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Development of methods by regional and international standards organizations for testing and measuring the contents and emissions of electronic nicotine and non-nicotine delivery systems

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ABBREVIATIONS

AFNOR	French Standardization Association
BSI	British Standards Institution
CEN	European Committee for Standardization
CFP	Cambridge filter pad
COP	Conference of the Parties
CORESTA	Cooperation Centre for Scientific Research Relative to Tobacco
CRM	CORESTA Reference Method
DAD	diode array detection
DNPH	2,4-dinitrophenylhydrazine
ENDS	electronic nicotine delivery systems
ENNDS	electronic non-nicotine delivery system
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
ISO	International Organization for Standardization
LC-MS	liquid chromatography-mass spectrometry
LLE	liquid-liquid extraction
NMR	nuclear magnetic resonance
OES	optical emission spectrometry
SOP	standard operating procedure
TobReg	WHO Study Group on Tobacco Product Regulation
TSNA	tobacco-specific nitrosamine
WG	working group
WHO FCTC	WHO Framework Convention on Tobacco Control

EXECUTIVE SUMMARY

Electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) are available in various flavours and with various nicotine contents; e-cigarettes are the most common of these products, although others are also available. Emissions of nicotine and other harmful components differ among users and devices in which e-liquid is aerosolized, depending on the composition of the e-liquid and according to how e-cigarettes are used. Standard methods are important for testing products, for either monitoring or regulatory reporting to ensure comparison of products between laboratories and countries and over time. To gather information on the methods available for these products, the Seventh Session of the Conference of the Parties (COP) to the WHO Framework Convention on Tobacco Control (WHO FCTC) requested that a report on the development of methods by regional and international standards-development organizations for the testing and measuring of contents and emissions of these products be submitted at either its Eighth or its Ninth session. This paper was commissioned by WHO to inform development of the COP report.

Article 9 of the WHO FCTC recommends that verification methods be developed independently of product manufacturers. Knowledge and expertise of independent laboratories, such as members of the WHO Tobacco Laboratory Network (WHO TobLabNet) can be used for this purpose. Methods may be based on existing standards for tobacco filler and cigarette smoke, although these must be adapted, as e-liquids usually do not require the sample extraction procedures necessary for tobacco. For determination of compounds in emissions of ENDS/ENNDS, the aerosol trapping method must be suitable for the high content of aerosolizing agents, such as propylene glycol and glycerol, in emissions. The sensitivity of the analytical instrumentation should also be considered because of differences in target analyte concentration. As product regulation in most jurisdictions is focused on reducing the attractiveness of products, development of methods for substances with limited relevance for cigarettes, especially flavourings, should be a priority. More research and further development of independent laboratory capacity is also necessary, to ensure sufficient capacity to evaluate the large number of products marketed around the world.

1 INTRODUCTION

Electronic nicotine delivery systems (ENDS), of which e-cigarettes are the most common, have generated global attention over the past decade because of their proliferation in various markets, their popularity and attractiveness to minors. In ENDS, electric energy is most commonly used to heat a liquid to generate a nicotine-containing aerosol intended to be inhaled by the user. Devices based on the same principle but that do not contain nicotine are referred to as electronic non-nicotine delivery systems (ENNDS).

ENDS and ENNDS are available in various sizes and shapes. Initially, ENDS were designed to resemble cigarettes. Subsequently, larger ENDS with refillable tanks became available and also products that resemble cigars, pipes or hookahs. Recently, prefilled cartridge products resembling USB sticks have become popular, especially among young people (1). The different products include a battery to heat a coil and vaporize a liquid matrix in order to deliver an aerosol (also referred to as “vapour”) to the user. Typically, the liquid matrix is a solution containing propylene glycol alone or in combination with glycerol (vegetable glycerol, often referred to colloquially as VG), nicotine and other constituents, in particular flavourings. The solution and resulting aerosol may contain various nicotine concentrations and other chemicals such as flavourings to enhance product appeal. Besides nicotine, other minor tobacco alkaloids, tobacco-specific nitrosamines (TSNAs), metals and other components have been reported in e-liquids (2–4). Harmful substances, including carbonyls, volatile and semi-volatile organic compounds, TSNAs and metals, have been reported in ENDS aerosols (4–6).

Further, new substances with little or no relevance for testing of tobacco cigarettes may be important analytical targets for ENDS/ENNDS. For example, ENDS have recently been marketed that contain so-called “nicotine salts”, indicating that an organic acid has been added to decrease the pH of the e-liquid (7). Several salts and acids are currently used in e-liquids (7), and at least six types of acid are used to create nicotine salts in e-liquids, lactic acid, benzoic acid and levulinic acid being most frequently identified. The types and concentrations of salts and acids used in e-liquids may differentially alter sensations in the throat and upper airways and the overall pattern of inhalation of aerosols by decreasing the pH (8). In addition, a variety of aromas are added to e-liquids to make them more attractive and palatable, and some of these substances are of toxicological concern. Toxicologically relevant substances can also be formed from the constituents of e-liquids (9), and such reactions can be facilitated by heat or by the metal coil that

acts as a catalyst (10). Some of these products (e.g. formaldehyde, acetaldehyde and acrolein) are on the WHO Study Group on Tobacco Product Regulation (TobReg) priority list of toxicants (11), while others are not. It is highly recommended that methods for the determination of organic acids, relevant aroma substances and reaction products be included in standardization.

National regulatory authorities have taken various approaches to addressing the public health impact of ENDS and ENNDS. Some have banned or regulated ENDS, as recommended by WHO or guidance or recommendations of the Conference of the Parties to the WHO Framework Convention on Tobacco Control (WHO FCTC) (12). ENDS and ENNDS products are available in more than 100 countries. While these products are banned in over 30 countries, they are currently unregulated in most (13). Because of the wide variation in the products and the absence of internationally agreed approaches and methods for testing the products, surveillance and scientific risk assessment remain challenges.

The Conference of the Parties (COP) to the WHO FCTC has attempted to address some of the regulatory challenges associated with ENDS and ENNDS and to bridge gaps in scientific information. The Secretariat of the WHO FCTC was requested at COP7 to invite WHO to

report on the development of methods by regional and international standards-development organizations for the testing and measuring of contents and emissions of these products, at either the eighth or the ninth session of the COP, as applicable (Decision FCTC/COP7(9)) (14).

This background paper will form the basis of the WHO report to the Ninth and Tenth Sessions of the Conference of the Parties to the WHO FCTC. Currently, only a few standardized methods are available for the determination of the components of e-cigarette contents and emissions (see below). Several groups, however, are developing standardized methods. In this background paper, we provide an overview of the methods available in the literature and method development activities for ENDS/ENNDS in TobLabNet and other international and regional standardization bodies. Its aim is to update the report to the seventh Conference of the Parties (15). Extension of existing standard operating procedures (SOPs) by the WHO TobLabNet to ENDS/ENNDS is covered in terms of their applicability and/or adaptability. Further, regional and international standardized methods and published methods on ENDS/ENNDS are discussed.

2 METHODS

A comprehensive review was conducted of published TobLabNet methods for measuring the analytes of interest in ENDS and ENNDS. Published methods and methods being developed by national, regional (such as the French national standardization body, Association Française de Normalisation (AFNOR) and international standardization bodies, such as the International Organization for Standardization (ISO), were also reviewed. The scientific literature in PubMed and Web of Science and the grey literature were searched with the terms “e-cigarettes”, “electronic nicotine delivery system“, “ENDS”, “electronic non-nicotine delivery system”, “ENNDS”, “content”, “emission”, “methods” and “standardization”. Methods for analysis of the toxicants and analytes of interest, such as sugars, tobacco-specific nitrosamines (TSNA), heavy metal and carbonyl compounds, in ENDS and ENNDS were identified in journal articles. The identified sources were initially screened by title and abstract, where applicable, and the papers of interest were considered for full review. Further, a questionnaire was sent to TobLabNet members to identify the methods used in their laboratories for analysing toxicants in ENDS and ENNDS. The methods identified from all these sources are discussed below.

The draft publication was reviewed by assigned reviewers, including the Chair of TobLabNet, and by the WHO Secretariat. Recommendations based on the synthesized evidence are provided below.

