Impact of the revised European Tobacco Product Directive on the quality of e-cigarette refill liquids in Belgium.

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ABSTRACT

Introduction: Since its introduction, the e-cigarette has become a commonly used consumer product. In this study, we investigate whether regulatory changes had an impact on the quality of refill liquids (e-liquids) available on the Belgian market through analysis of their chemical composition. Hence, the nicotine concentration accuracy was investigated in samples before, during and after the implementation of the revised Tobacco Product Directive (TPD) as an indicator of good manufacturing practices. This is, however, not enough to assure the quality. Therefore, extra criteria were also assessed based on TPD requirements.

Methods: By using in-house validated methods, a total of 246 e-liquids purchased prior (2013-2015), during (2016) and after (2017-2018) the implementation of the TPD revisions, were analyzed for the presence of nicotine, nicotine-related impurities, volatile organic compounds (VOCs), caffeine and taurine, and the flavours diacetyl and acetylpropionyl.

Results: Although not all manufacturers managed to produce and label their products accurately, nicotine labelling discrepancies have decreased over time. Moreover, also the number of e-liquids, containing high risk VOCs (10% in 2016 *versus* none of the samples in 2017-2018), caffeine (16% in 2017 *versus* 5% in 2018) and diacetyl and acetylpropionyl (55% in 2017 *versus* 27% in 2018 of sweet flavoured samples) diminished over time.

Conclusion: Our results demonstrate that the overall quality of the e-liquids has improved after the implementation of the revised TPD. However, the results also show that periodic quality control might be required to ensure further compliance to the TPD.

Implications: This study clearly demonstrates that the implementation of the revised TPD has improved the quality of the e-liquids on the Belgian market. However, there are still e-liquids that are not in agreement with the TPD due to nicotine concentration label discrepancies, presence of e-liquid impurities and controversial flavours diacetyl and acetylpropionyl or the additive caffeine.

1. INTRODUCTION

Since its introduction, more than 10 years ago, the number of e-cigarette users is only increasing. Indeed, a study performed in the UK demonstrated that in 2018 almost 2/3 of tobacco smokers have tried the e-cigarette and 20% of them have continued to use e-cigarettes [1,2]. To assure the quality and safety of these consumer products, the European Parliament approved in 2014 a revision of the Tobacco Products Directive 2014/40/EU (TPD) [3] that includes since then also a set of regulations concerning e-cigarettes. Since the TPD revision, nicotine-containing e-cigarettes are classified as tobacco products. In Belgium this means that they are no longer considered as a medicine, suspending their *de facto* ban that existed until then.

The revised TPD includes a set of more stringent measures concerning promotion and packaging/warning labels of e-cigarette products, limited maximum volumes for cartridges and e-liquids. In addition, manufactures are required to notify the competent authorities before placing their products on the EU market. Moreover, stricter requirements are put forward for the ingredients present in the e-liquids [3], rendering their chemical characterization essential to be able to monitor compliance to the TPD and to control the quality of the available products on the market.

Our strategy to assess the quality of e-liquids is first of all based on the nicotine concentration accuracy. This is an indicator of good manufacturing practices during production of e-liquids. Nicotine in e-liquids and e-cigarette aerosols has been extensively investigated and studies performed prior to the implementation of the TPD report a number of discrepancies between the actual nicotine concentration in the e-liquid and the labelled concentration [4–9].

The nicotine concentration accuracy is, however, not enough to assure the quality of these products. Therefore, extra criteria need to be assessed, based on the 3 main requirements mentioned in the TPD concerning the e-liquid ingredients. These include 1) the use of high purity ingredients 2) prohibition of additives associated with energy and vitality and 3) the used ingredients may not pose any health risk in heated or unheated form.

