0.34	H226, H315, H319, H335
Ethyl Acetate	NL GR	23.2	1.1 1.5	0.17	H225, H319, H336*
Ethanol (F)	NL GR	23.1	31 26	2.8	H225* H319; H350, H371,
Maltol (F)	NL GR	22.8	1.3 2	0.22	H302, H319
Ethyl Vanillin (F)	NL GR	19.4	6.8 8.7	0.88	H302, H315, H319
Furaneol (F)	NL GR	19.3	2 2.5	0.27	H302, H314, H317, H319
Methyl cyclopentenolone	NL GR	18.3	2		H302
Cis-3-hexenol (F)	NL GR	17.8	1.5		H226, H319
Isoamyl Acetate (F)	NL GR	16.3	2.3		H226*
Ethyl 2-Methyl Butyrate (F)	NL GR	16.0	2.2		H226
Acetic Acid	NL GR	15.7	1.2 1,2	0.13	H226, H314*
Triacetin (F)	NL GR	14.4	5.6		None
Benzyl Alcohol (F)	NL GR	14.2	3.3 4.6	0.5	H 302* H319
Menthol (F)	NL GR	12.1	18		H315, H319
Hexyl Acetate (F)	NL GR	10.3	1		H226
Sucralose (F)	NL GR	8.3	11		None

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
(*)Harmonised Classification (ECHA web site) All the other classifications are the H phrase most frequently attributed by Applicants reported on the ECHA web site
(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.*, 2019). 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 'nicotine booster' by consumers in order to add nicotine to hand-made e-liquid mixes (Havermans *et al.*, 2019). 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) 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 were 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.*, 2019)

6.5 Assessment of Health Risks

In order to evaluate the health risks related to the use of electronic cigarettes, the SCHEER follows different lines of evidence. The SCHEER is of the opinion, that mainly chemicals present in the aerosol 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 considered also 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 know (yet) 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 to when using electronic cigarettes, specific information on consumer behaviour is necessary like the frequency of use, number of puffs, puff duration, puff volume and puff interval. The

SCHEER compiled available information on prevalence rates, smoking behaviour and on smoking protocols to estimate exposure to different chemicals for electronic cigarette users. Exposure can be measured, or it can be calculated on the base 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 most recent "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. There was wide variation among EU Member states in the proportions of ever users of electronic cigarettes: the proportion of adults who were regular electronic cigarette users in 2017 ranged from 4.7% in the UK to 0.2% in Bulgaria.

Use in young populations, 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).

Smoker protocols – how a specific user uses an electronic cigarette, smoking 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 smoking behaviour were selected (DeVito and Krishnan-Sarin, 2018; Evans and Hoffman, 2014). The recent (2018) review of DeVito and Krishnan-

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

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 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.*, 2015) 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. 11 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; Robinson *et al.*, 2018; Lee *et al.*, 2018 a; Lee *et al.*, 2018b; Kosmider *et al.,* 2018; 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 smoking protocols 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.

6.5.2 Exposure assessment

A large number of devices and liquids are available on the market with frequent addition of new ones. 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 a smoking machine and as

measured in aerosol from electronic cigarette users. 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.*, 2014; Jensen *et al.*, 2015). Emitted (inhaled) aerosol is highly concentrated and contains mainly submicrometric-size particles. 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 (Chad *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 production of aerosol 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.*, 2014; 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).

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. 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).

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 mm. 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*)*.*

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

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

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 whether the particles 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 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):

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 already was 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) recently 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).

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 however will 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 retained by users of electronic cigarettes after inhalation. Because electronic cigarettes are only active when users take a puff, electronic cigarettes do not continue to smoulder between puffs. 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.*, 2019), 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 production 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).

6.5.2.3 Quantification of aerosol concentrations

Electronic cigarette users

The quantification of the aerosol composition is frequently done by simulating the use of electronic cigarettes under controlled conditions in so-called smoking machines. Experimental variables are the puff volume, puff flow rate, puff frequency, the type and temperature of the smoking device, and the voltage of the battery. The most controlled studies are discussed below.

