Chemical Composition of Electronic Cigarette E-Liquids: Overview of Current Evidence of Toxicity

KULHÁNEK, A.[1], BAPTISTOVÁ, A.[1,2]

1 | Charles University, First Faculty of Medicine and General University Hospital in Prague, Department of Addictology, Prague, Czech Republic
2 | Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

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SUMMARY: The term "electronic cigarette" includes a broad range of different electronic devices with which to inhale aerosols. The e-liquid vaporisation process comprises a chain of several chemical reactions which result in hundreds of chemical compounds. The composition and concentration of chemicals contained in e-liquids or, more precisely, in the resulting aerosol, are difficult to identify and quantify. This review article includes a summary of current evidence about the toxicity of selected chemical substances contained in electronic cigarette liquids. Forty initial sources were selected in online databases on the basis of a non-systematic literature review using a set of keywords. This overview presents selected categories of e-liquid ingredients according to their functions and chemical composition. The most frequent substances include bulking agents (propylene glycol and glycerol). So far, the least-explored category consists of a wide range of flavourings. A separate category is represented by nicotine, the cause of the development of physical dependence. Another, highly diverse group of compounds is chemical substances formed as by-products of vaporisation. Knowledge of the toxicity of e-cigarettes is essential to assessing the degree of associated risks, as well as to establishing appropriate regulatory and public health measures.

Keywords | Electronic Cigarettes – ENDS – Toxicity – Aerosol – Nicotine – E-Liquid

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Corresponding author | Adam Kulhánek, PhD, Charles University, First Faculty of Medicine and General University Hospital in Prague, Department of Addictology, Apolinářská 4, 128 00 Prague 2, Czech Republic **adam.kulhanek@lf1.cuni.cz**

1 INTRODUCTION

Electronic cigarettes (also called e-cigarettes) represent a heterogeneous group of electronic devices designed to heat liquids (e-liquids). As shown in *Figure 1*, the device consists of a battery, an atomiser (heating element) with a metal heating coil, an e-liquid cartridge (tank), and a mouthpiece (Brown & Cheng, 2014). The vaporisation of the e-liquid takes place when it is aspirated in the absorption medium (cotton, cotton wool, or siliceous wicks) with the subsequent introduction of e-liquid to the pre-heated heating element. The vaporisation temperature varies between 100 °C and 350 °C, depending on the electric resistance of the heating coil metal (Zhao et al., 2016). Heating the e-liquid leads both to the development of gaseous phase and to the development of an aerosol as a heterogeneous mixture of solid and liquid particles dispersed in a gas, i.e. a mixture of smoke and mist. The amount of aspirated aerosol can differ substantially among users and depends on a number of factors. For a healthy adult, inhaling once from an e-cigarette amounts to approximately 500 ml (National Academics of Sciences and Medicine 2018). The resulting chemical composition is influenced especially by the following factors: 1) the composition of the e-liquid, 2) the materials used in the individual e-cigarette components, and 3) the device output and heating temperature. The aerosol dose is influenced especially by the device power output and method of inhaling. The vaporisation of e-liquid in electronic cigarettes therefore represents a cascade of several chemical reactions, which result in hundreds of chemical substances whose composition and concentration are exceedingly difficult to analyse (Pepper, Byron, Ribisl, & Brewer, 2017).

This article acts as a basic overview of the chemical substances contained in the liquids of electronic cigarettes, more precisely in the aerosol created by heating the e-liquid, grouped according to their functions and chemical nature.

2 METHODS

A non-systematic literature review was performed to identify key sources providing an overview of the chemical composition of liquids for electronic cigarettes and of the effects of selected chemical substances. The search process was performed using the PubMed (NCBI), Web of Science, Scopus, SciFinder, and Reaxys online databases. The search criteria were publications written in English and published between 2000 and 2020. A combination of the following keywords was applied to search for resources: “electronic cigarette”, “ENDS”, “electronic nicotine delivery system”, “e-cigarette constituents”, “e-liquid”, “liquids”, “e-cigarette aerosol”, “flavourings”, “toxicological study”, “in vitro toxicity”, “in vivo toxicity”, “chemical composition”, “inhalation”, and “nicotine”. A total of 47 sources were selected, predominantly research articles, reports, and guidelines. Additionally, four chemical literature sources older than the selection criteria were included to describe the effect of selected chemical substances.