3 TESTING AND MEASURING THE CONTENTS AND EMISSIONS OF ENDS/ENNDS

3.1 Compounds to be measured

When developing a method for analysis of ENDS/ENNDS, the first step is to specify the purpose of the method in terms of the regulatory aim. Also, the intention of the analysis should be clarified, such as whether it is to determine the components, impurities or reaction products in the e-liquid directly or in emissions. The compounds to be determined could be selected to address toxicity or their effects on the addictiveness or attractiveness of the product. On the basis of such considerations, TobReg has published a non-exhaustive list of priority toxic contents in the emissions of tobacco products (Table 1) (11), some of which have also been detected in emissions of ENDS/ENNDS. Substances with carcinogenic, mutagenic and reprotoxic properties should not be used in e-liquids. Groups of interest in e-liquid contents and emissions include nicotine, glycerol, propylene glycol, TSNAs, polyaromatic hydrocarbons, volatile organic compounds, phenolic components, metals and flavourings. Regulators should also consider whether only identification of compounds is necessary or whether they should also be quantified. A decision should be made with regard to analytical properties, such as accuracy and specificity for their specific aims.

Table 1. TobReg priority list of toxic contents and emissions of tobacco products

Compound group	Components
Alkaloids	Nicotine
Aldehydes	Acetaldehyde, acrolein, formaldehyde, crotonaldehyde, propionaldehyde, butyraldehyde
Aromatic amines	3-Aminobiphenyl, 4-aminobiphenyl, 1-aminonaphthalene, 2aminonaphthalene
Hydrocarbons	Benzene, 1,3-butadiene, isoprene, toluene
Polycyclic aromatic hydrocarbon	Benzo[a]pyrene
TSNAs	4-(Methylnitrosamino)-1-(3- pyridyl) -1-butanone, <i>N</i> -nitrosoanornicotine, <i>N</i> -nitrosoanatabine, <i>N</i> -nitrosoanabasine
Phenols	Catechol, <i>m</i> -, <i>p</i> - and <i>o</i> -cresol, phenol, hydroquinone, resorcinol
Other organic compounds	Acetone, acrylonitrile, quinoline, pyridine
Metals and metalloids	Cadmium, lead, mercury, arsenic ^a
Other constituents	Ammonia, carbon monoxide, hydrogen cyanide, nitrogen oxides

Source: WHO Study Group on Tobacco Product Regulation (11)

^a Added after the extended list was published in document FCTC/COP/6/14

3.2 Adaptation of methods for determination of compounds in cigarettes to methods for determination of compounds in e-cigarettes

Validated methods are available for determining several of the compounds listed above in cigarette tobacco contents or emissions, and some methods already exist for determining these compounds in ENDS/ENNDS or their emissions. These reports can be found on the websites of, for example, WHO TobLabNet (<https://www.who.int/groups/who-tobacco-laboratory-network>), ISO (<https://www.iso.org>), CEN (<https://www.cencenelec.eu>) and CORESTA (<https://www.coresta.org>).

Extraction methods from tobacco are not required for determining compounds in e-liquids. In most cases, simple dilution and stabilization of the compounds are sufficient. Because of the high viscosity of e-liquids, however, special precautions are required in weighing and pipetting e-liquids to avoid high variation or even incorrect results.

To generate emissions, specific settings should be selected for the puffing protocol and the device (if applicable). These settings can vary among different types of e-cigarettes and should be agreed upon.

To determine the components of emissions from ENDS/ENNDS, dedicated equipment is required for aerosol generation, test procedures and trapping. Depending on the trapping system used, extraction methods and measurement techniques similar to those for cigarette emission testing might be used or adapted for testing e-cigarette emissions. To determine whether currently available validated methods for cigarette emissions are applicable or adaptable for measuring ENDS/ENNDS emissions, the concentrations of several of the harmful components in ENDS/ENNDS emissions must be compared with those in cigarette smoke (16). Accordingly, more sensitive analytical instrumentation should be considered.

3.3 Prioritization of compounds for analysis and relevance of the priority list of toxicants to ENDS

Various procedures can be used to prioritize the methods to be developed for testing ENDS/ENNDS, depending on product attractiveness or addictiveness or to reduce toxicity.

The attractiveness of ENDS/ENNDS is enhanced by sugars and flavourings in e-liquids, which thus play a key role in the selection of e-liquids, especially by young people (17). Fagan et al. (18) found significant correlations between sugar content in e-liquids and carbonyl emissions. These findings and their potential effect on product attractiveness indicate that development and validation of

methods for determining sugar in e-liquids should be a high priority. Goldenson et al. (19) reported that sweet-flavoured (as compared with non-sweet and flavourless) e-liquids have greater appeal and received higher sweetness ratings by young adults. Nicotine received greater throat hit ratings than placebo but did not significantly increase the appeal of e-liquids nor interact with flavours in the appeal of e-liquids. Krüsemann et al. (20) developed an e-liquid “flavour wheel” and provided a guideline for systematic classification of e-liquids according to their marketing descriptions. Application of the flavour wheel in research on e-liquids will improve data interpretation, increase the comparability of studies and guide policy-makers in setting rules for regulating e-liquid flavourings. Use of the flavour wheel and the preferences of young adults, specific flavourings could be prioritized for method development.

With regards to addictiveness, nicotine is the most relevant component in e-cigarettes. In the European Union, maximum concentration of nicotine in e-liquids is limited to 20 mg/mL (21). Determination of the presence or the concentration of nicotine in e-liquids for ENDS or ENNDS is highly important. The method published by WHO (TobLabNet SOP11 (22)) for the determination of nicotine in e-liquids can be used to fulfil regulatory requirements for correct declaration of the nicotine content on packages. Further requirements for the determination of the ratio of nicotine salt to free-base is indicated.

For evaluation of the toxicity of ENDS/ENNDS, analysis of the priority chemicals listed in Table 1 is a good start. Toxic carbonyl compounds have been detected in many studies. For instance, Conklin et al. (23) reported that the levels of carbonyls (formaldehyde, acetaldehyde, acrolein and crotonaldehyde) depended on the propylene glycol:glycerol ratio and the presence of flavourings in e-liquids. Development, validation and standardization of a method for determination of carbonyls in ENDS/ENNDS emissions is a high priority. Other priority list compounds and (potentially) hazardous compounds that are less relevant for tobacco cigarettes have been detected in ENDS/ENNDS emissions as discussed below, and a dedicated priority list for toxic compounds in ENDS/ENNDS emissions is recommended based on current literature and further research.

4 ACTIVITIES OF WHO TOBLABNET

WHO TobLabNet was established in 2005 to connect government, university and other independent laboratories globally to develop analytical testing methods for tobacco products (24). The main aim is to strengthen national and regional capacity for testing of and research on the contents and emissions of tobacco products, in accordance with Article 9 of the WHO FCTC. WHO TobLabNet therefore develops and validates methods for testing nicotine and tobacco products without the involvement of the tobacco industry.

As of June 2021, WHO TobLabNet counted 50 members in 33 countries in all six WHO regions. WHO TobLabNet is coordinated by the No Tobacco Unit of the Health Promotion Department of WHO.

4.1 Role of WHO TobLabNet in method development

As stated in Article 9 of the WHO FCTC,

each Party shall, where approved by competent national authorities, adopt and implement effective legislative, executive and administrative or other measures, for the testing and measuring of the contents and emissions of tobacco products and for the regulation of these contents and emissions.

Paragraph 2.4 of the Partial guidelines for implementation of Articles 9 and 10 (25), requires that laboratories used by Parties for compliance purposes should be either governmental laboratories or independent laboratories that are not owned or controlled, directly or indirectly, by the tobacco industry. In addition, such laboratories should be accredited according to ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories) by a recognized accreditation body. Parties may also consider making use of governmental or independent laboratories located in other countries. Currently, organizations, such as the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), the European Committee for Standardization (CEN) and the International Organization for Standardization (ISO) work at global level on standardization of methods for the determination of the contents and emissions of e-cigarettes. Some standardization bodies are strongly influenced by the tobacco and ENDS/ENNDS industries. Published documents by regional and local standardization bodies (technical reports and so-called “voluntary standards”)

often recommend components to be measured (26) or even accepted levels of harmful components (27), which cannot be decided by the industry. With respect to Article 5.3 and the requirements of Article 9 of the WHO FCTC, regulatory measures shall be taken independently of industry involvement. Therefore, methods for determining the contents and emissions of ENDS/ENNDS for regulatory purposes should be developed by laboratories that are independent of tobacco and ENDS/ENNDS manufacturers. It is therefore essential to strengthen the independent laboratory network, WHO TobLabNet, further, including building more independent laboratory capacity and government support to the laboratories.