Visser *et al.* (2014 and 2015) used a smoking 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	14	0.001	0.142	0.051	mg/puff
nicotine					
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					
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

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.*, 2014; 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 a smoking 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	Machine smoking (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	Machine smoking (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:

Reference	Methodology for carbonyl trapping/analysis	Type of electronic cigarette(s)	Liquid(s) used	carbonyl emissions
				up to 82.9 µg/l aerosol No correlation with flavourings
Flora *et al.*, 2017	Machine smoking (puff volume: 55 ml, puff duration: 4 seconds, puff interval: 30 seconds, direct trapping in DNPH-solution, HPLC	6 types of first-generation electronic cigarettes	Not reported	formaldehyde: 70-14100 ng/puff acetaldehyde: 30-13610 ng/puff acrolein: up to 4110 ng/puff crotonaldehyde: up to 40 ng/puff formaldehyde emissions rises sharply above 350 C
Ogunwale *et al.*, 2017	Machine smoking (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
Sleimann *et al.*, 2016	Machine smoking (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	Machine smoking (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	Machine smoking (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	Machine smoking (puff volume: 70 ml, puff duration 3 seconds, puff interval: 10 seconds, direct trapping in DNPH-solution, HPLC	First-generation electronic cigarette		formaldehyde: 0.48-2.5 µg/l aerosol acetaldehyde: 0.58-1.52 µg/l aerosol acrolein: 0.4-2.1 µg/l aerosol
Farsalinos *et al.*, 2015	Machine smoking (puff volume: 60 ml, puff	New generation rebuildable tank	Glycerol (45%) propylene	formaldehyde: up to 1100 ug/puff

Reference	Methodology for carbonyl trapping/analysis	Type of electronic cigarette(s)	Liquid(s) used	carbonyl emissions
	duration 4 seconds, puff interval: 30 seconds, direct trapping in DNPH-solution, HPLC	electronic cigarette,	glycol (45%, water (8%)	acetaldehyde: up to 450 ug/puff acrolein: up to 100 ug/puff Much higher levels at dry puff conditions
Tayyarah and Long, 2014	Machine smoking (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	Machine smoking (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.*, 2014	Machine smoking (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	Machine smoking (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 at 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.* (2014) 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* (2015) analysed TSNAs, using a second-generation device and three commercial e-liquids. No TSNAs were detected in the aerosol. Goniewicz *et al.* (2014) 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 a smoking 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 a smoking 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/m3, respectively (Klager *et al.*, 2017)

Metals

Goniewics *et al.* (2014) analysed the aerosols generated by a smoking 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.* (2015) 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 smoking 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 ML, *et al.*, 2014. 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.*, 2014), 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 2014; 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.*, 2015). 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 showing 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.

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

Compound	Maximum aerosol concentration Visser *et al.*, 2014 and 2015 (µg/l)	Maximum aerosol concentration other studies[1] (µg/l)		
		Margham, 2016	Olmedo, 2017	Halstead *et al.*, 2019
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	nm		
NNN	0.0038	0.00098		
NAT	0.0012	0.000236		
NAB	0.0001	nm		
NNK	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

nm= not measured [1] Other studies than Visser *et al.* in this section 6.5.2.3.

The higher carbonyl levels in several studies most probably are generated under dry puff conditions and can be considered unusable for the risk assessment.

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. 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. Exposure results are summarised in table 6. 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**		**Median**	**unit**
		min	max		
carrier liquid and nicotine	17	<LOQ	2140	108	ng
nicotine					
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					
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 total TSNAs	9	<LOQ	77	28	pg
Metals					
copper	3	<LOQ	2.92	<LOQ	ng
all other metals	3	<LOQ	<LOQ	<LOQ	ng

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.

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).

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 however scarce, reported in different units and related to highly different exposure scenario's, device designs, topography, and liquid compositions. The consistency of the data selected therefore is judged to be low.

The weight of evidence for second-hand exposure assessment is judged to be weak to 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.

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 (Khlystov and Samburova, 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 not a 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, or 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 measured 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. About 60 mg is fatal for humans. Death from respiratory paralysis occurs after only a few minutes.

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.*, 2017).

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 SCENIHR Opinion on Tobacco additives 1 (2016).

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. However, as said, their toxicity after inhalation, the major route of exposure for electronic cigarette users, is largely untested. It has been reported that they may be potentially harmful (Zare *et al.*, 2018): indeed when reviewing the health impact of flavour in 7 studies, several e-liquids resulted as potentially allergenic (Hutzler *et al.*, 2014). Most importantly, other can cause airway resistance (Pisinger and Dossing, 2014) and respiratory irritation (Tierney *et al.*, 2016).