3 BASIC COMPOUNDS IN E-LIQUIDS AND AEROSOL OF E-CIGARETTES

The liquid for electronic cigarettes (also called e-liquid) contains especially a mixture of propylene glycol (PG) and glycerol in various mixing ratios (hundreds of mg/mL). The mixtures can also contain nicotine (up to tens of mg/mL), depending on whether they are nicotine-containing or nicotine-free e-liquids. The liquid also contains tens of other different substances with various functions. These can affect the proper formation of smoke or have an influence on taste, etc. These substances are e.g. aldehydes, trace elements, volatile organic compounds (VOC), phenols, polycyclic aromatic hydrocarbons (PAH), and heavy metals (*Table 1*). The concentration of these substances amounts to

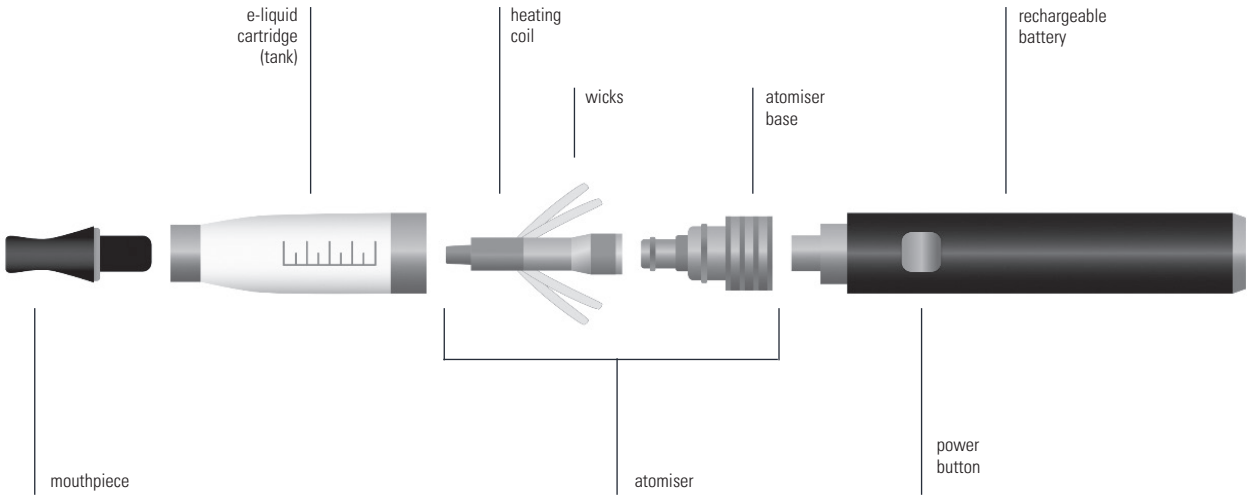


Figure 1 | Construction of an electronic cigarette

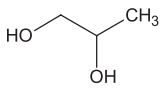
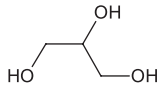
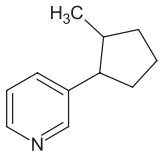
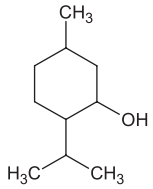
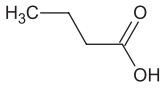
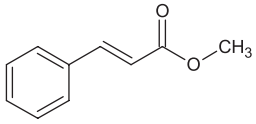
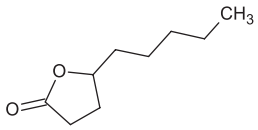
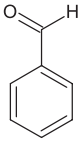
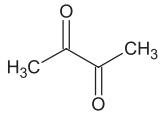
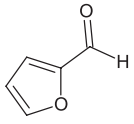
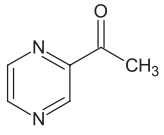
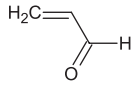
Trivial Compound Name	Structural Formula	Category	Functions
Propylene Glycol		Bulking agent	One of the main components of e-liquid
Glycerol		Bulking agent	One of the main components of e-liquid
Nicotine		Constituent causing physical dependence	Highly addictive neurotoxin
Menthol		Flavouring	Induction of mint-like taste
Butyric acid		Flavouring	Induction of cheese-like taste
Methyl cinnamate		Flavouring	Induction of sweet taste
gamma-Nonalactone		Flavouring	Induction of coconut butter-like taste
Benzaldehyde		Flavouring	Induction of fruit-like taste
Diacetyl		Flavouring	Induction of butter and caramel-like taste
Furfural		Flavouring	Induction of pastry-like taste
2-Acetylpyrazine		Flavouring	Induction of popcorn-like taste
Acrolein		Toxic substance formed by vaporization	Compound formed during glycerol oxidation
Formaldehyde		Toxic substance formed by vaporization	Compound formed during glycerol and propylene glycol oxidation

Table 1 | Selected chemical constituents of e-liquids

one ng/mL or less. The same concentration levels may be exhibited by other, undesirable substances, such as pesticides and various unknown impurities. The aerosol inhaled by the user may contain up to several hundreds of different compounds (Pepper et al., 2017), as some chemical substances are formed by thermal decomposition during evaporation (Beauval et al., 2017; Herrington & Myers, 2015).