4.2 Adaption of WHO TobLabNet SOPs for cigarettes to use for ENDS/ENNDS

To date, WHO TobLabNet has published 11 validated methods for determining the contents and emissions of cigarettes and e-liquids. The current validated WHO TobLabNet methods for the determination of nicotine, TSNA, aldehydes, volatile organic compounds and benzo[*a*]pyrene in cigarette emissions can be adapted for determination of the components of ENDS/ENNDS emissions. Particular attention should be paid to trapping efficiency, measurement range, interference and product variation and stability.

In addition to the measurement methods validated by TobLabNet, puffing topography used for cigarette emission testing can be adapted for ENDS/ENNDS. The main items to be adapted or added to the SOP for ENDS/ENNDS emission generation are connection of ENDS/ENNDS to smoking or vaping machines, activation of ENDS/ENNDS with a certain air flow or by pushing a button and specific characteristics of puffing topography according to product type (e.g. cig-a-like, pod e-cigarettes, ENDS/ENNDS with direct-to-lung inhalation).

After adaptation of methods to use for ENDS/ENNDS, they should be validated and tested in a collaborative study before they become official WHO TobLabNet SOPs.

4.3 Development of new WHO TobLabNet methods for ENDS/ENNDS

In May 2019, WHO requested members of TobLabNet to provide information on the methods they used to determine the contents and emissions of ENDS/ENNDS and to provide copies of the methods used to WHO. Only a few laboratories reported use of methods other than the ISO methods for determination of nicotine, glycerol and propylene glycol in e-liquids. Because of the small number

of responses, the outcomes are not representative and are not summarized in this document, although all the reported methods are discussed.

In 2020, the European Joint Action on Tobacco Control, in collaboration with WHO, began to validate an analytical method for the determination of nicotine, glycerol and propylene glycol in e-liquids in a collaborative study, independent of tobacco industry and e-liquid manufacturers' laboratories. TobLabNet laboratories were invited by WHO to participate in this collaborative study. The validated SOP has been designated TobLabNet SOP11 (22) and is available to countries for regulatory purposes. It will be translated into other United Nations languages.

5 METHODS BEING DEVELOPED BY INTERNATIONAL STANDARDS ORGANIZATIONS

Several international bodies are working on standardization of methods for ENDS/ENNDS. The activities of five organizations are summarized, namely CORESTA, ISO, CEN, the British Standards Institution (BSI) and the French Standardization Association (AFNOR). It should be noted that, unlike WHO TobLabNet, none of these organizations is free of the influence of the tobacco industry;¹ however, government laboratories and institutions can participate in these standardization bodies to counterbalance industry influence and introduce their independently developed methods for standardization.

“Publicly available specifications” are documents designed to allow efficient, rapid standardization in response to market demand or at the request of sponsors. In principle, application of publicly available specifications is voluntarily, but they may become mandatory by agreement or demand in regulatory instruments. These procedures do not necessarily involve government institutions or other regulating agencies. Usually, publicly available specifications are reviewed after an initial period of 2 years and may then be proposed as basis for European or international standards.

5.1 Methods published by CORESTA

CORESTA published a method for aerosol collection and sample preparation for e-cigarette emission testing (28) and a method for the determination of glycerol, propylene glycol, water and nicotine in the aerosol of e-cigarettes by gas chromatography (29). The method defined a set of puffing topography parameters (puff volume, 55 mL; puff duration, 3 s; puff interval, 30 s; rectangular puff profile), which do not, however, take into account the differences in puff topography that are relevant to the new generations of e-cigarettes such as ENDS for direct-to-lung inhalation). The CORESTA method also describes aerosol collection on a glass-fibre filter, whereas, as discussed below, other trapping techniques might be more applicable for ENDS/ENNDS.

¹ See the business plans of CORESTA (<https://www.coresta.org/who-we-are-29290.html>), ISO (https://www.iso.org/home.isoDocumentsDownload.do?t=1939tNKe1shwCgOV9mUP8sYZma83WPcAqNReBgWKWcuJvIRKy7gzz0Kx36XC_Pb5&CSRFTOKEN=IRBQ-TZU6-YJFX-LSOU-X76H-CQXO-KAUR-G6AF), CEN (<https://standards.cencenelec.eu/BPCEN/1958025.pdf>), BSI (<https://www.bsigroup.com/en-GB/about-bsi/media-centre/press-releases/2015/july/E-cigarettes-guidance-published/>) and AFNOR (<https://www.afnor.org/en/news/vaping-afnor-publishes-method-characterizing-emissions/>).

As a liaison member of ISO TC/126, CORESTA (30) develops methods for physical or chemical measurements in tobacco products and e-cigarettes, including proficiency testing, collaborative studies and conduct reviews, leading to publication of recommended methods and technical reports. To date, CORESTA has published the following methods and reports related to e-cigarettes:

- CORESTA Reference Method (CRM) 84. Determination of glycerin, propylene glycol, water, and nicotine in the aerosol of e-cigarettes by gas chromatographic analysis (06/2017)
- CRM 96. Determination of formaldehyde and acetaldehyde in e-vapour product aerosol (02/2021)
- Guide 18. Sample handling and sample collection of e-cigarettes and e-vapour generating products (11/2016)
- Guide 22. Technical guide for the selection of appropriate intense vaping regimes for e-vapour devices (02/2018)
- Guide 25. Technical guide for aerosol collection and considerations when testing e-vapour product technologies (05/2019)
- Guide 26. Technical guide for designing e-vapour product stability studies (09/2019)
- Guide 28. Technical guide for setting method LOD and LOQ values for the determination of metals in e-liquid and e-vapour aerosol by ICP-MS (11/2020).

5.2 Methods published by CEN

In Europe, a technical committee (CEN/TC 437) within CEN was founded by AFNOR² to perform standardization work based on Tobacco Products Directive 2 of the European Commission. The work of the committee started in 2015, and, at the time of writing, it had held eight plenary meetings. The committee consisted of five working groups (WGs):

- WG 1. Terminology and definitions
- WG 2. Requirements and test methods for electronic cigarette devices. The results were combined in technical specification CEN/TC 17287, and the working group was disbanded.
- WG 3. Requirements and test methods for e-liquids. The following activities are ongoing:

² CEN Technical Board decision

- PWI00437001. General principles for manufacturing, filling and holding e-liquids for prefilled containers or products
- PWI00437002. General principles and requirements for testing for quality and nicotine levels of e-liquids
- E-liquid ingredients
- User information
- WG 4. Requirements and test methods for emissions, with activities in
 - PWI. Intense vaping regime;
 - prEN17375:2019. Reference e-liquids;
 - PWI00437005. Method to measure consistent emission of nicotine;
 - PWI00437009. Requirements and test methods for emissions.
- WG 5. Extractables and leachables

Additionally, at the eighth plenary meeting, a new work item was proposed, “Child safety requirements and test methods for electronic cigarettes”. CEN has published a technical report (CEN/TR 17236) on constituents of interest for determination in emissions of ENDS/ENNDS and a standard specifying a reference e-liquid for emission testing (EN 17375).

Because of limited technical experience and the small number of participating laboratories in CEN TC/437, and also because of the advantage of standardizing methods globally, the CEN technical committee decided to transfer development and validation of standardized methods to ISO technical committee TC/126 on “Tobacco and tobacco products”.

