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# WHO study group on tobacco product regulation

Report on the scientific basis of tobacco product regulation:
Sixth report of a WHO study group

World Health Organization

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Rio de Janeiro, Brazil, 9–11 December 2015

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# Abbreviations and acronyms

| | |
|---|---|
| CDC | Centers for Disease Control and Prevention (USA) |
| CFP | Cambridge filter pad |
| CI | confidence interval |
| CO | carbon monoxide |
| COP | Conference of the Parties |
| CORESTA | Cooperation Centre for Scientific Research Relative to Tobacco |
| ENDS | electronic nicotine delivery systems |
| FCTC | Framework Convention on Tobacco Control |
| FEMA | Flavor and Extract Manufacturers Association (USA) |
| FID | flame ionization detection |
| GC | gas chromatography |
| GRAS | generally recognized as safe |
| HPLC | high-performance liquid chromatography |
| IARC | International Agency for Research on Cancer |
| ISO | International Standards Organization |
| MS | mass spectrometry |
| NNAL | 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol |
| NNK | 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone |
| NNN | *N*′-nitrosonornicotine |
| PAH | polycyclic aromatic hydrocarbon |
| ppm | parts per million |
| RIVM | National Institute for Public Health and the Environment (Netherlands) |
| SOP | standard operating procedure |
| TobLabNet | Tobacco Laboratory Network |
| TobReg | WHO Study Group on Tobacco Product Regulation |
| TPM | total particulate matter |
| TSNA | tobacco-specific nitrosamine |
| VOC | volatile organic compounds |

# 1. Introduction

Effective tobacco product regulation is an essential component of a comprehensive tobacco control programme. It includes regulation of contents and emissions by mandated testing, disclosure of test results, setting limits, as appropriate, and imposing restrictions on packaging and labelling. Tobacco product regulation is covered under Articles 9, 10 and 11 of the WHO Framework Convention on Tobacco Control (WHO FCTC) and in the partial guidelines on implementation of Articles 9 and 10.

The WHO Study Group on Tobacco Product Regulation (TobReg) was formally constituted by the WHO Director-General in 2003 to address regulatory gaps. Its mandate is to provide evidence-based policy recommendations on tobacco product regulation to the Director-General. TobReg is composed of national and international scientific experts on product regulation, treatment of tobacco dependence and laboratory analysis of tobacco ingredients and emissions. The experts are from countries in all six WHO regions.

As a formalized entity of WHO, TobReg submits technical reports to the WHO Executive Board through the Director-General to draw the attention of Member States to the Organization's work in tobacco product regulation. The technical reports are based on unpublished background papers that have been discussed by TobReg.

The eighth meeting of TobReg was held in Rio de Janeiro, Brazil on 9–11 December 2015. The discussions covered priorities for tobacco product regulation and addressed the request of the COP of the WHO FCTC at its sixth session to:

- prepare a report based on scientific evidence on specific characteristics of cigarettes, including slim and "super-slim" designs, filter ventilation and innovative filter design features such as flavour-delivering mechanisms in capsules, to the extent that those characteristics affect the public health objectives of the WHO FCTC, for consideration by TobReg at its first meeting after the sixth session of the COP;
- assess options for regulating electronic nicotine and non-nicotine delivery systems in order to achieve the objectives outlined in resolution FCTC/COP6(9) and to consider methods for measuring the contents and emissions of these products;
- assess, within two years, whether the standard operating procedures (SOPs) for determining nicotine, tobacco-specific *N*-nitrosamines (TSNAs) and benzo[*a*]pyrene in cigarette contents and emissions are applicable or adaptable, as appropriate, to tobacco products other than cigarettes, including waterpipe smoke and smokeless tobacco; and

- prepare reports on the toxic contents and emissions of waterpipe and smokeless tobacco products.

At the meeting, a background paper on the aerosol of electronic nicotine delivery systems (ENDS) was also discussed, which is published separately.[1] TobReg also discussed the prevalence and use of menthol in tobacco products and, after the meeting, published an advisory note[2] containing evidence-based conclusions and recommendations for policy-makers and regulators, including for a ban on menthol (and its analogues, derivatives and precursors) in cigarettes.

TobReg hopes that the conclusions and recommendations in this report and the advisory note will be helpful to countries in implementing the product regulation provisions of the WHO FCTC.

[1] http://www.who.int/tobacco/industry/product_regulation/eletronic-cigarettes-report-cop7-background-papers/en/

[2] http://apps.who.int/iris/bitstream/10665/205928/1/9789241510332_eng.pdf?ua=1

# 2. Cigarette characteristics and design features

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**Contents**

## 2.1 Introduction

This report was prepared in response to a request by the COP to the WHO FCTC at its sixth session (Moscow, Russian Federation, 13–18 October 2014) to the Convention Secretariat to invite WHO to prepare a report based on scientific evidence on specific cigarette characteristics of interest, including slim and "super-slim" designs, filter ventilation and innovative filter design features, including flavour-delivering mechanisms such as capsules, to the extent that those characteristics affect the public health objectives of the WHO FCTC, for consideration by the WHO FCTC COP Working Group on Articles 9 and 10 at its meeting in February 2016. With respect to the design features of slim and super-slim cigarettes, the report should cover cigarette circumference and length in relation to nicotine delivery and exposure.

The report addresses cigarette characteristics that influence user perception, user behaviour and the delivery of toxic constituents. Typical characteristics of cigarettes are the tobacco blend, additives, tobacco weight, density, cigarette paper, filter type, filter ventilation and cigarette geometry (circumference, length) *(1)*. Recently, cigarettes have been marketed with new design features, such as filter flavour capsules, special filters and coloured paper. The main purpose of adding such characteristics to cigarettes is to increase their attractiveness and addictiveness *(2)*, which can be achieved by reducing their negative aspects (e.g. throat irritation), increasing their positive aspects (e.g. improved draw and mouth feel), appealing to new users and target groups, increasing the convenience and ease of use and increasing perceptions of lower risk or safety. Certain ingredients may also increase the addictive potential of a product, for instance by improving nicotine delivery. Many new products have been marketed or are being investigated by the tobacco industry that are claimed to lower the concentrations of some toxicants, for instance with more efficient filters or treated tobacco.

This section covers the following topics:

- the cigarette characteristics that influence user perception and behaviour (e.g. attractiveness, risk perception, ventilation, pressure drop, flavour, design and shape, including cigarette diameter or diameter-to-length ratio) (section 2.2);
- the cigarette characteristics that affect the delivery of toxic emissions (e.g. tobacco type, tobacco blend, amount of tobacco, ventilation, paper porosity, filter type) (section 2.3);
- design features and additives that modify smoke pH and addictiveness (section 2.4);
- innovations that could influence perceptions and/or emissions (e.g. flavour capsules, new filter design) (section 2.5); and
- areas of research that would inform scientific evaluation of the public health impact of design characteristics (section 2.6).

The literature search was conducted mainly in the PubMed database and with the SciFinder search tool, which retrieves data from the Medline and CAplus databases. Relevant articles cited in publications and reports were also included. In addition, the Internet was used to identify websites that provide product characteristics and marketing information and to search major tobacco manufacturers' websites, tobacco industry document repositories, blogs and news articles.

## 2.2 Cigarette characteristics that influence perception and use

### 2.2.1 Overview

Cigarette characteristics can influence nicotine delivery *(3)* and smokers' sensory experience, which have been shown to influence a wide range of smoking-related behaviour, from initiation, to progression, tobacco dependence and smoking satisfaction in highly dependent smokers. The combination of nicotine delivery and sensory cues is critical in determining smoking satisfaction *(4, 5)*, psychological reward *(6)* and reduced craving *(7)*. For instance, a perception of a "lighter" feel and taste of the smoke from cigarettes with highly ventilated filters may be an important factor in their wide acceptability, due to better palatability, a perception of reduced risk or both *(8–10)*.

The influence of cigarette product design on user perceptions and smoking behaviour has been investigated by academic and government researchers as well as the tobacco industry, resulting in a substantial knowledge base *(11)*. Internal research conducted by the industry, some of which is now publically available, is of particular interest, because modifications of cigarette design to

achieve effective nicotine delivery and specific sensory characteristics have been used by manufacturers to establish brand and sub-brand identity and to enhance the consumer appeal of products. Therefore, a review of relevant internal industry research is important for designing effective policies and regulations on cigarette characteristics *(12)*. We summarize here the most important cigarette characteristics that have been shown to affect user perception and behaviour.

### 2.2.2 Cigarette characteristics that influence user perception

#### 2.2.2.1 Cigarette and filter tipping paper, decorative elements

Several studies have been conducted on the effect of the appearance of cigarettes on consumers' response. They indicate that elements such as the colour and pattern of filters and paper affect perceptions of the attractiveness and relative harm of cigarettes *(13–16)*. Moodie et al. *(14)* showed that pink cigarette paper may be more appealing and give young women perceptions of a pleasant taste and less harm. In contrast, dark colours generally had little appeal and gave perceptions of strong taste and greater harm; however, a pleasant aroma from a dark-coloured cigarette could enhance its appeal and the perception of taste and decrease the perception of harm. An exploratory study by Ford et al. *(16)* in a group of 15-year-old participants showed that white filter tips and decorative elements on the filter tipping paper, including the font style of brand names, can generate interest, provide novelty, communicate a positive image and lead to an overall perception of attractiveness. These findings indicate that cigarette appearance can be exploited as a promotional tool. For instance, it has been suggested that white tipping paper on cigarettes with ventilated filters was designed to reinforce the perception of a safer product, in contrast to most full-flavoured cigarettes, which have cork-coloured filter tips *(17)*. Recent innovations that include such elements of cigarette appearance as cigarette paper and filter colours are further discussed in sections 2.5.3 and 2.5.4.

#### 2.2.2.2 Filter ventilation

***Perception of reduced harm***

The composition and ventilation of cigarette filters and the effects of these features on emission content are described in detail in section 2.3.3. Many smokers are unaware that low-yield cigarettes have ventilated filters, which dilute cigarette smoke with air *(17, 18)*. Filter ventilation changes users' sensory responses to cigarette smoke and affects their perception of the harm associated with low-yield cigarettes. Specifically, filter ventilation in low-yield cigarettes leads smokers to perceive that the smoke tastes lighter and is less irritating than that of regular cigarettes, which powerfully supports their belief that the tar and nicotine intakes from such cigarettes are lower *(8, 10, 19)*. For instance, O'Connor et al. *(20)* found

that the degree of filter ventilation was consistently associated with the perceived lightness ($P < 0.001$) and smoothness ($P = 0.005$) of cigarettes. Cummings et al. *(17)* showed that many Marlboro Lights smokers believed incorrectly that light and ultra-light cigarettes were less harmful than higher-tar, full-flavoured cigarettes. Only 11% of Marlboro Lights smokers in that study knew that their exposure to tar and other constituents from "light" cigarettes is about the same as that from full-flavoured cigarettes. It has also been shown *(10)* that many smokers agree that "light" cigarettes are not less harmful in general, but they still believe that they reduce their exposure, because of their sensory experience.

Sensory experiences can lead users to perceive reduced exposure when smoking low-yield cigarettes, independently of any descriptive term or colour coding on the cigarette pack *(8–10, 21)*. Longitudinal studies show that the removal of brand descriptors such as "light", "mild" and "low tar" has not had a sustained impact on smokers' perceptions, as many continue to believe or rationalize that "lighter" cigarettes are less harmful *(22, 23)*. For instance, significant proportions of smokers in Australia (55%), Canada (43%) and the United Kingdom (70%) continue to believe that low-yield cigarettes offer some health benefit as compared with regular cigarettes. While the introduction of new terms ("smooth", "fine") and pack colours to suggest "lightness" or "smoothness" by manufacturers contributes to sustaining this misperception *(24–26)*, smokers are also partly encouraged by the perception that light cigarettes are "smoother" on the throat and chest than regular cigarettes *(9)*.

#### *Perception of draw*

Increased filter ventilation in "lower-delivery" cigarettes and the resulting reduction in chemosensory impact can also make smokers dissatisfied because of changes in "perception of draw" or the greater perceived effort required to inhale a sufficient amount of smoke from the cigarette. Substantial research on this phenomenon has been conducted by the tobacco industry, which shows that the perception of draw from smoking cigarettes with ventilated filters can be improved by increasing the levels of nicotine, volatile aldehydes, ammonia and other constituents and additives in smoke (reviewed in *(4)*). The effects of ammonia and other additives on smoke characteristics are discussed in more detail in sections 2.4.2 and 2.4.3.

### 2.2.2.3 Physical dimensions, including slim and "super-slim" cigarettes

The length and circumference of cigarettes influence their appeal and perceptions of harm. Longer, slimmer cigarettes are widely acknowledged to increase the perception of stylishness and to appeal generally to women *(12, 14)*; and research conducted by the tobacco industry suggests that these characteristics have been exploited in targeting women. For instance, Philip Morris observed that

fashion-conscious female smokers associated slim, long, light-tasting cigarettes with increased femininity and with weight control *(27)*. Lorillard consumer research also indicated that female smokers of slim 100-mm cigarettes perceived the style as both feminine and graceful and milder and longer lasting *(27)*. A recent study showed that longer cigarettes were often perceived by smokers as attractive and of high quality *(15)*. In addition, Ford et al. *(16)* showed that slim and super-slim cigarettes were perceived as less harmful by 15-year-olds. The draft European Commission Tobacco Products Directive proposed that cigarettes < 7.5 mm in diameter be banned to reduce the possibility that cigarette appearance will mislead consumers about the harm they cause *(28)*. The ban was not, however, included in the final Tobacco Products Directive *(29)*.

#### 2.2.2.4 Flavours

Flavoured tobacco products generally appeal to young adults and adolescents and are often marketed towards them *(30–32)*. In a study of university students who smoked flavoured and unflavoured cigarettes, flavoured cigarettes elicited greater positive expectancy than unflavoured cigarettes, even among nonsmokers *(33)*. For instance, Camel Exotics elicited greater positive expectancy than Camel Lights ($F_{(1421)} = 38.4$, $P < 0.001$) in experimental smokers, regular smokers and nonsmokers, although only a modest effect was seen in committed nonsmokers when analysed separately ($F_{(1249)} = 5.4$, $P < 0.05$). Significantly less negative expectancy was observed for flavoured than for unflavoured brands. Thus, Camel Lights were rated more negatively than Camel Exotics ($F_{(1421)} = 8.2$, $P < 0.01$), and the effect did not depend on smoking status. Logistic regression analysis showed that positive expectancy predicted "intention to try" each brand by regular smokers and by susceptible and experimental smokers. For example, study participants were 2.4 times more willing to try Camel Exotics than Camel Lights. These findings are consistent with the view that flavoured cigarettes serve as "starter" products *(32)*.

The sensory qualities of menthol, the most common flavouring additive, may result in a perception of smoothness, increasing the appeal of smoking *(33)*. Flavours such as menthol, spearmint, peppermint, chocolate, apricot, coconut and marshmallow have been used to address concern about after-taste and the aroma preferences of women *(27)*.

Research thus shows that aromatized cigarettes are used mainly by women and young people, people who are aware of smoking-related health risks and those who perceive that some cigarettes are less harmful than others *(30, 34, 35)*.

The WHO FCTC advises countries to prohibit or restrict ingredients that may be used to increase attractiveness *(36)*. Some countries have already promulgated legislation to decrease the attractiveness of products by regulating flavours. Brazil (RDC ANVISA No. 14) and Canada (Bill C-32) have prohibited

most flavours, whereas other countries restrict use in a product or package to a concentration that will not result in a strong non-tobacco flavour, such as fruit or sweets. The Food and Drug Administration in the USA has banned additives, artificial and natural flavours (other than tobacco and menthol) and herbs and spices that impart a characterizing flavour to cigarettes *(37)*. The new European Union Tobacco Product Directive also prohibits a characterizing flavour other than one of tobacco in cigarettes and roll-your-own tobacco *(38)*, in which a characterizing flavour is defined as a "clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herb, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product."

### 2.2.3 Cigarette characteristics that influence user behaviour

#### 2.2.3.1 Filter ventilation

Filter ventilation and subsequent smoke dilution with air result in compensatory smoking, such as drawing larger puffs, inhaling more deeply and blocking filter vents to prevent smoke dilution *(39)*, because most smokers seek to optimize their nicotine intake, with the perceived chemosensory impact, to achieve rewarding sensations and to avoid the aversive sensations associated with nicotine withdrawal *(40, 41)*. Smokers also block filter vents with their fingers or lips, although many smokers of light and ultra-light cigarettes are unaware that they are doing so *(18, 42)*. Such compensation is likely to be complete for most smokers who switch from higher- to lower-yield cigarettes *(41)*. Smoking cigarettes with substantially reduced smoke nicotine yields from very-low-nicotine tobacco blends does not, as opposed to filter ventilation, lead to compensatory smoking *(43)*.

It has been demonstrated that the ratio of smoke intake to tar and nicotine delivery is nonlinear; larger, more intense puffs change the concentration of smoke constituents more drastically by reducing their retention on cigarette filters and decreasing smoke dilution *(44)*. Smokers who believe that they are smoking a product with lower delivery of harmful emissions may actually increase their exposure by changing their behaviour, such as blocking filter vents or taking larger puffs. This is particularly relevant for smokers of highly ventilated cigarette brands. Such "brand elasticity" allows smokers to effectively regulate nicotine delivery by adjusting their puffing behaviour. It also presents a major problem for measuring the actual nicotine and tar delivery of a brand. Cigarette brands vary in elasticity, and more elastic ones appear to have the greatest market share *(44)*.

Industry researchers have long known that smokers adjust their puffing behaviour to maintain a fairly constant daily dose of nicotine when they switch to cigarettes formerly marketed as light or ultra-light *(17)*. Furthermore, tobacco industry documents show that filter ventilation was the main approach in engineering low-yield cigarettes, with other design features such as more porous

paper *(10)*. These features tend to encourage stronger puffing by smokers and negate any potential reduction in exposure from smoking low-yield cigarettes *(39, 45–47)*. For instance, Strasser et al. *(46)* estimated that smokers who block filter vents may be increasing their exposure to cigarette smoke constituents by 30%. Hammond et al. *(44)* showed that smokers who switched to low-yield cigarettes increased their total smoke intake per cigarette by 40% ($P$ = 0.007), with no significant change in their salivary cotinine levels. The compensatory changes were stable, with no observable decrease over 5 days. Self-reported smokers of "light" cigarettes also perceived themselves as less addicted, were more likely to have ever attempted to quit than regular smokers and had stronger intention to quit but less confidence in their capacity to do so. The absence of any reduction in exposure of smokers of low-yield cigarettes to nicotine and other smoke constituents has been convincingly demonstrated in many studies with biomarkers of exposure *(41, 48–51)*. Together, these findings provide strong in-vivo evidence of behavioural compensation for filter ventilation of cigarettes.

#### *Pressure drop*

Resistance to draw, or "pressure drop", is proposed as one of the major determinants of puff duration and volume *(47, 52–55)*. As chemosensory impact defines smokers' perception of achieving a satisfying volume of smoke, an insufficient impact in the mouth and upper respiratory tract will drive smokers to continue increasing their puff intensity until they feel an adequate draw *(4)*.

#### *Carbon-containing filters*

The presence of carbon in cigarette filters may affect the levels of some smoke constituents that contribute to the perception of draw and therefore lead to changes in smoking intensity. In a study by Rees et al. *(57)*, Marlboro Lights smokers were switched to carbon-filtered Marlboro Ultra Smooth and non-carbon Marlboro Ultra Lights cigarettes for 48 h each. Larger puff volumes were taken of the carbon-containing cigarettes than either Marlboro Lights (difference in puff volume, 2.4–13.6 mL in two study groups; overall $P$ = 0.006) or Marlboro Ultra Lights (difference in puff volume, 2.4–3.6 mL; overall $P$ = 0.007).

### 2.2.3.2 Physical dimensions

Studies in which smokers smoked cigarettes of full or partial length suggest that length may affect smoking behaviour, such as puff duration and volume *(52–55)*. In one study, smoking full-length cigarettes was associated with more puffs and self-reported smoking "satisfaction" than smoking half-, quarter- or eighth-length cigarettes. In the same study, smokers smoked fewer cigarettes but took more puffs of full-length research cigarettes manufactured with high (2.0 mg) or low (0.2 mg) nicotine than quarter-length versions of the same cigarettes *(56)*. In

a study of nationally representative data from the National Health and Nutrition Examination Survey on serum cotinine and urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol (NNAL) concentrations in smokers of regular-sized, king-sized and long or ultra-long cigarettes, those who smoked long or ultra-long cigarettes had higher measures of smoking intensity and addiction (e.g. time to first cigarette, number of cigarettes smoked per day) and significantly higher tobacco biomarker levels than smokers of regular- or king-sized cigarettes (geometric mean serum cotinine, 263.15 ng/mL versus 173.13 ng/mL or 213.79 ng/mL; urinary NNAL, 0.48 ng/mg creatinine versus 0.34 ng/mg or 0.33 ng/mg, respectively) *(52)*.

#### 2.2.3.3 Flavours

Flavours in cigarettes not only have potential marketing appeal to some population groups (e.g. young people, women, certain ethnic groups) and nonsmokers but may also mask the harshness of smoke, making inhalation easier. In a pilot study of differences in puff topography and cigarette ratings among 20 university student smokers of Camel Light and Camel Exotic Blend cigarettes (with similar tar, nicotine and filter ventilation) *(58)*, participants took smaller puffs on Exotic Blend than on Camel Light cigarettes (42 mL vs 48 mL, $P < 0.001$), but the difference in total smoke volume was not significant (613.9 mL vs 630.7 mL, $P = 0.79$), and no increase was seen in carbon monoxide (CO: 6.2 vs 6.2 ppm, $P = 0.90$). When participants rated each cigarette on characteristics such as strength, irritation and taste, they rated Exotic Blend cigarettes as being most different from their usual brand, but the taste ratings did not differ. These results suggest that adding flavours to cigarettes does not significantly influence how they are smoked by established smokers.

One flavour that could change smoking behaviour is menthol, although the results of many studies are inconclusive or conflicting *(33, 59)*. Some indicated that daily cigarette consumption or puffing intensity were greater with menthol cigarettes *(60, 61)*, while others found that the puff frequency *(62, 63)* and volume smoked *(63)* were similar to those of smokers of non-mentholated cigarettes. Strasser et al. *(64)* found that menthol has a minimal impact on smoking behaviour, biomarkers of exposure and subjective ratings; however, smokers of mentholated cigarettes smoked their first cigarette of the day sooner than smokers of non-mentholated cigarette, implying greater dependence on nicotine with use of mentholated cigarettes *(61)*. Smokers of mentholated cigarettes attempted to quit more often but had less successful quitting rates, which suggests that mentholated cigarettes are more addictive than non-mentholated ones *(65, 66)*. Other studies have shown that menthol cigarettes are used disproportionately by young people, probably because of their taste, sensory properties and easier inhalation *(65)*. While there are few, inconclusive data on the role of menthol

cigarettes in initiation of smoking *(67)*, studies indicate that adolescents smoke more mentholated than non-mentholated cigarettes, suggesting that these cigarettes are preferred during early tobacco use *(68)*.

## 2.3 Cigarette characteristics that affect the content of smoke emissions

Manufacturers can introduce or manipulate many variables to affect the composition of tobacco smoke *(69)*. Traditional, tobacco-burning cigarettes, novel products and product features (reduced ignition propensity cigarettes, potentially reduced exposure cigarette products and denicotinized tobacco) were recently addressed in a WHO technical report *(70)*. It is difficult to determine the contribution of each cigarette characteristic to the adverse health effects of tobacco use; a general recommendation is to focus research on reducing the levels of toxicants (per cigarette or "stick" or per milligram of nicotine). WHO has recommended mandated lowering of nine toxicants in cigarette smoke – *N*'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), acetaldehyde, acrolein, benzene, benzo[*a*]pyrene, 1,3-butadiene, CO and formaldehyde – on the basis of their toxicity and the feasibility of lowering their concentrations *(71)*.