Although nearly all e-cigarettes (or e-liquids) contain PG and glycerol as carrier compounds, in 2014 nicotine salts containing a nicotine base and a weak organic acid (benzoic acid or lactic acid) were introduced onto the market (National Academies of Sciences and Medicine 2018). Through neutralisation this combination forms a nicotine salt which can be commonly found in tobacco leaves. Nicotine salt can bring some subjective advantages to e-cigarette users (such as a taste more similar to that of a regular cigarette, more efficient nicotine absorption, a less intensive feeling of a sore throat, and lower filling consumption).

3.1 Bulking agents

The bulking agents in e-liquids are usually propylene glycol (PG) and glycerol.

3.1.1 Propylene glycol

PG (IUPAC name propane-1,2-diol) is a clear, viscous, non-turbid, odourless, slightly sweet-tasting liquid. PG is easily miscible with water (under normal conditions). PG is used in cosmetics, pharmaceuticals, and in the food industry as a humidifier or bulking agent. According to the Food and Drug Administration (FDA), it has been classified as "generally known and safe" since 1973 (HHS, 2015). However, this list has been created for oral ingestion, not for inhalation. PG is partially metabolised to pyruvate in the liver through lactic acid, and partially excreted unchanged in urine (Nielsen & Ingvartsen, 2004).

Although preclinical studies proved that human exposure to inhaled PG may be safe for months (Robertson, Loosli, et al., 1947), allergic reactions and respiratory tract irritation immediately following the inhalation have been documented (Wieslander, Norbäck, & Lindgren, 2001). The long-term effects of PG aerosol inhalation in humans are not yet known. There are no studies which have assessed the PG concentration in blood following the use of an electronic cigarette (National Academies of Sciences and Medicine, 2018).

3.1.2 Glycerol

Glycerol (preferred IUPAC name propane-1,2,3-triol) is a clear, viscous, hygroscopic, odourless, sweet-tasting liquid with good solubility in water. Glycerol can be extracted from natural oils and fats (glycerol is also called "vegetable glycerin/glycerine" or "VG") as one of the products of reactions in the petrochemical industry. Like PG, it is commonly used in the pharmaceutical and food industries, and according to the FDA it is classified as "generally known and safe" (HHS, 2017). Glycerol is an endogenous substance which is used as a precursor for glucose metabolism. Free glycerine can be found in blood plasma in concentrations between 0.05 and 0.1 mmol/L (Nelson, Harmon, & Robergs, 2011). Glycerol is predominantly metabolised in the liver and excreted in urine (Lin, 1977). As mentioned earlier, glycerol and PG are the main constituents of electronic cigarette liquids, while the glycerol content in e-liquids can vary between 30 and 50%. Acute toxicity of glycerol aerosol was observed e.g. in rats (five male animals) (CIR, 2015). The animals were subjected to glycerol which was heated to 200 °C and converted to aerosol with a concentration of 11.0 mg/L. The average lethal time of 50% of the animals (LT_{50}) was 423 minutes. Studies of repeated doses of glycerol aerosol administered to rats have also been conducted (Renne et al., 1992). The result of these toxicological studies is "no observed adverse effect level" (NOAEL) = 1,000 mg/m^3 (six hrs/day, five days/week, two weeks) and at 0.167 mg/dm^3 (six hrs/day, five days/week, 13 weeks) (Renne et al., 1992).

The Occupational Safety and Health Administration (OSHA, 2006) determined the provisional dose limit value 10 mg/m^3 for the duration of eight hours for all organic "mists" (usable for PG and glycerol).

3.2 Constituent causing dependence

Nicotine (IUPAC name 3-[(2S)-1-Methylpyrrolidin-2-yl] pyridine) is a clear, tasteless, odourless, colourless to yellowy liquid (normal conditions). Nicotine is hygroscopic and is well soluble in alcohol and ether. Nicotine is slightly basic (pK_a = 8.5). It forms solid nicotine salts soluble in water. Nicotine is an optical isomer, and it occurs naturally almost exclusively as a left-handed enantiomer. This pyridine alkaloid occurs naturally in tobacco plants (Benowitz et al., 2009).