5.3 Methods published by ISO

Within ISO/TC 126 (Tobacco and tobacco products), sub-committee SC3 on Vape and vapour products was created, with the following working groups:

- WG 1. Substances in liquids of e-cigarettes, ISO 20714:2019-08; standard is published, working group disbanded
- WG 2. Routine analytical e-cigarette vaping machine – Definition and standard conditions. ISO 20768:2018. This standard defines the parameters and specifies the standard conditions, the technical requirements and the puffing regimen for generation and collection of aerosol from e-cigarettes.
- WG 3. Analytical methods for the testing of emissions of vapour products, with the projects:
 - ISO 24197, Vapour products – Determination of e-liquid vaporised mass and aerosol collected mass

- ISO 24198, Vapour products – Analytical method to measure metals of e-vapor product emissions
- ISO 24199, Vapour products – Determination of nicotine in vapour product emissions – Gas chromatographic method
- ISO 24211, Vapour products – Determination of selected carbonyls in vapour product emissions

A dedicated method for the determination of nicotine, glycerol and propylene glycol in e-liquids is available (ISO 20714:2019-08) (31). The method describes determination of components by weighing the e-liquid, followed by dilution and measurement by gas chromatography–flame ionization detection. No detailed information on progress in the working groups is available, as this information is available only to members of the groups.

5.4 Methods published by BSI

Not only international standardization organizations like ISO and CEN (within Europe) but also national standardization bodies are preparing proposals for standardization of methods for the determination of components of e-liquid or e-cigarette aerosol. For instance, the BSI has published a list of proposals for testing e-cigarette contents and emissions:

- NP 24211: Vapour products – Analytical method to measure carbonyls of e-vapor product emissions
- NP 24199: Vapour products – Analytical method to measure nicotine of e-vapor product emissions
- NP 24198: Vapour products – Analytical method to measure metals of e-vapor product emissions
- NP 24197: Vapour products – Analytical method to measure mass of e-liquids vaporized
- NP 4352: E-liquid, vapour products and aerosol - Determination of nicotine, propylene glycol and glycerol content – Liquid chromatographic method
- N 251: Electronic cigarettes and e-liquids – Determination of nicotine delivery consistency over defined puff sequences of a number of e-cigarettes of identical type
- N 242: E-liquid Ingredients

5.5 Methods published by AFNOR

The French national standardization body, AFNOR (Association *Française* de Normalisation) is also standardizing national methods for testing e-cigarette contents and emissions. AFNOR has so far published three voluntary standards on e-cigarettes and e-liquids, with three main sections:

- XP D90-300-1: Requirements and test methods for electronic cigarettes
- XP D90-300-2: Requirements and test methods for e-liquids
- XP D90-300-3: Requirements and test methods concerning emissions

Although these standards are voluntary, they can be proposed by AFNOR to international standardization bodies as new internationally standardized methods.

6 ANALYTICAL METHODS FOR ENDS/ENNDS DESCRIBED IN THE SCIENTIFIC LITERATURE

The content and emissions of ENDS/ENNDS have been measured by various research groups to generate data for risk assessment and to determine the factors that influence the composition of the aerosol. These reports can be found on the websites of, for example, WHO TobLabNet (<https://www.who.int/groups/who-tobacco-laboratory-network>), ISO (<https://www.iso.org>), CEN (<https://www.cencenelec.eu>) and CORESTA (<https://www.coresta.org>). Many studies have addressed issues in the analysis of ENDS/ENNDS or compounds that are relevant for ENDS/ENNDS but not for cigarette smoke. Ideally, one would start with non-targeted screening and toxicological assessment to prioritize components to be measured in e-cigarette contents and emissions. Strongin (32) reviewed e-cigarette chemistry and analysis in 2019; these and new methods could be the basis for standardization and for a list of priority analytes. A non-comprehensive discussion of analytical approaches described in the scientific literature is given below.

6.1 Aerosol generation

An important aspect of the study of aerosol generation is determining the most appropriate puffing regime, as each device type might require a different regime. E-cigarettes are used in various ways by consumers, as described in some publications (33–38) and the seventh report of TobReg (11). The puffing topography of users depends not only on the device and settings but also on the nicotine and flavouring content of the e-liquid and user experience (33–38), and the wide variation in published puffing parameters (volume, 9–250 mL; duration, 1–6 s; puff interval, 17–70 s) might mean that more than one testing topography is required (e.g. average and worst case) for regulatory purposes or product classification. Additionally, Korzun et al. (39) reported that nicotine emissions are strongly influenced by coil composition. McAdam et al. (40) reported the creation and analysis of a large database of “vaping” videos on social media, a “near-natural” setting. They showed similar mean puff durations for three categories of “vaping” devices, including direct-to-lung and mouth-to-lung puffing behaviour, and concluded that the wide variety of puffing durations among users of ENDS/ENNDS would make it difficult to identify a single yet representative machine-puffing regime for laboratory studies. They recommended a puff duration of 5.6 s (representing the 95th percentile puffing behaviour of ENDS/ENNDS users) as appropriate for a more intense puffing regime in addition to the standardized

puff duration of 3 s. As reported by DeVito et al. (41), puff duration and puff number are better determinants of the nicotine delivery of e-cigarettes than puff volume and velocity. Talih et al. (42,43) established a model for predicting aerosol generation and nicotine delivery based on design characteristics and puffing topography. Puffing duration was found to be an important factor but not puffing volume or flow rate. They demonstrated that the heating time of the coil of an e-cigarette is usually equal to the puffing duration, which implies that it might be more important to adjust the puffing duration than the puffing volume.

Another practical problem in studying ENDS/ENNDS aerosol generation is the connection of the device to a “vaping” machine. Some devices have oddly shaped mouthpieces (e.g. rectangular) that do not allow an airtight connection with flexible tubes. Connections with heat-shrinkable tubes have been tested for this purpose (44).

6.2 Determination of nicotine salts and organic acids

The current standard methods for determining nicotine in cigarette tobacco and e-liquids cannot distinguish between nicotine and nicotine salts. Further, methods should be developed for determining organic acid concentrations in e-liquids, given the importance of nicotine salts.

Duell et al. (45) reported a method for determining the ratio of free-base nicotine to protonated nicotine with ^1H -nuclear magnetic resonance (NMR) technology. Gholap et al. (46) compared various approaches to determining the percentage of free-base nicotine in flavoured e-liquids. E-liquids were diluted, and free-base nicotine was determined either by applying the Henderson-Hasselbalch equation or with high-performance liquid chromatography (HPLC) after liquid–liquid extraction (LLE) (47) to separate free-base from protonated nicotine, or an approach without dilution (direct determination with ^1H -NMR spectroscopy (48)) was applied. They discussed the advantages and shortcomings of each method and concluded that more investigation is required to decide on an appropriate approach for determining free-base nicotine in e-liquids.

Harvanko et al. (7) reported that at least six types of acid are being used to create nicotine salts in e-liquids, lactic, benzoic and levulinic acids being the most frequently identified. The authors analysed e-liquids by liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS). In LC–MS analysis, e-liquids were diluted in water and methanol. Before GC–MS analysis, the acids were converted to their methyl esters. Organic acids were not quantified. In another study, headspace–solid phase micro-extraction online-coupled with GC–MS was used to quantify benzoic acid from e-liquid and

ENDS aerosol collected on Cambridge filter pads (CFPs) after addition of sulfuric acid and a saturated sodium chloride solution (44).

6.3 Determination of flavouring components

Flavouring compounds, which make products, such as e-cigarettes and tobacco products appealing (49-51), are among the most commonly used ingredients in e-liquids (50,51) and are mainly determined by GC or LC methods with mass detection.

Tierney et al. (52) conducted quantitative analysis of 90 flavourings by simple dilution with methanol followed by GC-MS analysis. Aszyk et al. (53,54) determined various flavouring components after dilution of e-liquid with acetonitrile and water, followed by LC-MS or GC-MS analysis; 88 flavouring components can be determined by using both methods. Erythropel et al. (55) diluted e-liquids with methanol and analysed them with GC-MS to quantify flavourings and the associated acetals, which are formed from reaction of flavourants with propylene glycol or glycerol after mixing with the e-liquid. Acetals were also investigated in aerosols collected with liquid nitrogen-chilled cold finger traps before GC-MS analysis.