For the first criterion this means that the impurities present in e-liquids need to be investigated. Aside from the ingredients intentionally added to e-liquids, the main focus of e-cigarette research has so far been the analysis of harmful and potentially harmful constituents. These include: (i) thermal degradation components formed during heating such as formaldehyde, acetaldehyde and acrolein, (ii) leachables from the e-cigarette device such as metals, but also (iii) impurities related to main e-liquid ingredients such as nicotine-related impurities, tobacco-specific nitrosamines (TSNAs), ethylene glycol (EG), diethylene glycol (DEG) and volatile organic solvents (VOCs) [10–19]. The latter group is thus indirectly mentioned in the TPD. In this study, the e-liquids are investigated for the presence of nicotine-related impurities and VOCs.

The second ingredient-related requirement is the prohibition of certain additives. E-cigarettes, containing lifestyle medicines (e.g. phosphodiesterase-5 inhibitors and slimming products) as well as drugs (e.g. MDMA) have been encountered [20,21]. In fact, a whole new niche of e-liquids has emerged in which vitamins and supplements such as vitamin B12 and melatonin (VitaCig, InhaleHealth) are added in order to achieve potential health benefits via vaping. The additives evaluated in this study are the stimulants caffeine and taurine.

The third criterion does not exclude explicitly a specific ingredient or component, but includes all possible harmful ingredients, such as the added flavourings. E-liquids are available in more than 7000 flavours [22], which could include potential dangerous inhalation toxicants (e.g. diacetyl-acetylpropionyl) [23–25]. Furthermore, it stands to reason that all these different flavours, represent a big challenge for regulating authorities in order to assess the health risk related to the inhalation of these components [26]. In our study, we assessed the presence of diacetyl and acetylpropionyl in the flavoured e-liquids.

Regardless of the implementation of the TPD, there is still no clear overview or systematic inspection of the quality of the different e-liquids available on the market. By investigating the chemical composition of e-liquids, according to the abovementioned strategy, a comparison of the quality of

the different e-liquids is possible. The nicotine concentration accuracy was investigated in samples acquired before (2013-2015), during (2016) and after (2017-2018) the implementation of the revised TPD. Additionally, nicotine stability in e-liquid matrices was investigated for up to 9 months. The other components (nicotine-related impurities, VOCs, the additives caffeine and taurine and the flavours diacetyl and acetylpropionyl) were only investigated in samples acquired during and after the implementation of the revised TPD and will be compared with data from previous studies performed before the implementation of the TPD.

2. MATERIALS AND METHODS

2.1 Sample collection

A total of 246 e-liquids were analysed; 159 originating from Belgian vapeshops, 23 samples were bought online (prior to the implementation of the TPD), 3 Do-It-Yourself (DIY) samples and 61 samples originated from the Federal Agency for Medicines and Health Care Products (FAMHP). The latter samples were either obtained upon inspections of different vaping shops in Belgium or were seized postal packages ordered by individuals through the internet. More details about the sample collection is provided in the Supplementary Material. All samples were stored at 4°C and protected from light. The target components were not analysed for all acquired samples. More details about which samples were investigated for which target component is given in Supplementary Table S1.

2.2 Chemical characterisation of the e-liquids

Nicotine and the **nicotine-related impurities** were screened with liquid chromatography-tandem mass spectrometry (LC-MS/MS), followed by quantification with ultra-high performance liquid chromatography with a diode array detector (UHPLC-DAD), as described and validated (with the total error approach and with the total error set at 10% maximum) in [27].

The VOCs were analysed by means of headspace-gas chromatography mass spectrometry (GC-MS). Hereto, e-liquid samples were simultaneously screened for the presence of volatile components that are classified as **residual solvents** by the ICH and the potential toxic flavourings **diacetyl and**

acetylpropionyl. The screening was performed on an Agilent 6890 N gas chromatograph coupled to an Agilent 5973N single quadrupole mass spectrometer and equipped with a G188A static headspace sampler (Agilent Technologies, Palo Alto, USA). The samples were diluted by dissolving 1g e-liquid sample in 10ml water. The identification was performed in full scan mode (from 25 to 400 m/z) while the quantification was executed in selected ion monitoring (SIM) mode. See Supplementary Material for further GC method details.