Besides possible toxic effects after inhalation, these chemicals may confer a characterising flavour to the e-liquid meaning a clearly noticeable smell or taste as for maltol, menthol or vanillin, thus contributing to attractiveness of electronic cigarettes. Flavourings can stimulate electronic cigarette use, especially among vulnerable groups such as non-smoking adolescents, thereby increasing exposure to potentially toxic ingredients. Indeed, the flavours by providing a specific and standardised taste, makes an e-liquid unique and recognisable among the large variety of available brands, thus binding the consumer (Havermans *et al.*, 2019). This was confirmed by a survey conducted in 2017 and related to ~20 000 e-liquids marketed in the Netherlands, identifying 245 unique flavour descriptions, reflecting flavour preference of electronic cigarette users (Havermans *et al.*, 2019).

Addictiveness is another possible negative effect associated to electronic cigarette use to which the composition of e-liquid can contribute. Indeed, it can be achieved, for example, by adding chemicals increasing the bioavailability of nicotine, altering the pH of the liquid or facilitating the inhalation, as in the case of additives with local anaesthetic effects such as menthol.

Menthol is a multifunctional additive. It is an effective anaesthetic, antitussive agent that may increase the sensation of airflow and inhibit respiratory rate, thereby allowing increased lung exposure to nicotine and other e-liquid ingredients. It may increase the absorption and lung permeability of aerosol, thereby increasing nicotine uptake while decreasing the irritation from nicotine. This action may increase the likelihood of nicotine addiction in adolescents and young adults who experiment electronic cigarettes and make it more difficult to quit (SCENIHR, 2016).

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).

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 produces toxic aldehydes. Although a direct relationship between enhanced aldehyde levels and flavour compound concentration has been reported (Khlystov and Samburova, 2016), 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 production of aldehydes has been associated to oxidative stress (Lerner *et al*, 2015; Muthumalage *et al*, 2018) and inflammatory responses (Gerloff *et al*, 2017; Leigh *et al*, 2016).

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.

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[9].

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.

[9] 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

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.

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 which 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 possible 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 and hazard classification of nicotine and tobacco-specific nitrosamines, carbonyl compounds and metals have been collected from international bodies or organisations. Therefore this information is considered to provide strong evidence. For chemicals with 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 / Compounds	IRRITANT (skin and eye membranes)	IRRITANT (respiratory tract[1]/GIT mucosa[2])	CNS (neuro-toxicity)	CVD (heart-rate and blood pressure)	Genotoxicity/ Carcinogenicity (nasal cavity, liver, lung)	Other (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	X	X[1]			X	
Acetaldehyde	X	X[1]	X		X	
Acrolein	X	X[1]				
Flavourings (**)	X					
Metals:						
Chromium VI		X[1], X[2]			X	X[1]
Copper		X[1]	X			
Zinc		X[2]				
Tin			X			
Lead			X	X		X[2]

(*) – irritant effects to skin&eye have been notified in ECHA C&L Inventory 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 (H317) and/or serious eye damage (H319).

6.5.4 Human evidence for health impacts of electronic cigarettes

The health impacts of electronic cigarette's use are still difficult to be established due to the lack of long-term data from epidemiological studies or clinical trials. However, since 2016, the World Health Organization (WHO)[10] 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*

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

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.

Acute effects

If assessed, acute mouth / throat irritation, and cough are reported by a sub-group of users (Polosa et al., 2011; Palamidas et al., 2017), these effects are not attributed to the nicotine content (Palamidas et al., 2017). It is speculated that these effects are caused by hyperventilation, which is associated with long puffing time (Morjaria *et al.*, 2011).

Palamidas *et al.* studied short term use of nicotine electronic cigarettes 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. More-over, short-term use of nicotine-free electronic cigarettes increased Raw and decreased sGaw among healthy never smokers (Palamidas *et al.*, 2017).

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[11]. 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 relevant 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. The EHN underlined 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. Finally, EHN recommends that health professionals should inform patients and the public of the risks related to electronic cigarette use. 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, Moheimani *et al.*, 2017), (atomized) nicotine impacts

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

vasculature (Zhang *et al.*, 2018) and arterial stiffness (Vlachopoulos *et al.*, 2016) similarly to conventional tobacco smoking, ultimately inducing hypertension (Moheimani *et al.*, 2017), 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 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 (Moheimani *et al.*, 2017), 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, Moheimani *et al.*, 2017) 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 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 cigarette use. The acute sympathomimetic effect of nicotine containing electronic cigarette can possibly be associated with increased cardiac risk populations with and without known cardiac disease. (Moheimani *et al.,* 2017).