Nicotine is distributed through blood vessels and enters the brain by passing through the blood-brain barrier. By inhaling, nicotine arrives to the nicotine acetylcholine receptors within 10–20 seconds, and the elimination half-life is approximately two hours. Nicotine is metabolised in the liver to pharmacologically inactive cotinine (up to 15%) and is excreted primarily in urine. Both absorption and excretion are influenced by pH (Benowitz et al., 2009).

Generally, nicotine can be regarded as a highly addictive neurotoxin (Benowitz, Hukkanen, & Jacob, 2009). In the brain, it binds to α4β2 acetylcholine nicotine receptors, which activate the reward system in the brain through the ventral tegmental area and the nucleus accumbens (Benowitz, 2010). The resulting dopamine projection to the prefrontal cortex is behind the development of addiction to nicotine. This substance also affects the cardiovascular system, causes vasoconstriction, and increases both pulse and blood pressure (HHS, 2014). Nicotine passes through the placenta and affects the foetus. It can also be found in the milk of breastfeeding female smokers. The smoker's exposure to nicotine from electronic cigarettes

is varied. According to DeVito and Krishnan-Sarin (2018), the amount of the substance that is absorbed depends on the product characteristics (i.e. the device type and output and type and amount of e-liquid, as well as the heating temperature) and on the method of inhalation by individual users (i.e. duration of vaping and drawing strength, as well as non-intended use of the device). On the basis of a detailed review (National Academies of Sciences & Medicine, 2018), the authors reported that nicotine levels in e-liquids can vary within a range of 0–87.2 mg/mL. It is not known how the individual e-liquid flavourings affect the absorption of nicotine by the organism (National Academies of Sciences & Medicine, 2018). The authors in National Academies of Sciences & Medicine (2018) further state that there is substantial evidence that experienced adult users may receive a dose of nicotine similar to that from traditional cigarettes. Databases and safety sheets consistently state that the lethal oral dose for adult human is 60 mg or less (30–60 mg). In addition, recent sources report an LD_{50} (orally) for nicotine that is many times lower (0.5-1 g) (Mayer, 2014). However, nicotine poisoning is not common in adults because ingestion causes vomiting at first (Angermann, Sugar, Miller, & Omaye, 2019).

3.3 Flavourings

According to Hsu, Sun, & Zhu (2018), more than 15,000 unique e-liquid flavourings have been identified on the global market. These flavourings comprise various organic chemical substances. Often, they are not included in the leaflet or subjected to long-term tests. If they are tested, then they are tested for oral administration, not for aerosol inhaling. The basic types of substances used in e-cigarette flavourings can be identified. According to the National Academies of Sciences and Medicine (2018), these are alcohols (geraniol, menthol, thymol, eugenol), acids (butyric acid, valeric acid), esters (ethyl butyrate, methyl cinnamate, methyl silicate), lactones (gamma nonalactone, delta decalactone), aldehydes (citral A, benzaldehyde, cinnamaldehyde, vanillin), ketones (diacetyl, acetylpropionyl), oxygenous heterocyclic compounds (furfural, 5-methylfurfural, maltol), and nitrogeneous heterocyclic compounds (2-acetylpyrazine).

In this context, it is usually not clear which substances or groups of substances are formed as a result of the vaporisation of the flavouring. For example, carbohydrates which are admixtures adding a sweet taste to the e-liquid degrade to aldehydes and oxygenous heterocyclic compounds (furals) when heated (Farley, Seoh, Sacks, & Johns, 2014).

Another example of this would be the use of cinnamaldehyde. This substance is used not only to provide a cinnamon flavour, but also for sweet-tasting flavours such as caramel, fruits, and condiments. The study of Behara et al. (2014) states that there is a direct correlation between cinnamon-tasting flavourings and cytotoxicity. In another study, the same authors (Behar et al., 2016) state claims that even in low concentrations in electronic cigarette liquids, this substance is toxic and has an adverse effect on cellular processes. These authors claim, furthermore, that cinnamaldehyde has the potential to disturb homeostasis in the respiratory tract.