Caffeine was screened in e-liquids samples with GC-MS (GC Agilent Technologies 7890A, MS 5975C). The samples were diluted by dissolving 1g e-liquid sample in 10 ml methanol. Positive samples were further quantified by UHPLC-DAD using an Acquity UPLC™ system (Waters, Milford, USA) equipped with a photodiode array detector with a Waters Acquity BEH RP18 2.1 mm × 100 mm, 1.7 µm column and a Van Guard BEH pre-column (2.1 mm × 100 mm, 1.7 µm). The samples were diluted by dissolving approximately 1 g e-liquid sample in 10ml water. The used methodology is further described in the Supplementary Materials.

Taurine was screened by employing non-targeted LC-MSn. Prior to injection, 1 g e-liquid sample was diluted into 10ml water. Next, 2µl of the diluted sample was injected onto a Dionex UltiMate 3000 Rapid Separation LC (RSLC) system (Thermo Scientific, Sunnyvale, CA, USA) coupled to an amaZon™ speed ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). More details are given in the Supplementary Materials.

2.3 Nicotine stability in e-liquid matrices

A stability study was set up to assess the influence of matrix type and light exposure on the stability of nicotine in e-liquids. Hereto, a subset of 18 e-liquid samples were spiked with a nicotine standard to obtain a final concentration of 3mg/g and 6mg/g preparations. Four different e-liquid matrices were included: 100% propylene glycol (PG) – 100% glycerol (G) and 50/50 PG/G and 100% 1,2-propandiol (PD). The samples were stored for up to 9 months at 25 ± 2°C and 60 ± 5% relative humidity, in

agreement with the long term climate type II conditions in the ICH guidelines [28]. Monitoring of the climate conditions was done using a Libero THi1 V3.24 datalogger. Additionally, in order to investigate the influence of light exposure, duplicates of all samples were prepared of which one was kept in the dark and the other not. Nicotine concentrations and its related impurities were determined after preparation of the sample and 1, 3, 6 and 9 months after storage.

2.4 Statistics

The conformity of the samples between groups (vapeshop, internet) and before, during and after the implementation of the TPD were performed by using the Pearson Chi square test. Correlations were determined by using Pearson's correlation coefficient. All comparisons were two-tailed, and a p value of <0.05 was considered statistically significant.

3. RESULTS

3.1 Nicotine concentration label accuracy

Nicotine was analysed in so-called "zero-liquids" retrieved between 2013 and 2018. Zero-liquids are conform the TPD if the measured nicotine concentrations are below the detection limit (0.5µg/ml). As can be seen from Figure 1A, 47% of the claimed zero-liquids collected prior to the TPD implementation contained nicotine either in traces or in higher amounts [<LOQ – 4.2 mg/g] (See Supplementary Table S2). The zero-liquids collected in 2016 i.e. during TPD implementation, demonstrate a high % of non-conformity. More specifically, 50% of the internet zero-liquids contained nicotine compared to 38% of the zero-liquids obtained from the vapeshops. This might indicate that the quality of internet-bought samples are of lesser quality than the e-liquids from vapeshops. The third set of samples, collected post TPD implementation and acquired from vapeshop inspections by the national health authorities in 2017 and 2018, demonstrate a higher level of conformity ($p < 0.05$) since only 4 zero-liquids were found to contain nicotine on a total of 35 samples (11%) [<LOQ].

Next, the conformity of nicotine-containing e-liquids was investigated by comparing the labeled *versus* the measured nicotine concentration. The results of the samples collected in 2016 show that 15% of the investigated samples have an actual nicotine concentration that deviates more than 20% from the labelled concentration whilst in the 2017-2018 sample set this was only 7% (Figure 1B). The high number of non-conformity in 2016 is mainly due to the DIY- and internet samples ($p < 0.05$). For the samples coming from the vapeshops, no significant trend change could be observed from 2016 to 2018. Interestingly, the non-conform samples acquired from the vapeshops, contained less nicotine than labeled, whereas the non-conform DIY samples and the samples acquired from the internet contained a higher nicotine concentration than claimed (See Supplementary Table S3).