### 2.3.1 Tobacco

The tobacco blend is the cigarette component that most heavily influences the delivery of various chemicals in the smoke emissions *(72)*. The properties of each type of tobacco influence its filling power (the ability to form a firm cigarette rod with a given moisture content), burn rate, tar and nicotine deliveries, amounts of chemicals in smoke, flavour and aroma and smoulder rate *(73–79)*. Bright tobacco, also known as flue-cured or Virginia tobacco, has a lower nitrogen content (i.e. less nicotine) and a higher sugar content than other varieties. At a given circumference, Virginia-blend tobacco cigarettes yield a higher puff count than American-blend cigarettes *(80)*. Cigarettes with flue-cured tobacco are heavier than those made with burley tobacco, so that more puffs can be taken from a given butt length *(81)*. As the amount of flue-cured tobacco in a blend is increased, the tar and CO yields also increase *(82)*; more formaldehyde is delivered in smoke from bright tobacco than from burley tobacco *(83)*. In most tobaccos, the concentration of NNN exceeds that of NNK; in bright tobacco; however, the NNK concentration exceeds that of NNN *(83)*.

Burley and Maryland tobaccos are air-cured and typically have higher nicotine contents but lower sugar contents. Burley tobacco has notably higher concentrations of nitrate and TSNAs than other tobacco types *(84)*. Oriental tobacco is sun-cured; it is often included in blended varieties because of its aromatic properties *(81)*. It has higher levels of phenol than flue-cured, burley

or Maryland tobacco *(85)*. Maryland tobacco has lower yields of tar, nicotine, phenol and benzo[*a*]pyrene than burley, oriental or flue-cured tobacco *(85)*.

Expanded, puffed and freeze-dried tobaccos are processed to increase their filling power *(86)*. They are treated with various volatile materials, which are then quickly removed, so that the tobacco cell structure greatly expands *(79)*. These modified tobaccos are used to reduce the amount of tobacco required to "fill" a cigarette; however, they alter the levels of some smoke emissions. For example, the nicotine level in the smoke of cigarettes containing expanded tobacco leaf is lower than that in the smoke of a cigarette made without such material *(86)*. With an increasing amount of expanded tobacco in a blend, the ratio of CO to carbon dioxide and the vapour phase aldehydes (acetaldehyde, acrolein) increases, and particulate-phase components decrease *(69, 82)*. Smoke from cigarettes made with expanded stems has more CO, nitrogen oxides, formaldehyde, tar, benz[*a*] anthracene and benzo[*a*]pyrene than smoke from cigarettes made of puffed tobacco, expanded tobacco or freeze-dried tobacco *(85)*.

Reconstituted tobacco is made of tobacco by-products, including tobacco dust ("fines"), ribs and stems, which are extracted and then re-formed into a pulp with adhesives, fibres to provide structure, and chemicals such as humectants and flavours before being dried to various densities *(81, 87, 88)*. Reconstituted tobacco costs less than tobacco leaf and has greater filling power, resulting in less dense tobacco filler, which contributes to a faster burn rate and fewer puffs per cigarette. These factors reduce the delivery of tar and nicotine in smoke *(87, 89)*. The chemistry of the smoke from cigarettes made from reconstituted tobacco depends on whether it is made exclusively of stems or of a blend of stems and other tobacco-derived material. Stem-only reconstituted tobacco smoke has higher levels of nitrogen oxides, acetaldehyde and polycyclic aromatic hydrocarbons (PAHs) than reconstituted tobacco made with stems and other tobacco materials; however, the levels of tar, nicotine, CO, hydrogen cyanide and PAHs are lower in the smoke of cigarettes made from either type of reconstituted tobacco (stem only or stems and other tobacco materials) than from those that do not contain reconstituted tobacco *(86)*.

### 2.3.2 Paper

Cigarette paper controls combustion – free or static burn rate (i.e. the amount of cigarette consumed between puffs) and smoulder rate – and strongly affects the puff count and smoke yield of cigarettes under machine-testing conditions *(79, 81)*. The controllable factors in cigarette paper that affect smoke emissions and composition are fibre composition; filler type, level and distribution; thickness and bulk density (standard paper or that with thicker bands used for reduced ignition propensity, "fire-safe" cigarettes); porosity (described below) and the type and level of chemicals or additives *(90)*.

Cigarette wrapper paper may alter smoke composition by directly contributing wrapper components or combustion components to mainstream smoke; by diffusion of smoke components through the wrapper; by diffusion of air through the wrapper; by altering the linear velocity, volume and distribution of the airstream in and around the burning cone and by altering the amount of tobacco burnt per puff *(69)*.

The most common means of reducing smoke yields, after filter ventilation, is changing paper porosity *(91)*. Porosity, which is the permeability of paper to oxygen and smoke gases when under a pressure differential, affects the burn rate, puff count and the amount of tobacco burnt per puff. The porosity of paper is controlled by the size (void volume) of the openings (pores) created by the bonded structure of cellulose fibres and calcium carbonate. Paper porosity can affect taste, delivery and variation in smoke dilution *(80, 90, 92)*. The porosity of cigarettes in the USA typically ranges from 30 to 50 units in the system defined by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) *(79)*.

Paper porosity influences the burn temperature of a cigarette. As porosity increases, the coal temperature decreases *(93)*, and the cigarette burns faster because of an increased static burn rate. The result under machine-smoking conditions is that more tobacco is consumed between puffs, fewer puffs are taken, and nicotine, tar and CO yields are reduced *(82, 91, 94)*. Very volatile smoke constituents such as CO readily diffuse through the porous wrapper, so that they are delivered in lower concentrations than less volatile constituents *(94)*. Delivery of benzo[*a*]pyrene decreases as paper porosity increases, as less tobacco is consumed during puffing and more is burnt in the interval between puffs *(81)*.

### 2.3.3 Filter

Most commonly used cigarette filters are made of cellulose acetate, paper or a combination of the two *(81)*. Crimped cellulose acetate fibre ("tow") is used in about 90% of all filters *(98)*.

Cigarette filters help to control cigarette pressure drop, absorb vapours and remove particulate matter from smoke. Filtration occurs by one of three mechanisms: mechanical trapping of particles, condensation followed by adsorption from the gas phase or transfer via the gas phase between particles and the filter *(96)*. Acetate filters show negative selectivity for nicotine, and the average particle size of nicotine is smaller than in unfiltered cigarettes *(95, 96)*. Consequently, more nicotine may be emitted in the mainstream smoke of cigarettes with acetate filters than in the smoke of unfiltered cigarettes, and the smaller particle size may mean that a larger percentage of the inhaled particulate matter travels further into the lungs *(83)*.

Fibrous filters significantly reduce the levels of semi-volatile and nonvolatile substances in smoke, slightly reduce the levels of vapour-phase compounds but do

not reduce those of gases *(97)*. Studies of machine-smoked cigarettes indicate that the smoke constituents removed by cellulose acetate filters include: water (60–75%), cresols (70–75%), particulate matter (35–40%), volatile *N*-nitrosamines (≤ 75%), acrolein (reduced to "a limited extent") and, notably, phenol (70–80%) *(85, 91, 98)*.

#### 2.3.3.1 Filter ventilation

Filter ventilation is defined as air entering a cigarette through the portion of tipping paper that does not overlap the tobacco rod *(99)*. Filter ventilation is achieved through a combination of a porous plug wrap and perforated or porous tipping paper. The degree of ventilation or dilution achieved depends on the porosity of the plug wrap, the perforation or porosity of the tipping-paper and the location of the perforations *(81)*. Ventilation ranges from about 10% in some full-flavoured cigarettes to 80% in very low-delivery brands *(100)*. The design feature of filter vent holes is easily defeated by smokers, who knowingly or unwittingly block them with their lips or fingers when they take a puff *(10)*. The information presented here refers to the theoretical aspects of filter ventilation as a design feature and that derived from studies of machine-smoked cigarettes with unobscured filter vents. When highly ventilated cigarettes are machine smoked under more intense conditions (larger puff volume, vents blocked), their emission levels may be equal to or exceed those from less ventilated, full-flavour cigarettes machine smoked under less intense International Organization for Standardization (ISO) conditions with the filter vents unblocked *(101)*.

Filter ventilation allows more complete combustion of tobacco and greater retention of particulate matter by the cellulose acetate in the filter *(85, 86)*. Both particulate delivery and vapour- or gas-phase delivery are reduced, generally in direct proportion to the degree of ventilation *(81)*. The effects of ventilation are not, however, entirely due to dilution of the smoke; the emissions of some compounds are increased or decreased, while those of others, including total nicotine, are relatively unchanged *(34)*.

#### 2.3.3.2 Absorbent filtration materials, charcoal

Cigarette filters may contain filtering aids, such as charcoal and other solid or liquid additives, for selective filtration of emissions *(81)*. Carbon granules, silica gel and alumina are examples of solid adsorbent materials used in filters *(95)*.

Carbon effectively adsorbs chemicals with boiling-points between 0 and 100 °C (e.g. acetaldehyde, acrolein and hydrogen cyanide) and can remove some chemicals with boiling-points up to 150 °C *(98)*. Depending on the smoking machine conditions, carbon (charcoal) filters can significantly reduce the levels of semi-volatile and vapour-phase compounds in smoke and slightly reduce the levels of non-volatile compounds *(97, 102)*. The levels of compounds of lower molecular mass that occur in significant amounts in the vapour phase (e.g. phenol,

cresols, hydroquinone) are reduced to a greater extent by charcoal filtration than are those of compounds of higher molecular mass and significantly lower volatility (e.g. benzo[*a*]pyrene, TSNAs) *(103)*. Charcoal filters usually do not reduce the levels of low-molecular-mass gases in smoke *(97)*, although charcoal coated with a mixture of metallic oxides is reportedly effective in removing acidic gases *(81)*.

The efficiency of removal depends on the amount of charcoal, the smoking machine conditions (smoking intensity) and the age of the charcoal filter *(97, 103)*. For example, hydrogen cyanide retention by a standard carbon filter decreases with the age of the cigarette, from about 38% at 0 weeks to about 25% at 8 weeks *(97)*. When charcoal-filtered cigarettes (about 45 mg charcoal) were smoked under more intense smoking machine conditions, the tar, nicotine and CO emissions and the reduced emissions of volatile constituents measured under less intense ISO smoking conditions were no longer significantly lower than in the smoke of cellulose acetate-filtered cigarettes, because insufficient charcoal was present. Filters with more charcoal (120 or 180 mg) resulted in significant reductions under both intense and less intense smoking conditions *(103)*.

Synthetic high-activity carbon spheres with a different pore structure from natural carbon have been used in the filters of experimental cigarettes, alone and in various combinations with treated tobacco and alternative filter ventilation. The cigarette circumference varied from 17 mm to 24.6 mm *(104)*. Slimmer cigarettes had less charcoal in the filter (17-mm cigarettes with a filter length of 27 or 33 mm and 20.4 or 30.6 mg charcoal, respectively, versus 24.6-mm cigarettes with a filter length of 27, 33 or 37 mm and 48, 72 or 88 mg charcoal, respectively). The smoking machine-generated tar yields of the larger cigarettes decreased as the carbon load increased; however, the tar yield of the slimmer, 17-mm cigarettes increased. The yields of many volatile constituents of smoke were significantly reduced as the carbon load increased, especially isoprene, acetaldehyde and acetone, with smaller reductions in pyridine, formaldehyde and styrene. The yields of hydrogen cyanide and 1,3-butadiene did not change significantly in the 17-mm cigarettes as carbon loading increased. The emissions of volatile smoke constituents from the slimmer cigarettes with activated carbon filters were higher than those from the wider cigarettes because of the greater smoke velocity in slimmer cigarettes and the lower activated carbon content. The reductions in volatile chemicals levelled off with the two highest charcoal loads, which the authors attributed to a limit in the amount of high-activity carbon that is effective in reducing the yields of some toxicants in a cigarette filter *(104)*.

### 2.3.4 Physical dimensions

#### 2.3.4.1 Diameter and circumference

The usual diameter of a conventional cigarette is 7.5–8.0 mm, although slim varieties may measure 5 or 6 mm *(83)*. The amount of tobacco consumed depends on

the circumference of the cigarette, and tar and CO yields increase as the circumference increases *(105)*. The emissions to smokers from cigarettes with cellulose acetate filters and a smaller circumference decrease accordingly *(83)*.

#### 2.3.4.2 Length

Cigarette length generally falls into one of four categories: "regular", 68–70-mm unfiltered; "king size", 79–88-mm filtered; "long", 94–101-mm filtered and "extra-long" 110–121 mm filtered *(83)*. Decreasing the cigarette circumference while keeping the packing density constant reduces the amount of tobacco available for burning and allows greater use of oxygen during combustion *(85, 86)*. As the circumference of a cigarette decreases, less tobacco is available for consumption, with a corresponding decrease in some smoke emissions *(106)*.

Some chemicals are filtered through the tobacco rod as smoke is drawn through the unburnt portion of the cigarette column *(98)*. Most smoke constituents, notably semi-volatile compounds, are formed during transit through the tobacco rod, as combustion products move from the burning zone at the lit end of the cigarette to a zone of lower temperature and lower oxygen downstream pyrolysis and distillation. For example, PAHs are formed in the lower-temperature regions of a burning cigarette. The smoke is condensated and filtered by the tobacco as it moves towards the mouth end of the cigarette *(107)*. Filtration of nicotine by the tobacco rod decreases with decreasing rod length of filtered and unfiltered cigarettes, whereas filtration of smoke condensate by the tobacco rod is considered to be independent of the length of the rod *(108)*.

#### 2.3.4.3 Packing density

The mediating effect of cigarette length on smoke composition depends on the packing density of the tobacco *(69)*. Increasing packing density provides more tobacco mass to burn during puffs, with a corresponding increase in chemical emissions in mainstream smoke. As described above, however, some smoke constituents are filtered as smoke is drawn through the tobacco rod. In one study of cigarettes of different packing densities that were machine smoked to predetermined lengths, the yields of nicotine and smoke condensate were lower in cigarettes with higher packing density and higher in cigarettes with lower packing density *(108)*.

#### 2.3.4.4 Implications for super-slim cigarettes

As the circumference of a cigarette decreases, less tobacco is available for consumption, with corresponding decreases in some smoke emissions *(106)*, as noted for cigarettes with circumferences smaller than the regular 24.8–25.5 mm (e.g. ≤ 23 mm) *(85)*. Decreasing circumference results in decreases in both total delivery and per puff delivery under machine-smoking conditions *(79)*.

Decreasing cigarette circumference, while keeping the packing density constant, reduces the amount of tobacco available for burning and allows a larger volume of oxygen consumption during combustion. This reportedly results in reductions in the yields of some smoke emissions, including tar, nicotine, CO and several volatile smoke constituents. For example, as the circumference decreases from 26 to 21 mm, the amount of CO per puff decreases by about 20% and that of benzo[*a*]pyrene by about 40%; however, with the same design parameters, the level of hydrogen cyanide in mainstream smoke is relatively unchanged as the circumference decreases. Nicotine delivery in mainstream machine-generated smoke decreased from 1.56 mg from a cigarette with a circumference of 26 mm to 1.21 mg from one with a circumference of 23 mm *(85, 86, 109)*.

In a recent study of the emissions of a large number of chemical constituents from six machine-smoked, super-slim, flue-cured tobacco cigarette varieties sold in Canada (diameter, 5.3–5.4 mm; circumference, 16.7–17 mm; length, 83–99 mm; and tobacco weight, 296–371 mg), the levels of all chemicals except formaldehyde, ammonia and phenols were lower than in a standard-size research cigarette, owing to the smaller quantity of tobacco and the reduced puff count. The increase in formaldehyde emissions from the super-slim cigarettes was attributed to an increased ratio of circumference to cross-sectional area, which facilitated oxidation reactions by allowing more tobacco to come into contact with ambient air during a puff. Decreased circumference is also thought to increase the combustion temperature, which contributes to higher emissions of phenols *(109)*. Decreasing cigarette circumference also increases flow rates, which reduces the time for the smoke to pass from the coal to the mouth end of the cigarette (residence time) and decreases the filtration achieved by the tobacco rod and retention by the filter *(110)*.

Factors that reduce filtration by the tobacco rod and retention by the filter may result in higher smoke emissions. The velocity of smoke in super-slim cigarettes is more than twice that in cigarettes of standard circumference *(110)*. As smoke velocity increases, particulate retention decreases, and there is less time for diffusion of gas-phase chemicals through the paper. Smoke velocity negatively affects particle retention and vapour adsorption in a cigarette filter *(110, 111)*. The effect of smoke velocity on adsorption of vapour-phase chemicals depends on the amount and the properties of the chemical (molecular mass and reactivity) and on the contact time with adsorbent materials *(110)*. For example, filter retention of hydrogen cyanide decreases steeply as the circumference decreases and tobacco weight is held constant, suggesting that corresponding increases in air velocity with decreasing circumference influence the formation of chemicals such as hydrogen cyanide that are distributed between the particulate and the gas phases *(98, 112)*. When experimental blended-tobacco super-slim cigarettes with unventilated carbon filters (15–90 mg per filter) were machine-smoked, about

twice as much carbon was required to retain about 50% of a smoke constituent when the super-slim was smoked under Canadian intense conditions than when it was smoked under ISO conditions *(110)*.

The complexity and interrelatedness of cigarette design features on smoke delivery make it difficult to propose specific design standards. More information is required on the consequences of changing design features. Furthermore, variations in the components of individual cigarettes are poorly understood, making it difficult to estimate interactions among them *(79)*. Thus, it might be appropriate to focus on the design features and product characteristics that most influence use behaviour, such as puff volume. While it is generally recognized that some well-known design features, such as filter vents, can lead to compensatory smoking, other features, such as the porosity of the plug wrap and tipping paper and properties of the tobacco rod, also affect smoke dilution and delivery and thus allow smokers to get more nicotine and other smoke emissions for a fixed volume of smoke. Tobacco manufacturers can, however, adjust other design features in order to compensate for changes that alter emissions, such as maintaining tar and nicotine delivery levels when they switch to paper that complies with fire standards *(113, 114)*. Consequently, product standards intended to lower the delivery of emissions should be based on delivery outcomes and not on changes in design that are anticipated to achieve such reductions.

## 2.4 Design features and additives that modify smoke pH and addictiveness

### 2.4.1 Overview

Nicotine, the primary addictive substance in tobacco, determines smoker "satisfaction" and the "physiological" strength of cigarette smoke *(72, 87)*. The addictiveness of nicotine is enhanced in various ways, such as by increasing the amount of total nicotine present in smoke, increasing uptake and controlling "smoothness" for optimal inhalation. In the tobacco leaf, nicotine is present mainly in the protonated salt form, but higher pH can increase deprotonation *(115)*. The unprotonated (volatile) or free base form of nicotine is more "physiologically effective" than the protonated (non-volatile) form *(116)* and is more rapidly available, by two mechanisms: because it is present in the volatile phase of smoke, it does not have to diffuse out of the smoke particle; and the unprotonated form is more lipophilic and can therefore diffuse rapidly across cell membranes and be taken up more quickly into the bloodstream *(117, 118)*.

The unprotonated nicotine fraction – but not the total amount of deliverable nicotine – is influenced largely by the alkalinity of cigarette smoke. Cigarette smokers experienced greater electrophysiological and subjective responses to the smoke of cigarettes with nicotine as base than with nicotine

as the citrate *(116)*. Industry documents indicate that unprotonated nicotine must be present to ensure a favourable sensory effect, termed the "impact", of cigarette smoke *(119–122)*. Opposing positions on the effect of smoke pH on unprotonated nicotine have been published, however, and attempts have been made to study the effect empirically. Calicutt et al. *(123)* found no significant difference in nicotine transfer among the test cigarettes analysed, which differed only in ammonia content. Varying the ammonia content of cigarettes would, however, affect only free nicotine and not total nicotine delivery. The total amount of nicotine absorbed is less pertinent than the rate of nicotine absorption, as the human body effectively absorbs most of the nicotine introduced by smoking. van Amsterdam et al. *(124)* examined nicotine uptake in venous blood samples from subjects who smoked test cigarettes with different measured levels of ammonia in the filler (0.89 and 3.43 mg/g). No difference was seen in "nicotine exposure"; however, the first sample was taken only 2.5 min after the last puff, which would not reflect absorption of free-base nicotine.

### 2.4.2 Ammonia, sugars and reconstituted tobacco

Ammonia has been described as an "ameliorant", an "impact booster" and a "satisfaction promoter" *(125)*. It is an active species, capable of causing complex changes when added to a tobacco blend *(126)*. The addition of ammonia and ammonia precursor compounds such as diammonium phosphate to tobacco increases the amount of unprotonated nicotine in both particulate matter and vapour *(127)*. Ammonia or diammonium phosphate is used in the production of reconstituted sheets, as it reacts with pectins and forms stable complexes with nicotine. The complexes decompose at the high temperatures typically reached during smoking, thereby increasing the transfer of nicotine from the filler material to the smoke, a characteristic known as "nicotine transfer efficiency" *(128)*. Increasing the temperature at which nicotine is released could increase the levels of unprotonated nicotine because the hydrolysis of nicotine is temperature-dependent *(129, 130)*. Ammonia stimulates the taste receptors, olfactory endings and the trigeminal nerve, giving a sensation described as "mouth feel" *(131)*. It reacts immediately with acids present in smoke, acting as an ameliorant. Binding of acids that could form salts with nicotine could liberate more free nicotine during pyrolysis *(132)*. Industry documents on tobacco smoke describe the total basic fraction (pyrazines, pyridines and alkaloids) and the total acidic fraction (organic acids, phenyl acids, phenolic acids and fatty acids), the larger fraction being basic. In the manufacture of reconstituted tobacco sheet and during casing, diammonium phosphate can react with reducing sugars to produce the Maillard-reaction products deoxyfructazines *(133)*, and pyrolysis of these products gives several pyridines and pyrazines that contribute to both the taste and the alkalinity of smoke *(134, 135)*. Hundreds of other bases have been identified in tobacco smoke,

most of which are nitrogen heterocycles associated with smoke flavours, probably formed during reactions involving ammonia and sugars that may also contribute to a basic smoke pH *(136, 137)*. Amino acids present at high levels in burley tobacco can also react with sugars to create similar, weakly basic compounds *(138, 139)*. A major pyrolysis product of sugar is acetaldehyde, which is thought to act synergistically with nicotine and increase addiction to cigarette smoke *(128, 140)*.

### 2.4.3 Other ingredients

Ammonia is not the only additive capable of deprotonating nicotine and forming Maillard reaction products with sugars: several other bases present in smoke create conditions favourable for the formation of unprotonated nicotine. Industry documents indicate that the urea–urease system is also used to raise the pH of the smoke by breaking urea down to ammonia by pyrolysis *(141, 142)*. Inorganic cations such as potassium and calcium can also raise the pH of smoke. As diammonium phosphate has been banned in some countries, other bases, such as calcium carbonate, are used to enhance nicotine delivery *(142)*. The levels of alkali metals like potassium and calcium can be manipulated by the use of fertilizers or curing practices or added directly to a tobacco blend, so that it is difficult to differentiate between native and added amounts in routine analysis. Calcium and sodium carbonates can also be added to cigarette filters to increase smoke pH, possibly eliminating the need for adding bases to tobacco filler *(143)*. A basic filter can liberate trapped nicotine, delivering volatile nicotine to smoke *(144)*. If smoke is perceived as too harsh, smokers might inhale less deeply; additives like laevulinic acid and liquorice may make smoke smoother and therefore more appealing and easier to inhale *(145)*. Additives like cocoa and menthol may not increase smoke pH but have been implicated as potential bronchodilators, thereby increasing the depth and volume of inhalation and facilitating total nicotine absorption *(146)*. Further, combustion products of cocoa might have monoamine oxidase inhibition properties, with an anti-depressant effect, which could contribute to the addictiveness of smoking in the presence or absence of nicotine *(142)*.

