Conference paper

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Electronic cigarettes – an important progress or just another risk for health?

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Abstract: Liquids for electronic cigarettes and the vapor generated from them were examined by chemical and biological methods in order to reveal potential risk factors and their acceptability for consumers. Although the majority of the liquids on the market appear to be safe, some aroma compositions have been identified as possibly toxic for human vein epithelial cells, similar to tobacco smoke, thus indicating potential health risks and suggesting suitable test procedures before marketing the liquids.

Keywords: e-liquids; electronic cigarettes; Eurasia 2018; human cells (HUVEC); toxicology; vaping vs. smoking.

Introduction

In the past decade, electronic cigarettes and the chemical substances used in them, the so-called e-liquids, have enormously developed in technology and variety, leading to a billion-dollar market. The main reason for this development is the hope that e-cigarettes are a suitable substitute for tobacco-smoking, diminishing the health risks associated with smoking without invoking the painful experiences associated with quitting it. The large variety of flavors provided by the e-liquid producers has facilitated the transition from smoking to 'vaping' as the use of e-cigarettes is commonly called, as it is based on evaporation at moderate temperatures instead of combustion at much higher temperature. Although studies available until now suggest that much less health risks are caused by vaping compared to smoking [1], the large variety of aromas used in the liquids and of devices available demand much more detailed and critical evaluation of potential risks. We have, therefore, decided to perform chemical and biological tests of the vapor inhaled and of the liquids themselves, under standardized conditions with numerous e-liquids, in particular the most-selling ones on the market.

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Analytical procedures

Generation of vapor

A device was constructed to generate vapor from liquid under controlled standard conditions. It uses a common e-cigarette of type Eleaf Pico mod and Eleaf atomizer with Ti coil, with average coil temperature set to 240 °C, fire interval to 30 s and firing time to 2 s. The vapor output of the device is connected from drip tip to an impinger containing distilled water or acetonitrile, in which the vapor is bubbling through the solvent. A second impinger follows and connects to a vacuum pump with flow regulation, which is started 1 s before firing and then runs for 4 s. After 100 cycles of firing the solution formed in the first impinger is used for analysis. For the biological experiments, 300 cycles of firing were performed to collect the vapor condensate. In this process, the impinger contained the buffer solution used in the experiments. A schematic drawing of the apparatus is shown in Fig. 1.

Chemical analysis

The liquids and the vapor collected in the impinger were analyzed using chromatographic methods GC (Agilent 6890 N with HP-5MS column), combined with mass spectroscopy (MS Agilent 5973 Inert). HPLC-UV was employed for the quantification of nicotine in the original liquids.

Particular attention was payed to compounds considered as potentially noxious, i.e. aldehydes and carbonyls, but also – after having obtained biological data – to some other toxic compounds occasionally used in aroma mixtures, e.g. estragol and thujones. For the quantification of the DNPH-derivatives of formaldehyde, acetaldehyde, acrolein and diacetyl, standard solutions in the range from 5 to 2500 ng/ml were prepared and treated under the same reaction conditions for calibration.

Volatile compounds of the vapor were also extracted using a SPME fiber (2-cm triphasic divinylbenzene/carboxen/polydimethylsiloxane fibre). The fibre was successively desorbed in an Agilent GC5890 equipped with a Agilent MSD5975C quadrupole mass analyser (Varian CP-Select 624 CB 60 m, i.d. 0.32 mm column, Injector temperature 250 °C/splitless).

Concentrations were quantified by calibration curves obtained for each component using pure compounds. Deuterated nicotine was used as internal standard.

Samples have been measured at least twice. Limit of quantification (LOQ) are reported in Table 1. The estimate uncertainty is ±30 %.