Hutzler et al. (56) screened e-liquid flavours qualitatively by LLE with ethyl acetate and addition of hydrochloric acid for acidic extraction or ammonia for basic extraction, followed by GC-MS analysis. Eddingsaas et al. (57) analysed three popular e-liquid flavours (cinnamon, mango, vanilla) by qualitative GC-MS after dilution with methanol. In the same study, components of the emissions of flavoured e-liquids were determined after trapping the components on CFPs and impingers, followed by GC-MS analysis. Nineteen components were observed in emissions that were not present in un-aerosolized e-liquid. Different components were found with the three sampling methods (e-liquid dilution and trapping of aerosol on a CFP or with impingers), implying that more than one method should be used. The authors suggested that further investigations should be conducted with different trapping mechanisms, such as those mentioned above and others, such as thermal desorption tubes and solvent extraction resins.

6.4 Determination of carbonyl compounds

While some aldehydes are added as flavourings, they can also be generated in ENDS/ENNDS aerosol due to thermal decomposition of glycerol and propylene glycol. Many research groups (56-64) have detected carbonyl compounds in ENDS/ENNDS aerosols, including formaldehyde, acetaldehyde, acrolein, crotonaldehyde and butyraldehyde. The aldehydes identified by the US Food and

Drug Administration and other aldehydes are usually analysed after derivatization with 2,4-dinitrophenylhydrazine (DNPH) and HPLC–diode array detection (DAD) analysis. Several trapping systems are used to test emission of the aldehydes, such as impingers with DNPH solution, DHPH solid-phase extraction cartridges and a carboxen cartridge combined with a CFP.

Uchiyama et al. (65) described a method for the determination of aldehydes, including glyoxal and methylglyoxal, consisting of a combined trapping system of coupled silica cartridges impregnated with hydroquinone and DNPH, followed by HPLC–DAD of the eluate. Stephens et al. (66) published a method for determining aldehydes with HPLC–DAD and confirmation by LC–MS. In this study, e-cigarette emission was trapped in a 10-mL syringe filled with 0.75 g of silica wool, which was found to be 94% more efficient than CFPs for trapping the vaporized liquid mass and to retain more condensate before reaching saturation. Trapping systems with quartz or silica wool might therefore have advantages as compared with other trapping systems, such as CFPs, cartridges or impingers. Depending on the components to be determined, collected emission components can be extracted simply by centrifuging quartz or silica wool, which has the advantage that the same analytical trapping method is used for determining the components of both e-liquids and e-cigarette emissions. Further research should be conducted to determine the most practical trapping system that avoids interference from the high amounts of propylene glycol and glycerol in the aerosol.

6.5 Determination of sugars

Most flavours in e-liquids can be categorized as sweet (19). Sugars can be added to enhance the sweet flavour of e-liquids. Fagan et al. (18) determined D-glucose, fructose and sucrose in e-liquids with an enzymatic kit combined with UV reading at 340 nm by a microplate reader in a 96-well plate. The published method for sugar determination in tobacco (67) is an alternative, less labour-intensive method, which could be adapted for e-liquids by simply diluting the e-liquid with water. In this method, sugars are analysed by HPLC with an electron light-scattering detector. Refractive index or charged aerosol detectors are other possible options for the analysis of sugars.

6.6 Determination of TSNAs

As nicotine in e-liquids is often extracted from tobacco, other components of cured tobacco may be present as contaminants in e-liquids; therefore, special attention must be paid to harmful components in e-liquids, including TSNAs (68,69).

Lee et al. (70) compared dilution of e-liquid with an ammonium acetate solution with dilution with acetonitrile, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) measurement for the determination of TSNAs. In general, dilution with acetonitrile gave better recovery than ammonium acetate. In the highest recovery test, however, dilution in acetonitrile resulted in recovery of up to 300%; no explanation was given in the publication. In the same study, TSNAs were determined in e-cigarette aerosol after collection on a CFP and on quartz wool. TSNAs were extracted with ammonium acetate. Quartz wool resulted in better recoveries than CFP trapping. Farsalinos et al. (71) analysed TSNAs in e-liquids after dilution with water and in e-cigarette aerosol with the same method used by Lee et al. (70). The recoveries of TSNAs in the aerosol, calculated in relation to spiked TSNAs in the e-liquid, were about 100% for all TSNAs. Kim & Shin (72) determined the TSNA content in e-liquids with LC-MS/MS to compare sample clean-up by solid-phase extraction and LLE. The best recovery (75–83%) was found after LLE with methylene chloride at pH 9.

E-cigarette aerosol can be trapped for the determination of TSNAs on CFPs. If recovery is inadequate, other trapping systems (e.g. quartz wool) should be investigated.

6.7 Determination of metals

More research should be conducted to identify the trapping method that collects all metals in the most practical way. Metal contamination of e-liquids and ENDS/ENNDS emissions can originate from impurities in chemicals used in the e-liquid, inappropriate handling during production or from metal components used in pre-filled e-cigarette cartridges, refillable tanks and the heating element (73,74). Belushkin et al. (4) determined metals in e-liquids by microwave digestion followed by inductively coupled plasma (ICP) and MS measurement. Hess et al. (75) described an alternative method, in which e-liquid was diluted with 1% HNO₃ and 0.5% HCl, followed by ICP-MS measurement. Aerosol was trapped for metal determination by various techniques. Belushkin et al. (4) used an electrostatic precipitation generator to collect particulate matter onto a glass tube, and the collected mass was extracted in methanol, evaporated and digested in a microwave. The gaseous-phase metals were trapped in an impinger containing a 10% v/v nitric acid solution, and the impinger solution was added to the same vessel and subjected to microwave digestion. In all these techniques, care must be taken to not introduce metal contamination from glass collection or other materials.

Ting et al. (76) used impingers containing 5 mL of 1% nitric acid (pH 3–4) to trap the metals in e-cigarette aerosol. Williams et al. (73) applied puff-by-puff aerosol collection in a solution, followed by analysis by ICP-MS and optical emission spectrometry (OES). ICP-MS is the method most widely used for measuring metals. ICP-OES or atomic absorption may also be used, depending on the required sensitivity of the instrumentation.

6.8 Determination of other potentially harmful components

Other possibly harmful components of e-liquids and e-cigarette aerosol are aromatic amines, polycyclic aromatic hydrocarbons and volatile organic compounds. Wagner et al. (77) determined aromatic amines and benzo[*a*]pyrene in e-liquids and e-cigarette aerosol with the same sample treatment. For determination of the compounds in e-liquids, the e-liquid was diluted with water and toluene, followed by addition of deuterated internal standards. Benzo[*a*]pyrene was analysed in the organic layer by GC-MS with no further treatment. Before determination of aromatic amines, the extracts were derivatized and loaded onto an SPE device; the eluent was then analysed by GC-MS.

Wagner et al. (77) also analysed volatile organic compounds in e-liquids and e-cigarette aerosol. The compounds were determined in e-liquids by diluting them with methanol followed by GC-MS analysis and in aerosol by trapping them in a cryogenically cooled impinger trap (–70 °C) after removal of propylene glycol and glycerol on CFPs.

Further research should be conducted to identify other relevant compounds that should be determined in e-liquids and emissions of ENDS/ENNDS.

7 SUMMARY

E-liquids for ENDS/ENNDS are available in various flavours and with various nicotine contents (2). The emission of nicotine and other harmful components may differ among consumers, depending on how they use e-cigarettes, which, in turn, is influenced by the device in which the e-liquid is aerosolized.

To determine the content of the components of e-liquids, validated TobLabNet methods for the determination of cigarette tobacco filler contents must be adapted to the characteristics of particular ENDS and ENNDS. Some of the procedures for extracting components of cigarette tobacco filler are unnecessary for e-liquids, and simple dilution in an appropriate solvent will usually be sufficient. The same techniques can be used for measuring e-liquid contents, with adaptation of the measurement range and the required sensitivity. Further, method variation will have to be determined for new applications.

International and national standardization bodies are currently working on methods for determination of the contents and emissions of ENDS/ENNDS.³ Additionally, many researchers have published methods for the determination of different groups of compounds, providing valuable information for method standardization. WHO TobLabNet recently published SOP 11 for the determination of nicotine, glycerol and propylene glycol in e-liquids (22). In accordance with Article 9 of the WHO FCTC, the Partial Guidelines on Articles 9 and 10 (25) and WHO guidance, methods for determining the contents and emissions of ENDS/ENNDS should be developed independently from product manufacturers.