### 2.4.4 Tobacco blend and physical characteristics

Differences in blends, inclusion of expanded tobacco and the position of tobacco leaves on the stalk can all alter the pH and chemistry of smoke, without chemical additives *(147, 148)*. At a slightly acidic smoke pH (6.5–7.0), about 7% of nicotine is absorbed into a smoker's system; less is absorbed at a pH < 6.6 *(131)*. Flue-cured and American-blend cigarettes are slightly acidic, with a pH of 5.7–6.2. The pH of the smoke of cigarettes made with air-cured tobaccos is 6.5–7.8 *(86)*, whereas that of smoke from a burley cigarette may be > 7.5. Both the total nicotine delivered and the resulting smoke pH of burley tobaccos are strongly influenced by stalk

position: leaves at higher stalk positions contain more nicotine and are more basic. Cigarettes made only with burley tobaccos may have increased unprotonated nicotine delivery; however, they may be perceived as harsh by smokers. The addition of pH-reducing sugars can mask the harshness, resulting in control of unprotonated nicotine delivery from the blend *(149)*. Expanded tobacco produced with ammonium carbonate releases ammonia into the smoke on burning, without the addition of ammonia *(142)*. Expanded tobacco includes stems, which have a higher nitrate content than leaves and substantially influence the smoke pH, as nitrate is partially reduced to ammonia during smoking *(86)*. Certain characteristics of cigarettes, such as more porous paper and filter ventilation, could also raise the smoke pH. Although both smoke pH and nicotine content increase with increasing tip ventilation, the mechanisms are poorly understood. Air drawn through filter ventilation holes could act as a "drying gas", reducing the water content of the aerosol particles and effectively increasing the pH, thereby favouring formation of unprotonated base nicotine in the gas phase *(138)*. The burning rate of tobacco may also be affected by tip ventilation *(139)*, or the tar:nicotine ratio could change with increased ventilation *(150)*. Both mechanisms would raise the smoke pH and therefore the level of unprotonated nicotine.

### 2.4.5 Measuring "smoke pH"

As pH cannot be measured in a smoke aerosol, smoke pH is usually measured in an aqueous solution *(149)*. This measure was used to compare differences between brands by the tobacco industry for years and was useful for tracking changes made to the acidic and basic properties of cigarettes to achieve sensory effects *(151)*. Current, non-industry methods for measuring unprotonated nicotine in mainstream smoke include headspace analysis of particulate matter collected on a Cambridge filter pad (CFP), gas chromatography (GC)–mass spectrometry (MS) of samples collected in bags *(152, 153)* and analysis of collected particulate matter by nuclear magnetic resonance spectroscopy *(154)*.

All the methods for analysing a dynamic reaction like the partitioning of nicotine between phases in the cigarette rod, cigarette filter and smoke aerosol have drawbacks. At best, relative differences between brands can be identified. Nonetheless, the tobacco industry has relied on such relative measurements since ammonia technology became the intense focus of industry research decades ago.

## 2.5 Innovations that could influence either perception or delivery

### 2.5.1 Overview

In this section, we describe innovations that could influence perceptions or delivery, which have either recently been marketed or are being developed, according

to publications in the scientific literature and other sources, such as websites, tobacco industry documents and patents.

A background paper for the seventh meeting of WHO TobReg on the evolution of new tobacco products *(155)*, including products that potentially "modify risk", described notable alterations to traditional products on the market, such as menthol capsules in filters and organic cigarettes with no additives. A new line of very-low-nicotine cigarettes has been introduced, with a nicotine emission of < 0.04 mg but a "normal" level of tar when smoked under ISO machine conditions. The paper also described technologies in development, including several types of treated tobacco and novel filters. Many of these developments were claimed to result in reduced exposure, but most of the studies used as a basis for such claims were performed and published by the industry. Tobacco substitute sheet materials dilute the amount of tobacco in a blend, and treatment of the tobacco blend reduces the levels of components that are precursors of toxicants, such as proteins. Modified filters reportedly reduce the levels of toxic smoke components in mainstream smoke by reacting with or selectively filtering smoke components; examples include amine resin, which reacts with aldehydes and hydrogen cyanide, and charcoal filters. Selective reduction of some mainstream smoke toxicants was reported with most of these products, but in some cases the levels of other toxicants increased. As smokers must inhale sufficient nicotine to sustain their addiction, toxicant levels should be expressed per nicotine level; however, nicotine emission levels were often not reported. Some of these products were reported to be less toxic in vitro or to give lower levels of biomarkers of exposure *(155)*. Consumers, however, generally found these products to be less acceptable than traditional cigarettes. It is therefore difficult to assess the net effect of these new technologies. The concerns should be kept in mind when evaluating the new tobacco industry research described in section 2.5.5.

Sections 2.5.2–2.5.5 summarize innovations introduced since October 2013, when the literature search for the background paper *(155)* was finalized.

### 2.5.2 Reduced-nicotine cigarettes

Unlike cigarettes that are designed (e.g. with filter ventilation) to yield less nicotine in the smoke, as measured with the ISO smoking method, reduced-nicotine cigarettes have less nicotine in the tobacco filler. "Magic" reduced-nicotine cigarettes (which emit 0.04 mg nicotine per cigarette) recently became available in tobacco shops in Spain, bearing the claim that they contain no nicotine. In accordance with European regulations that require cigarette manufacturers to list the nicotine yield directly on each pack of cigarettes and to round the yield to the nearest 1/10 place, Magic 0 packs prominently feature the words "0.0 mg nicotine" *(156)*.

Recently, use of denicotinized cigarettes was tested as a complement to standard smoking cessation treatment, consisting of behavioural support

combined with pharmacotherapy (varenicline or nicotine replacement therapy). Abstinence from cigarettes was significantly higher with nicotine-free cigarettes than with standard treatment after 1 (70% vs 53%) or 4 (58% vs 43%) weeks but not after 12 weeks (39% vs 31%) *(157)*. In a study of 840 smokers of five or more cigarettes a day, smokers who switched to cigarettes with a lower nicotine content were smoking fewer cigarettes per day (about 16) after 6 weeks than those who smoked cigarettes with a normal nicotine content (several types were tested; about 22), and no significant compensation by smoking more intensely was observed *(43)*. Nevertheless, the participants commonly smoked cigarettes outside the study, which probably obviated any reduction in exposure to nicotine. The researchers are conducting further studies with different approaches, such as a gradual vs an immediate reduction to very-low-nicotine cigarettes and combining such cigarettes with nicotine patches.

### 2.5.3 Coloured cigarette paper

Some cigarette brands have coloured paper (Fig. 2.1). These include Ziganov Colours (pink, dark pink, yellow, green and purple), Ziganov Black, Sobranie Cocktails, Fantasia, Black Devil, Pink Elephant, Nat Sherman Fantasia and Vanity Fair. Coloured cigarette tubes are available for roll-your-own cigarettes *(158)*.

Fig. 2.1. Examples of coloured cigarettes

A web post states that Sobranie Cocktails "… are five separate bright pastel shades with a gold foil filter, and are the same ring gauge as standard cigarettes, unlike Nat Sherman's Fantasias, which are slimmer and use deeper, primary colours" *(159)*. This type of cigarette is "… particularly made for ladies with its slim features and bright colours which attracted many women to this popular brand."

Few studies are available on perceptions of the colour of cigarettes, in contrast to cigarette pack design. As discussed in section 2.2.2.1, brightly coloured cigarettes can create significant interest and are generally perceived as appealing, pleasant tasting and less harmful *(14)*, whereas black cigarette paper may have low appeal and be associated with a strong taste and greater harm.

The WHO FCTC advises countries to prohibit or restrict features that make tobacco products more attractive to consumers, including coloured cigarette paper. "Colouring agents are added to various components of tobacco products to make the resulting product more appealing. Attractively coloured cigarettes (e.g. pink, black, denim blue) have been marketed in some countries. Examples of colouring agents include inks (e.g. imitation cork pattern on tipping paper) and pigments (e.g. titanium dioxide in filter material)" *(36)*.

### 2.5.4 Specialty filters

Many filter types are available from cigarette material suppliers, suggesting a demand from the tobacco industry. For instance, the company Hauni Maschinenbau offers 18 types that differ in visual effect, filtration properties, taste enhancement and interactivity *(160)*. Various elements and combinations can be used, such as charcoal, hollow shapes in e.g. the form of a heart and coloured filters. Tobacco, flavour capsules or herbal or botanical granules can be added to filters. Different tastes can be achieved by inserting flavoured thread or spraying flavour directly into the filter tow. Flavoured thread can be coloured "to create a more unique appearance".

Essentra Filter Products also has a wide range of filters available in different product ranges, e.g. sensory (capsules, flavour thread, direct application on filter), earth tones (faster degradation in the environment), performance (high filtration efficiency, also selectively for e.g. vapours) and visual differentiation ("…use visual appearance to indicate a flavour, a particular product attribute, a brand logo or indeed just to visually differentiate your brand") *(161)*. Coloured flavour threads that can be used to add ingredients such as menthol are described as a "visual indicator of taste delivery technology". For instance, DJ Mix Flavoured Cigarettes in the USA have not only a coloured package but also the same colour applied to the filter to reflect product flavours (e.g. red for strawberry and green for apple). Marlboro Black Freeze (Mexico) has a menthol stripe running through the middle of the filter and the same symbolic stripe printed on paper.

The new European Tobacco Products Directive 2014/40/EU *(29)*, in Article 7 on regulation of ingredients, prohibits the use of flavourings, tobacco or nicotine in filters and cigarette paper: "Member States shall prohibit the placing on the market of tobacco products containing flavourings in any of their components such as filters, papers, packages, capsules or any technical features

allowing modification of the smell or taste of the tobacco products concerned or their smoke intensity. Filters, papers and capsules shall not contain tobacco or nicotine."

Flavour capsules were already described in the background paper on novel tobacco products *(155)*. According to industry reports, flavour capsules in cigarette filters, which can be crushed to release a burst of flavour, are a significant growth segment *(162)*. Capsules typically contain menthol or similar flavours, such as lemon mint, and are available in many different types of cigarettes; sometimes, two differently flavoured capsules are present in one filter. A study among smokers in Australia, Mexico and the USA showed that flavour capsules are most attractive to young people, use of cigarettes with flavour capsules is growing, they are associated with misperceptions of relative harm, and young people differentiate brands *(162)*. A focus group study among young female nonsmokers and occasional smokers showed that they perceived flavour-capsule cigarettes very positively *(14)*. They appreciated the novelty and liked the fact that the taste could be switched from "normal" to menthol. Just as research shows that cigarette packs can influence perceptions of appeal, harm and taste, this study suggests that the actual cigarettes can also do so.

Two recent studies of the effects on mainstream smoke of a crushed menthol capsule in Camel Crush found no change in the yields of particle-phase constituents. Gordon et al. *(163)*, using a real-time detector, found not only the expected increase in menthol delivery but also increased yields of several gas-phase constituents, notably five volatile organic compounds (VOCs), acetaldehyde, acrylonitrile, benzene, 1,3-butadiene and isoprene. Dolka et al. *(164)*, at Philip Morris, however, found no such increases when using cooled impingers with methanol to sample gas-phase components.

### 2.5.5 Tobacco industry research on delivery through special filters and with treated tobacco

Techniques are being developed for producing reduced-toxicant emission cigarettes, including filter adsorbents, blend tobacco treatments and tobacco substitute sheets. British American Tobacco examined the effects of modifying filter ventilation, varying cigarette circumference and active charcoal filter length and loading and combinations of these features *(104)*. An air-dilution mechanism, called "split-tipping", was developed in which a gap between two separated sections of tipping paper, exposing an area of the filter, is wrapped with a band of porous paper. This band minimizes the loss of effective filter ventilation that occurs at the high flow rates encountered during human smoking and facilitates the diffusional loss of volatile toxicants. The results showed that the ratio of these toxicants to nicotine emissions in mainstream smoke was reduced, except in the test cigarettes with 1 mg of tar.

Another paper from British American Tobacco described assessment of the genotoxicity and cytotoxicity in vitro of the particulate matter generated from experimental cigarettes with 50% blend tobacco, 15% tobacco substitute sheet, polymer-derived activated charcoal and split-tipping *(165)*. In comparison with control cigarettes that had a standard cellulose acetate filter, tipping paper and typical tobacco blends (3R4F, a US-style blended product, and M4A, a flue-cured product), bacterial mutagenicity and mammalian genotoxicity were reduced with the experimental cigarette, whereas there was no significant difference in cytotoxicity.

A study funded partly by Guangdong Tobacco Industrial Company *(166)* described use of specific filter additives and molecularly imprinted polymers with nicotinamide as the template on a silica surface for the adsorption of TSNAs in mainstream cigarette smoke. The levels of TSNAs were reduced by up to 41% as compared with those in the cigarette smoke of the control group. This study would appear to be selective, as the tar levels remained the same and nicotine levels were not reported.

A study from Cultex Laboratories GmbH and Japan Tobacco Inc. showed that smoke from K3R4F cigarettes with integrated charcoal filter tips were less toxic to cilia in normal bronchial epithelial cells than regular K3R4F cigarette smoke, when machine-smoked under standard ISO conditions *(167)*. VOCs, which were removed by the charcoal filter tip, affect cilia formation in primary bronchiolar epithelial cells. Histopathological analysis of the exposed cultures showed fewer cilia-bearing cells, shorter existing cilia and, finally, disappearance of all cilia in cells exposed to cigarette smoke. In cultures exposed to charcoal-filtered cigarette smoke, small changes in cilia length were seen after four exposures, but the effects were reversed after a 2-day recovery period.

A patent issued to Philip Morris describes the development of a tobacco smoking mixture and a cigarette wrapper containing high-temperature ammonia-release agents *(168)*. The ammonium compounds were claimed to be present "in an amount effective to reduce the cytotoxicity of gas phase or particulate matter formed during smoking of the cigarette".

Although some of these new cigarette types were found to have lower machine yields of toxicants in mainstream smoke and reduced toxicity in vitro than conventional cigarettes, substantial scientific data would be required to conclude that they represent a lower health risk. In evaluating the efficacy of design changes in reducing human risk, consideration must be given to the acceptability of a product to consumers, its effect on their smoking behaviour and whether it actually results in reduced exposure as assessed by e.g. biomarkers.

## 2.6 Research that would inform scientific evaluation of the public health impact of design characteristics

As discussed above, a substantial body of evidence has established that cigarette attractiveness and addictiveness and the delivery of smoke toxicants to users are strongly associated with the physical characteristics and design features of cigarettes. The effects of certain characteristics have been studied in greater detail than others. For instance, the effects of filter ventilation on consumer perceptions, machine-generated emissions and the exposure of smokers have been extensively studied and reported, while there are limited data on the effects of flavours. Similarly, more data on the potential of reduced-nicotine cigarettes (< 0.4 mg/g in tobacco filler) to facilitate smoking cessation would be helpful. A systematic review of past and pending studies would be informative; however, it should not be used in any way to promote smoking. Because of the complexity of the interplay between consumer perceptions and behaviour and smoke chemistry, however, the available data do not necessarily provide a clear understanding of how certain physical features could be modified to reduce toxicant emissions and thus protect public health. Therefore, further research would inform the scientific basis for effective regulatory measures.

Given the complexity of the impact of cigarette physical characteristics, tobacco type and use of additives on human exposure and the fact that exposure is mediated by smokers' perceptions and behaviour, studies should take a comprehensive approach to determining how specific cigarette designs influence many outcomes, including machine smoke delivery, smokers' beliefs and smoking topography and the resulting exposure.

Studies of the effect of design features on emissions should always include nicotine levels. Any effect on emissions should be reported per milligram of nicotine, as smokers inhale sufficient amounts of nicotine to sustain their addiction *(2, 70)*. As free-base nicotine is the most bio-available form, international standards for measuring free-base nicotine or determining the ratio of free-base to protonated nicotine would be helpful. In addition, researchers should be aware that any manipulation of a product to reduce the content of one or more constituents may unintentionally increase the concentrations of other constituents. Research approaches to investigating how design features interrelate and affect mainstream smoke emissions include:

- systematic studies of individual design features case by case. For a few selected parameters, such as filter ventilation and cigarette dimensions, this approach could be applied in many testing laboratories. For other design parameters, like filter material or paper porosity, studies would have to be done in a well-equipped testing laboratory and might require the production of custom cigarettes with specific design features.

- extensive multivariate analysis of tobacco filler constituents, mainstream smoke emissions (under smoking protocols of varying intensity) and the physical properties of cigarette products on the market. This approach would allow identification of the design parameters that have the greatest influence on mainstream smoke emissions.
- in-depth, detailed statistical analyses of all relevant design features, parameters and specifications, with mainstream smoke emissions provided by cigarette manufacturers. This approach could be used if there is sufficient regulatory authority and would include checking of the results by an ISO 17025-accredited government laboratory or an independent contract laboratory as part of regulatory oversight.

Appropriate tools for studying the perceptions and behaviour of smokers and nonsmokers, in particular adolescents, include consumer surveys, focus group analyses and clinical (topography and biomarker analyses) investigations. Actual exposure could be estimated by measuring a relevant set of biomarkers in smokers. The results will show whether reductions in machine-measured yields of specific constituents reduce the exposure of smokers.

The health effects of exposure can be assessed in clinical studies, e.g. by measuring biomarkers of (early) effects. Alternatively, a set of relevant in-vitro assays for important smoking-related diseases could be used. In-vitro tests based on air–liquid interface cell models are promising, as they model the exposure of the airways to smoke.

It is important to monitor developments in the tobacco product market in order to remain informed about innovations that concern public health, by, for example, standard searches of websites, including social media, as well as field research.

## 2.7 Conclusions

The main purpose of cigarette design is to increase the appeal of the product (i.e. to make it more palatable, attractive or less harmful), to reduce the negative aspects of the product, to ensure that smokers experience satisfaction in using the product and to attract the interest of young people and novice users. Cigarette characteristics that increase their appeal include those that influence a user's perception of the cigarette's appearance or whether they can "customize" it. The decorative elements of cigarettes directly and substantially affect the appeal of the cigarette by suggesting strength, novelty or reduced harm, particularly to women and young smokers. These elements are some of numerous innovations that have been introduced by manufacturers. Given that the sole purpose of such features is to attract new consumers, they can lead to misperceptions of health risk. Limiting cigarette appearance to standard features, i.e. white paper, standard tipping paper colour and standard print of cigarette brand, could be expected to protect public health.

Most of the other physical characteristics of cigarettes have complex and sometimes opposite effects on multiple outcomes. For instance, filter ventilation results in lower machine-generated emissions per cigarette and perceptions of lighter taste and greater safety by smokers. Higher filter ventilation is an example of a physical characteristic that can change smoking behaviour, resulting in similar or higher exposure to toxic and carcinogenic emissions than would result from smoking less ventilated cigarettes. Filter vents are a design feature that is easily manipulated by smokers to obtain higher nicotine and smoke emissions from a cigarette. Porous tipping paper and cigarette wrappers and the properties of tobacco blends are other design features controlled by manufacturers that allow a smoker to unwittingly take more smoke from a cigarette. Cigarette dimensions are also associated with a complex interplay of outcomes. Thus, slim cigarettes contain less tobacco for burning and can therefore result in lower overall exposure of smokers per cigarette; however, slim cigarettes appeal to women with their stylish, attractive, high-quality appearance and are perceived as being less harmful, which is a public health concern. Furthermore, the exposure of smokers of these slim cigarettes to constituents such as hydrogen cyanide and formaldehyde may not be lower than from cigarettes of standard circumference.

Research has been conducted not only on ventilated filters and slim cigarettes but also to support the more general hypothesis that manufacturers use tobacco blend properties and pressure drop (paper porosity, filtration, filter retention) as a product design strategy to develop cigarettes with an "elasticity" that allows smokers to obtain the amount of nicotine they desire and sensory "satisfaction". Most cigarettes have some elasticity, especially "ultra-low" cigarettes, whereas full-flavour brands have less. Under machine smoking conditions, elasticity appears as nonlinear increases in toxic emissions with increasingly intense puffing.

Cigarette design, such as filter additives that reduce emissions of selected chemicals in smoke, can modify sensory cues, resulting in changes in smoking behaviour. It has been shown that smokers take larger puffs when smoking cigarettes with charcoal filters. Adding chemicals to the smoke as flavours can also influence sensory cues. While there is some evidence that smokers perceive a flavoured cigarette as novel and take smaller puffs, the overall design of the cigarette means that they are exposed to harmful smoke emissions like CO as much as when they are smoking a tobacco-flavoured cigarette. The interplay between perceptions, behaviour and measures of exposure is complex. Perhaps the best example is use of mentholated cigarettes, which is reported to be associated with stronger addiction and fewer successful attempts to quit smoking; however, the results of studies on the influence of mentholation on smoking behaviour are mixed.

Established smokers habitually use cigarettes to obtain nicotine. The satisfaction they experience when smoking is due to the sensation of tobacco smoke entering their mouths ("impact"), followed by rapid absorption of nicotine

from the lungs to the brain within seconds of inhalation. The unprotonated (un-ionized) form of nicotine is reported to be taken up from smoke more effectively, and it reaches the brain more rapidly than in the protonated (ionized) state. Several design features and additives can influence the proportion of nicotine that is in the unprotonated form. Alkalinizing agents increase the amount of unprotonated nicotine while increasing "mouth feel" and improving taste by forming products from reactions with acids and reducing sugars in smoke. Mouth feel and taste act as cues to smokers to modulate their smoking behaviour in response to the physiological "strength" of the smoke.

Many innovations for changing perceptions or smoke emissions have focused on tobacco blend and filter technologies, because of their roles in controlling delivery and use behaviour. Non-traditional methods of adding flavours, such as flavour capsules and flavour threads, create appeal by their novelty and brand differentiation. Flavour capsules are a significant growth segment for the tobacco industry and are particularly attractive to young people. While some new technologies have been encouraging, such as reducing selected toxicants, the gains are frequently offset by increased amounts of other toxicants or poor consumer acceptability. The combination of filter additives and treated tobacco has been explored by the tobacco industry as a means of reducing emissions of toxicants. Internal industry documents suggest that laboratory assessment of these cigarettes show reduced toxicity; however, it is not known whether any of these technologies has been reviewed by regulators or used in commercial products in unregulated markets. Recent studies of low-nicotine cigarettes in a market where standard-nicotine cigarettes were available showed that smokers' behaviour changed (they smoked fewer cigarettes per day) and that they were significantly more likely to abstain from smoking cigarettes; however, the abstinence was no better than after standard cessation treatment 12 weeks later, and smokers frequently smoked cigarettes with standard levels of nicotine.

## 2.8 Recommendations

The ultimate goal of research on cigarette design is to ensure that any ensuing regulatory measures simultaneously reduce the attractiveness and addictiveness of cigarettes and the harm associated with their consumption, as already recommended in the partial guidelines for implementation of articles 9 and 10 of the FCTC *(36)*. This can be achieved by standardizing cigarette appearance; eliminating design features and ingredients that make cigarettes more appealing to new or novice smokers or more difficult for established smokers to quit; reducing the addictiveness of cigarettes by lowering their nicotine level or the biological availability of nicotine; and reducing exposure to harmful emissions by a combination of selective filtration and modifications to cigarette dimensions, packing density and tobacco blend.

On the basis of the conclusions, the following specific policy and research recommendations are proposed.

### 2.8.1 Policy recommendations

1. Require manufacturers to disclose information on all the design features, parameters, specifications and levels of contents and emissions levels of current and emerging products. Examples include cigarette paper, capsules in cigarettes filters and cigarette dimensions.
2. Prohibit filter ventilation and any other design characteristic that allows cigarette elasticity (increased puff volume by smokers, especially of lower-tar varieties); and prohibit filter capsules, slim cigarettes and any other product attribute that increases its attractiveness, smoke emissions or addictiveness.
3. Require lowering of all toxic emissions (per mg nicotine), according to the approach set out by TobReg *(71)*.