Recent findings demonstrate that volatile liquids containing nicotine may induce adverse cardiovascular effects attributed to its toxic impact on myocardial cells. Most electronic cigarettes containing nicotine have a basic pH > 9, which seems to enhance the dosage of nicotine delivered (Stepanov and Fujioka, 2015). 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 (Moheimani *et al.*, 2017, 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

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). 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). 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. However, the effects of long-term use particularly in relation to lung cancer remain to be determined in epidemiological investigations (Chun *et al.*, 2017, Murthy, 2017).

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, it was reported lack of evidence 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 sex 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.*, 2017). 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.*, 2017). While respiratory exposure was more frequent among paediatric patients, ocular exposure was more frequent among adults (Vardavas *et al.*, 2017). 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.*, 2017).

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, 2017). However, a limited number of studies (Ballbe *et al.*, 2014, Flouris *et al.*, 2013), mimicking real-life situations, regarding the impacts of passive smoking due to electronic cigarettes currently exists (Shearston *et al.*, 2019), evaluating primarily the effects upon airborne nicotine levels, serum cotinine, lung function, complete blood counts and inflammatory marker levels (Shearston *et al.*, 2019). Of these, solely a single study which evaluates the effects of regular passive smoking exposure due to electronic cigarettes within the home, demonstrating increased levels of ambient air nicotine and biomarkers of nicotine (Ballbe *et al.*, 2014).

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 impacting 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, 2017). 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. In 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 populations, 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 victims' pant pocket and ignites the victims 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 it's 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-work '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 form the United States and the rest from EU MS.

Only 10 entries refers 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 and 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 by a recall of the products from end users.

The remaining entries are classified as risks coming from 'chemical products' and generally refers 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 form the content and for that reason they were withdrawn from the market. However, the lack of child-proof fastening and opening mechanism was described also 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 connected 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 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 TPD and 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 beside being not compliant with 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 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 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, acute effects of electronic cigarette use are mouth/throat irritation, and cough and is reported by a sub-group of users, this effect seems not to be related to the nicotine content and the overall incidence was low. 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 prioritized for the risk assessment (Section 6.5.5.1). The preferred approach for the risk assessment will be explained in Section 6.5.5.2. Risk assessments will be presented based on simulations and based on 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.* (2020) 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.* 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 also will 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), often taken from an animal experiment 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 2015a) 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: fifteen inhalations per day, 1 puff per 4 minutes, with a total daily use duration of sixty minutes.
2. Average user: sixty inhalations per day, 1 puff per 2 minutes, with a total daily use duration of 120 minutes.
3. Heavy user: five hundred 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 maybe is not worst case 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 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, also air will 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 smokers 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. Systemic effects on the cardiovascular system are considered possible since the alveolar concentrations calculated are higher than effect concentrations in human volunteer studies. 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. 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. 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 pulmonary retention (0% for local effects, 50% for systemic effects), that exhalation of the chemical may not have been complete in the first exhalation but may continue with subsequent exhalations, and taking into account ventilation. 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. 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.*, 2015; 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 these quotients are based under the assumption of continuous exposure and therefore likely to be overestimated.

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 which are mainly being assessed for oral exposure through food.

Tierney *et al.* (2016) analysed flavour chemicals in 2 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 are occurring 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 4 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. Scungio *et al.* (2018) evaluated the excess lifetime carcinogenic risk (ELCR) of substances on particulate matter in second-hand smoke 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, 3 random clinical trials (RCT) and 9 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).

6.5.5.6 Conclusions

On risks for electronic cigarette users

In its report on "Electronic Nicotine Delivery Systems and Electronic Non-Nicotine Delivery Systems (ENDS/ENNDS)" published in August 2016 the WHO (WHO, 2016) stated: "Based mostly on the levels and number of toxicants produced during the typical use of unadulterated ENDS/ENNDS made with pharmaceutical-grade ingredients, it is very likely that ENDS/ENNDS are less toxic than cigarette smoke. However, ENDS/ENNDS are unlikely to be harmless, and long-term use is expected to increase the risk of chronic obstructive pulmonary disease, lung cancer, and possibly cardiovascular disease as well as some other diseases also associated with smoking. The magnitude of these risks is likely to be smaller than from tobacco smoke although there is not enough research to quantify the relative risk of ENDS/ENNDS over combustible products".