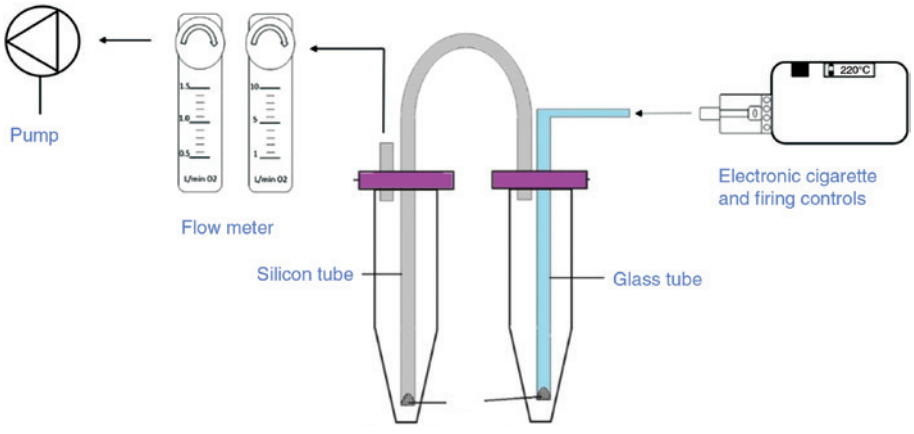

Fig. 1: Schematic drawing of the device for vapor generation.

Table 1: Limits of Quantification (LOQ) for metal ions in vapor, given in µg for 200 puffs.

Metal	Limit of Detection in µg/200 puffs
Sb	0.1
Ni	0.3
Cr	0.3
Cd	0.1
Pb	0.1
As	0.1
Zn	1
Cu	0.1
Al	1
Sn	0.3
Ti	1
Hg	0.0

Concentration of metals in e-liquid vapor was below LOQ in all cases.

In a separate series of experiments, the nicotine content of the vapor as a function of the coil temperature was examined, although it seems well established that nicotine is not the main health risk in smoking and vaping [1]. On the contrary, nicotine seems to have a number of health-promoting properties documented in numerous studies on Parkinson and Alzheimer disease [2, 3], COPD, asthma and heart conditions [4–6], Colitis Ulcerosa [7, 8], and psychiatric syndromes [9–12].

Another series of analysis was focused on metals in the vapor as possible emissions from the coil in the vaporizer or the tank material, using ICP/MS. In particular, possible contaminations by Ni, Cr, Cd, Pb, Zn, Ti, Sb, Cu, Al, Hg and As were investigated.

Further details of the analytical work are given in Ref. [13].

Biological analysis of cell toxicity

Analyses were performed with e-liquids added to the cell medium and with the cell medium plus buffer solution containing the vapor condensate. Human umbelical cord endothelial cells (HUVEC/TERT2) were used as biological samples. This assay is mimicking the toxic effect of e-liquids or vapor condensate on blood vessels in the body.

Cytotoxic substances can damage the cell membrane leading to the release of cytosolic lactate dehydrogenase (LDH) and immediate cell lysis, which was determined after 24 h of incubation. General toxic effects were measured by resazurin assays after 48 h of incubation. Normal cells gradually reduce resazurin forming resorufin, which can be quantified by fluorescence measurements (Ext. 560 nm, Emi 590 nm). Any cytotoxic substances will lower the metabolic activity of the cells, resulting in a reduction of the fluorescent signal, which is proportional to the metabolic activity of the cell culture.

Results and discussion

Chemical analysis

The most important organic vapor contents generally considered as noxious are carbonyls. If not present as flavors, they can apparently form in very minor concentrations under the vape conditions employed in our experiments (which mimick the common usage of e-cigarettes). The concentrations found for Acrolein and

Acetaldehyde are below the maximal allowed value for permanent exposure at factory work (https://roempp.thieme.de/roempp4.0/do/data/) and should not pose, therefore, a relevant risk for the user of e-cigarettes. The Formaldehyde and Diacetyl concentrations ameasured were up to 10 times higher than the working place limits, which still does not seem critical as vapor content.