3.2 Impurities: Nicotine-related impurities

Analysis of the nicotine specified impurities, present in the 2nd (2016) and 3rd (2017-2018) sample set demonstrate that the most abundant impurities present are nicotine-N-oxide and myosmine (see Figure 2). Also cotinine, nornicotine, anatabine and anabasine were found in some nicotine-containing e-liquids. The only impurity that could not be detected was β-nicotyrine. In Supplementary Table S4 the measured concentration for each alkaloid impurity is given in detail. Until now the limits used for comparison of the nicotine alkaloids are those from the European Pharmacopoeia (Ph. Eur.). These limits are established for quality control purposes of nicotine used as an active pharmaceutical ingredient. These can be regarded as the minimal purity requirements for nicotine. The limits set for each specific impurity may not exceed the threshold of 0.3% relative to nicotine and a total relative concentration threshold of 0.8% is allowed. In Figure 2, the relative concentration of the quantified impurities is given for each sample. The limit of 0.3% was exceeded for 20 e-liquids samples. In 16 of these samples, nicotine-N-oxide was the responsible alkaloid impurity, followed by anatabine and anabasine in 6 and 3 of the 20 samples, respectively. The other detected impurities were all present in relative concentrations below the Ph. Eur. limit. The limit for the total relative concentration of impurities was exceeded by 6 e-liquid samples that also exceeded the 0.3% limit. The samples from 2016 contained more impurities and in higher concentrations as compared to samples from 2017 and

2018. Especially the samples acquired from the internet contained more impurities compared to the samples purchased in vapeshops.

3.3 Impurities: Residual solvents

The presence of **residual solvents** was investigated in 128 samples (2016-2018). Ethylbenzene, xylene, naphthalene, hexane and isopropanol were identified and confirmed in a total of 13 e-liquid samples using the HS-GC-MS screening. The results are summarized in Supplementary Table S5. Napthalene, xylene and ethylbenzene were present in quantities lower than 0.5 ppm. Hexane and isopropanol, regarded of lower risk to human health, were present in concentrations between 7.69 – 22.49 µg/g and 0.13 – 66.72 mg/g, respectively.

3.4 Additives: Caffeine and Taurine

The additives caffeine and taurine were screened in 112 samples bought after the implementation of the TPD. Taurine was not present in any of these samples. Caffeine, on the other hand, was identified in 12 of the e-liquid samples tested (Figure 1C) with more positive samples in 2017 compared to 2018. The concentration varied from <LOQ (1µg/ml) to 29 µg/ml (see Supplementary Table S6).

3.5 Harmful ingredients: Diacetyl and Acetylpropionyl

The amount of diacetyl and acetylpropionyl, putatively present in 56 sweet-flavoured e-liquids (2016-2018), was assessed by HS-GC-MS. Sweet-flavoured e-liquids include fruit, dairy (butter, cheesecake, yogurt) and brown (caramel, vanilla coffee, chocolate) flavourings similar to the sample selection of Allen et al. in 2016 [23]. As shown in Figure 1D, diacetyl was present in 11 samples, acetylpropionyl in 2 samples and both compounds were simultaneously present in 2 sweet flavoured samples [29]. It was noticed that the positive e-liquids belong mainly to the brown flavours (e.g. caramel, chocolate and coffee-associated flavourings). The positive samples were further quantified and the concentrations varied from 5-287 µg/ml and 31-115 µg/ml for DA and AP, respectively (see Supplementary Table S7).

3.6 Nicotine - stability study

The concentration of nicotine was analysed during 9 months in the different spiked e-liquids. At a first glance, nicotine seems stable in all matrices (Figure 3). However, an increase in the impurities nicotine-N-oxide and myosmine, was observed over time (Figure 4). A possible explanation for this rather contradictory results is the fact that the used UHPLC-DAD method is sensitive enough to quantify nicotine-impurities with high sensitivity and precision in the lower concentration ranges (µg/ml), while the precision and thus the power of the method to detect significant small differences in the higher concentration range (mg/ml) of nicotine concentration is rather low. The nicotine-N-oxide concentration exceeds the Ph. Eur limit after 3 months, while myosmine remains within the limits of specified impurities. This effect is more pronounced in a 1,3-propanediol e-liquid matrix. No effect was observed on nicotine stability when the e-liquid samples were exposed to light.