### 2.8.2 Research recommendations

1. Continue research on the design characteristics of tobacco products and innovations in that area, including their impact on:

- the perceptions and behaviour of smokers, former smokers and people who have never smoked, in particular adolescents;
- emissions, normalized per mg of nicotine except for reduced-nicotine cigarettes (< 0.4 mg nicotine per g tobacco in filler);
- toxicity; and
- exposure.

2. Develop and validate a standard method for measuring free-base nicotine levels or determining the ratio of free base to protonated nicotine.
3. Continue research on potential use of reduced-nicotine cigarettes as a smoking cessation strategy. A systematic review on past and pending studies may be informative, although it will be important to ensure that it is not used in any way to promote smoking.

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# 3. Possible application of WHO Tobacco Laboratory Network standard operating procedures to evaluation of electronic nicotine delivery systems

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**Contents**

## 3.1 Background

This section provides recommendations on the application of existing and pending WHO Tobacco Laboratory Network (TobLabNet, http://www.who.int/tobacco/global_interaction/toblabnet/en/) Standard Operating Procedures (SOPs, available at http://www.who.int/tobacco/publications/prod_regulation/en/) to the analysis of the content and emissions of Electronic Nicotine Delivery Systems (ENDS), as requested by the seventh session of Conference of the Parties (COP) to the WHO Framework Convention on Tobacco Control (WHO FCTC, http://www.who.int/fctc/cop/cop7/FCTC_COP7_9_EN.pdf?ua=1). The recommendations in this section may also be appropriate for electronic non-nicotine delivery systems (ENNDS) after determination of suitability for that matrix (e.g., appropriate measurement range, interference, etc.).

ENDS consist of a battery that heats a coil and vaporizes a liquid matrix (content) to deliver an aerosol (emission), also referred to as a vapour. For the purposes of this report, the aerosol from the mouth end of the ENDS device is referred to as "first-hand aerosol" (FHA), the liquid matrix is referred to as "e-liquid" and when the e-liquid contains nicotine the device is referred to as Electronic Nicotine Delivery Systems (ENDS). "E-cigarette" is used when referring to cigarettes-shaped ENDS or when quoting from a source that uses this word.

The vaporization temperature of ENDS is a function of the battery voltage and the current through the coil *(1)*. The e-liquid is usually a solution containing propylyene glycol alone or in combination with vegetable glycerol, nicotine, flavourings and other constituents, such as caffeine. The e-liquids and resulting first-hand aerosols usually contain nicotine in a wide range of concentrations and other chemicals added to enhance appeal. Nicotine, minor tobacco alkaloids,

tobacco-specific nitrosamines (TSNAs), flavourings, metals, VOCs, phenolic compounds, and solvents have been reported in e-liquids. Carbonyls, VOCs, TSNAs and metals have been reported in ENDS aerosol *(2)*.

ENDS may be disposable or reusable and may have features (e.g. variable voltage ranges) that allow the user to "customize" the delivery and chemical composition of the aerosol. Initially, ENDS were designed to resemble cigarettes in size and shape. The new generations of ENDS are larger, have refillable tanks and may resemble cigars, pipes or hookahs (waterpipes) or not look like any tobacco product at all *(2–4)* (Fig. 3.1). ENDS are sold worldwide *(5)*, sometimes regulated as tobacco products, sometimes as consumer products and sometimes as pharmaceutical products. However, other countries have banned ENDS, refills that contain nicotine and even, ENNDS *(90)*.

Fig. 3.1. Electronic nicotine delivery systems

In contrast to conventional tobacco cigarettes, for which there are reference materials (e.g. CORESTA Monitor and Kentucky research cigarettes), there are currently none for ENDS, and there are no methods based on human use for machine generation of ENDS aerosol for analysis. The patterns of use (topography) of ENDS products have been examined in only a few studies *(6, 7)*, and the issue is further complicated by the diversity of ENDS products. CORESTA (https://www.coresta.org/), an international association of tobacco product manufacturers, tobacco industry institutes and laboratories, published a recommended method for machine generation of aerosol *(8)* for ENDS that contain "electronic components which vaporize a liquid to generate an aerosol carried by the air drawn through the device by the user. [The device] could be

designed either as a single piece or as a modular, multiple component product for disposable, rechargeable and/or refillable use." The products reportedly covered by the method are those that meet the above definition and also "products described as e-cigarettes, e-cigars, e-shisha, e-pipes and other related product categories'. The CORESTA method is not based on measures of human puffing topography that reflect actual use or behaviour.

Several companies have begun to manufacture and sell automated machines to generate ENDS aerosol (e.g. Cerulean, Milton Keynes, United Kingdom; and Borgwaldt GmbH, Hamburg, Germany). A machine designed to generate ENDS aerosol for analytical purposes should provide a source of electrical power for the device, and research should address the requirements for the power source. The available equipment and methods are optimized for cigarette-like devices (e-cigarettes); therefore, additional equipment or modifications to the method might be required for newer designs of ENDS, notably the larger "tank' varieties.

## 3.2 General methodological considerations in the evaluation of ENDS

Quantitative methods for chemical analysis of any product depend on the nature of the matrix in which measurements are made. The ENDS matrices (e-liquid or first-hand aerosol) are less chemically complex and less varied in composition than conventional tobacco products (tobacco filler and mainstream tobacco smoke). Standardized measures of mainstream cigarette smoke from conventional cigarettes apply to a particular brand under standard conditions specified by ISO *(10)*, the Federal Trade Commission in the USA *(11)*, the Centers for Disease Control and Prevention (CDC) in the USA *(12)*, CORESTA *(13, 14)*, Health Canada *(13)*, the Commonwealth of Massachusetts Department of Public Health in the USA *(15)* and WHO *(16)*. The smoking regimens designed for analysing conventional tobacco cigarettes differ only slightly amongst the less intense standard methods (e.g. ISO and Federal Trade Commission); others (e.g. Canadian Intense and Massachusetts) simulate larger puff volumes and the vent-blocking behaviour of smokers, which can give widely different results than with the less intense methods. All the methods generally include specific temperature and humidity-controlled conditioning of cigarette samples, machine smoking of cigarettes in a specified regime (i.e. puff volume, duration and interval) to a butt length determined for each product (23 mm, the filter length plus 8 mm or the filter overwrap plus 3 mm) and open, partially blocked, or completely blocked filter ventilation. The findings of studies on human ENDS use topography *(6, 7)* raise questions about whether standard and "intense" regimes (analogous to ISO and Canada Intense machine smoking regimens for tobacco cigarettes) are appropriate and the corresponding modifications to the procedure or equipment used to analyse emissions from different ENDS products.

Machine-generated mainstream tobacco smoke samples are usually analysed, after sample preparation, by GC–MS or flame ionization detection (FID), liquid chromatography with ultraviolet–visible spectrophotometry, liquid chromatography with MS or inductively coupled plasma-MS.

## 3.3 Nicotine

ENDS products deliver nicotine, an addictive chemical, via the respiratory system. The nicotine concentration listed on the labels of ENDS cartridges and refill e-liquid may be significantly different from the values measured in the liquid *(3, 18)*. Sleiman and colleagues recently reported the levels of nicotine in ENDS liquid, measured by headspace GC with mass-selective detection (HS-GC/MS), from commercial ENDS products purchased at retail stores in California, USA. The levels were 20.4, 25.4 and 32.1 mg/mL for e-liquids with marketed nicotine levels of 18, 24, and 18 mg/ mL, respectively *(1)*.

### 3.3.1 Nicotine in ENDS liquid

Two factors should be considered when measuring the nicotine content of e-liquids. The first is the insolubility of propylyene glycol and vegetable glycerol in the hexane extraction solution used in the TobLabNet method for measuring nicotine in tobacco filler. They are more soluble in the isopropanol extraction solution used in the standard ISO method for measuring tar, nicotine and CO in smoke *(10)*. Consequently, the standard ISO method for analysis of nicotine trapped on a Cambridge Filter Pad (CFP) is more appropriate for analysis of nicotine in e-liquid than is TobLabNet SOP-04. Alternatively, WHO SOP-04 could be used with a more miscible extraction solvent. An important consideration in the analysis of nicotine in ENDS e-liquid is that the upper level may greatly exceed that in tobacco cigarette smoke extracts, even those generated under intense smoking machine conditions (e.g. about 36 mg/mL versus 0.3 mg/mL; CDC, unpublished data). Accordingly, the isopropanol extraction volume must be adjusted so that the nicotine concentrations in the e-liquid samples fall within the calibration range.

Secondly, it has been noted that knowing the total amount of nicotine in a tobacco product is not sufficient to understand its effect on users *(19)*. Nicotine can occur in either the protonated or the unprotonated (also referred to as unionized or "free" nicotine) state. Absorbed nicotine in the unprotonated state reaches the brain more quickly than that in the protonated state, which is an important factor in the addiction potential of the chemical. Addition of alkalinizing agents increases the proportion of nicotine that is in the readily absorbed unprotonated form *(20)*. The pH of e-liquids can be measured by the procedure commonly used for measuring the pH of smokeless tobacco, with timed measurements taken with a pH meter. The pH of some e-liquids has been

reported to be greater than the pKa of nicotine *(3, 21)* suggesting a substantial amount of the nicotine is unprotonated.

### 3.3.2 Nicotine in ENDS aerosol

The smoking regime (standard or intense) is usually specified in methods for measuring nicotine in tobacco smoke, while the results of both regimes capture a range of possible smoking topographies among conventional cigarette smokers. The ISO smoking regime involves smoking cigarettes with the ventilation holes unblocked, a 35-mL puff volume, a 2-s puff duration, a 60-s puff interval and enough puffs to reach a butt length equivalent to the filter length plus 8 mm or the filter overwrap plus 3 mm (whichever is longer), whereas the Canadian "intense" and the WHO smoking regimes specify a 55-mL puff volume, a 2-s puff duration, a 30-s puff interval and 100% blockage of the cigarette filter ventilation holes. The resulting mainstream smoke total particulate matter (TPM) is extracted in isopropyl alcohol and analysed by GC–FID *(10)*. For e-cigarettes, CORESTA has recommended a "vaping" (automated machine generation of e-cigarette aerosol) regime *(8)* to generate aerosol, with a 55-mL puff volume, a 3-s puff duration, a 30-s puff interval and no specified puff count, although at least 50 puffs per session are considered to generate adequate TPM on a CFP for determination of nicotine (*22*; CDC, unpublished data). The CORESTA method reportedly covers ENDS products designed for single use, disposable units and modular, multi-component products such as rechargeable and/or refillable devices (i.e. tank systems). If the method is verified by independent laboratories, it might obviate modifications to the procedure and equipment for analysing emissions from different product designs. The puffing parameters in the WHO SOP-01 intense machine smoking method are similar to those in the CORESTA method and could be modified for aerosol generation until sufficient data on product design variables and ENDS use behaviour become available to design a protocol for generating ENDS aerosol that is more representative of how the products are used.

The CORESTA method does not specify the analytical platform for quantitative measurement of nicotine in the collected aerosol. As combustion is not expected to occur when ENDS are operated under non-intense conditions, the composition of the collected aerosol resembles that of the liquid. The analytical platforms used in the WHO SOPs for analysis of tobacco smoke extract could be applied to ENDS aerosol captured on a CFP. A study in which ENDS aerosols were collected on CFPs with a downstream adsorbent trap indicated that nicotine is present in aerosol particles and that more than 98% of the nicotine was captured by the CFP *(22)*. The conventional detection schemes could be extended for use in analysing ENDS aerosol, especially as it is significantly less complex than tobacco smoke and the chemicals are soluble in isopropyl alcohol. A preliminary comparison of the CORESTA e-cigarette regime with a standard testing protocol for tobacco cigarette mainstream smoke showed that

the CORESTA method provided reliable quantification of nicotine in a limited sample of ENDS products (CDC, unpublished data); similar results are expected with WHO SOP-01. Additional ENDS configurations such as e-pipes and e-hookahs should be evaluated in the future.

Nicotine in tobacco smoke is usually measured in conjunction with "tar" (TPM minus nicotine and water) and CO. As in mainstream tobacco smoke, ENDS TPM, consisting of solvents, water, nicotine and other aerosol contents, is captured on a CFP during machine generation of ENDS aerosol *(22)*.

## 3.4 Tobacco-specific nitrosamines

TSNAs are formed mainly during the curing, fermentation and combustion of tobacco and are found in all types of tobacco product *(23)*. WHO has recommended mandated lowering of TSNAs, specifically NNN and NNK, which are potent human carcinogens, in tobacco and tobacco smoke *(24)*.

### 3.4.1 Tobacco-specific nitrosamines in ENDS liquid

While some TSNAs are formed during combustion of tobacco from alkaloid precursors, they are mainly present in cured tobacco in cigarette filler and transferred directly to mainstream smoke during the combustion of tobacco *(25)*. As the nicotine in e-liquid is extracted from tobacco, any TSNAs in ENDS e-liquid are probably impurities introduced during nicotine extraction.

Laugesen *(26)* analysed the liquid in Ruyan® e-cigarette cartridges and found a nicotine content that varied from 0 to 16 mg per cartridge. Of the four TSNAs, only NNK was detectable in all cartridges. NNN was found at higher levels than NNK but was detectable only in the nicotine-containing cartridges; 0.260 ng NNK was detected in the "zero nicotine" cartridge. The levels of NNN and NNK increased with increasing concentrations of nicotine. In another study, NNN and NNK were found in the refill e-liquids of brands sold by 11 companies and purchased in the Republic of Korea *(27)*, while Westenberger *(28)* found no detectable levels of TSNAs in 10 varieties of cartridge e-liquids for two brands purchased in the USA. Researchers at the National Institute for Public Health and the Environment (RIVM) in the Netherlands found detectable, but low levels of TSNAs in nearly all ENDS liquids by ultra-performance liquid chromatography coupled with tandem MS. A very small fraction of ENDS liquids contained up to 150 ng/mL of individual nitrosamines and 285 ng/mL total TSNAs *(29)*. The higher concentrations found might be due to the use of tobacco extracts as a flavour, as all the liquids in which they were found were labelled "with tobacco flavour".

A comprehensive study of NNN and NNK levels in tobacco filler from cigarettes sold in 14 countries indicated total TSNA at a concentration of 0.087–1.9 mg/g *(30)*. Thus, the levels of TSNAs in ENDS liquid, when present, are much lower than in cigarette tobacco filler.

### 3.4.2 Tobacco-specific nitrosamines in ENDS aerosol

In the WHO SOP for determination of TSNAs in mainstream cigarette smoke under ISO and intense smoking conditions *(31)*, cigarette smoke particulate matter is collected on a CFP, extracted with ammonium acetate and analysed in a high-performance liquid chromatography (HPLC) tandem MS system. Cigarette smoke is created by combusting tobacco filler, whereas aerosol from ENNNDS is created by heating e-liquid at temperatures that depend on the device parameters. As the tobacco cigarette smoke matrix is much more complex than ENDS aerosol matrix, containing about 8000 chemicals *(32)*, the SOP should be applicable for the analysis of TSNAs in ENDS aerosol. In one study, however, the maximum levels of TSNAs detected in ENDS aerosol were 28.3 ± 13.2 ng per 150 puffs for NNK and 4.3 ± 2.4 ng per 150 puffs for NNN. With fewer puffs (e.g. *15*), the estimated levels were 2.83 ng NNK and 0.43 ng NNN. Even if the minimum volume of extraction solution (10 mL) in the TobLabNet TSNA method were used, the NNK level would be 0.28 ng/mL and that of NNN 0.043 ng/mL, which are lower than the reporting limit of the method, 0.5 ng/mL *(33)*. Thus, a higher puff count (e.g. *50*) should be used to optimize the conditions for measuring TSNAs in ENDS aerosol. Comparisons of emissions "per unit" (i.e. per stick for a tobacco cigarette and per unit for a single-use ENDS product) or "per session" might give different results but would still be expected to be substantially lower than those in the mainstream smoke of tobacco cigarettes.

## 3.5 Benzo[*a*]pyrene

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of carcinogens formed during incomplete combustion of organic materials such as tobacco. Benzo[*a*]pyrene is a widespread environmental pollutant, a human carcinogen and the most thoroughly studied member of this class of compound *(34, 35)*. WHO has recommended lowering of benzo[*a*]pyrene levels in mainstream tobacco smoke *(24)*.

### 3.5.1 Benzo[*a*]pyrene in ENDS liquid

In several studies of harmful chemicals in ENDS e-liquid, no significant quantities of PAHs were found. Kavvalakis et al. *(36)* found no PAHs in e-liquid samples on the Greek market, and Leondiadis found no PAHs in Nobacco brand refill e-liquids *(37)*. The study by Laugesen *(26)* is one of only a few in which PAHs were found above the limit of detection. Four PAHs, anthracene, phenanthrene, 1-methyl phenanthrene and pyrene, were detected in a hexane extract of 0 mg nicotine, Ruyan® e-liquid. The authors calculated the amount of each PAH as a percentage of the amount of the PAH in the smoke of an equivalent number of tobacco cigarettes, assuming that consumption of the e-liquid was equal to

smoking 20 tobacco cigarettes in one day. The levels detected, 7, 48, 5 and 36 ng per cartridge, respectively, were estimated to correspond to average deliveries of < 1% of that delivered by 20 tobacco cigarettes. The four PAHs are classified by the International Agency for Research on Cancer (IARC) in Group 3, inadequate evidence for carcinogenicity in humans and inadequate or limited evidence in animals *(37)*. Benzo[*a*]pyrene was not detected.

### 3.5.2 Benzo[*a*]pyrene in ENDS aerosol

In a study of environmental deposition, ENDS aerosol was introduced into a sampling bag with a large quantity of dilution air. Most PAHs, including benzo[*a*] pyrene, were not present above the limit of detection, and benzo[*a*] pyrene was found at levels similar to those in blank samples *(38)*. Tayyarah and Long *(39)* found no quantifiable levels of PAHs in ENDS aerosols, and Romagna et al. *(40)* detected no PAHs in environmental air when comparing emissions from ENDS and conventional cigarettes. Lauterbach and Laugesen *(41)* reported that the level of benzo[*a*]pyrene in Ruyan® ENDS aerosol (more than 300 puffs of aerosol) from 16-mg nicotine cartridges was below the reporting limit. PAHs might have to be monitored if tobacco–ENDS "hybrid" products become available.

The methods used for analysing PAHs in ENDS aerosol matrix in the aforementioned studies were not explicitly stated. In most studies, the methods appear to differ minimally or not at all from those used for conventional cigarette smoke. Sample preparation for analysis of benzo[*a*]pyrene by the CDC method *(32)* appeared to be similar to that in the TobLabNet method *(42)*. Preliminary studies at CDC (unpublished data) showed minimal differences between PAH calibration curves prepared for the ENDS propylyene glycol–glycerol matrix and standard tobacco cigarettes, suggesting that the method is applicable. Most methods for preparing samples for analysis of benzo[*a*]pyrene include extraction with nonpolar solvents and clean-up by silica solid phase extraction. The applicability of sample generation and preparation methods to ENDS analysis should be tested before a method is considered appropriate.

## 3.6 Additional analytes

### 3.6.1 Carbonyls

"Carbonyls" is a collective term for aldehydes and ketones. Studies of conventional tobacco cigarettes indicate that humectants form short-chain carbonyls and other toxic chemicals when exposed to high temperatures. For ENDS, the temperature of the heating coil, which is in contact with the e-liquid, depends on the puff duration, the puff frequency and the heat transfer properties around the coil *(1)*. It is currently considered that thermal decomposition of solvents is the predominant source of carbonyls in ENDS aerosol. Glycerol dehydrates

at about 280 °C to form acrolein, which undergoes reactions to formaldehyde and acetaldehyde. Contact of liquids such as propylyene glycol and vegetable glycerol in e-liquid with heated atomizer nichrome wire has been proposed as a source of carbonyls *(43)*. Carbonyls are of public health concern, as some have been evaluated as known or probable human carcinogens, and propylyene glycol is thermally degraded to propylene oxide, which is carcinogenic in laboratory animals *(23, 25, 44, 45)*. Formaldehyde, acetaldehyde and acrolein are present at notable levels under certain conditions, especially in later puffs, in ENDS aerosol when glycerol and propylene glycol are heated and in the absence of other e-liquid constituents, such as nicotine or flavourings *(1)*. In a recent study of ENDS aerosol from flavoured and unflavoured e-liquids, however, Khlystov and Samburova showed that carbonyl formation also depends on the concentration of flavourings, independently of e-liquid solvents *(46)*.

Some carbonyls (e.g. formaldehyde) have been detected in the particulate and gas phases of ENDS aerosol (N. Kunugita, personal communication). Recently, Sleiman and colleagues *(1)* found trace levels (ng/mL) of formaldehyde, acetaldehyde and acrolein in e-liquid. When the liquid was aerosolized, there was a notable, voltage-dependent increase in the levels of formaldehyde, acetaldehyde and acrolein. Between the first five puffs ("initial") and the puffs captured between the 30th and 40th puffs ("steady state"), the level of formaldehyde increased from 2900 ng/mg of liquid consumed to 8950 ng/mg at 3.8 V and from 7250 ng/mg of liquid consumed to 48 200 ng/mg at 4.8 V. Larger increases with increased puff count were observed for acetaldehyde and acrolein (from 230 ng/mg of liquid consumed to 1820 ng/mg at 3.8 V and 740 ng/ mg of liquid consumed to 19 080 ng at 4.8 V, and from 90 ng/mg of liquid consumed to 1700 ng/mg at 3.8 V and 400 ng/ mg of liquid consumed to 10 060 ng at 4.8 V, respectively). Other carbonyls found at levels above the limits of detection were crotonaldehyde, methacrolein, butrylaldehyde, benzaldehyde, valeraldehyde, p-tolualdehyde and hexaldehyde. Formaldehyde, acetaldehyde, acrolein, propionaldehyde, benzaldehyde and glyoxal were measured in ENDS aerosol at levels of micrograms per gram of e-liquid with flavourings. In contrast, in the same ENDS devices, unflavoured e-liquid produced detectable levels of only glyoxal and benzaldehyde *(46)*.

The parameters chosen for generating aerosol from ENDS strongly determine the amounts of carbonyls found. Independent variables including the battery voltage, puff volume, puff duration, coil number, placement, resistance, wick design and length, solvent, e-liquid viscosity and air flow resistance may affect the rate at which carbonyls are formed.

An SOP for carbonyls in mainstream tobacco smoke is being validated by TobLabNet. Briefly, it is based on trapping the carbonyls in smoke with a combination of an absorbent and a filter, followed by extraction, derivatization and analysis by HPLC with photodiode array detection. Acrolein cannot be

analysed with a standard 2,4-dinitrophenylhydrazine cartridge because the derivative is unstable and decomposes in the cartridge during sample collection *(47–51)*. In the hydro-quinone–2,4-dinitrophenylhydrazine method *(52)* and the CX-572 methods (45, 52), acrolein does not appear to decompose, because carbonyls, including acrolein, are collected on the sorbent hydroquinone or the CX-572-cartridge.

When it is validated, the WHO SOP for carbonyls can be expected to be applicable to the analysis of carbonyls in ENDS aerosol. Because other ingredients such as flavourings can contribute interference *(53)*, steps should be taken to ensure analytical validity and suitability. Extreme testing conditions (e.g. very high battery voltage) might yield amounts of carbonyls that exceed the levels to which a user would usually be exposed *(54)*. Additional investigation is required to standardize the device parameters during aerosol generation.

### 3.6.2 Solvents

Although propylene glycol and vegetable glycerol are commonly termed "humectants", these compounds function in e-liquids as solvents and form droplets during aerosolization that, when existing in the e-liquid, carry nicotine and flavour compounds in the aerosol to facilitate inhalation *(55)*. The solvents may be used alone or a mixture of the two *(56)*. A few e-liquids contain low-molecular-mass polyethylene glycols, either pure or in a mixture with propylene glycol or glycerol. Polyethylene glycol-400 is used because it is liquid at room temperature and because it is readily available in high purity, as it is used as an excipient in pharmaceutical products *(57)*.