Nicotine is released to the vapour, and the amount of it is a function of both the coil temperature and its concentration in the liquid, and thus the user of an e-cigarette can easily use both temperature control (for regulated devices) or choice of nicotine concentration to adjust the amount of nicotine to be taken in.

The measured metal concentrations in vapor are below the limits of quantification, listed in Table 1, and hence cannot be considered a risk, provided standard coils and common heating temperatures are employed.

Biological analysis

Sample selection

A total of more than 40 commercially available e-liquids was investigated, selecting the most popular, best-selling ones of European and American market and liquids with most popular flavors from a variety of pro-

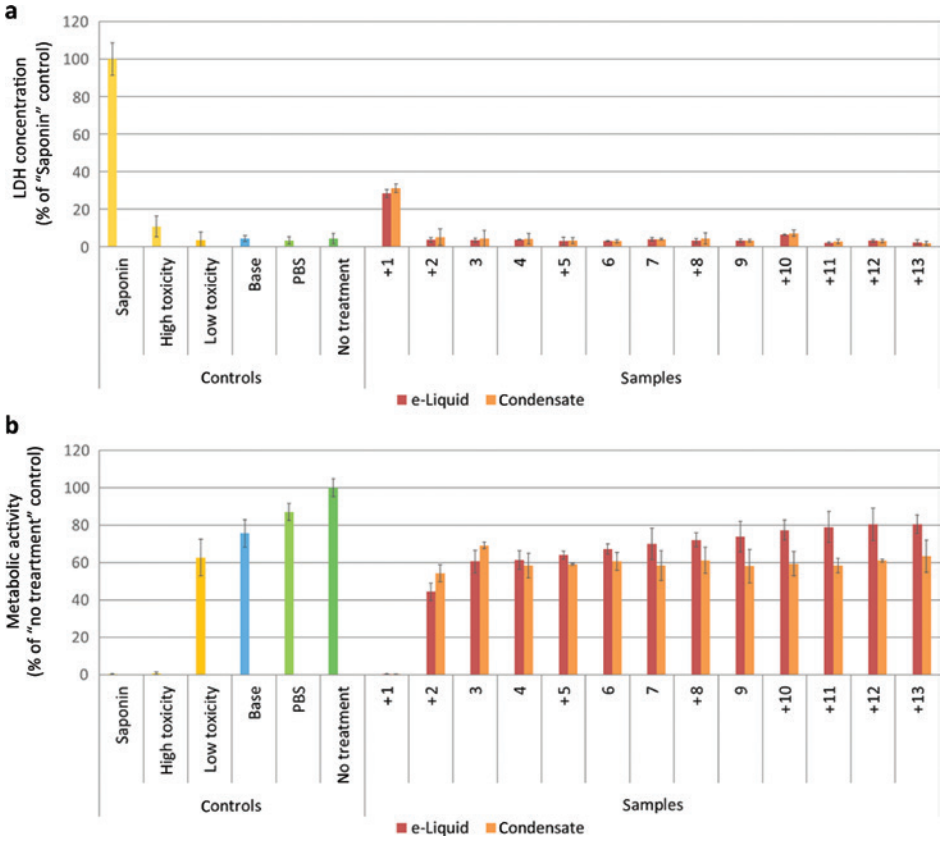

Fig. 2: Comparison between the cell toxicity caused by e-liquids and the respective e-liquid condensates on human umbilical cord endothelial cells (HUVECs). (a) Quantification of lactate dehydrogenase (LDH) release upon cell lysis after 24 h of treatment of HUVECs with e-liquids or e-liquid condensates. (b) Metabolic activity of HUVECs evaluated by the quantification of Resazurin turnover after 48 h of treatment of HUVECs with e-liquids or e-liquid condensates. **Saponin** (0.03 %)was used as cell lysis control. **High toxicity** and **low toxicity** controls are e-liquids with high and low toxic effects on HUVECs, respectively. **Base**… treatment with e-liquid PG/VG base without flavors; **PBS**… Phosphate saline buffer treatment; **1–13…** treatment with popular commercially available e-liquids; Liquids marked with a cross (+) contained 20 mg/ml nicotine. The studies were performed in technical triplicates and were repeated in 2–4 independent experiments.

ducers. In addition a series of liquids based on herb extracts as aromas, and two additives commonly used as sweetener and cooler in e-liquids were selected for these experiments.