4. DISCUSSION

Similar to pharmaceutical ingredient content analysis in medicines, **nicotine** content analysis is seen as a quality indicator for e-cigarettes. Our data suggest that labelling discrepancies have decreased over time. Nevertheless, not all manufacturers manage to manufacture and label their products correctly. Label discrepancies might have several causes. The "zero-liquids" found with nicotine traces are probably due to contamination during manufacturing and handling. The presence of quantifiable amounts of nicotine (>1mg/g) is, however, more likely because of mislabeling. Several studies also suggest that poor storage conditions or unstable e-liquid formulations can lead to degradation of nicotine [33]. This could explain the observation of e-liquids with a nicotine concentration lower than the claimed concentration. However, in our 9 month stability study the nicotine concentration did not vary more than 10% of the initial concentration making this explanation unlikely.

From a regulatory point of view, zero-liquids that contain traces of nicotine do not comply with the TPD labelling criteria. However, the biological effects and potential associated harms due to exposure

to these nicotine traces has not yet been assessed. The effect of the nicotine e-liquid concentration on the nicotine exposure by vaping is not as significant as other vaping parameters (device, vaping behavior,…) [8]. Hence, poor labelling accuracy of nicotine-containing e-liquids is more a matter of misleading the consumer about the actual nicotine content than it is a safety concern as the impact of inhaling a lower or slightly higher nicotine dose is unlikely to be harmful for a nicotine addict [8]. However, it has been suggested that even these small nicotine label discrepancies might be of significant concern when teenagers (<18 years) and persons trying to quit their nicotine addiction are unintentionally exposed [30].

According to the TPD, avoidable impurities should be limited in e-liquid products [3]. Therefore, next to the quantity of nicotine also its quality was investigated. Nicotine can either be extracted from the tobacco plant or chemically synthesized. The latter is the most expensive and to our knowledge not often used for e-liquids. Yet, due to the nicotine extraction from tobacco plants, **tobacco-related impurities** are unavoidable in the nicotine extract.

The presence of nicotine-related impurities in e-liquids is also linked to the stability of nicotine in the e-liquid matrix. Previous stability studies showed results comparable to ours for the impurity nicotine-N-oxide. Liu et al (2017) showed that the formation of nicotine-N-oxide is temperature and humidity dependent [31]. Flora et al. (2016) conducted stability studies up to 6 months on nicotine cartridges packed in blisters. A significant increasing trend could be observed for nicotine-N-oxide, nornicotine, myosmine and cotinine, though the found concentrations were not as pronounced as observed in our stability study [32]. The different results between the studies can probably be explained by the final packaging in which the e-liquid is stored. In the study of Flora et al. cartridges are stored in a blister packaging with back foil which protects against external factors such as humidity and light and is also more airtight compared to the in-house packed samples that were used in our stability study.

The presence of nicotine-N-oxide is unavoidable in e-liquid matrix because of the N-oxidation of nicotine that occurs spontaneously over time. The biological effects of nicotine-N-oxide are not

extensively studied. It is, however, known that it is a major metabolite of nicotine that is reduced back to nicotine in the body, and thus leads to recycling of nicotine [33]. Myosmine is not only found in tobacco plants, but is also found in nuts, cereals and other foods [34]. The myosmine concentrations found in the investigated e-liquids were, however, in the relatively higher ranges (µg/g) as compared to concentrations found in food (ng/g). Unlike the other tobacco-related impurities, myosmine is reported as a potential genotoxic compound [35]. Therefore, whilst investigating the potential genotoxicity of this component in more detail, it should be avoided in e-liquids.

One should note that the majority of the e-liquid samples did not contain the impurities anabasine, anatabine, cotinine and nornicotine and that these impurities were not formed over the time of 9 months in our stability study. Nevertheless, these impurities are also an interesting indicator of the quality of the e-liquid. Thus, manufacturers should take measures to constrain the presence and the rapid formation of these nicotine-impurities in e-liquids and as well provide well-supported expiration dates on their products so that the consumer is sure that the quality of their product complies to the TPD.