Rainey et al. *(58)* demonstrated that GC–FID and GC–MS can be used to measure these chemicals in tobacco, although GC-MS was recommended for full chromatographic resolution of glycerol and triethylene glycol. However, the suitability of GC–FID for comprehensive analysis of solvents in e-liquids is supported by a report from the RIVM, in which this method was used to quantify propylene glycol, glycerol, polyethylene glycol, diethylene glycol and nicotine *(29)*.

The method was shown to be suitable for e-liquids and offers the advantage of including nicotine. It is recommended as a starting point for a comprehensive method that includes the solvents of interest, chemically related contaminants such as ethylene glycol and diethylene glycol and, additionally, nicotine. A standard containing polyethylene glycol molecules in the molecular mass range of interest is required for quantification of polyethylene glycol. Such standards are commercially available (e.g. Sigma Aldrich 81396).

Solvents in ENDS aerosol can be collected on a standard 44-mm CFP. Staff at R.J. Reynolds observed that more than 98% of the glycerol and propylene glycol in ENDS aerosol is captured on a CFP *(22)*. Experiments with a wide range of e-liquids (RIVM, personal communication) indicate that the amount

of TPM collected on the filters corresponds closely to the amount of liquid lost. These finding have been replicated and confirmed in the CDC tobacco laboratory (unpublished data). They are important because they indicate that the glass-fibre filters used for conventional cigarette analysis efficiently retain solvents, which are the most abundant chemicals present in aerosol TPM generated from e-liquids. The solvents can be extracted from the filter with methanol and the extract directly injected onto a GC by the same method used for the analysis of e-liquids. This approach is recommended as a basis for a detailed protocol for the quantification of solvents in ENDS aerosol.

One concern is that e-liquids often contain a large number of flavour components *(53)*, which may co-elute with the solvents and interfere with their quantification. As many different flavour components could be present in e-liquids, it would be time-consuming to optimize the chromatographic method to ensure complete separation of solvents. Use of a more selective approach, GC–MS, instead of GC–FID would be advantageous in this respect. Any method to be validated should be applicable to a wide range of product types, including those that are highly flavoured.

Several authors have reported GC–FID or GC–MS methods for the quantification of humectants in tobacco *(58)*. TobLabNet SOP-06 for the determination of vegetable glycerol, propylene glycol and triethylene glycol in tobacco filler has been validated *(59)* and provides both GC–FID and GC–MS variants of the method. The SOP is expected to be applicable to the analysis of solvents in ENDS e-liquid and aerosol. It has been suggested that the method for measuring nicotine in ENDS e-liquids could be adapted for simultaneous determination of solvents (glycerol and propylene glycol) and nicotine.

Further method development is required to optimize the determination of solvents in ENDS e-liquid and aerosol, taking into account possible interferences. Adjustment of existing methods to determine glycerol, propylene glycol and e-liquid contaminants simultaneously should be considered.

### 3.6.3 Volatile organic compounds

Some VOCs are potent carcinogens and therefore potential targets of policy and regulation to mitigate the toxicity of tobacco products. For example, benzene and 1,3-butadiene in mainstream tobacco smoke are included as priorities in Articles 9 and 10 of the WHO FCTC *(24)*. A few reports have been published on the analysis of VOCs in ENDS refill e-liquids, cartridges and aerosols. Laugesen *(26)* found xylene and styrene in ENDS e-liquid cartridges, and the China National Tobacco Quality Supervision and Test Centre found several VOCs at levels of parts per million in refill e-liquids for ENDS, including benzene, styrene, ethylbenzene and toluene *(60)*, some of which are classified as carcinogenic or possible carcinogenic to humans by IARC *(23, 35, 61)*. Goniewicz et al. *(33)*

detected toluene and *m*- and *p*-xylene in ENDS aerosol, the content of toluene being 0.2–6.3 mg per ENDS (150 puffs). VOCs may originate from tobacco extracts, solvents or other sources. The differences in the levels found may be due to the different nature of the samples (aerosol or e-liquids) or differences in the sensitivity of analytical methods used.

The SOP for VOCs in mainstream tobacco smoke is being validated in TobLabNet. It is expected to be applicable to the analysis of VOCs in ENDS e-liquid and aerosol.

### 3.6.4 Phenolic compounds

Phenolic compounds are on the initial WHO list of 18 priority toxicants and also on the non-exhaustive priority list of 39 toxic contents and emissions of tobacco products *(24)*. Most analytical studies on phenolic compounds have focused on cigarette smoke, and few studies are available on their presence in e-liquids or ENDS aerosol. *p*- and *o*-dihydroxybenzene, phenol and *m*-, *p*- and *o*-cresol were detected in refill e-liquids at a total amount of 0.5–5 μg/g. No relation was found between the amount of nicotine and the amount of phenols, implying that phenolic compounds originate from ingredients other than the nicotine source *(56)*.

HPLC with fluorescence detection is the most commonly used method for determining phenolic compounds in cigarette smoke. Both Health Canada *(62)* and CORESTA *(63)* have recommended methods for analysing selected phenolic compounds in mainstream cigarette smoke with this method. Generally, it is expected that the methods commonly used to determine phenolic compounds in tobacco emissions could be extended to the less chemically complex e-liquids and ENDS aerosols. The corresponding SOPs should be established.

### 3.6.5 Metals

Metals were not included in the original priorities for tobacco and mainstream tobacco smoke in Articles 9 and 10 of the WHO FCTC. ENDS devices, however, contain several metallic parts, including wiring, a heating element, solder connections and structural components. The metallic elements commonly found in the alloys used in ENDS devices include chromium, nickel, aluminum, iron, lead, tin and gold. Metals could also be introduced into e-liquids during manufacture, as contaminants during extraction of nicotine from tobacco plants. Metals in e-liquid and aerosol have been identifed by several independent laboratories using inductively coupled plasma-MS and scanning electron microscopy *(29, 33, 64)*. Williams et al. *(64)* used this method to identify amorphous and fibrous particles in e-liquid.

Inductively coupled plasma-MS is a highly sensitive, versatile technique for the analysis of metals in a variety of matrices, and its suitability for the analysis of e-liquids has been demonstrated *(29, 33, 64)*. Metals may occur in e-liquids in

the form of small metallic particles or dissolved as ions *(64)*. As different forms vary widely in their bioavailability and toxicity, any method should distinguish between the forms. The different species can be separated by HPLC, and it is recommended that an HPLC-inductively coupled plasma-MS method be developed for analysis of metals in e-liquids. An additional sample preparation step may be necessary to dissolve metallic particles. For safety reasons, it should be noted that the reaction between nitric acid (often used for dissolving metals) and glycerol (a common component of e-liquids) may yield nitroglycerine, which is an impact- and friction-sensitive explosive. A safe, efficient sample preparation procedure is therefore imperative.

WHO has not prepared a SOP for metals in tobacco or mainstream tobacco smoke. Several researchers reported using quartz CRFs to collect metals in e-liquids *(29, 64)*; however, others have noted that CFPs already contain significant amounts of metals, which could contribute to a high baseline level *(65)*. Quartz CFPs can be leached with dilute hydrochloric acid and nitric acid before use to reduce background levels of metals *(65)*. A possible alternative to CFPs for collecting aerosol for the analysis of metals is Whatman 47 mm QMA grade filters (catalogue No. 1851-047), which have been found to contain low background levels of metals *(22)*. Their slightly greater diameter will require manufacture of appropriately sized filter holders. It is recommended that precautions be taken to ensure accurate measurements of metals in e-liquid and in the collection and analysis of ENDS aerosol.

### 3.6.6 Flavours

Flavourings in tobacco or tobacco smoke were not included in the original priorities for tobacco and mainstream tobacco smoke in Articles 9 and 10 of the WHO FCTC. E-liquids are available in over 7500 unique flavours, and new flavours are being introduced daily *(66)*. Most e-liquid flavourings have been found to be "generally regarded as safe" (GRAS) when ingested, but GRAS certification does not apply to chemicals that are heated at high temperatures and inhaled; therefore, certification has not been issued for flavourings inhaled with ENDS aerosol *(67)*. Although evidence is emerging of health effects resulting from inhaling ENDS first-hand aerosol *(68, 69)*, the role of flavourings is largely unknown. Nevertheless, some classes of flavour compounds reported in e-liquids pose potential health risks *(70)*.

Farsalinos et al. *(71)* found diacetyl and acetyl propionyl, chemicals which impart a characteristic buttery flavour, in 69% of the refill e-liquids and aerosols of sweet-flavoured varieties. Voltage had no apparent effect on the levels of diacetyl in ENDS aerosol, with 438 ng/mg of ENDS liquid consumed at 3.8 V versus 433 ng/ mg of e-liquid consumed at 4.8 V) *(1)*. While the measured concentrations of diketones were significantly lower than in conventional cigarettes, a number of

products tested contained acetyl propionyl and diacetyl at concentrations greater than occupational exposure limits. Exposure to diacetyl is associated with severe respiratory illness, including bronchiolitis obliterans, or "popcorn lung". The first documented case of "popcorn lung" due to use of flavoured e-liquid was reported recently *(72)*.

Other common flavour additives are also of concern. For example, cinnamon-flavoured e-liquids contain cinnamaldehyde and 2-methoxycinnamaldehyde at concentrations that are toxic to cultured cells *(73)*, and a direct correlation was found between the number and concentration of cinnamon flavour chemicals in the e-liquids and toxicity *(74)*. A number of e-liquids list pyrazines as additives. These compounds have been used to make inhalation easier and to reduce the harshness associated with nicotine in conventional cigarettes (*75, 76)*. It is possible that they also ease the use of ENDS by novice smokers *(77)*. Sweet- or "candy"-like flavours may make ENDS products attractive to children or novice users *(78)*.

The literature on measurement of flavour additives in e-liquids and aerosol is limited. A recent survey of 18 flavourings in three commercial e-liquids found that one marketed as "classic tobacco" contained detectable levels of vanillin, while two others ("bubblicious" and "mojito mix") had detectable levels of seven flavour compounds *(1)*. The most commonly used techniques for analysing products are HPLC, GC–MS and GC–MS/MS. Diketone compounds like diacetyl and acetyl propionyl were determined on an HPLC–MS platform *(71)*. Other flavours in e-liquids, including menthol, vanillin, methyl anthranilate, benzaldehyde and piperonal, were quantified by GC–MS and GC–MS/MS *(3, 79)*. Most of the methods used to analyse ingredients and toxicants in tobacco can be extended to e-liquids and aerosols. For the analysis of e-liquids with large numbers of different flavourings, chromatographic separation must be assured for methods with non-specific detectors.

## 3.7 Recommendations for extension of methods

A matrix for considering extension of the WHO SOPs based on the reported and observed presence of toxicants in ENDS e-liquid or aerosol was presented at a meeting of the WHO collaborating centres for tobacco product testing and research in Manila, Philippines, in September 2015. An updated version of the matrix is presented in Table 3.1.

Table 3.1. Proposed decision matrix for extension of current and pending WHO standard operating procedures

| | Applicability of current TobLabNet SOP to proposed matrices | |
|---|---|---|
| **Current method** | **E-liquid** | **Aerosol** |
| Nicotine in tobacco (filler) | ? | No |
| Tobacco-specific nitrosamines in mainstream smoke | ? | ? |
| Benzo[*a*]pyrene in mainstream smoke | No | No |
| Nicotine in mainstream smoke | ? | Yes |
| Humectants in tobacco (filler) | Probably | Probably |
| Volatile organic compounds in mainstream smoke[a] | No | Probably |
| Carbonyls in mainstream smoke[a] | No | Probably |

[a] Method under development

Many factors, notably voltage and e-liquid composition, can influence the chemical composition of first-hand aerosol. For example, the coil temperature, which is a major factor in formation of emissions, can reportedly vary widely between devices for a given battery and vaping behaviour *(1)*. CORESTA Recommended Method No. 81 *(8)* and WHO SOP-01 with a fixed number of puffs (≥ 50 to ensure adequate TPM on the CFP) are adequate as standardized, publicly available regimes for machine generation of ENDS aerosol for the limited purpose of evaluating application of the WHO SOPs to analysis of nicotine, TSNAs, and benzo[*a*]pyrene in samples of disposable and refillable ENDS. Reports generated during method extension should contain a statement that "The [CORESTA/WHO] method was used for convenience; the method is not based on how ENDS are used by consumers and its use does not constitute an endorsement of the method as appropriate for all current or future ENDS product configurations."

The puffing regime of CORESTA method No. 81 includes a puff volume of 55 ± 0.3 mL, a puff frequency of one every 30 ± 0.5 s, a flow rate of 18.5 mL/s, a puff profile of rectangular (or "square") shape, a puff duration of 3 ± 0.1 s and a counted, recorded puff count. The CORESTA-recommended method states that it is applicable to a variety of single-use and refillable ENDS (e-cigarettes, e-cigars, e-shisha, e-pipes); therefore, it could be used for the "cigalike" products that are used in method extension studies. The WHO SOP for intense smoking of cigarettes specifies a puff volume of 55 ± 0.1 mL, a puff frequency of 30 s and a puff duration of 2 s. As the WHO SOP was developed for tobacco cigarettes, it does not specify a flow rate.

The analytical smoking machine used in method verification should be capable of drawing a fixed volume of air, contain devices to control the puff volume, puff duration and puff frequency, be mechanically and electrically reliable, be capable of sufficient compensation, be able to produce a rectangular puff profile and be capable of taking clearing puffs after termination of smoking.

The machine should count puffs at each port. Depending on the product, actuation should start no later than 0.1 s or when the flow rate rises to > 50% of peak flow 0.1 s after starting the puff and shall not be stopped later than 0.1 s after the puff is finished and terminated by the operator or a sensor. The ENDS device holder should be leak-free and impermeable to air and aerosol. The pressure drop in the instrument should not exceed 300 Pa, and the temperature and the relative humidity in the room should be maintained constant ± 2 °C and ± 5%, respectively, throughout the session, as for tobacco cigarette machine smoking. The aerosol trap holders should be airtight, with non-hygroscopic, chemically inert end caps; the retaining efficiency of the filter should be 99.9% of all particles with a diameter ≥ 0.3 μm of a dioctyl phthalate aerosol at 140 mm/s velocity; the content of the binder should not exceed 5% as mass fraction; and the pressure drop should not exceed 250 Pa after completion of aerosol collection.

For extension of any new method or cross-matrix method, recovery of the targeted analyte should be measured at low (e.g. 25%), medium (e.g. 50%) and high (e.g. 75%) spike levels, corresponding to the reportable analytical range (e.g. acceptable at 100 ± 10% recovery), to determine whether flavourings or liquid formulations are biasing the results for the target analyte. Only new products should be used for testing purposes.

### 3.7.1 Nicotine

The ENDS conditioning method in ISO 3402 and a modification of ISO standard procedures specified in SOP-04 should be modified, as the nicotine in ENDS is in liquid form in a closed container.

In cigarettes, tobacco leaves are wrapped in paper and are easily affected by the medium and the conditions in which they are stored. It should be determined whether conditioning of ENDS or e-liquid cartridges is required. The content of nicotine in a sample will depend on the ENDS brand and model; therefore, extraction and the range of the calibration curve will have to be optimized in terms of the liquid volume and nicotine concentration to be analysed. The concentration of nicotine in e-liquid usually ranges from 0 to about 36 mg/mL, the upper range being much higher than in cigarette smoke extracts generated under intense smoking machine conditions (0.3 mg/mL) (CDC, unpublished data). The analyte volume spiked into the extraction solution should be adjusted (e.g. approximately 0.25–0.5 mL) to be within the existing calibration range. As propylene glycol and glycerine are present as nicotine solvents, the recovery of nicotine after extraction should be assessed, as these compounds are not soluble in some solvents (e.g. hexane). Nicotine can be analysed in e-liquid after extraction with isopropanol, as in the standard ISO method for tar, nicotine and CO in smoke or with an appropriate modification of WHO SOP-04. The analytical specifications should be recalculated for the new matrix containing propylene glycol and/or glycerol.

The range of nicotine levels observed in ENDS aerosol are comparable to those reported in tobacco smoke. Adapters or special holders may be required to accommodate diverse ENDS designs and configurations. A study of a variety of ENDS products (tank, refillable, disposable) in the United Kingdom *(71)* found no statistical relation between the concentration of nicotine in the liquid and that in the aerosol; however, voltage was not measured, although it has been shown to affect nicotine levels in machine-generated aerosol *(80, 81)*. Voltage settings are under discussion; they should account for the maximum delivery to consumers (analogous to the Canadian "intense" cigarette smoking machine regimes for conventional tobacco cigarettes) and use of "pre-heating" options recommended by the manufacturer.

### 3.7.2 Tobacco-specific nitrosamines

Very low levels of TSNAs have been found in e-liquid and aerosol, and they varied widely by brand *(33, 39, 82)*. For example, Goniewicz and colleagues *(33)* found NNN at levels of 0.8–4.3 ng and NNK at 1.1–28.3 ng in the total aerosol of 10 of 12 ENDS purchased in Poland. As there was no tobacco in the ENDS tested in the Goniewicz study, the appreciable levels of TSNAs in aerosol may be due to direct transfer from the e-liquid. It has been hypothesized that TSNAs in e-liquid are contaminants of nicotine extraction. A review of WHO SOP-03 for TSNAs in mainstream tobacco smoke suggests that the chemistry of ENDS aerosol is compatible with the WHO SOP and that either aerosol TPM collected on CFPs or e-liquid could be analysed.

As noted above, however, the reported levels of TSNAs in ENDS aerosol are below the reporting limit of the WHO SOP for TSNAs in tobacco smoke. If "hybrid" ENDS product designs that incorporate tobacco are introduced, TSNA levels in aerosol may be higher.

### 3.7.3 Benzo[*a*]pyrene

WHO TobLabNet SOP-05 for the analysis of benzo[*a*]pyrene in tobacco smoke could be adapted for ENDS aerosols, although the benzo[*a*]pyrene concentration in aerosol is expected to be much lower than that in cigarette smoke. Consequently, the number of CFPs to be analysed in one flask, the volume of the extraction solvent as well as the range of the calibration curve would have to be adjusted accordingly. As there are high concentrations of propylene glycol and glycerol in ENDS aerosols, the recovery of benzo[*a*]pyrene from the propylene glycol–glycerol matrix should be assessed when cyclohexane is used as the extraction solvent. If recovery in cyclohexane is low, other extraction solvents should be tested to determine the solubility of propylene glycol and glycerol. The analytical calibrations should be recalculated for the new matrix.

As there are few or no PAHs in e-liquid or ENDS aerosol *(26, 36–39)*, it is recommended that liquid or aerosol from ENDS not be analysed for benzo[*a*] pyrene, as the results will not significantly inform public health or regulatory decision-making.

### 3.7.4 Volatile organic chemicals

The SOP for VOCs in mainstream tobacco smoke is being validated in TobLabNet and could be adapted to the analysis of ENDS aerosols. Besides 1,3-butadiene and benzene, other harmful VOCs may be present in e-liquids and aerosols, such as toluene, styrene and ethylbenzene. The concentrations of VOCs in aerosols may, however, be much lower than in tobacco mainstream smoke *(33)*. Therefore, the number of puffs, the type of carbon molecular sieve, the volume of the extraction solvent and the range of the calibration curve should be adjusted accordingly.

### 3.7.5 Carbonyls

Carbonyls are generated during vaporization of e-liquids and have been widely reported in ENDS aerosol. In most studies, carbonyls were found in trace amounts or much lower levels than in tobacco cigarette smoke *(43)*. The choice of solvent, device design (e.g. refillable, single-use) and voltage should be considered.

"Dry puffing", when the wick is not in contact with sufficient liquid because the cartridge is empty or the coil is overheating, can lead to the formation of toxic chemicals *(83)*; however, this phenomenon is not thought to represent common ENDS consumer use patterns *(84)*. The analytical specifications in the pending WHO SOP for carbonyls in mainstream tobacco smoke should be evaluated and modified as necessary to account for potential ENDS-specific emissions and their concentrations in ENDS aerosol. These include glyoxal and methyl glyoxal, which have been reported in ENDS aerosols but not in tobacco cigarette smoke *(85)*.

Toxic and carcinogenic carbonyls have been detected in aerosols *(86)* and are thus potential targets of policy and regulation to mitigate the toxicity of tobacco products. Consequently, extension of the pending SOP for analysis of carbonyls in tobacco smoke to analysis of carbonyls in ENDS aerosol is recommended.

## 3.8 Research that will inform future regulatory use of data on ENDS

- Identify or develop standard ENDS research products.
- Identify or develop standardized research materials for testing ENDS batteries.

- Review and refine the specifications of commercial ENDS aerosol generating machines.
- Develop ENDS device holders and trapping system(s) for a variety of ENDS.
- Determine whether current analytical methods are applicable for a variety of ENDS and how they should be modified to provide accurate, reproducible, robust measurements.
- Define the critical aspects of ENDS use topography, including puff duration, frequency, volume and count.
- Determine which product design variables (e.g. variable voltage, battery power, heating coil temperature settings) should be specified in an aerosol generating regime.
- Determine "standard" and "intense" aerosol generation methods that reflect ENDS use behaviour and, for the "intense" method, adjustments of products design variables, which will inform regulatory decision-making.
- Assess whether separate regulatory limits are appropriate for early- versus later-generation products or for different kinds of ENDS (e.g. e-cigars, e-waterpipes).
- Survey the extent of impurities in solvents and nicotine extracts to determine whether routine testing of impurities is warranted.
- Determine whether the pH of ENDS aerosol can be derived or inferred from that of e-liquid with procedures similar to those developed for smokeless tobacco.
- Assess interference, recovery, matrix comparisons and the appropriate range of the calibration curve for all analytical methods.

## 3.9 Conclusions

A series of chemicals have been detected in ENDS e-liquids and aerosols. Considering the prevalence of ENDS use and the evolving nature of these products the application of existing and pending WHO TobLabNet SOPs to the analysis of ENDS e-liquid and aerosol is justified.

Whereas carbonyls are generated when e-liquid solvents and flavourings are exposed to elevated temperatures, benzo[*a*]pyrene and TSNAs in current products are attributed to impurities in nicotine extracts; they are therefore not routinely detected and, when present, are found at very low levels. TSNA levels increased with increasing nicotine level in a study of cartridges sold for an ENDS brand by one manufacturer, and there are several independent reports

that the TSNA levels in machine-generated aerosol are much lower than those in the smoke of tobacco cigarettes and below the reporting limit of the WHO SOP for TSNAs in mainstream cigarette smoke. Requiring manufacturers to use nicotine that is certified free of contaminants should eliminate TSNAs in e-liquid and aerosol.

Routine testing for TSNAs and benzo[*a*]pyrene in ENDS is not warranted because they do not contain tobacco. However, validated methods will allow regulators and researchers to screen e-liquid at their discretion and new "hybrid" products as they emerge. Extension of the WHO SOPs for the analysis of TSNAs and benzo[*a*]pyrene in mainstream tobacco smoke to ENDS will provide researchers and regulators with analytical methods suitable for future configurations and design variations in which tobacco is included, which could result in higher levels of these toxicants. Major transnational tobacco companies have launched such "hybrid" products called heat-not-burn products. Examples are IQOS which releases a nicotine-containing vapor [2], Vype which passes a nicotine-containing vapor through tobacco [3], and Ploom which delivers a vapor that passes through a capsule of granulated tobacco [4]. Examples are iQOS which releases a nicotine-containing vapor *(91)*, Vype which passes a nicotine-containing vapor through tobacco *(92)*, and Ploom which delivers a vapor that passes through a capsule of granulated tobacco *(93)*.

It would be advisable to measure nicotine and toxicants (e.g. metals) of public health or regulatory significance that are either frequently detected or present at more than trace levels in e-liquid and aerosol to better characterize potential exposure. Many aspects of the design of ENDS devices affect the composition of the aerosol, including the heating coil resistance, wick design and material, reservoir design and airflow openings.