Toxicity of e-liquids and vapor condensates

Figure 2 shows the results of this comparison, and the differences between both experiments are within methodical limits almost identical. This seems to be of particular interest for any further testing of liquids for their biological acceptability, as it will reduce the procedure to the more simple liquid application instead of the much more expensive study of generated vapour condensates.

Nicotine containing liquids are marked with a '+' sign below their identifying numbers. Inspection of the results for 12 mg/ml nicotine-containing and nicotine-free liquids leads to the conclusion that nicotine is not responsible for any observed cell toxicity.

Figure 3 displays a selection of the biological activity of a number of e-liquids, in the upper part showing the percentage of disintegrated cells after 24 h of exposure to liquid/condensate compared to saponin as control substance, and in the lower part the metabolic activity of the exposed cells after 48 h in comparison to untreated cells and those exposed to the pure PG/VG base and those cultivated in pure medium with buffer system added.

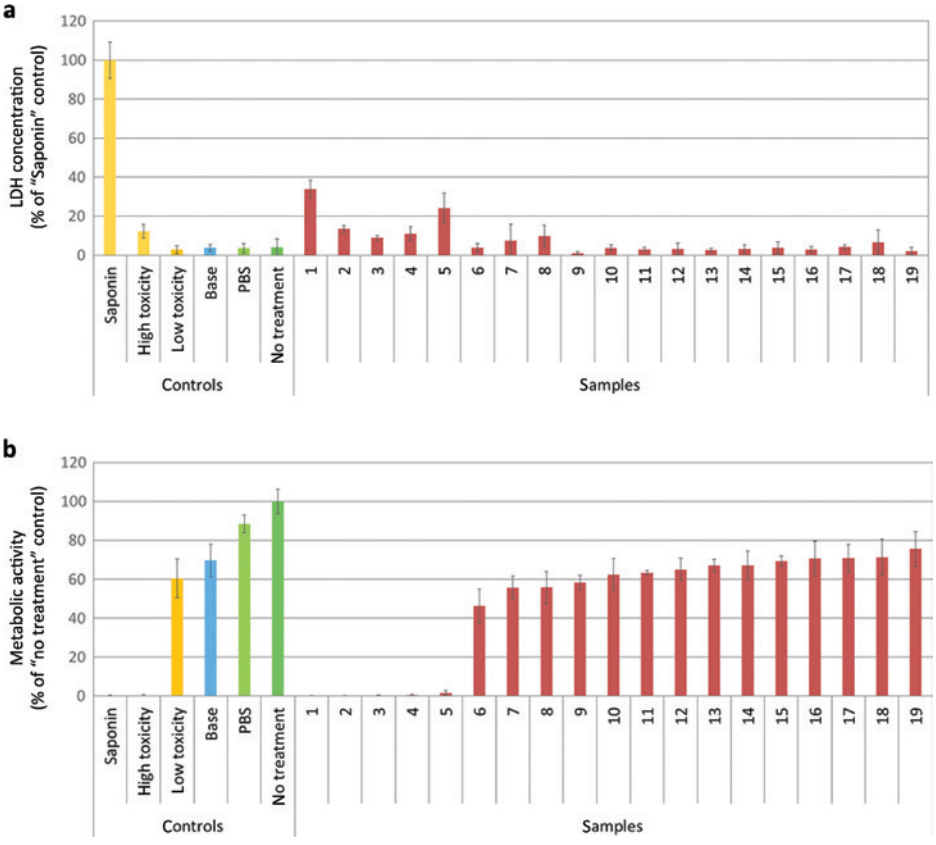

Fig. 3: Toxicity evaluation of commercially available e-liquids on human umbilical cord endothelial cells (HUVECs). (a) Quantification of lactate dehydrogenase (LDH) release upon cell lysis after 24 h of treatment of HUVECs with e-liquids or vapor condensates. (b) Metabolic activity of HUVECs evaluated by the quantification of Resazurin turnover after 48 h of treatment with e-liquids or e-liquid condensates. **Saponin** (0.03 %)was used as cell lysis control. **High toxicity** and **low toxicity** controls are e-liquids with high and low toxic effects on HUVECs, respectively. **Base**… treatment with PG/VG e-liquid basis without flavors; **PBS**… Phosphate saline buffer treatment; **1–19…** treatment with a series of commercially available e-liquids. The measurements were performed in technical triplicates and were repeated in 2–5 independent experiments.

These diagrams clearly prove that the majority of the liquids investigated is rather non-toxic for the cells, but that there are still over 20 % of them causing cell death and/or damage to the cell metabolism. A similar picture was obtained with a second series of 25 liquids from other producers.

A comparison of 2 non-toxic and 2 toxic e-liquid vapor condensates with tobacco cigarette smoke condensate was made, including dilution series from 20 to 0.08 µl/ml. The results of this investigation are illustrated in Fig. 4. Whereas dilution to a fifth of the original concentration removes the toxicity of the e-liquid vapor, cigarette smoke condensate has to be diluted at least by a factor of 100 to lose its toxic influence on the cells. This finding provides another strong argument for the ongoing discussion about smoking vs. vaping.

Which liquids are toxic?

All liquids proven toxic to the cells contain at least one of the following of flavors: cinnamon, absinth, anise or vanilla. Consequently, the chemical analysis of these liquids was extended to identify the responsible toxic components. This search identified cinnamaldehyde, estragol, thujones and vanillin as the most likely culprits, and this finding agrees well with previous investigations on the toxicity of aromas in e-liquids [14].