Other impurities that can be avoided are the **VOCs**. We specifically investigated VOCs that are not intentionally added. High risk VOCs such as naphthalene, ethylbenzene and xylene were present in the investigated samples in concentrations well below the maximum allowed limits for pharmaceutical products [36]. Isopropanol, however, was present in concentrations above the ICH limit, but below the oral toxicity limits [37], nevertheless they also exceed the current airborne occupational hazard limit (400 ppm) [38]. Other VOCs with a higher toxicity profile, including benzene and toluene, were not found in the investigated samples, contrary to previous reports [19,39]. These previous studies indicated an overall higher percentage of positive samples for VOC, with a concentration range similar to our findings. The VOCs detected in e-liquids are mainly contaminants of the used ingredients such as the nicotine and flavouring extracts, which might implicate doubtful manufacturing practices. This should be limited by using high purity ingredients as required by the TPD. Next to the presence of

these VOCs in the e-liquids, some suggest that VOCs could be formed by the heating process during vaping of the e-cigarette [40]. Therefore VOCs are contaminants that could be found in higher concentrations in the e-cigarette vapours compared to the e-liquid.

Diacetyl and **acetylpropionyl** are two controversial flavourings used in e-cigarettes. Both are examples of flavours that are generally considered as safe (GRAS) in food, but their inhalation is reported to be associated with respiratory inflammatory diseases. From our analysis, it can be seen that there is a positive evolution ongoing, as the number of positive samples for DA and AP was lower compared to samples from previous studies [23,24] where respectively 90% and 75% of the samples contained one of both diketons. Compared to our study, we found that in 2017, 9 samples out of the 26 sweet flavoured e-liquids (25%) were either positive for DA, AP or both, while in 2018 this was the case for 6 out of 30 sweet flavoured samples (20%). More recently, LeBouf et al. found that 70% of the brown flavours contained either DA or AP, which is still more than the 54% in the respective sample set (15 samples out of 26 brown flavoured e-liquids) [19]. The TPD does not explicitly prohibit the use of these flavourings. In fact, the TPD is unclear about flavours used in e-cigarettes. To assure the safety of consumers, a next step in the regulation of e-cigarettes should therefore encompass inhalatory risk assessment of flavourings in order to compile lists of restricted/prohibited flavouring substances.

Similar measures were taken to regulate the use of additives in e-cigarettes such as **caffeine** and **taurine**. The main reason of the prohibition of caffeine and other stimulants in e-liquids in the EU is that they might give the perception that these e-cigarette products may be used for lifestyle purposes instead of nicotine replacement therapy. Consequently, they could also contribute to the normalization of cigarette use, although this has not been investigated yet. The caffeine concentrations found in the e-liquids in this study were lower than in previous investigations [41]. Also, the number of caffeine-associated flavoured e-liquids compliant to the TPD is higher than seen during previous investigations, nevertheless there are still samples of 2018 that contained caffeine. The potential health risks of the inhalation of caffeine through e-cigarettes are currently unknown.

These do not only depend on the found concentration in the e-liquids, but also on the amount that is actually transferred from the e-liquid to the vapour and in particular on the local effects of caffeine on the respiratory tract and lungs.

Taken together, our study results clearly demonstrate that the implementation of the revised TPD has improved the quality of the e-liquids on the Belgian market. There are, however, certain limitations that could be addressed in future research. First, due to insufficient amounts, not all e-liquid samples could be investigated for each component. Also, a follow-up of e-liquids of the same brand before and after the implementation of the TPD would allow an even more accurate conclusion, however this was not possible because the brands analysed before the TPD were not available on the Belgian market after the implementation of the TPD. The third limitation concerns the restriction of the quality parameters chosen, namely the levels of nicotine, nicotine-related impurities, VOCs, additives caffeine and taurine, and the flavours diacetyl and acetylpropionyl. Although these parameters are the most important ones, including also nitrosamine and heavy metals impurities would have been even more complete, but requires yet other analytical instruments. Lastly, this study only focused on the e-liquids. Emission studies on e-cigarette aerosols would, however, have made it also possible to perform a preliminary safety evaluation of the poor-quality e-liquids identified.