For example, the level of nicotine in ENDS aerosol under initial (during the first five puffs) and steady-state conditions (30th to 40th puffs) and at two voltage settings ranged from 13.1 µg/mg to 23.9 µg/mg of e-liquid consumed with a battery setting of 3.8 V, and 7.6 µg/mg to 22.7 µg/mg of e-liquid consumed with a battery setting of 4.8 V *(1)*. These features should be fully specified in any standardized ENDS device used to validate analytical methods. In addition, the pH of e-liquid, which can be expected to influence the amount of nicotine present as rapidly absorbed unionized (free) nicotine, has not been fully characterized.

In developing an "intense" aerosol generation method that approximates an upper limit of the device, research should be conducted on product design variables. The availability of standardized ENDS devices with well-documented critical design parameters would facilitate the development of additional analytical methods for ENDS. Research should address which aspects of device design are the most important. "Hybrids" products such as Heat-Not-Burn products may be associated with substantially different use behaviour and reach

higher heating temperatures, which can qualitatively and quantitatively influence smoke emissions, including possible generation of CO.

Extension of the SOP for humectants in tobacco filler to detect and quantify impurities in propylyene glycol, glycerol and polyethylenes (e.g. ethylene glycol and diethylene glycol) will assist investigations into the prevalence of such impurities, which raise concern about toxicity that is not present with propylyene glycol or glycerol alone. Analysis of nicotine could be combined with analysis of solvents, so that both can be determined in a single GC–FID run. Sample preparation in this case would consist of dilution of the liquid with a suitable solvent such as methanol *(29)*.

The Flavor and Extract Manufacturers Association (FEMA) in the USA issued the statements *(67)* that: "FEMA GRASTM status for the use of a flavor ingredient in food does not provide regulatory authority to use the flavor ingredient in e-cigarettes in the US" and "E-cigarette and flavor manufacturers and marketers should not represent or suggest that the flavor ingredients used in e-cigarettes are safe because they have FEMA GRASTM status for use in food because such statements are false and misleading." New and existing methods for the analysis of flavourings in e-liquids and aerosols should be evaluated individually to ensure data quality. As many flavourings contain a ketone or aldehyde moiety, the large amounts of flavourings in e-liquids could interfere with the analysis of carbonyl compounds when a non-specific detector is used. This could be an advantage if certain flavourings and short-chain carbonyls are quantified in a single run. If interference proves problematic for routine analysis, a compound-specific detector (MS) could be used.

In view of the highly variable nicotine yield of different devices and because users adjust their behaviour to modify the nicotine yield, a different machine method for generating aerosol should be developed that reflects changing ENDS use behaviour. ENDS use behaviour varies among users. It has been reported as two to four puffs per minute, a puff volume of about 50 mL, puff durations of 2–8 s, inter-puff intervals of 18–30 s and a puff flow rate of about 20 mL *(1)*. Reports of variations in puffing regimes *(88, 89)* indicate that several parameters should be assessed, including puff duration, frequency, volume and count as well as battery power, to better approximate use behaviour. The procedure or equipment for assessing different product designs might have to be modified in accordance with the results of studies on ENDS use and of discussions on possible standard and "intense" aerosol generation regimes. Further, the requirements of the ENDS power source should perhaps be specified to set voltage, power or temperature settings for the heating coil. Various batteries are available that can be combined with different devices. They may be unregulated (DC, with lower voltage as the battery runs down) or regulated. Regulated batteries can be designed to provide a fixed voltage, fixed power or even a fixed temperature of the heating element;

recent high-end models of ENDS batteries measure and regulate the temperature of heating coils made of certain metals (e.g. titanium). A laboratory power supply that imitates the battery could be used. Research should be conducted to define the electrical specifications of the power source, including regulation of the voltage, power and temperature, the voltage, power and temperature for maximum output, the allowable ripple current and voltage and ripple frequency. Additional research and consensus are therefore needed on aerosol generation regimens and instrumentation for future testing of aerosols.

Several scientists *(78, 90)* have found that the patterns of ENDS use differ widely from those for conventional tobacco cigarettes. Users are thought to adjust their behaviour to maximize nicotine yield, achieving plasma levels of nicotine and cotinine similar to those of tobacco cigarette smokers *(78)*. It has been observed that the puff duration from ENDS is significantly longer than that from ordinary tobacco cigarettes. It is not clear to what extent use behaviour affects the chemical composition of the vapour; however, puff duration and puff frequency are reported to influence the temperature of the coil *(1)*. The operating temperature of ENDS cannot be predicted from battery and coil characteristics alone *(1)*, and more research is needed to inform this area as ENDS devices evolve.

In summary, the marketing and promotion of ENDS and their subsequent popularity and availability to consumers through retail sales in most countries and over the Internet warrant monitoring of the chemical composition of e-liquids and aerosols, including measurements of nicotine, solvents and carbonyls, with current methods. Metals should be measured to determine whether they represent a health risk, and validated methods should be developed for routine analysis if a risk is identified. Routine measurement of TSNAs is not warranted for current products if policy-makers and regulators require certification of the quality of the nicotine extract. Measurement of benzo[*a*]pyrene is also not warranted at this time. Introduction of "hybrid" products such as heat-not-burn products, however, might warrant additional testing of tobacco-derived toxicants like TSNAs and PAHs. Flavour compounds, phenolics and VOCs should be considered in future discussions, as they are present in e-liquid and aerosol and may influence their potential toxicity.

## 3.10 Recommendations

- Sufficient data are available from independent laboratories to support extension of existing and pending WHO SOPs for nicotine, humectants (solvents), carbonyls, benzo[*a*]pyrene and TSNAs in ENDS e-liquid and aerosol.
- Routine measurement of TSNAs is not warranted for current products if policy-makers and regulators require certification of the qual-

ity of the nicotine extract. Measurement of benzo[*a*]pyrene is also not warranted at this time.

- Emergence of "hybrid" products such as heat-not-burn products may require additional testing of tobacco-derived toxicants like TSNAs and PAHs.
- It is recommended that the pH of the e-liquid be measured to establish the range of pH in e-liquids, as this information may contribute to investigations of the addictive potential of the nicotine delivered to ENDS users *(21)*.
- Metals should be measured to determine whether they represent a potential health risk; if so, validated methods should be developed for their routine analysis.
- Flavour compounds, phenolics and VOCs should be considered in future discussions, as they are present in e-liquid and aerosol and may influence the toxicity of the products.
- Development of an "intense" aerosol generation method to approximate an upper limit of the device should systematically include the relative importance of product variables and should establish a standardized ENDS device that can be used to compare aerosols.
- The applicability of the CORESTA method or a SOP (e.g. SOP 01) for an intense smoking machine method for tobacco cigarettes to all current and future ENDS product designs remains to be determined. As use data are established and as products evolve, the choice of regime for generating aerosol must be reevaluated.
- For cross-matrix verification of a method, the slopes of the calibration curves for each analyte in each matrix should be compared to evaluate the equivalence of the method for each applicable matrix.
- As part of method development or extension to new sample matrices, a recovery study with low medium and high spike levels is recommended to ensure applicability.
- Sample preparation techniques should be investigated to ensure their compatibility with e-liquid solvent matrices (propylene glycol and glycerol). In some instances, the miscibility of the extraction solvent and the matrix solvent may cause insufficient extraction.
- Testing procedures should require the use of new, unused products and follow any actuation or pre-heating recommendations provided by the manufacturer.

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# 4. Waterpipe toxicant content and emissions

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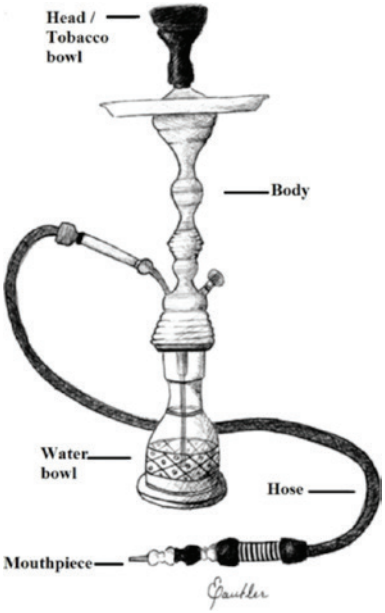
**Contents**



## 4.1 Introduction

Broadly defined, a "waterpipe" is an instrument commonly used to smoke tobacco, characterized by a container in which smoke bubbles through a column of water. Use of variants of the waterpipe has been reported in indigenous cultures in the Americas, Africa and Asia, even before the introduction of tobacco *(1)*. In recent years, a variant of the waterpipe used in south-west Asia and North Africa – often referred to as "narghile", "shisha" or "hookah" – has become widely popular, attracting young and new tobacco users around the globe. Fig. 4.1 illustrates the main features of this type of waterpipe. The head (fired clay), body (metal), water bowl (glass) and corrugated hose (leather or nylon stretched over a wound flexible wire coil, or more recently, plastic tubing) are the primary elements from which it is typically assembled, and each is manufactured in a variety of sizes. The overall height of a common waterpipe can vary from approximately 40 cm to more than 1 m and the length of the hose from 75 to 150 cm.

Fig. 4.1. Narghile waterpipe



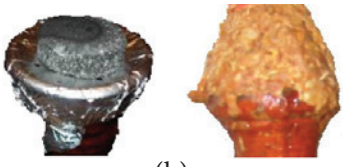

Source: reference 2

Because the tobacco preparation has high moisture and humectant contents, it does not burn in a self-sustaining manner, and lumps of burning charcoal are placed on top of the tobacco to keep it alight. The charcoal is periodically replenished or adjusted to maintain the smoke strength desired by the smoker. Usually, a pile of burning charcoal is kept in a nearby firebox for this purpose, particularly in restaurants and cafés where waterpipes are provided. Waterpipe users may also use quick-lighting charcoal briquettes to avoid preparing and maintaining a firebox every time they smoke. Interestingly, the weights of charcoal and *maassel,* a heavily flavoured tobacco mixture, consumed during a session are comparable *(3)*.

When a smoker sucks from the hose, air is drawn over and heated by the charcoal in the head. The hot air and charcoal combustion products then pass through the tobacco, from which smoke is produced. The smoke thus contains wood charcoal fumes in addition to the fumes emanating from the tobacco preparation. The smoke continues from the head through the central conduit in the body and then bubbles through the water before entering the hose. Thus, by the time the smoke reaches the mouthpiece, it has been humidified and cooled to room temperature. Adding to the sensory experience of inhaling a cool, humid, sweet aerosol, users feel and hear the action of the bubbler as they smoke the waterpipe.

The two most common waterpipe configurations are referred to as *maassel* and *ajami*. In the *maassel* configuration, a relatively deep (approximately 3 cm) head is filled with 10–20 g of *maassel* ("honeyed" in Arabic), which consists by weight of up to 65% humectant *(4)* (mainly glycerol), the balance consisting of tobacco, water, flavourings and other additives. Hundreds of flavours are available on the market, mimicking an array of fruits, sweets, beverages, spices, flowers and herbs. The *maassel* is covered with an aluminium foil sheet perforated for the passage of air (Fig. 4.2a), and burning coals are placed on top of the aluminium foil. In the second configuration, that of the more traditional "unflavoured" *ajami* tobacco (commonly referred to as *tombac*, or "tobacco" in Arabic), smokers mix a small amount of water with dry, shredded tobacco to make a mouldable matrix, which they shape into a mound on top of a shallow clay head (Fig. 4.2b); the coal is placed directly on the moistened tobacco. The *maassel* configuration is the most prevalent worldwide.

Fig. 4.2. Waterpipe heads: (a) maassel configuration with tobacco underneath foil; (b) ajami configuration with tobacco on top of the head and no foil to separate charcoal from tobacco



(a) (b)

Tobacco-free versions of *maassel* have appeared in shops and on-line recently, which are commonly marketed as a "healthy" option. The toxicant delivery profile and biological activity of the smoke produced with these products was, however, found to be essentially identical to the tobacco-containing versions, apart from the absence of nicotine (see section 4.3).

## 4.2 Puff topography and emissions testing regimens

Unlike cigarettes, narghiles allow relatively high puff volumes, largely because of their low resistance to draw, which is very similar to free inhalation. Puff volumes of the order of 1000 mL are common, in contrast to the volumes of 30–50 mL for cigarettes. Thus, a single narghile puff may displace as much smoke as is drawn during the consumption of an entire cigarette. A typical smoking session consists of hundreds of puffs over about 1 h, for a cumulative inhaled volume of about 100 L *(5)*. In addition, unlike cigarettes, waterpipes are smoked to no well-defined end point until they are considered to have been "consumed"; in general, a smoker simply stops when smoking is no longer appealing, whether because of a change in flavour, a sense of satiation or a change in social circumstances (e.g. the end of a dinner during which a narghile was used).

Laboratory characterization of toxicant emissions produced in a smoking machine requires specification of puff topography parameters, such as puff number, volume and duration and interpuff interval, because toxicant emissions are strongly influenced by the puffing parameters used to smoke a given product *(3, 6, 7)*. Several studies of waterpipe puff topography have been reported, in various populations and in both clinical laboratory and natural environments. These studies are summarized in Table 4.1, which shows a mean puff volume of 500–1000 mL, a puff duration of 2–3 s and an interpuff interval of 10–35 s. The variations among studies shown in Table 4.1 probably reflect the influence on puff topography of factors such as years of experience, smoking frequency and setting. Some experimental data suggest that waterpipe puff topography is influenced by the nicotine content of the product smoked; in a blinded experiment, experienced waterpipe users were found to puff more intensively when they were given a nicotine-free waterpipe product *(14)*. Experimental data also show that puff topography is affected by the degree of nicotine dependence *(11)*. Such variations notwithstanding, it is noteworthy that the puff volumes taken during waterpipe smoking are more than 10 times greater than those taken during cigarette smoking. It is clear, therefore, that cigarette puff topography parameters cannot be used in waterpipe machine smoking tests.

Table 4.1. Reported measurements of waterpipe puff topography

| | Waterpipes | | | | | | | Cigarettes |
|---|---|---|---|---|---|---|---|---|
| Study | Shihadeh et al. *(5)* | Maziak et al. *(8)* | Katurji et al. *(9)* | Cobb et al. *(10)* | Alzoubi et al. *(11)* | Pulcu & McNeil *(12)* | Brinkman et al. *(13)* | Djordjevic et al. *(6)* |
| Location | Beirut, Lebanon | Aleppo, Syrian Arab Republic | Beirut, Lebanon | Richmond, VA, USA | Irbid, Jordan | Istanbul, Turkey | Columbus, OH, USA | Westchester, NY, USA |
| Setting | Café | Laboratory (30 min) | Café | Laboratory (45 min) | Laboratory | Laboratory (30 min) | Laboratory | Laboratory |
| No. of participants | 52 | 61 | 61 | 54 | 59 ´ 2 | 20 | 35 | 77 |
| Interpuff interval (s) | 17.0 | 12.6 | 15.2 | 35.4 | 12.4/8.0 | 11.7 | 26.2 | 18.5 |
| Puff volume (mL) | 530 | 511 | 590 | 834 | 520/480 | 1040 | 640 | 44.1 |
| Puff duration (s) | 2.6 | 3.2 | 2.8 | Not reported | 2.3/2.7 | 3.5 | 4.5 | 1.5 |
| Total no. of puffs | 171 | 169 | 169 | 75 | 157/199 | 120 | 71 | 12.1 |
| Total volume (L) | 90.6 | 79.1 | 130 | 61.6 | 82.6/91.8 | 114 | 45.4 | 0.523 |

Cigarette topography from Djordjevic et al. (*6*) shown for comparison

To date, the most commonly used puff topography regimen for analytical studies of waterpipe tobacco smoke is that of the Beirut method *(9)*, which specifies 171 puffs of 2.6-s duration, 530-mL volume and 17-s inter-puff interval. This method is based on two field campaigns in cafés in the Beirut area where waterpipes were provided and was validated by measuring "tar", nicotine and CO in smoke sampled in real time from waterpipes as they were smoked by café patrons *(5, 9)*.

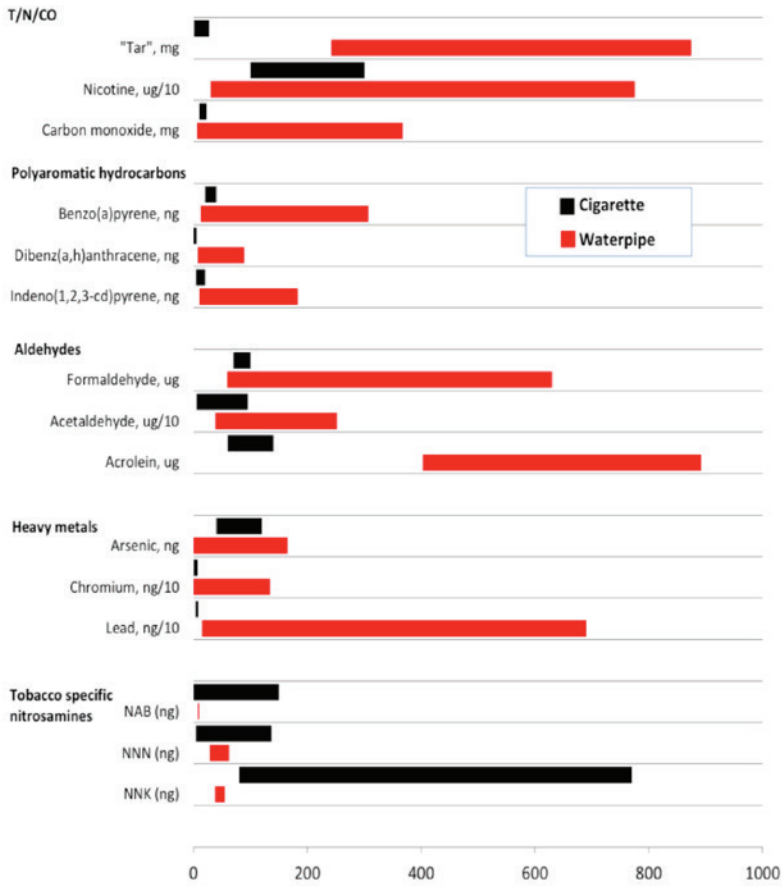
## 4.3 Toxicant content and emissions

Laboratory studies during the past decade have begun to elucidate the chemistry of waterpipe smoke with modern analytical methods, reliable machine smoke generation and sampling protocols. A recent review of the scientific literature showed that approximately 300 chemical species have been identified and 82 quantified in waterpipe smoke *(15)*. In addition to the addictive drug nicotine, the quantified species include carcinogens such as TSNAs, PAHs, benzene, furans and heavy metals, as well as other important toxicants such as volatile aldehydes, nitric oxide and CO.

Like cigarette smoke, waterpipe smoke includes constituents that are simply transferred from the raw material (e.g. heavy metals, nicotine, TSNAs), constituents that are chemically synthesized during smoking (e.g. CO, nitric oxide) and constituents that are both transferred and synthesized *in situ* (e.g. PAHs) *(16)*. Furthermore, because burning charcoal is usually used as the heat source during waterpipe smoking, the smoke contains toxicants emitted from the charcoal in addition to those from the tobacco product itself. Thus, the composition of both the charcoal and the tobacco preparation can influence smoke constituents. A large fraction of the PAHs and heavy metal content of waterpipe smoke may be accounted for by the PAH content of raw charcoal *(16)* and the metal content of the *maassel* products *(17, 18)*, respectively. These constituents were found to vary by product, suggesting that regulation to limit the toxicant content might be feasible.

Because published reports on waterpipe toxicant yields are specific to particular combinations of charcoal and tobacco product, puffing protocol and waterpipe design, the reported toxicant contents vary widely. Nonetheless, as noted in an extensive review of the toxicants and biological activity of waterpipe tobacco smoke *(15)*, all studies to date point to the same conclusion, that, during a typical waterpipe use session, the user will draw large doses of toxicants, ranging from less than one to tens of cigarette equivalents, depending on the toxicant (see Fig. 4.3). These toxicants are linked to addiction, heart and lung diseases and cancer in cigarette smokers and can result in similar outcomes in waterpipe users.

Fig. 4.3. Reported levels of mainstream smoke toxicants produced during a single 1-h waterpipe use session and during smoking of a single cigarette




Source: reference *2*; data on cigarettes from references *18* and *19* and on waterpipes from references *3*, *20* and *21*

Reports of toxicant emissions in waterpipe smoke have been corroborated by biomarker assays in smokers, which show that users are systemically exposed to CO, nicotine, PAHs and TSNAs *(22–27)*. In addition, differences in systemic exposure patterns to toxicants between cigarette and waterpipe smokers mimic the differences found in measured toxicant emissions with these smoking methods; e.g., on a nicotine-normalized basis, waterpipe smokers have greater exposure to CO and PAHs and lower exposure to TSNAs than cigarette smokers. Such agreement between markers of exposure and measured toxicant yields gives confidence in the findings to date that waterpipe smoke contains and delivers large doses of toxicants.

Increasing awareness of such findings may be a factor in the appearance of tobacco-free *maassel* preparations that are marketed as products "for the health-conscious user". Except for nicotine, the smoke produced by use of tobacco-free *maassel* products has essentially the same toxicant profile and biological activity as that of conventional tobacco-based products (Table 4.2 and *17*, *28*, *29*).

Table 4.2. Direct comparisons of mainstream smoke toxicant yields from tobacco-based and tobacco-free waterpipe products

| | **Waterpipe preparation (mean ± 95% confidence interval)** | | |
|---|---|---|---|
| **Toxicant** | **Tobacco** | **Non-tobacco** | ***P*** |
| "Tar" (mg) | 464 ± 159 | 513 ± 115 | NS |
| Nicotine (mg) | 1.04 ± 0.30 | < 0.01 | < 0.001 |
| CO (mg) | 155 ± 49 | 159 ± 42 | NS |
| Nitric oxide (mg) | 437 ± 207 | 386 ± 116 | NS |
| Polyaromatic hydrocarbons (ng) | | | |
| Fluoranthene | 385 ± 74 | 448 ± 132 | NS |
| Pyrene | 356 ± 70 | 444 ± 125 | NS |
| Benz[*a*] anthracene | 86.4 ± 15.2 | 113 ± 46 | NS |
| Chrysene | 106 ± 16 | 124 ± 36 | NS |
| Benzo[*b*+*k*] fluoranthenes | 64.7 ± 11.3 | 72.9 ± 12.6 | NS |
| Benzo[*a*]pyrene | 51.8 ± 12.9 | 66.1 ±17.8 | NS |
| Benzo[*ghi*] perylene | 33.6 ± 10.2 | 39.6 ± 10.7 | NS |
| Indeno[1,2,3-*cd*] pyrene | 47.3 ± 10.7 | 44.3 ± 10.4 | NS |
| Carbonylic compounds (µg) | | | |
| Formaldehyde | 58.7 ± 21.6 | 117.6 ± 78.7 | NS |
| Acetaldehyde | 383 ± 121 | 566 ± 370 | NS |
| Acetone | 118 ± 36 | 163± 68 | NS |
| Propionaldehyde | 51.7 ± 15.3 | 98.4 ± 65.0 | NS |
| Methacrolein | 12.2 ± 4.4 | 20.4 ± 9.7 | NS |

Adapted from Shihadeh et al. (*29*)
NS, not significant
Smoke was generated by reproducing human puffing with machine smoking during 62 *ad libitum* smoking sessions by 31 waterpipe users, each of whom completed two sessions in a controlled clinical setting: one with their preferred tobacco-based product and one with a flavour-matched tobacco-free product.