The priority list of relevant toxicants in cigarette smoke can be extended only partly to ENDS/ENNDS (11). A new list based on current knowledge of the content and emissions of ENDS/ENNDS is recommended. High priority should be given to methods for determining flavourings in e-liquids, as these are important factors in product attractiveness and potential addiction. Thus, the highest priority would be validation of method for measuring commonly used flavourings (e.g. methyl cyclopentenolone, vanillin, furaneol, menthol) in e-liquids. The puffing topographies of different device types should be standardized by WHO TobLabNet,

³ See the business plans of CORESTA (<https://www.coresta.org/who-we-are-29290.html>), ISO (https://www.iso.org/home.isoDocumentsDownload.do?t=1939tNKe1shwCgOV9mUP8sYZma83WPcAqNReBgWKWcuJvIRKy7gzz0Kx36XC_Pb5&CSRFTOKEN=IRBQ-TZU6-YJFX-LSOU-X76H-CQXO-KAUR-G6AF), CEN (<https://standards.cencenelec.eu/BPCEN/1958025.pdf>), BSI (<https://www.bsigroup.com/en-GB/about-bsi/media-centre/press-releases/2015/july/E-cigarettes-guidance-published/>) and AFNOR (<https://www.afnor.org/en/news/vaping-afnor-publishes-method-characterizing-emissions/>).

followed by method development and validation for measuring nicotine and aldehyde emissions. The ratio of free-base to nicotine salt and measurement of other compounds, such as organic acids and metals, in e-liquids and ENDS/ENNDS aerosols should also be considered. Further investigation of trapping methods is also necessary. Alternative approaches to CFPs and impingers (e.g. trapping on quartz wool followed by centrifugal extraction) should be evaluated for their suitability for testing ENDS/ENNDS emissions and e-liquids. Development and validation of such methods, especially in collaborative studies, depends on the capacity of participants, the support of governments and available funding.

8 CONCLUSIONS AND RECOMMENDATIONS

In the absence of long-term experience, consumer products must be assessed and regulated on the basis of the available scientific evidence in order to protect consumers. Regulators worldwide are developing regulatory frameworks for ENDS/ENNDS, and some regulatory measures have already been taken (78). The Third Conference of the Parties to the WHO FCTC mandated TobLabNet to validate new methods for testing a number of prioritized constituents of cigarette tobacco and cigarette smoke, in addition to existing standards and to those under development by ISO at that time. All the methods that have been validated to date are available to countries and can be adapted to testing ENDS and ENNDS. A published method for determination of nicotine, glycerol and propylene glycol is also available. Some recommendations based in the review of literature are listed below.

- **It is recommended** that verification methods be developed, validated and standardized, independently of product manufacturers to ensure that marketed products comply with regulatory requirements. Standard methods are important for testing products in different laboratories, for monitoring or for regulatory reporting, as a standard protocol for testing allows comparison of products among laboratories and countries over time.
- **It is recommended** that all these methods be used for regulatory purposes to strengthen implementation of Articles 9 and 10 of the WHO FCTC.
- **It is also recommended** that WHO TobLabNet knowledge and expertise be leveraged for the development and validation of further test methods for regulation of ENDS/ENNDS, to further strengthen independent laboratory capacity.
- More research **is recommended** on the most suitable methods for determining components of interest in e-liquids and the emissions of ENDS/ENNDS. Standardization nevertheless requires a strong network of independent laboratories participating in collaborative studies.
- **It is strongly recommended** that the WHO TobLabNet extend its work in developing, validating and standardizing methods for determining the content and emissions of other products, including ENDS/ENNDS, to provide legislators with methods that are independent of tobacco and related industries.

REFERENCES

1. Vallone DM, Cuccia AF, Briggs J, Xiao H, Schillo BA, Hair EC. Electronic cigarette and JUUL use among adolescents and young adults. *JAMA Pediatr.* 2020;174(3):277–86. doi:10.1001/jamapediatrics.2019.5436.
2. Lisko J, Tran H, Stanfill S, Blount B, Watson C. Chemical composition and evaluation of nicotine, tobacco alkaloids, pH, and selected flavours in e-cigarette cartridges and refill solutions. *Nicotine Tob Res.* 2015;17(10):1270–8. doi:10.1093/ntr/ntu279.
3. Grana RA, Ling PM, Benowitz N, Glantz S. Electronic cigarettes. *Cardiology patient page. Circulation.* 2014;129(19):e490–2. doi:10.1161/CIRCULATIONAHA.114.007667.
4. Belushkin M, Djoko DT, Esposito M, Korneliou A, Jeannet C, Lazzerini M et al. Selected harmful and potentially harmful constituents levels in commercial e-cigarettes. *Chem Res Toxicol.* 2019;12:20. doi:10.1021/acs.chemrestox.9b00470.
5. Herrington JS, Myers C. Electronic cigarette solutions and resultant aerosol profiles. *J Chromatogr A.* 2015;1418:192–9. doi:10.1016/j.chroma.2015.09.034.
6. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control.* 2014;23:133–9. doi:10.1136/tobaccocontrol-2012-050859.
7. Harvanko AM, Havel CM, Jacob P, Benowitz NL. Characterization of nicotine salts in 23 electronic cigarette refill liquids. *Nicotine Tob Res.* 2020;22(7):1239–43. doi:10.1093/ntr/ntz232.
8. Leventhal AM, Madden DR, Peraza N, Schiff SJ, Lebovitz L, Whitted L et al. Effect of exposure to e-cigarettes with salt vs free-base nicotine on the appeal and sensory experience of vaping: a randomized clinical trial. *JAMA Netw. Open.* 2021;4(1):e2032757. doi:10.1001/jamanetworkopen.2020.32757.
9. Paine JB, Pithawalla YB, Naworal JD, Thomas CE. Carbohydrate pyrolysis mechanisms from isotopic labeling: Part 1: The pyrolysis of glycerin: Discovery of competing fragmentation mechanisms affording acetaldehyde and formaldehyde and the implications for carbohydrate pyrolysis. *J Anal Appl Pyrolysis.* 2007; 80:2. doi:10.1016/j.jaap.2007.03.007.
10. Bekki K, Uchiyama S, Ohta K, Inaba Y, Nakagome H, Kunugita N. Carbonyl compounds generated from electronic cigarettes. *Int J Environ Res Public Health.* 2014;11(11):11192–200. doi:10.3390/ijerph111111192.
11. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: Seventh report of a WHO study group (WHO Technical Report Series, No. 1015). Geneva: World Health Organization; 2019.
12. Electronic nicotine delivery systems and electronic non-nicotine delivery systems (ENDS/ENNS). Report to the Conference of the Parties to the WHO Framework Convention on Tobacco Control, Seventh session (FCTC/COP7/11). Geneva: World Health Organization; 2016.

13. Tobacco e-cigarettes. What are the policy options for regulating ENDS? Geneva: World Health Organization; 29 January 2020 (<https://www.who.int/news-room/q-a-detail/tobacco-e-cigarettes>).
14. Electronic nicotine delivery systems and electronic non-nicotine delivery systems. Report to the Conference of the Parties to the WHO Framework Convention on Tobacco Control, Seventh session. Geneva: World Health Organization; 2016 (https://www.who.int/fctc/cop/cop7/FCTC_COP7_9_EN.pdf?ua=1).
15. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: Sixth report of a WHO study group (WHO Technical Report Series, No. 1001). Geneva: World Health Organization; 2017.
16. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23:133–9. doi:10.1136/tobaccocontrol-2012-050859.
17. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: Eighth report of a WHO study group (WHO Technical Report Series, No. 1029). Geneva: World Health Organization; 2021 (<https://www.who.int/publications-detail-redirect/9789240022720>).
18. Fagan P, Pokhrel P, Herzog TA, Moolchan ET, Cassel KD, Franke AA et al. Sugar and aldehyde content in flavoured electronic cigarette liquids. *Nicotine Tob Res*. 2018;20(8):985–92. doi:10.1093/ntr/ntx234.
19. Goldenson NI, Kirkpatrick MG, Barrington-Trimis JL, Pang RD, McBeth JF, Pentz MA et al. Effects of sweet flavourings and nicotine on the appeal and sensory properties of e-cigarettes among young adult vapers: Application of a novel methodology. *Drug Alcohol Depend*. 2016;168:176–80. doi:10.1016/j.drugalcdep.2016.09.014
20. Krüsemann EJZ, Boesveldt S, de Graaf K, Talhout R. An e-liquid flavour wheel: a shared vocabulary based on systematically reviewing e-liquid flavour classifications in literature. *Nicotine Tob Res*. 2018;21(10):1310–9. doi:10.1093/ntr/nty101.
21. Directive 2014/40/EU of The European Parliament and of the Council, on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC. Brussels: European Commission; 2014 (<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014L0040> (https://ec.europa.eu/health/sites/default/files/tobacco/docs/dir_201440_en.pdf)).
22. Standard operating procedure for determination of nicotine, glycerol and propylene glycol in e-liquids (WHO TobLabNet SOP 11). Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/bitstream/handle/10665/340503/9789240022744-eng.pdf?sequence=1&isAllowed=y>).