Another group of aromas used in e-liquids comprises menthol flavourings. These aromas contain, among other substances, the substance called pulegone. Pulegone is a carcinogenic substance which, when administered orally, causes liver cancer, pulmonary metastases, and other tumours in rodents (Jabba & Jordta, 2019). For this reason, the FDA withdrew authorization for the use of pulegone as a synthetic flavouring substance for use in food in 2018 (FDA, 2018). The study by Jabba and Jordta (2019) proved that users of pulegone-containing e-cigarettes may receive a daily dose of this substance that is higher than that of smokers of normal cigarettes. This study also warned that there are no studies available regarding the inhalation toxicity of this substance.

Diacetyl, systematic name butane-2,3-dione, is a substance that occurs naturally in butter, which is why it is often added as a flavouring to food. Together with related substances (2,3-pentadione, acetoin), it is added to electronic cigarettes to achieve a butter-like taste. While diacetyl is recognised as safe and generally known in food according to GRAS, this is not true if it is inhaled. For instance, in 2000 (Kreiss et al., 2002), obliterative bronchiolitis (irreversible loss of lung function) developed in eight former employees in a popcorn factory. Allena et al. (2016) examined 51 e-liquid flavourings and diacetyl was found in 39 of them. At least one of the previously mentioned substances was found in 47 of them.

3.4 Toxic substances formed by vaporisation

The vaporisation process and contact between the e-liquid and the heating coil involve the formation of unwanted products. These include, for example, carbonyl compounds and metals.

3.4.1 Carbonyl compounds

Various studies (National Academies of Sciences and Medicine, 2018) proved that toxic carbonyl compounds are formed (through thermal decomposition) when the e-liquid is heated. These are especially formaldehyde, acetaldehyde, acrolein, and glyoxal. Furthermore, they include acetone, propanal, methylglyoxal, glycidol, and 2-methylbenzaldehyde.

Formaldehyde, a substance usually detected in the highest concentrations, is classified as a human carcinogenic substance (Class 1). Acetaldehyde is classified as a potential human carcinogenic substance (Class 2B) (IARC, 1999), and, for instance, glycidol as a potential human carcinogenic substance (Class 2B) (National Toxicology Program, 2011).

The amount of carbonyl compounds varies between various manufacturers, as well as between individual e-cigarettes

of the same brand. The quality and quantity of the carbonyl compounds formed by vaporisation and in the subsequent reactions depend especially on the type of e-liquid used, on the heating temperature (more precisely, on the voltage used), and on the product design, as well as the age of the e-cigarette. As regards the type of e-liquid, the quality and quantity of carbonyl compounds depends on whether PG or VG is used as a base and whether flavourings are used. Geiss et al. (2016) confirmed that PG oxidises primarily to acetaldehyde, whereas VG typically oxidises in acrolein. Formaldehyde is formed by the oxidation of both PG and VG. For VG, this reaction is rarer and oxidation to acrolein and acetaldehyde was only observed with higher coil temperatures.

Although in most cases the detected level of carbonyl compounds is significantly lower than in normal cigarettes, cases of remarkably high levels of these compounds, especially formaldehyde, have been documented. For example, Geisse et al. (2016) claim that within the daily inhalation dose of 3 g of e-liquid the highest possible dose of formaldehyde amounted to 22 mg per day. This value exceeds the highest permissible dose according to OSHA (2011) by ten times. Animal research was performed e.g. by Canistro et al. (2017). The authors of this study confirmed changes in lungs and oxidation stress in the brown rats they tested (four weeks of aerosol inhalation). Although the study involved very high concentrations, which cannot be achieved by normal e-cigarette users, it demonstrates a certain level of risk which the aerosol inhalation involves. The temperatures at which the e-liquid vaporises and carbonyl compounds, feared to cause chronic health issues, especially a higher risk of developing cancer (Jensen, Luo, Pankow, Strongin, & Peyton, 2015), are formed, typically range from 150 °C to 350 °C. These authors also found that a battery power setting of 5 V leads to excessive decomposition of PG and thus to an increase in the content of formaldehyde in the aerosol. In this case, the formaldehyde is up to 14.4 mg per day, which is much more than when using conventional cigarettes (3 mg of formaldehyde per 20 cigarettes). However, in normal use (a power setting of 4 V), the amount of formaldehyde is 32% lower than in the case of using combustible cigarettes (Farsalinos, Voudris, Spyrou, & Poulas, 2017).