5. CONCLUSION

E-cigarettes are not a new phenomenon anymore, nevertheless hitherto there was no overview of the quality of the different e-liquids available on the market. In this study, the major aim was to assess the compliance of currently available e-liquids to the TPD by analysis of the nicotine, nicotine-related impurities, VOCs, additives caffeine and taurine, flavours diacetyl and acetylpropionyl levels. In addition, we also investigated whether the recent changes in the TPD have affected the quality of e-cigarette liquids by testing samples acquired from specialized vapeshops as well from the internet before, during and after the implementation of the revised TPD.

Our results demonstrate that since the implementation of the revised TPD the quality of zero-liquids has improved. However, nicotine label discrepancies are still common. Therefore continuous monitoring of the e-liquid market remains important, together with measurements to assure good e-liquid products (in the context of harm reduction) such as the use of high quality starting materials, good manufacturing practices and stability testing for appropriate storage packaging. Thus, overall the quality of the e-liquids nowadays are better than before the implementation of the TPD. Vapeshop samples are generally more compliant to the TPD than internet samples and DIY samples. These findings support the Belgian legislation to retain the prohibition of the internet sale of e-liquids.

It has to be mentioned that these results shown here only reflect the e-liquids itself since the aerosols of e-cigarettes were not investigated. Indeed, there are many aspects that still need to be explored: the process of heating, the interactions between the different components, the formation of other (hazardous) components, inhalatory toxicity studies and the effect of mixture toxicology. Thus, while we can state that the analytical methodologies for chemical characterization are widely established, the standardization of emission studies is still in its infancy. Therefore, considerably more work needs to be done for the harmonization of emission studies of e-cigarette aerosols.

DECLARATION OF INTERESTS

None declared.

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FIGURE CAPTIONS

Fig 1. Overview of the chemical characterization of the investigated e-liquid samples. **A.** Conformity of the tested zero-liquids (no nicotine present (NEG), nicotine present (POS)). **B.** Label accuracy of the nicotine concentration. The first category (green) contains samples that deviate less than 10% from the claimed nicotine concentration. These samples are regarded as conform as the error of the analytical method (10%) is taken into account. The second category (orange) is the grey zone that contains samples with a nicotine concentration deviating between 10% and 20% from the labelled concentration. The third category (red) contains samples with measured nicotine concentrations deviating more than 20% of the claimed nicotine concentration. **C.** Caffeine in e-liquids (no caffeine present (NEG), caffeine present (POS)). **D.** Diacetyl (DA) and acetylpropionyl (AP) in sweet-brown flavoured e-liquids.

Fig 2. Presence of nicotine-related impurities in the investigated e-liquid samples. Nicotine impurities content (specified impurity (mg)/ nicotine (mg) %) of e-liquids, with individual specified impurity limit of 0.3% and total specified impurities limit of 0.8%. Samples exceeding the Ph.Eur. threshold are not conform (Myosmine (MYO), anabasine (ANAB), anatabine (ANAT), nornicotine (NOR), cotinine (COT), nicotine-N-oxide (NNO)).

Fig 3. **Stability of nicotine in e-liquid matrices.** The relative difference of nicotine at 1,3,6 and 9 months versus the initial concentration ± SD is shown for the different matrices. As there was no significant difference between samples stored in the dark or exposed to light, the pooled averages ± SD are shown.

Fig 4. Formation of nicotine-related impurities in e-liquid matrices. Left: The influence of the different e-liquid matrices on the formation of nicotine-N-oxide and myosmine for propylene glycol (PG), glycerol (G), PG/G and 1,3-propanediol (PD). Right: The influence of light exposure on the formation of nicotine-N-oxide and myosmine for the PG/G matrix.