23. Conklin DJ, Ogunwale MA, Chen Y, Theis WS, Nantz MH, Fu XA et al. Electronic cigarette-generated aldehydes: The contribution of e-liquid components to their formation and the use of urinary aldehyde metabolites as biomarkers of exposure. *Aerosol Sci Technol*. 2018;52:1219–32. doi:10.1080/02786826.2018.1500013.
24. Information sheet on WHO TobLabNet methods for measuring priority contents and emissions in tobacco and related products. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/bitstream/handle/10665/331427/WHO-HEP-HPR-2020.1-eng.pdf?sequence=1&isAllowed=y>).
25. Partial guidelines for implementation of Articles 9 and 10 – Regulation of the contents of tobacco products and regulation of tobacco product disclosures. Geneva: World Health Organization; 2017 (https://www.who.int/fctc/treaty_instruments/guidelines_articles_9_10_2017_english.pdf?ua=1).
26. Electronic cigarettes and e-liquids – Constituents to be measured in the aerosol of vaping products (CEN/TR 17236). Swedish Institute for Standards; 2018 (<https://www.sis.se/en/produkter/agriculture/tobacco-tobacco-products-and-related-equipment/sis-centr-172362018/>).
27. Electronic cigarettes and e-liquids – Part 2: Requirements and test methods for e-liquids (XP D90-300-3). La Plaine Saint Denis: Association française de normalisation; 2016 (<https://www.boutique.afnor.org/en-gb/standard/xp-d903003/electronic-cigarettes-and-e-liquids-part-3-requirements-and-test-methods-for/fa186224/1595>).
28. CRM 81. Routine analytical machine for e-cigarette aerosol generation and collection – definitions and standard conditions. Cooperation Centre for Scientific Research Relative to Tobacco; 2015 (https://www.coresta.org/sites/default/files/technical_documents/main/CRM_81.pdf).
29. CRM 84 Determination of glycerin, propylene glycol, water and nicotine in the aerosol of e-cigarettes by gas chromatography. Cooperation Centre for Scientific Research Relative to Tobacco; 2021 (https://www.coresta.org/sites/default/files/technical_documents/main/CRM_84-April2021.pdf).
30. Cooperation Centre for Scientific Research Relative to Tobacco (www.coresta.org).
31. ISO 20714:2019. E-liquid – Determination of nicotine, propylene glycol and glycerol in liquids used in electronic nicotine delivery devices – Gas chromatographic method. International Standards Organization; 2019 (<https://www.iso.org/standard/68905.html>).
32. Strongin RM. E-cigarette chemistry and analytical detection. *Annu Rev Anal Chem*. 2019;12(1):23–39. doi:10.1146/annurev-anchem-061318-115329.
33. Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Voudris V. Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: Implications for research protocol standards definition and for public health authorities’ regulation. *Int J*

- Environ Res Public Health. 2013;10:2500–14. doi:10.3390/ijerph10062500.
34. Son Y, Mainelis G, Delnevo C, Wackowski OA, Schwander S, Meng Q. Investigating e-cigarette particle emissions and human airway depositions under various e-cigarette-use conditions. *Chem Res Toxicol*. 2020;33(2):343–52. doi:10.1021/acs.chemrestox.9b00243.
 35. Voos N, Smith D, Kaiser L, Mahoney MC, Bradizza CM, Kozlowski LT et al. Effect of e-cigarette flavours on nicotine delivery and puffing topography: results from a randomized clinical trial of daily smokers. *Psychopharmacology*. 2019;237(2):491–502. doi:10.1007/s00213-019-05386-x.
 36. Ramôa CP, Hiler MM, Spindle TR, Lopez AA, Karaoghlanian N, Lipato T et al. Electronic cigarette nicotine delivery can exceed that of combustible cigarettes: A preliminary report. *Tob Control*. 2016;25(e1):e6–9. doi:10.1136/tobaccocontrol-2015-052447.
 37. St Helen G, Shahid M, Chu S, Benowitz NL. Impact of e-liquid flavours on e-cigarette vaping behavior. *Drug Alcohol Depend*. 2018;189:42–8. doi:10.1016/j.drugalcdep.2018.04.032.
 38. Lee YH, Gawron M, Goniewicz ML. Changes in puffing behavior among smokers who switched from tobacco to electronic cigarettes. *Addict Behav*. 2015;48:1–4. doi:10.1016/j.addbeh.2015.04.003.
 39. Korzun T, Lazurko M, Munhenzva I, Barsanti KC, Huang Y, Jensen RP et al. E-cigarette air-flow rate modulates toxicant profiles and can lead to concerning levels of solvent consumption. *ACS Omega*. 2018;3:30–6. doi:10.1021/acsomega.7b01521.
 40. McAdam K, Warrington A, Hughes A, Adams D, Margham J, Vas C et al. Use of social media to establish vapers puffing behaviour: Findings and implications for laboratory evaluation of e-cigarette emissions. *Regul Toxicol Pharmacol*. 2019;107:104423. doi:10.1016/j.yrtph.2019.104423.
 41. DeVito EE, Krishnan-Sarin S. E-cigarettes: Impact of e-liquid components and device characteristics on nicotine exposure. *Curr Neuropharmacol*. 2018;16:438–59. doi:10.2174/1570159X15666171016164430.
 42. Talih S, Balhas Z, Salman R, El-Hage R, Karaoghlanian N, El-Hellani A et al. Transport phenomena governing nicotine emissions from electronic cigarettes: Model formulation and experimental investigation. *Aerosol Sci Technol*. 2017;51(1):1–11. doi:10.1080/02786826.2016.1257853.
 43. Talih S, Balhas Z, Eissenberg T, Salman R, Karaoghlanian N, El Hellani A et al. Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: Measurements and model predictions. *Nicotine Tob Res*. 2015;17(2):150–7. doi:10.1093/ntr/ntu174.
 44. Mallock N, Trieu HL, Macziol M, Malke S, Katz A, Laux P et al. Trendy e-cigarettes enter Europe: Chemical characterization of JUUL pods and its aerosols. *Arch Toxicol*. 2020;94:1985–94. doi:10.1007/s00204-020-02716-3.