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), and taking into account the moderate to strong weight of evidence for the exposure assessment for users of electronic cigarettes, 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

  - These substances are all identified as irritants.
  - 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.
  - The model studies revealed low MoEs for irritative effects for individual chemicals and these will be even lower in an additive approach.
  - The alveolar concentrations of nicotine calculated are higher than or comparable to effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour.
  - 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, while the risk was considered not to be excluded or uncertain for average and light users.

- The overall weight of evidence for risk of long-term systemic effects on the cardiovascular system is strong. The lines of evidence are the following:
  - Heart rate and blood pressure effects were identified as hazards for nicotine (and lead).
  - 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.
  - Based on human evidence, there is a moderate and growing evidence for harmful health effects for electronic cigarette users, especially, for cardiovascular disease.
  - The alveolar concentrations of nicotine calculated in the model studies are higher than effect concentrations in studies with human volunteers exposed repeatedly to nicotine vapour.

- 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:
  - Nitrosamines, formaldehyde and acetaldehyde have been identified as genotoxic and carcinogenic.
  - The human evidence is very limited and does not allow a conclusion.
  - In the model calculations, exposure to the nitrosamines increased the calculated risk of tumour development in the respiratory tract, especially, in heavy users. It is assumed that this risk will increase due to cumulative exposure to these chemicals.
  - The formaldehyde-induced damage to the respiratory epithelium can be a precursor to tumour formation and 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.

- 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.

- The overall weight of evidence for risk of other long-term adverse health effects, such as pulmonary disease and CNS- and reprotoxic effects, plausible based on the hazard identification and limited human evidence, cannot be established due to lack of consistent data.

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

of aldehyde 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), and taking into account the weak to moderate weight of evidence for the second-hand exposed persons, 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:
  - This irritation is mainly due to exposure to glycols. Glycols are identified as irritants.
  - The model studies revealed low MoEs for irritative effects from propylene glycol.
  - MoEs for nicotine do not point at a risk for respiratory irritation.
  - Exposure of bystanders to glycerol or aldehydes is negligible or orders of magnitude lower than for electronic cigarette users.

- 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:
  - Heart rate and blood pressure effects were identified as hazards for nicotine.
  - In the model calculations, the MoEs for cardiovascular effects are low.
  - 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.

- The overall weight of evidence for a carcinogenic risk due to cumulative exposure to TSNAs is weak to moderate. The lines of evidence are the following:
  - Nitrosamines have been identified as genotoxic and carcinogenic.
  - The MoEs calculated for the carcinogenic risk from TSNAs are low.
  - Human evidence is lacking.

Further research is needed whether children and adolescents have higher risk than adults when regularly second-hand exposed 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, 7 reviews were found in the period 2016-2019 that covered this topic. It needs to be noted that most of the included studies have been carried out in the US. The SCHEER is aware, that US data may 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 given as far as possible.

Electronic cigarettes are rapidly becoming a new trend among adolescents (Perikleous, *et al.*, 2018). In the US, they have become the most common tobacco products used by

youth, driven in large part by marketing and advertising by electronic cigarette companies (Fadus, *et al.* 2019, Walley, *et al.* 2019). A 2016 review already showed that adolescents were nearing complete awareness of electronic cigarettes (Greenhill, *et al.* 2016). US current use among high school students increased from 1.5% in 2011 to 20.8% in 2018 (Fadus, *et al.* 2019, Walley, *et al.* 2019). This leads to concern that electronic cigarettes may be exposing a significant number of youth to nicotine who would have not otherwise be using tobacco, and additionally a "gateway" effect for combustible cigarettes and cannabis use has been suggested (Fadus, *et al.* 2019). Among adolescents, older age, male gender, conventional smokers, peer influence, daily smoking, and heavier smoking are the most common characteristics of electronic cigarette users (Perikleous, *et al.* 2018). In the EU, according to the "Special Eurobarometer 458" (May 2017), 15% of the respondents have at least tried electronic cigarettes and 2% use them regularly. Among young people (15-24), ever use is higher than average (25%), but no data are reported on current use per age group. However, these responses are from early 2017, and new data with a focus on youth use are warranted, given the dynamic electronic cigarette market, and the increase among youth use reported in the US. A recent review on the prevalence of electronic cigarette use among the general adult and young populations 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.* 2019). 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.[12]

[12] 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 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).