Figure 1

Figure 2

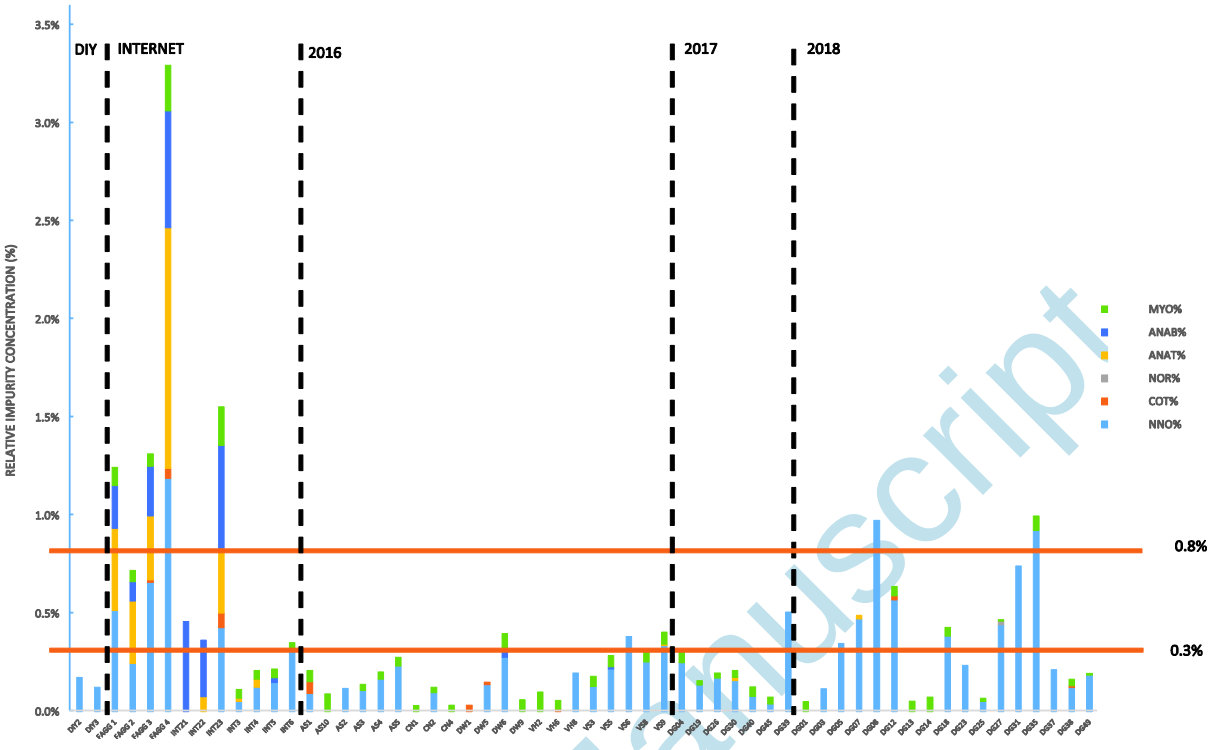

Figure 3

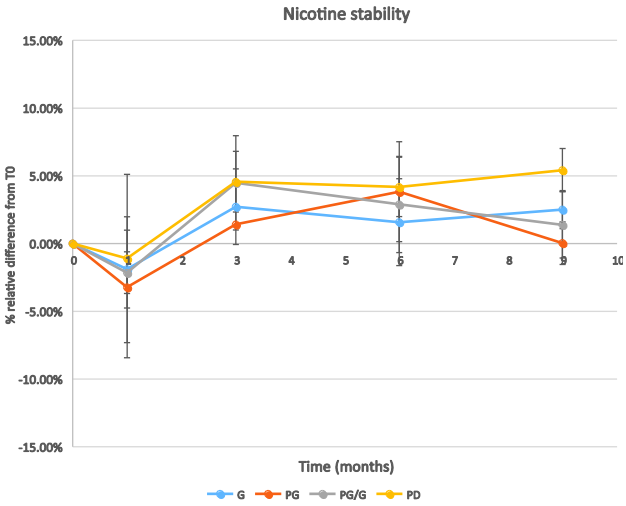

Figure 4