Since *maassel*, the heavily flavoured form of waterpipe tobacco, was introduced in the early 1990s *(43)*, very little research has been conducted to identify and quantify the flavourings in these tobacco products. The number of manufacturers and the number and variety of flavours available have increased steadily in the past 20 years, in conjunction with the popularity of this form of tobacco smoking *(44)*. Several flavourings were identified in the mainstream smoke from *maassel*

in quantities up to 1000 times greater than in mainstream cigarette smoke, including vanillin, ethyl vanillin and benzyl alcohol *(45)*. Using a non-targets analysis approach, Schubert et al. *(33)* tentatively identified 79 volatile flavourings, and quantitatively confirmed the presence of 11, in the headspace of a variety of waterpipe tobaccos from Egypt, India, Jordan and the United Arab Emirates. Flavours in tobacco products can be directly harmful by increasing the toxicity of the inhaled smoke. One example is cinnamon flavoured e-liquids. Behar et al. *(47)* showed that the cytotoxicity of e-cigarette emissions correlated strongly with the concentration of cinnamaldehyde in the e-liquid that was vaped. Another example is sweet flavour additives such as fructose and glucose. Soussy et al. *(48)* showed that these sugars decompose thermally during e-cigarette vaping to form 5-hydroxymethylfurural and furfural. Although not yet rigorously investigated, the same decomposition pathways are plausible for waterpipe tobacco, as it can contain up to 70% by weight of sugars, and both 5-hydroxymethylfurural and furfural were measured by Schubert et al. *(46)* in mainstream waterpipe tobacco smoke. Perhaps the greater potential contribution of flavours to adverse health effects in new and established tobacco smokers is increasing the appeal of smoking. Flavours may cause harm indirectly by lowering the barrier to initiation of use of tobacco products, by smoothing or sweetening the harshness of tobacco smoke, making it easier to inhale. Cross-sectional data on a nationally representative sample of young people (≤ 17 years) in the USA indicated a positive correlation between reporting that one's first tobacco product was flavoured and current tobacco use *(49)*. Recent preliminary longitudinal data from the same study show that young people who first use a flavoured tobacco product are significantly more likely to be tobacco users at one-year follow up than if their first-use product was unflavoured.[3]

While second-hand smoke is not a focus of this report, it should be noted that environmental exposure to waterpipe smoking also poses a significant health hazard. In controlled laboratory experiments, large quantities of volatile aldehyde species, CO, PAH and nanoparticles are emitted directly into the environment from the waterpipe head during smoking *(50)*. It has been estimated that during the course of a one-hour use session, a single waterpipe user will generate toxicant emissions equivalent to 2 to 10 cigarette smokers during the same one hour period, depending on the toxicant in question. Reports of observations in natural settings where waterpipes are used also show that waterpipe smoking results in high ambient concentrations of fine particulate matter (PM2.5) *(18, 51–53)*.

[3] Villanti AC. Are youth and young adults who first try a flavored tobacco product more likely to continue using tobacco? Findings from the PATH study. Presented at the annual meeting of the Society for Research on Nicotine and Tobacco, Florence, Italy, 9 March 2017.

## 4.4 Influence of testing protocols on measurements of toxicant emissions from waterpipes

Protocols for testing tobacco product emissions should include procedures for sampling and preparing waterpipe tobacco products and generating, collecting and quantifying toxicants in the mainstream smoke from these products. Such protocols, the activities that they should include and the questions they should address are summarized in Table 4.3.

Table 4.3. Procedures, activities and variables that require specification in tobacco product testing protocols

| Testing protocol | Activity | Specific testing variables |
|---|---|---|
| Tobacco sampling and waterpipe preparation | Homogenization | Number of purchased units? Sticks or twigs removed? Storage conditions? Stability in storage? |
| | Conditioning | Conditioned tobacco or as is from newly opened manufacturer's packaging? If conditioned, for how long and at what temperature and humidity? |
| | Tobacco packing | Clean head with solvents or water? Pack tobacco loosely or tightly? Aluminium foil perforation pattern? Quantity of tobacco? |
| | Pipe and hose cleaning | Clean with organic solvents and/or water? Use fresh hose each time? Check air infiltration rate? |
| Sample generation | Puffing regimen | Single- or multi-stage puffing? High-resolution reproduction of human puffing? Puff volume, duration and frequency? |
| | Heat source | Charcoal or electric? Amount and timing of charcoal application? Type of charcoal? |
| | Smoking machine | Puffing mechanism? Puff waveform? |
| Sample collection | Particulates and semivolatiles | Type and size of filter? How many filters are sufficient? |
| | Gas phase and volatiles | Impingers, sorbents, canister or bag collection? |
| Toxicant quantification | Extraction | Which solvent? Which clean-up method? Which surrogate standard? |
| | Quantification | Which internal standard? Which instrumental method? |

As there are no protocols for testing waterpipe emissions, however, there have been no studies on the influence of such protocols on toxicant emissions. In the absence of standard protocols, research groups have used a wide variety of equipment and procedures to study waterpipe emissions. While studies of toxicant emissions cannot be compared, data collected by research groups are available for estimating the influence of certain variables. In particular, the influence of puffing regimen, heat source, tobacco temperature and bowl water have been investigated and are discussed briefly below. The effects of other variables such as tobacco conditioning (loose versus tight packing into the bowl), sample generation (e.g. heat source ignition timing, puffing mechanism), smoke collection conditions (e.g. filter type, number and diameter for the particle phase; impingers, sorbent or real-time collection) and toxicant quantification methods still require investigation. Some methods for quantifying toxicants in cigarette tobacco and emissions have been modified for waterpipe emissions, but none has been validated with reference materials or inter-laboratory studies, and thus more work is required.

Mainstream constituents of waterpipe tobacco smoke have been analysed with machine smoking and various equipment, including commercially available waterpipes (e.g. *3*), a specially designed waterpipe smoking machine equipped with a laboratory waterpipe (e.g. *21*) and a research-grade waterpipe *(13)*, as shown in Table 4.4. Commercially available waterpipes were generally smoked with radial positive displacement vacuum pumps, such as rotary vane, diaphragm, piston or scroll pumps, operating at a constant flow rate. Computer-controlled solenoid or manual valve switching control was used for puffing (e.g. *3*, *17*, *44*). In the "shisha smoker" and the research-grade waterpipe, smooth action, single-stroke piston displacement is used to generate the puff, which more closely approximates the human diaphragm than vacuum pumps. "Playback" smoking machines to reproduce human puffing behaviour in fine detail have also been used to study waterpipe emissions (e.g. *45*). How different waterpipe designs and puffing mechanisms affect the level of toxicants in mainstream smoke is not well understood.

Table 4.4. Waterpipe machine smoking regimens

| Waterpipe | Pump mechanism | Puff volume (L) | Puff duration (s) | Inter-puff interval (s) | Total no. of puffs | Total puff volume (L) | Smoking duration (min) | Coal no./ type/ diameter (mm) | Amount of tobacco (g) | Tray or foil (no. of holes) | Hose material |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beirut method, Black Single Pearl, Khalil Mamoon *(6, 42)* | Mechanical pump with digital solenoid control | 0.53 | 2.6 | 17 | 171 | 90.6 | 55.6 | 1.5C / 3 Kings / 33 mm | 10 | Foil (18) | Leather and plastic *(43)* |
| Modified Beirut method *(21)* | Pneumatic single-stroke cylinder | 0.53 | 2.6 | 17 | 171 | 90.6 | 55.6 | 1C / 3 Kings / 40 mm | 10 | Foil (18) | Plastic |
| Super shisha *(18)* | Vacuum pump, 6 L/min | 0.3 | 3 | 15 | 100 | 30 | 30 | 1C / Swift-Lite / 33 mm | 10 | Foil (19) | Not reported |
| Clay bowl *(43)*[a] | Mechanical pump and manual syringe every 10th breath | 1.0 | 5 | 25 | 100 | 100 | 50 | 1C / Swift-Lite / 33 mm | 8 | Tray (not reported) | Not reported |
| Research-grade waterpipe[b] | Single-stroke glass syringes | 0.72<br>0.46 | 4.6<br>3.6 | 16.4<br>28.7 | 32<br>42<br>74 | 23.0<br>19.1<br>42.1 | 11.2<br>22.6<br>33.8 | 1C / 3 Kings / 40 mm Electric heat source | 10 | Foil (18)<br>Tray (30) | Plastic |

[a] Waterpipe smoked for 3-min "warm-up" period before mainstream smoke sampling

[b] Kroeger RR, Brinkman MC, Buehler SS, Gordon SM, Kim H, Cross KM, et al. The impact of variation of hookah components on chemical and physical emissions. Presented at the annual conference of the Society for Research on Nicotine and Tobacco, Seattle, WA, USA, 6 February 2014.

### 4.4.1 Puffing regimen

Most testing of waterpipe emissions has been conducted with one of three types of puffing regimen: steady, periodic summary data modelling of human puffing behaviour in a waterpipe café *(46)*; multi-stage, steady, periodic summary data modelling of human puffing behaviour in a laboratory setting;[4] and high-resolution, time-resolved (10 Hz) "playback" puffing to mimic each person's behaviour precisely *(29, 45, 47)*. In the first case, with the Beirut method *(5, 48)*, the smoking machine is programmed with the average puff in a rectangular waveform and a fixed puff volume and duration, repeated at a fixed frequency. In the second case, a smooth parabolic waveform is used, with two waveform and frequency stages – one used for the first third of the smoking session (stage 1) and the other for the remainder (stage 2) – to account for the observation that smokers take larger, more frequent, intense puffs at the beginning of a waterpipe smoking session *(5)*.[2] In the third case, the puffing topography collected during a participant's smoking session is "played back" or uploaded to the smoking machine to replicate the session exactly. To compare the emissions in the first and third types of puffing regimen, Shihadeh and Azar *(45)* compared the tobacco consumed, tar, smoke temperature and CO yields. The periodic regimen resulted in 20% less CO in mainstream smoke, indicating that CO data generated during these regimens may be underestimates of actual exposure.

Using a single-stage periodic regimen, Shihadeh *(3)* also explored the influence of puff volume and frequency on waterpipe tobacco consumption and mainstream tar and nicotine delivery. Larger puff volumes resulted in increased consumption of tobacco, probably because of greater airflow through the coal and head and the resulting higher tobacco temperature. Larger puff volumes also resulted in more TPM (wet and dry) in mainstream emissions, even when normalized by the mass of tobacco consumed and total puff volume. Doubling the puffing frequency (by halving the inter-puff interval from 30 to 15 s) while holding the puff volume constant resulted in about 1.5 times more tar in mainstream emissions, even when tar was normalized by the mass of tobacco consumed; however, doubling the puffing frequency did not significantly change nicotine delivery.

### 4.4.2 Heat source

Several researchers have examined how the heating source influences the delivery of toxicants including furans, VOCs and PAHs. To better understand whether the primary source of specific toxic emissions is charcoal or *maassel*, researchers have conducted machine smoking with electric and charcoal heat sources and also

[4] Kroeger RR, Brinkman MC, Buehler SS, Gordon SM, Kim H, Cross KM, et al. The impact of variation of hookah components on chemical and physical emissions. Presented at the annual conference of the Society for Research on Nicotine and Tobacco, Seattle, WA, USA, 6 February 2014.

with charcoal alone (no *maassel*). Most furans were not detectable in emissions generated with charcoal alone, indicating that *maassel* may be the dominant source *(36)*. This is not the case for VOCs such as benzene and toluene, which are present in waterpipe smoke at similar levels whether the waterpipe head contains *maassel* or not *(49)*. To identify the dominant source of CO and PAHs in mainstream waterpipe smoke, Monzer et al. *(20)* used an electric heat source designed to match the spatial and temporal temperature distribution of quick-light charcoal. Emissions collected individually with each heat source showed that charcoal contributed most of the CO (90%) and benzo[*a*]pyrene (95%). In a comparison of a commercially available electric heater with quick-light charcoal, a research-grade waterpipe and a two-stage puffing regimen, Kroeger et al.[2] reported a reduction in the yields of fine-particle PAHs (50 time less) and nicotine (about four times less) in mainstream smoke and of CO (about 2000 times less) and benzene (about 1200 times less) in sidestream smoke.

#### 4.4.3 Temperature of tobacco

The concentrations of carbonyls such as acetaldehyde, formaldehyde, acetone and acrolein are strongly influenced by the peak temperature reached in tobacco, higher temperatures resulting in greater yields *(4)*. In turn, the peak temperature reached in tobacco is influenced by the concentrations of glycerol and propylene glycol, the primary humectants in waterpipe tobacco, greater humectant content resulting in lower temperatures *(36)*.

#### 4.4.4 Effect of water

Several studies have indirectly and directly addressed whether toxicants dissolve in the bowl water during puffing and are thus effectively "filtered" from mainstream waterpipe smoke. Indirect measures indicate that the concentrations of toxicants in mainstream waterpipe smoke depend on the presence of water in the bowl. The presence of water reduced the level of nicotine by 4.4 times *(3)* and the level of carbonyls by 3.7 times *(4)*. Schubert et al. *(49)* measured the phenol content of the bowl water directly and found that it contained detectable levels of two phenols: phenol and guaiacol (7.9 and 3.3 times more, respectively, in water than in smoke). Shihadeh *(3)* reported, however, that the level of tar was not significantly different when water was removed from the bowl.

### 4.5 Influence of waterpipe design on levels of emissions of waterpipe tobacco products

#### 4.5.1 Components and accessories

In developing a testing protocol for waterpipe emissions, it is useful to distinguish between waterpipe components and accessories. Components are defined as

necessary elements of the apparatus required for smoking tobacco in a waterpipe, whereas accessories are optional elements that may be incorporated into the apparatus but are not strictly required. The components and examples of some of the many accessories available, and the physical and chemical attributes that may affect waterpipe emissions, are shown in Table 4.5. The influence of overall waterpipe design and some components and accessories such as the hose, tray and foil on emissions is discussed briefly below. The influence of other components and popularly used accessories is unknown and requires investigation.

Table 4.5. Waterpipe components and accessories that may affect emissions

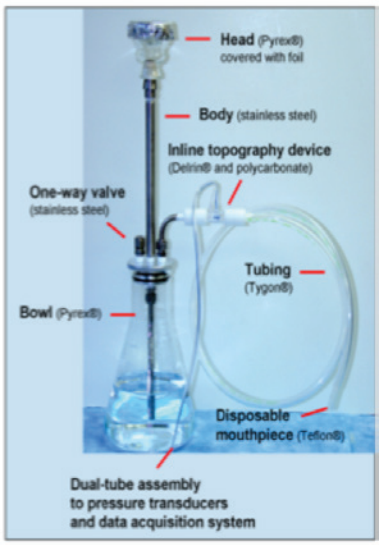
| Component | Purpose | Physical attributes |
|---|---|---|
| Head | Holds the tobacco | Construction material; geometry; connection or joint; location, diameter and number of airway holes; weight |
| Body | Transfers smoke from head to mouth of bowl | Construction material, geometry, connection or joint, immersion depth |
| Stem | Transfers smoke from mouth of bowl into water | Construction material, geometry, connection or joint, immersion depth |
| Bowl | Holds water | Construction material, shape (dimensions), volume |
| Water | Bubble formation | Volume, purity, pH |
| Hose | Transfers smoke from bowl to user | Construction materials, length, inner and outer diameter |
| Charcoal tray or foil | Barrier to reduce burning of tobacco | Fabrication material; thickness; shape (dimensions); area for holding charcoal; location, diameter and number of airway holes; weight |
| Hookah cream | Increases the amount of smoke or aerosol generated | Ingredients, mass used per mass of waterpipe tobacco, preparation method (layered or "stacked" or mixed evenly with the tobacco) |
| Bubble diffuser | Produces smaller bubbles, quieter puffing, reduced smoke harshness | Fabrication material; shape (dimensions); location, diameter and number of airway holes; length of stem covered when installed; type of sealing joint to stem |
| Mouthpiece | To prevent spread of germs during group smoking | Fabrication material, surface smoothness, length, inner and outer diameter |
| Wind cover | Shields charcoal from wind | Fabrication material; thickness; shape (dimensions); area for holding charcoal; location, diameter and number of airway holes; weight; type of sealing joint to head |

### 4.5.2 "Real-world" and research-grade waterpipes

Waterpipe emissions have been tested with either commercially available waterpipes (e.g. *3*, *46*) or waterpipes especially designed for use in an analytical laboratory (e.g. *13*, *21*). Commercially available waterpipes and their components vary widely in design and durability, including in the materials used to fabricate stems, bases, bowls and hoses, sealing joint designs and degree of leak-tight fit and the diameter of the flow path. All the variables can affect the net thermal energy transferred from the heat source to the tobacco, which in turn can affect the nature and concentration of the mainstream smoke particle phase *(4, 36)* and the smoker's puffing behaviour. The fabrication materials and design of any commercial waterpipe may change without notice, which could confound emissions testing.

Research-grade waterpipes, such as that shown in Fig. 4.4, were designed to address these issues. They are fabricated from inert materials that should minimize chemical adsorption to and desorption from surfaces and eliminate stray sources of chemicals from the waterpipe itself (e.g. metal solder and thermal degradation products). Research-grade waterpipes have benchmarked performance metrics for precision and accuracy *(13)* and inter- and intra-subject variability *(50)* and have been well accepted in terms of satisfaction and reward by experienced smokers in clinical studies *(13)*.

Fig. 4.4. Standardized research-grade waterpipe equipped with human puff topography data collection and acquisition




### 4.5.3 Waterpipe hose

In most studies of machine smoking, the waterpipe hoses were made of leather or plastic. Plastic hoses resulted in more than twice the amount of TPM and CO in mainstream smoke, largely because leather hoses infiltrate air *(43)* and result in water loss *(51)*. The nicotine levels were not significantly different.

### 4.5.4 Waterpipe tray versus foil

In most studies of machine smoking, either foil or a metal tray was used as the interface between charcoal and tobacco. Kroeger et al.[5] compared mainstream and sidestream emissions generated in a research-grade waterpipe equipped with a metal tray or foil and a two-stage puffing regimen. The concentrations of some

[5] Kroeger RR, Brinkman MC, Buehler SS, Gordon SM, Kim H, Cross KM, et al. The impact of variation of hookah components on chemical and physical emissions. Presented at the annual conference of the Society for Research on Nicotine and Tobacco, Seattle, WA, USA, 6 February 2014.

toxicants in the mainstream fine-particle phase were significantly lower, including those of the TSNAs NNN and NNK (two to three times lower) and the PAHs benzo[*a*]pyrene and pyrene (two to three times lower), when the metal tray was used; however, the concentrations of some sidestream gas-phase toxicants were significantly higher, including those of acetaldehyde, acetonitrile, acrylonitrile, benzene, 1,3-butadiene and isoprene (one to three times higher).

In summary, the testing protocols for waterpipe tobacco smoke emissions and waterpipe components and accessories can influence tobacco consumption and the identity and concentration of the resulting mainstream and sidestream emissions. A preliminary list, based on current knowledge, of protocol conditions and their effects on priority toxicants is shown in Table 4.6. Overall, the heat source has the greatest influence on mainstream and sidestream waterpipe smoke emissions.

Table 4.6. Waterpipe testing protocol conditions and influence on resulting toxic emissions

| Condition 1 | Condition 2 | Toxicants in MS, SS and BW | Toxicant level in condition 1 |
|---|---|---|---|
| Periodic puffing *(45)* | Playback puffing | MS CO | 1.2 times greater |
| | | MS TPM (dry) | Not significantly different |
| | | Tobacco consumption | 1.2 times greater |
| 300 mL puff volume *(3)* | 150 mL puff volume | Tobacco consumption | 1.4 times greater |
| | | MS TPM (wet)[a] | 3.8 times greater |
| | | MS TPM (dry)[a] | 3.2 times greater |
| | | MS nicotine | Not significantly different |
| 1 puff every 30 s *(3)* | 1 puff every 15 s | MS tar | 1.5 times greater |
| | | MS nicotine | Not significantly different |
| With tobacco | No tobacco | MS furans *(48)* | Contains ~100% of the furans |
| | | MS benzene *(36)* | Not significantly different |
| | | MS toluene *(36)* | Not significantly different |
| Quick-light charcoal | Electric heat source (*20*) | MS CO | Contains 90% of the CO |
| | | MS benzo[*a*]pyrene | Contains 95% of the benzo[*a*] pyrene |
| | Commercial electric coal[b] | MS nicotine *(3)* | 4 times higher |
| | | SS CO | 2000 times higher |
| | | SS benzene | 1200 times higher |
| Peak temperature reached in tobacco, 277 °C | Peak temperature reached in tobacco, 203 °C *(4)* | MS acetaldehyde | 3.3 times higher |
| | | MS acrolein | 1.3 times lower |
| | | MS formaldehyde | 1.2 times higher |
| With bowl water | Without bowl water | MS nicotine *(3)* | 4.4 times lower |
| | | MS acetaldehyde *(4)* | 3.9 times lower |
| | | MS acrolein *(4)* | 3.5 times lower |
| | | MS formaldehyde *(4)* | 2.8 times lower |
| In bowl water[b] | In MS waterpipe smoke | BW phenol | 7.9 times higher |
| | | BW guaiacol | 3.7 times higher |

| | | | |
|---|---|---|---|
| | | Tobacco consumption | 1.4 times higher |
| | | MS TPM (wet) | 2.4 times higher |
| Plastic hose *(43)* | Leather hose | MS CO | 2.4 times higher |
| | | MS NNN | 3.2 times higher |
| | | MS NNK | 1.8 times higher |
| | | MS pyrene | 1.9 times higher |
| | | MS benzo[*a*]pyrene | 2.6 times higher |
| | | SS acetaldehyde | 1.5 times lower |
| | | SS acetonitrile | 1.4 times lower |
| | | SS acrylonitrile | 1.5 times lower |
| | | SS benzene | Not significantly different |
| | | SS 1,3-butadiene | 1.9 times lower |
| Foil[c] | Tray | SS isoprene | 1.6 times lower |

MS, mainstream (active); SS, sidestream (passive); BW, bowl water after machine smoking

[a] TPM normalized by mass of tobacco consumed

[b] Measured directly in bowl water

[c] Kroeger RR, Brinkman MC, Buehler SS, Gordon SM, Kim H, Cross KM, et al. The impact of variation of hookah components on chemical and physical emissions. Presented at the annual conference of the Society for Research on Nicotine and Tobacco, Seattle, WA, USA, 6 February 2014.

## 4.6 Conclusions

Waterpipe puff topography varies by population and setting; however, too few studies have been conducted to draw conclusions about the extent of the variation. In all studies to date, the puff volume, flow rate and puff number were much larger than during cigarette smoking, and machine testing regimens must be adjusted accordingly.

Waterpipe tobacco smoke contains and delivers high concentrations of the toxicants associated with tobacco-related diseases, including nicotine addiction, lung disease, heart disease and cancer. Waterpipe smoke generated from tobacco-free products also contains and probably delivers high concentrations of the toxicants associated with tobacco-related diseases, including lung disease, heart disease and cancer.

Toxicant emissions depend not only on the tobacco product smoked but also on the combination of tobacco product, charcoal type, waterpipe design, waterpipe preparation method, puff topography and their interactions. In the current state of knowledge, protection of public health requires regulation of the characteristics and contents of tobacco products and charcoal.

The global resurgence of waterpipe smoking and the high exposure to toxicants associated with waterpipe use indicate that waterpipe smoking should be included in all tobacco control programmes and policies, including banning flavouring additives and indoor smoking.

## 4.7 Recommendations for regulators

- Require that manufacturers disclose the ingredients and contaminants (specified in Table 4.2) of tobacco and charcoal products marketed for waterpipe use (including *maassel*, herbal *maassel*, waterpipe stones and other products intended for mixing with tobacco or charcoal).
- Require manufacturers of products intended for waterpipe smoking, including tobacco and tobacco-free products, charcoal, waterpipe components (e.g. hose infiltration) and accessories (e.g. aluminium foil), to disclose to regulators their intent to market such products.
- Require points of sale of waterpipe products to maintain records of compliance of product with regulations, once regulations are adopted.
- Ban the use of flavour compounds in tobacco-based and tobacco-free waterpipe products.
- Include all forms of waterpipe use in indoor smoking bans.
- Communicate to users that used waterpipe water is hazardous because of its chemical and microbial content.

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# 5. Applicability and adaptability of the WHO Tobacco Laboratory Network standard operating procedures for cigarettes to waterpipe tobacco

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**Contents**



## 5.1 Introduction

This section includes recommendations on the application of existing and pending TobLabNet SOPs for waterpipe tobacco smoking, which were considered by the WHO FCTC COP Working Group on Articles 9 and 10 at its meeting in February 2016. The features of waterpipes used globally are described in section 4.