3.4.2 Metals

According to substantial evidence (National Academies of Sciences and Medicine, 2018), the aerosols from electronic cigarettes contain metals. These include metals such as cadmium, chromium, lead, and nickel. These metals can originate from the metal coil inside the e-cigarette heating unit, which heats the e-liquid, further e-cigarette components, or the e-liquid itself (Hess et al., 2017; Olmedo et al., 2018). However, the presence of metals has also been identified in the e-liquid itself but in lower doses compared to those e-liquids contaminated after contact with the heating coil (Zhao et al., 2020). Exposure to metals through inhalation from e-cigarettes could lead to serious health consequences. Inhaled compounds of nickel are, according to the International Agency for Research on Cancer (IARC, 2018), considered to be Class 1 carcinogenic substances for humans. Furthermore, nickel is often connected to a number of allergies, including in connection with electronic cigarettes (IARC, 2018). Lead and mineral compounds of lead are generally toxic; they affect most human bodily organs and accumulate e.g. in the bones and liver. According to the IARC classification, they are considered probable carcinogenic substances (IARC, 2000). Chronic lead intoxication causes anaemia and nephropathy. Compounds of cadmium have been included among Class 1 carcinogenic substances (IARC). These compounds cause both acute and chronic organism intoxications, and they accumulate in the liver and kidneys. Hexavalent chromium is toxic, too. It increases the risk of lung cancer and accumulates in the kidneys and bones (IARC, 2012). More metals cited in connection with e-cigarettes include tin, aluminium, magnesium, copper, and iron. It is very disturbing that the absorption of metals by the human organism is, similarly to most other compounds, significantly higher when they are inhaled. The toxicity is further influenced by the type of compound formed (more precisely, by the degree of oxidation of a given element). It can also be surprising that the total number of different metals is higher in e-cigarettes than in tobacco cigarettes. Additionally, the number of individual metal compounds was, except for compounds of cadmium, higher in e-cigarettes than in tobacco cigarettes. Both the product type and method of use increase the variability of the immediate concentration of metals determined in the aerosols from e-cigarettes (Nickel Institute, 2018). So far, very few studies have been conducted on the chemical form of metals in e-liquids (National Academies of Sciences and Medicine, 2018; Zhao et al., 2020).

4 CONCLUSION

Electronic cigarettes are a highly heterogeneous group of devices with varied composition and output, as well as high variability of e-liquids. Although the liquids comprise mainly PG and glycerol, the number and heterogeneity of the other constituents significantly influence the related health risks. In this paper, we have, on the basis of research on the current scientific evidence, presented selected categories of liquids for e-cigarettes and the aerosol formed from those according to their function and chemical composition (see Table 1). The major chemical substances which can form up to a half of the e-liquid are bulking agents (PG and glycerol). Another category, and so far the least explored one, consists of flavourings. Nicotine, the cause of the development of dependence, could form a category by itself. A highly diverse group of compounds is represented by chemical substances formed as by-products of vaporisation. In addition to these, the aerosol from e-cigarettes can contain tobacco-specific nitrosamines, free radicals, and volatile organic compounds, as well as a number of other substances whose description would go beyond the scope of this article.

The globally increasing prevalence of electronic cigarette use and the increasing variety of devices and e-liquids on the market mean that the toxicity of both the liquid filling and of the composition of the aerosol need to be tested more

thoroughly than ever before. The qualitative and quantitative analyses of e-liquids and aerosols are, however, limited by several different factors (such as the unavailability of standards for the individual chemical substances, and detection and determination limits with state-of-the-art instrumentation). For these reasons, the widespread implementation of individual components or identification and quantification of constituents is very complicated. Regarding the fact that a number of chemical processes take place during vaporisation (i.e. heating of the e-liquid by a metal coil), the results of e-liquid analyses performed on fillings in their liquid state can be misleading. Other obstacles to research on toxicity lie in the heterogeneity of the users' particulars and the technical parameters of the devices mentioned earlier. Given the increased concerns regarding the highly dynamic growth of the number of characteristic e-liquid flavours, the efforts of toxicological research should go in this direction. First, it is necessary to map the products available on the market continuously; second, it is essential to analyse the composition of e-liquids and to carry out toxicological research that assesses the influence of aerosol inhalation on the user's organism. Research and evidence-based knowledge about the toxicity of e-cigarettes are key to assessing the degree of risk and to setting up appropriate regulatory and public health measures within tobacco control policies.

Authors' contributions: AK and AB conducted a non-systematic review process. AB categorised and described the selected chemical substances. AK participated in the finalisation of the concept of the manuscript. Both authors contributed to the article and have approved the final version of the manuscript.

Declaration of interest: The authors of this article declare that they are in no conflict of interest.

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