In the EU, according to the "Special Eurobarometer 458" (May 2017), the most frequently mentioned reason (61%) for taking up electronic cigarettes was to stop or reduce tobacco consumption. Other reasons included electronic cigarettes being perceived as less harmful (31%), and lower cost (25%). Regarding the two most often-mentioned reasons, reducing tobacco consumption and being less harmful, more than three quarters of those aged 40 or over (76-78%) cite one of these as a reason, vs. 59% of those aged 15-24. Regarding product type, especially pod devices have 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 with 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 8 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). 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 Krusemann, *et al.,* 2018). 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 (Krusemann, *et al.,* 2018).

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 factor 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 the 76% 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 in 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) 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 "Special Eurobarometer 458" (May 2017), a relative majority are in favour of banning flavours in electronic cigarettes (40% in favour vs. 37% against). Interestingly, younger respondents (15-24) and electronic cigarette users (49% and 84% resp.) are more likely to oppose a ban on flavours in electronic cigarettes, 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 "Special Eurobarometer 458" (May 2017) also reports that the most popular flavour of electronic cigarette is fruit flavour (47%), followed by tobacco flavour (36%), menthol or mint (22%) and candy flavour (18%). Alcohol flavoured electronic cigarettes are the least popular, favoured by only 2% of respondents, while a small minority (3%) also mentioned other, unspecified, flavours. Tobacco-flavoured electronic cigarettes are much more popular among those aged 55 or more (66%) vs those aged between 15 and 24 (19%), whereas younger respondents are much more likely to prefer fruit-flavoured electronic cigarettes (72%, compared with 17% of the oldest cohort) and somewhat more likely to prefer candy-flavoured electronic cigarettes (22%, compared with 11%).

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 Krusemann, *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 Krusemann, *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 the 76% 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 desire to smoke (O'Connell, *et al.,* 2019).

The popular pod device utilizes protonated nicotine, which the company claims provides 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 2 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 not containing 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 the 76% 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 regards 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 9 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, 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. 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 had different survey methods, sample sizes, age groups and differed in 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 or 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 the 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 either are compiled of either 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 are a number of studies that 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.,* 2018c).

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 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.

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.*, 2018; 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 also was substantially higher than reported ever use among other age groups s (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).

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).

There is information 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%, 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 strong 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 the 25 by 2025 goals, and the WHO Regional Office for Europe has professed their ultimate goal to have 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. During this timeframe, experimentation with the use of electronic cigarettes for smoking cessation increased (3.7% to 9.7%), while on the contrary the use of pharmacotherapy (14.6% to 11.1%) and smoking cessation services (7.5% to 5.0%) declined across the EU (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 quit attempts and intention to quit, and use of cessation assistance among a cohort of smokers from eight European countries indicated that experimentation with electronic cigarettes 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 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 1year, 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."*

In addition, the European Heart Network reported that there is not sufficient evidence until now that electronic cigarettes' use is an effective mean for smoking cessation.

There is a lack of robust longitudinal data on the effect of electronic cigarettes on smoking cessation.

7. MINORITY OPINIONS

None.

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9. 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;
electronic 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;
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;
Trap, ion trap;
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 a constituent in cigarette smoke encompasses two areas of effort: sample preparation and instrumental analysis. Sample analysis involves sample extraction and sample collection from liquid and smoke/aerosol.

The analytical methods depend on the chemical compounds analysis, 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.

- **Propylene glycol** was found to be present in all liquids, because it was used as the solvent for nicotine and flavours. The agreement was considerably poorer for the remaining e-liquid ingredients, mainly flavours [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 differentiated for electronic cigarette-liquids and electronic cigarette aerosols, aerosol, smoke are presented in tables A1.1 to A1.3.

Table A.1.1: Methods for nicotine and nicotine-related compounds

Literature	Nicotine	TSNAs	Aldehydes	Metals	VOCs	Phenols	PAHs	Drugs	Alkaloids
8	LC/MS/MS								
11	UHPLC/DAD, GC/FID, GC/MS								
14	GC/TSD								

Literature	Nicotine	TSNAs	Aldehydes	Metals	VOCs	Phenols	PAHs	Drugs	Alkaloids
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 [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 nicotine-related compounds	Cartridge	HSGC-MS	28
	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

Analytes or classes of analytes	Matrices	Analytical techniques	References
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 ; 41

[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]

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

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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

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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).

		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 ecigarette 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, 2018)
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 ecigarette 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.*, 2018)

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