45. Duell AK, Pankow JF, Peyton DH. Free-base nicotine determination in electronic cigarette liquids by ¹H NMR spectroscopy. *Chem Res Toxicol.* 2018;31:431–4. doi:10.1021/acs.chemrestox.8b00097.
46. Gholap VV, Heyder RS, Kosmider L, Halquist MS. An analytical perspective on determination of free base nicotine in e-liquids. *J Anal Meth Chem.* 2020:6178570.
47. El-Hellani A, El-Hage R, Baalbaki R, Salman R, Talih S, Shihadeh A et al. Free-base and protonated nicotine in electronic cigarette liquids and aerosols. *Chem Res Toxicol.* 2015;28(8):1532–7. doi:10.1155/2020/6178570.
48. Duell AK, Pankow JF, Peyton DH. Free-base nicotine determination in electronic cigarette liquids by ¹H NMR spectroscopy. *Chem Res Toxicol.* 2018;31(6):431–4. doi:10.1021/acs.chemrestox.8b00097.
49. Krüsemann EJZ, Wenng FM, Pennings JLA, de Graaf K, Talhout R, Boesveldt S. Sensory evaluation of e-liquid flavours by smelling and vaping yields similar results. *Nicotine Tob Res.* 2020;22(5):798–805. doi:10.1093/ntr/ntz155.
50. Romijnders K, van Osch L, de Vries H, Talhout R. Perceptions and reasons regarding e-cigarette use among users and non-users: a narrative literature review. *Int J Environ Res Public Health.* 2018;15(6):1190. doi:10.3390/ijerph15061190.
51. WHO report on the global tobacco epidemic 2021: addressing new and emerging products. Geneva: World Health Organization; 2021 (<https://www.who.int/publications/i/item/9789240032095>).
52. Tierney PA, Karpinski CD, Brown JE, Luo W, Pankow JF. Flavour chemicals in electronic cigarette fluids. *Tob Control.* 2016;25:e10–5. doi:10.1136/tobaccocontrol-2014-052175.
53. Aszyk J, Kubica P, Kot-Wasik A, Namiesnik J, Wasik A. Comprehensive determination of flavouring additives and nicotine in e-cigarette refill solutions. Part I: Liquid chromatography–tandem mass spectrometry analysis. *J Chromatogr A.* 2017;1519:45–54. doi:10.1016/j.chroma.2017.08.056.
54. Aszyk J, Wozniak MK, Kubica P, Kot-Wasik A, Namiesnik J, Wasik A. Comprehensive determination of flavouring additives and nicotine in e-cigarette refill solutions. Part II: Gas-chromatography–mass spectrometry analysis. *J Chromatogr A.* 2017;1517:156–64. doi:10.1016/j.chroma.2017.08.057.
55. Erythropel HC, Jabba SV, DeWinter TM, Mendizabal M, Anastas PT, Jordt SE et al. Formation of flavourant – propylene glycol adducts with novel toxicological properties in chemically unstable e-cigarette liquids. *Nicotine Tob Res.* 2019;21(9):1248–58. doi:10.1093/ntr/nty192.
56. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J et al. Chemical hazards present in liquids and vapors of electronic cigarettes. *Arch Toxicol.* 2014;88:295–308. doi:10.1007/s00204-014-1294-7.
57. Eddingsaas N, Pagano T, Cummings C, Rahman I, Robinson R, Hensel E. Qualitative analysis

- of e-liquid emissions as a function of flavour additives using two aerosol capture methods. *Int J Environ Res Public Health*. 2018;5:323. doi:10.3390/ijerph15020323.
58. Han S, Chen H, Zhang X, Liu T, Fu Y. Levels of selected groups of compounds in refill solutions for electronic cigarettes. *Nicotine Tob Res*. 2016;18(5):708–14. doi:10.1093/ntr/ntv189.
 59. Farsalinos KE, Voudris V, Poulas K. E-cigarettes generate high levels of aldehydes only in “dry puff” conditions. *Addiction*. 2015;110(8):1352–6. doi:10.1177/1753465817744960.
 60. Flora JW, Meruva N, Huang CB, Wilkinson CT, Ballentine R, Smith DC et al. Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols. *Regul Toxicol Pharmacol*. 2016;74:1–11. doi:10.1016/j.yrtph.2015.11.009.
 61. Gillman IG, Kistler KA, Stewart EW, Paolantonio AR. Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. *Regul Toxicol Pharmacol*. 2016;75:58–65. doi:10.1016/j.yrtph.2015.12.019.
 62. Geiss O, Bianchi I, Barahona F, Barrero-Moreno J. Characterisation of mainstream and passive vapours emitted by selected electronic cigarettes. *Int J Hyg Environ Health*. 2015;218(1):169–80. doi:10.1016/j.ijheh.2014.10.001.
 63. Kosmider L, Sobczak A, Fik M, Knysak J, Zaciera M, Kurek J et al. Carbonyl compounds in electronic cigarette vapors: Effects of nicotine solvent and battery output voltage. *Nicotine Tob Res*. 2014;16(10):1319–26. doi:10.1093/ntr/ntu078.
 64. Uchiyama S, Senoo Y, Hayashida H, Inaba Y, Nakagome H, Kunugita N. Determination of chemical compounds generated from second-generation e-cigarettes using a sorbent cartridge followed by a two-step elution method. *Anal Sci*. 2016;32(5):549–55. doi:10.2116/analsci.32.549.
 65. Uchiyama S, Ohta K, Inaba Y, Kunugita N. Determination of carbonyl compounds generated from the e-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. *Anal Sci*. 2013;29:1219–22. doi:10.2116/analsci.29.1219.
 66. Stephens WE, de Falco B, Fiore A. A strategy for efficiently collecting aerosol condensate using silica fibers: Application to carbonyl emissions from e-cigarettes. *Chem Res Toxicol*. 2019;32:2053–62. doi:10.1021/acs.chemrestox.9b00214.
 67. Jansen E, Cremers J, Borst S, Talhout R. Simple determination of sugars in cigarettes. *J Anal Bioanal Technol*. 2014;5:6. doi:10.4172/2155-9872.1000219.
 68. Kim HJ, Shin HS. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography–tandem mass spectrometry. *J Chromatogr A*. 2013;1291:48–55. doi:10.1016/j.chroma.2013.03.035.
 69. Konstantinou E, Fotopoulou F, Drosos A, Dimakopoulou N, Zagoriti Z, Niarchos A et al. Tobacco-specific nitrosamines: a literature review. *Food Chem Toxicol*. 2018;118:198–203. doi:10.1016/j.fct.2018.05.008.

70. Lee YS, Kim KH, Lee SS, Brown RJC, Jo SH. Analytical method for measurement of tobacco-specific nitrosamines in e-cigarette liquid and aerosol. *Appl Sci*. 2018;8:2699. doi:10.3390/app8122699.
71. Farsalinos KE, Gillman G, Poulas K, Voudris V. Tobacco-specific nitrosamines in electronic cigarettes: Comparison between liquid and aerosol levels. *Int J Environ Res Public Health*. 2015;12(8):9046–53. doi:10.3390/ijerph120809046.
72. Kim HJ, Shin HS. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography–tandem mass spectrometry. *J Chromatogr A*. 2013;1291:48–55. doi:10.1016/j.chroma.2013.03.035.
73. Williams M, Bozhilov K, Ghai S, Talbo P. Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. *PLoS One*. 2017;12(4):e0175430. doi:10.1371/journal.pone.0175430.
74. Olmedo P, Goessler W, Tanda S, Grau-Perez M, Jarmul S, Aherrera A et al. Metal concentrations in e-cigarette liquid and aerosol samples: The contribution of metallic coils. *Environ Health Perspect*. 2018;126(2):027010. doi:10.1289/EHP2175.
75. Hess CA, Olmedo P, Navas-Acien A, Goessler W, Cohen JE, Rule AM. E-cigarettes as a source of toxic and potentially carcinogenic metals. *Environ Res*. 2017;152:221–5. doi:10.1016/j.envres.2016.09.026.
76. Ting CY, Ahmad Sabri NA, Tiong LL, Zailani H, Wong LP, Agha Mohammadi N et al. Heavy metals (Cr, Pb, Cd, Ni) in aerosols emitted from electronic cigarettes sold in Malaysia. *J Environ Sci Health A Tox Hazard Subst Environ Eng*. 2020;55(1):55–62. doi:10.1080/10934529.2019.1665950.
77. Wagner KA, Flora JW, Melvin MS, Avery KC, Ballentine RM, Brown AP et al. An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion. *Regul Toxicol Pharmacol*. 2018;95:153–60. doi:10.1016/j.yrtph.2018.03.012.
78. Electronic nicotine delivery systems. Report to the Conference of the Parties to the WHO Framework Convention on Tobacco Control, Seventh session (FCTC/COP6/10). Geneva: World Health Organization; 2014 (https://apps.who.int/gb/fctc/PDF/cop6/FCTC_COP6_10Rev1-en.pdf).

No Tobacco unit (TFI)
Department of Health Promotion
World Health Organization
20, Avenue Appia
1211 Geneva 27
Switzerland
hq-tfi@who.int
[https://www.who.int/teams/health-promotion/
tobacco-control/product-regulation](https://www.who.int/teams/health-promotion/tobacco-control/product-regulation)