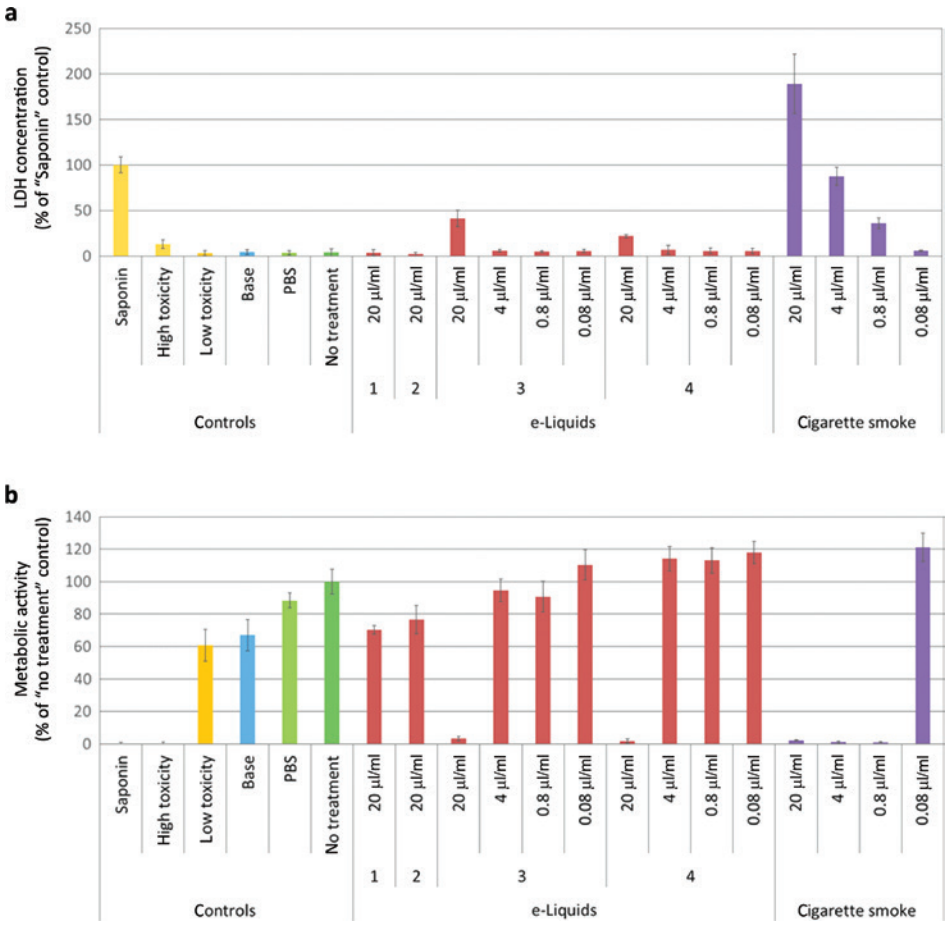

Fig. 4: Comparison of the toxicity of condensates from commercially available e-liquids compared to cigarette smoke condensate on human umbilical cord endothelial cells (HUVECs). (a) Quantification of lactate dehydrogenase (LDH) release upon cell lysis after 24 h of treatment of HUVECs with e-liquids or e-liquid condensates. (b) Metabolic activity of HUVECs evaluated by the quantification of Resazurin turnover after 48 h of treatment with e-liquids or vapor condensates. **Saponin** (0.03 %) was applied to lyse cells and was used as cell lysis control. **High toxicity** and **low toxicity** controls are e-liquids with high and low toxic effects on HUVECs, respectively. **Base**… treatment with e-liquid basis without flavors; **PBS**… Phosphate saline buffer treatment; **1 and 2…** two examples of non-toxic e-liquids; **3 and 4…** two examples of toxic e-liquids. The studies were performed in technical triplicates and were repeated in 2–6 independent experiments.

Differences in the toxicity of liquids with the above mentioned flavors from different producers could be caused by different concentration but also by the use of different aroma compounds.

Natural extracts and common additives as flavoring

As in the production of aromas traditionally extraction of herbs and fruits plays a major part, and as in the past few years extracts from herbs have become significant components of e-liquids, it seemed of interest to investigate liquids prepared with such extracts by the same methodology. For this purpose, extracts were prepared by Soxhlet extraction of dried herbs with 70 % ethanol (3 cycles) and subsequent concentration of the extract in vacuo 7 to 1. Valerian, lavender, ginseng, ginger, thyme and balm were employed, and the concentrated extract was used as 10 % flavor in PG/VG liquid. The tests with these liquids and their vapor condensate proved all of them as non-toxic to the cells and thus as acceptable for use in e-liquids. In this context, also two very common additives to e-liquids were investigated, namely *Sucralose* and *Koolada*, used as sweetener and cooler, respectively. Within the common concentration range applied, up to 5 %, they did not show toxic effects on our cells. Recent investigations performed by us indicate, however, that this may not be valid for evaporation temperatures considerably higher than 200 °C.

Conclusions

The results obtained so far indicate that the assumed reduction of health risks by changing from smoking to vaping is valid, but that certain vaping conditions and some of the aromas being used can induce new problems and potential risks. The performance of further studies of this kind seems extremely important, therefore, in order to confirm the advantages of vaping versus smoking, to avoid hazardous substances in flavoring and to create science-based directives and hence legislation. At present, legislation strongly differs from country to country, ranging from almost complete banning to financial support for changing from tobacco to electronic cigarettes, and studies of the type presented here should provide a good basis for future decisions and regulations. For marketing of e-liquids it is strongly recommended to have them tested by the procedure used in this study before release.

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