Laboratory characterization of toxicant emissions by use of a smoking machine requires specification of puff topography parameters such as puff volume, duration and inter-puff interval. Specification is necessary because toxicant emissions are strongly influenced by the puffing parameters used to smoke a given product *(1–3)*. Several studies of waterpipe puff topography have been reported in the scientific literature, covering various populations in clinical laboratory and natural environments. These studies are summarized in section 4, Table 4.1, which shows mean puff volumes of 500–1000 mL, puff durations of 2–3 s and inter-puff intervals of approximately 10–35 s. The variations among studies seen in the Table probably reflect the influence on puff topography of factors such as years of experience, smoking frequency and setting. Some experimental data suggest that waterpipe puff topography can be influenced by the nicotine content of the product smoked; in a blinded experiment, experienced waterpipe users puffed more intensively when they were provided with a nicotine-free waterpipe product *(4)*. Experimental data also show that puff topography is affected by the level of nicotine dependence *(5)*. Such variations notwithstanding, it is noteworthy that waterpipe smoking involves puff volumes more than 10 times greater than those of a cigarette and that a single waterpipe puff displaces approximately the same smoke volume as an entire cigarette. It is clear, therefore, that cigarette puff topography parameters should not be used in waterpipe machine smoking tests.

To date, the most commonly used puff topography regimen for analytical studies of waterpipe tobacco smoke is that of the Beirut method *(6)*, which specifies 171 puffs of 2.6 s duration, 530 mL volume and 17 s inter-puff interval, in addition to waterpipe design, preparation and charcoal addition procedures. This method was based on two field campaigns in cafés in the Beirut area in which waterpipes were served *(6, 7)* and was validated by measuring "tar", nicotine and CO in smoke sampled in real time from waterpipes as they were smoked by café patrons *(6)*. It is the only method to date that has been validated against human data.

## 5.2 Smoking methods

As noted in the previous section, methods designed for cigarette testing are not applicable to quantification of waterpipe emissions. Numerous factors unique to waterpipe smoking have been considered in investigations of emissions, which are discussed below.

### 5.2.1 Heat sources

Quick-lighting charcoal is the most popular heat source described in published research. After the charcoal has been lit with an open flame, it is placed on the head for 60 *(8)* to 100 s *(1)* before machine smoking is started.

Researchers have investigated two electric heat sources, one fabricated in the laboratory *(9)* and the other purchased commercially.[6] Temperature measurements in both studies at two locations, just under the heat sources and in the tobacco in the head, indicate that an electric heat source can mimic the behaviour of charcoal. The results with both devices indicate that the most the CO and PAHs come from the charcoal *(9)*. Kroeger et al.[2] also showed that most benzene comes from burning charcoal, and, with a different experimental approach, Schubert et al. *(10)* confirmed this result. The constituents of mainstream waterpipe tobacco smoke should be tested with both electric and charcoal heating sources so that toxicity can be properly attributed. We recommend that protocols for charcoal and electric heating sources be included in the adapted SOP or that separate SOPs be developed for charcoal emissions.

### 5.2.2 Head

Levels of constituents have been reported mainly for waterpipes with heads made of ceramic (e.g. *1*) or metal (e.g. *8*), but some research has been conducted with a waterpipe with a glass head *(11)*[1]. Each of these materials has different thermal conductivity, which will probably affect the temperature of the tobacco, which in turn may influence the variety and concentration of constituents in mainstream smoke, although this has not yet been rigorously proven. We recommend that the type and thickness of the head material and its dimensions, including the number and diameter of the holes in the head, be specified in the adapted SOP.

The emissions also depend on the amount of tobacco used. A head of standardized dimensions is therefore required, and the emissions per gram of tobacco used should be calculated. To be certain that the distance between the heating device and the tobacco does not vary, the head should be completely filled, with a special cover on which the heating device is placed.

### 5.2.3 Head covering

In most studies with machine-smoking, aluminium foil or a metal tray with holes was used to cover the head of the pipe so that the charcoal or other heat source dis not touch the tobacco. These two materials are likely to transfer heat to the tobacco with different efficiency, thereby affecting the toxic content of mainstream smoke. We recommend that the thickness and size of the foil or tray and the number and diameter of the holes in these coverings be specified in the adapted SOP. Depending on the heat source used, covering the head might reduce heat transfer too much, which might imply that tests should be performed without covering the head.

[6] Kroeger RR, Brinkman MC, Buehler SS, Gordon SM, Kim H, Cross KM, et al. The impact of variation of hookah components on chemical and physical emissions. Presented at the annual conference of the Society for Research on Nicotine and Tobacco, Seattle, WA, USA, 6 February 2014.

### 5.2.4 Water

The amount of water in the bowl should be specified and measured, because it is directly related to the pressure drop, or resistance to flow, that the smoker must overcome to inhale smoke through the hose. The smoker must, by sucking on the hose, create a vacuum in the bowl that is greater than that in the static head *(1)*. This is directly related to the size of the bowl and the distance between the bottom end of the stem and the water level. We recommend that the bowl dimensions, the length of the stem and the length of the stem that is covered by the bowl water be specified in the adapted SOP.

### 5.2.5 Hose

In most studies of machine-smoking, waterpipe hoses made of leather or plastic were used. Researchers have shown that, owing to air infiltration through *(12)* and water loss to *(13)* leather hoses, plastic hoses result in more than twice the amounts of TPM and CO generated in mainstream smoke, although the level of nicotine was not significantly different *(12)*. We recommend that the adapted SOP specify use of a plastic hose in order to reduce variation arising from the different porosity and humidity of leather and that the length and diameter of the hose also be specified, as these factors affect both flow resistance and particle deposition.

### 5.2.6 Filter

In mainstream cigarette smoke, the majority of nicotine (90–99%) is in the protonated form and thus attached to the smoke aerosol *(14, 15)*. Standard analyses of cigarette constituents involve collection of TPM onto a glass-fibre filter, which is extracted with a solvent and quantified by GC *(16)*. The mass of TPM generated during waterpipe tobacco smoking may be 10–100 times more than that from cigarette smoking *(17)*. Therefore, during waterpipe machine smoking, the filter must not be overloaded, as this will create too high a pressure drop, which may result in poor sample retention, damage to the filter and/or pump overload.

In routine testing, filter pads should not be changed during a machine smoking run, as this may jeopardize the integrity of the puff volume. The system cannot be checked for leaks after a filter change without modifying the machine smoking regimen, and a leak-tight system is critical for reproducible constituent analyses. Researchers reported 1–2.7 g of TPM in the mainstream smoke from a single waterpipe tobacco smoking session *(1, 8)*, of which about 60% is attributable to water. Nicotine is soluble in water, and comparison of machine smoking with and without water in the bowl indicates that approximately 75% of the nicotine is retained in the water *(1)*. The high water content of the waterpipe aerosol requires that hydrophobic filter media such as Teflon be avoided for

smoke sampling in order to avoid blockage; in a hydrophilic medium such as a glass-fibre wick, the moisture travels along the filter fibre. To the extent that nicotine is in the particle phase, filter sampling will be effective in trapping it, provided that the filter is not overloaded during machine smoking (i.e. that it becomes saturated such that liquid droplets are found on the back of the filter). The degree to which semi-volatile analytes that can partition between the gas and particle phases are retained on the filter may be affected by such variables as the particle size distribution, the hygroscopicity of the analyte and the duration of the smoking session *(18)*.

For the adapted SOP, it is recommended that mainstream smoke be split into a minimum of two equivalent streams and that two filter cartridges (92 mm in diameter) be installed for the duration of the waterpipe smoking session to ensure that particle loading remains within the carrying capacity of the filters. Breakthrough of semi-volatile chemicals due to different particle sizes and combinations of filters require more rigorous testing.

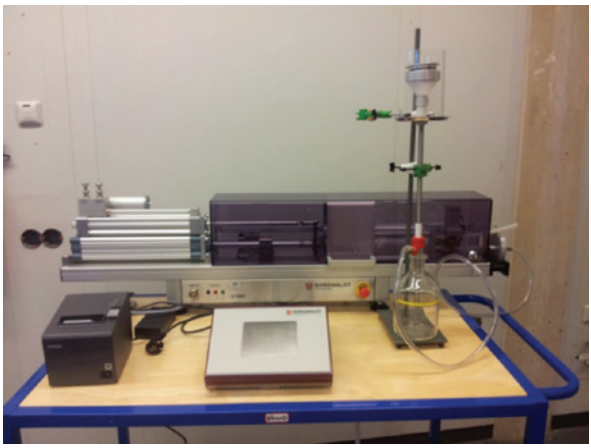
## 5.3 Smoking machines

In view of the differences in puffing parameters and mechanical design of waterpipes and cigarettes, analytical smoking machines designed for testing cigarette emissions cannot be used for testing those from waterpipe tobacco. To determine emissions from waterpipe tobacco, the smoking machine must consist of the same principal components as waterpipes; head, body, bottle and suction device. While the exact parameters of waterpipe smoking topography to be used in machine smoking of waterpipe tobacco remain to be specified, it is essential that the machines fulfil at least the following requirements:

- applicable for testing various types and amounts of waterpipe tobacco or molasses;
- accommodate different types of heating device (e.g. charcoal, electrical heating);
- have components that are chemically resistant, inactive and free of contamination, including all tubes, hoses and connectors;
- suitable for different bottle types and sizes;
- capable of drawing puffs up to a volume of at least 1000 mL;
- capable of connection to different trapping systems for particulate matter as well as for gaseous phase components;
- have pipes, hoses, collection devices and other components of defined length, diameter and position; and
- include device(s) for setting parameters, controlling the equipment and storing and printing data.

A working group within ISO/TC 126 is establishing definitions and standard conditions for a waterpipe tobacco smoking machine, and a smoking machine for generating waterpipe smoke has been made commercially available by Borgwaldt GmbH (Fig. 5.1). An analytical waterpipe smoking machine, the heating devices and the settings to be used for determining the emissions of waterpipe tobacco should all be adjusted according to future demands and regulations. The specific requirements of an analytical smoking machine for testing waterpipe emissions and their possible influence on emissions are described in section 5.4. It is recommended that a standard waterpipe design and puff profile be adapted for emission testing purposes.

Fig. 5.1. Analytical waterpipe smoking machine developed by Borgwaldt GmbH



## 5.4 Sampling of waterpipe tobacco

Currently, cigarettes are sampled for regulatory purposes mainly according to ISO 8243 *(19)*. This ISO standard describes sampling of cigarettes at one time or over a period of time, both for sampling at a point of sale and at the premises of the manufacturer or importer. This standard also establishes the confidence intervals for the amounts of tar, nicotine and CO emitted when cigarettes are smoked according the ISO regime. Sampling of roll-your-own or make-your-own tobacco products is described in ISO 15592 part 1 *(20)*, by the same procedures as for cigarette sampling.

In sampling for (regulatory) testing of tobacco products (including waterpipe tobacco), a representative sample of a specific product must be obtained, either at one time or over a period of time. When all the products available to consumers comply with regulations, sampling (and testing) should be done at one time. If the purpose is to check whether the product in general complies with the regulations, sampling over time is advisable, although each set of samples should still be tested.

The location of sampling (point of sale or premises of the manufacturer or importer) depends on the purpose: to determine whether products to be used by consumers are in compliance with regulations or whether the manufacturer or importer produces or imports waterpipe tobacco that is in compliance with regulations. As the intention of regulation is to protect consumers, sampling at points of sale is the best option, although a possible disadvantage is that a manufacturer or importer might claim that they are not responsible for the product after it leaves their premises. To avoid manipulation by the manufacturer or importer (preselection of samples that are in compliance), it is advisable to arrange sampling by a government agency or an independent organization.

These recommendations for sampling are also applicable to related products to be tested, such as charcoal.

## 5.5 Sample preparation

Sample preparation as described in this section means handling of a waterpipe tobacco sample from the moment it enters a test facility until the start of the test procedure(s). Additional preparation required for a specific test should be included in the procedures of that test. The main goal of sample preparation is to create a homogeneous, stable, representative sample for testing from the laboratory sample. Important procedures are mixing and conditioning of waterpipe tobacco.

As all individual sales units must comply with regulatory limits, each package of the product should be homogenized separately. Waterpipe tobacco may not be homogeneous and may contain components that might be discarded by consumers before smoking, such as large tobacco plant stems. Further investigation is needed to determine the influence of these components on the contents and emissions of waterpipe tobacco and how consumers deal with these components. The results will indicate whether these components should be included in or excluded from the test procedures.

The number of tests required for verification depends on the variation in the product that is allowed and the confidence intervals (CIs). For cigarettes, ISO 8243 (*19*) specifies that the average result requires the average of 20 test results, in each of which each 20 cigarettes are smoked on an analytical smoking machine for verification of their regulatory compliance. The number of homogenized packages of waterpipe tobacco to be tested remains be defined, taking into account variation among packages and acceptable CIs. The CIs of measurements can be determined in inter-laboratory validation studies. The CIs at one time in ISO 8243 (*19*) are 20% for tar and nicotine and 25% for CO. Depending on the variation among packages and the analytical variability of specific determination(s), the number of packages to be tested can be limited by setting a maximum acceptable CI.

As determination of waterpipe tobacco contents and emissions starts with weighing a certain amount, the moisture content is an important variable, as more water corresponds to less tobacco in the same product weight. In other tobacco products (cigarettes and roll-your-own tobacco), the relative humidity depends on the desired moisture content, with an average of about 13% in cigarettes and about 20% in roll-your-own tobacco *(20)*, corresponding to a relative humidity for conditioning of 60% and 75%, respectively, as specified in CORESTA-recommended method 42 *(21)*. As both product types are conditioned at 22 °C, there is no need to adjust the temperature according to the moisture content. For waterpipe tobacco, conditioning for stabilization might interfere in the determination of some components. For regulatory purposes, it can be decided that waterpipe tobacco should be analysed as sold to consumers.

Waterpipe tobacco is usually sold in sealed containers, which might increase the variation in results over time and between laboratories due to differences in water content and therefore different amounts of tobacco used in the determination. To minimize variation, regulatory limits can be set for the dry product, such that the water content must be determined or the waterpipe tobacco must be dried before analysis. Both options will require additional testing, increasing the cost of regulatory measurement of components of waterpipe tobacco. Alternatively, the water content of waterpipe tobacco might be determined at the same time as nicotine, as both components are soluble in isopropanol. The applicability of combined measurement should be investigated further.

The moisture content of waterpipe tobacco influences its emissions during smoking. To minimize variation over time and between laboratories in the emissions of waterpipe tobacco, the products should be be stable and smoked under defined conditions. Laboratory testing of a few waterpipe tobacco samples for water extractable with isopropanol showed a moisture content of 10–30%. Differences in relative humidity would require several conditioning steps; as this would be difficult to apply in practice, it is advisable to use one setting for relative humidity. In comparison with the moisture content of cigarettes and roll-your-own tobacco, a relative humidity of 75% would be suitable for conditioning waterpipe tobacco, rather than 60%. As only a few laboratories have access to conditioning equipment suitable for 75% relative humidity, waterpipe tobacco could be conditioned at 60% relative humidity and 22°C, as described in ISO 3402 *(22)*. The minimum and maximum duration of conditioning waterpipe tobacco should be investigated further and included in the SOP.

Currently, the influence of the temperature and the humidity of the environment during (machine) smoking of waterpipe tobacco is unknown. As both cigarettes and roll-your-own tobacco are smoked at the same temperature (22 °C) and humidity (60%), despite different moisture contents, it is recommended that waterpipe tobacco be (machine) smoked in the same conditions.

## 5.6 Determination of contents and emissions

TobLabNet has validated analytical methods for the determination of contents (three methods) and emissions (four methods) of cigarettes. Below, the applicability of these methods to waterpipe tobacco is discussed.

### 5.6.1 Contents of waterpipe tobacco

Of the three validated TobLabNet SOPs for determination of the contents of cigarette tobacco filler, those for humectants and nicotine are discussed in relation to their applicability to waterpipe tobacco.

#### 5.6.1.1 Humectants

TobLabNet SOP-06 for the determination of humectants in cigarette tobacco filler is validated for glycerol, propylene glycol and triethylene glycol. Glycerol and propylene glycol are present in cigarette tobacco at 0.5–4.0%, while triethylene glycol is present only occasionally in cigarette tobacco as a possible contaminant of the humectants used during production. In contrast, triethylene glycol was identified in 6 of 44 waterpipe tobacco products tested, and nearly all the products contained much higher levels of glycerol than cigarette tobacco *(23)*.

A similar extraction procedure for humectants in waterpipe tobacco was tested by Rainey et al. *(23)*, as described in TobLabNet SOP-06. This implies that there is no need to adapt the extraction procedure of TobLabNet SOP-06 for the determination of humectants in waterpipe tobacco.

Because of the much higher levels of glycerol in waterpipe tobacco, precautions should be taken in GC settings to avoid co-elution of glycerol and triethylene glycol. The calibration range of glycerol and propylene glycol should also be adjusted for the higher levels of these compounds in waterpipe tobacco.

#### 5.6.1.2 Nicotine

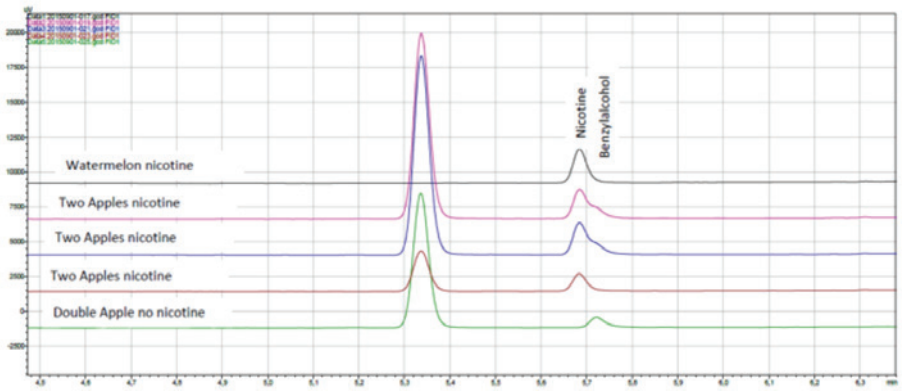
The determination of nicotine in cigarette tobacco filler is described and validated in TobLabNet SOP-04. In this method, nicotine is extracted from cigarette tobacco with water, a sodium hydroxide solution and hexane. During extraction, nicotine is transferred to hexane and is analysed by GC–FID.

The high levels of humectants in waterpipe tobacco might result in incomplete extraction of nicotine. This should be investigated by testing recovery of added nicotine dissolved in glycerol or propylene glycol or with different extraction solutions.

InTobLabNet SOP-04, nicotine is analysed by GC–FID. This technique is widely used for the analysis of nicotine and is applicable for the determination of nicotine in various matrices. Waterpipe tobacco, however, contains not only high levels of humectants but also various types and amounts of flavours, which might

contain chemical components that interfere with nicotine analysis (Fig. 5.1). Changing the chromatographic parameters to avoid co-elution of interfering flavours would be very time-consuming or almost impossible because of the huge number of different flavours used in waterpipe tobacco. A more practical approach would be to use GC–MS to achieve more reliable identification of nicotine and more reliable quantitative results.

Fig. 5.1. Chromatogram of nicotine determination in waterpipe tobacco with different flavours




### 5.6.2 Emissions of tar, nicotine and carbon monoxide

The determination of emission components depends on the type of smoking machine, the smoking protocol, trapping of components, extraction and preparation of the sample solution and measurement of specific components. In this section, the applicability of procedures for trapping components, preparing sample solutions and measuring components are discussed, with possible adjustments of the waterpipe smoking machine or protocol to avoid loss or interference.

For the determination of cigarette emissions, the trapping systems prescribed in the TobLabNet SOPs are:

- a CFP for tar, nicotine, benzo[*a*]pyrene and TSNAs;
- a gas sampling bag for CO; and
- a Carboxen cartridge for aldehydes and VOCs.

In general, the applicability of the TobLabNet SOPs for the determination of cigarette emissions to waterpipe tobacco emissions depends on the level of each component, the sensitivity of the equipment and the presence of components that interfere with trapping efficiency or instrument measurements.

The CFPs used for collecting the particulate phase of cigarette smoke, as described in ISO 3308 *(24)*, can collect particles with a diameter ≥ 0.3 μm with an

efficiency > 99.9%. Depending on the type of smoking machine used for smoking cigarettes (linear or rotary), ISO 4387 *(16)* notes that breakthrough of the filter pads might occur when more than 150 mg (linear) or 600 mg (rotary) particles per filter are trapped. The composition of waterpipe smoke will influence the collection of particles on the CFP. If the amount of total particulate matter from waterpipes is approximately the same as that from cigarettes, the same CFPs can be used for trapping. More research is required to determine whether the CFPs used for collecting cigarette smoke particles can also be used for collecting the particulate phase of waterpipe smoke.

The efficiency of the trapping devices to be used for collecting components of waterpipe smoke should be investigated with respect to the levels of the components in waterpipe smoke and machine smoking topography. If the trapping devices cannot collect all the components in one smoke run, methods will be required for replacing the trapping device during a run. This might include adjustment to the smoking machine by, for instance, by introduction of a multi-trapping system or by introducing pressure drop monitoring during smoking to determine when the trapping devices must be replaced. In the latter case, special precautions must be taken to prevent leakage when traps are replaced. Special CFP holders will be required, because at least two traps will have to be attached simultaneously.

If it is assumed that all the nicotine in waterpipe smoke is present in the particulate phase, the nicotine will be trapped on the CFPs. The composition of the TPM of waterpipe smoke will probably not interfere in the extraction of nicotine from the CFPs with isopropanol. The number of CFPs and the extraction volume should be further investigated to define the optimal conditions and quantifiable levels of nicotine.

When waterpipe tobacco has large amounts of flavours, they might also be present in the smoke. Further investigation is required to determine whether flavours are trapped on CFPs and thus interfere with the determination of nicotine or whether they remain in the gaseous phase of waterpipe smoke.

CO levels in waterpipe smoke are substantially higher than those in cigarette smoke *(25, 26)*. CO in cigarette smoke is collected in gas sampling bags provided by the manufacturers of smoking machines, which can hold 3 L (linear smoking machine) or 10 L (rotary smoking machine) of gas. The size of the gas collection bag should be adjusted to the machine smoking topography. Another option is to define the number of puffs to be collected in one bag. Precautions must be taken in measurement procedures because of the harmful effects of CO. To protect laboratory staff from exposure to CO, it is advisable that the waterpipe smoking machine be placed under an exhaust system and the gas collection bags be deflated in a safe environment by staff wearing personal alarm systems.

Laboratory tests show that the CO level in waterpipe tobacco emissions depends on the device used to heat waterpipe tobacco *(9)*. Almost no CO is

emitted when an electrical heating device is used. Thus, CO is produced from charcoal used to heat waterpipe tobacco and not from the tobacco itself. There is no standardized method for determining CO production and emission from charcoal.

## 5.7 Discussion

The WHO FCTC recognizes that regulation of tobacco products is required to prevent initiation and promote cessation of tobacco product use and to protect the public from secondhand exposure *(27)*. In 2003, the Scientific Advisory Committee on Tobacco Product Regulation *(28)* addressed tobacco product contents and emissions and recommended that upper limits be set for known toxic chemicals in the ingredients and emissions. Progress has been made in implementing this recommendation through collaboration between IARC and the WHO Tobacco Free Initiative. The aim of collaboration with IARC was to restrict emissions on the basis of their toxicity.

Although the yields of chemicals from machine-smoked cigarettes generated with standardized puffing regimens (ISO/FTC *(16)*, Massachusetts Benchmark *(29)*, Canadian Intense *(30)*) do not provide valid estimates of human exposure *(31)*, they do provide a framework for establishing and monitoring mandated thresholds for chemical yields. In this approach, products that exceed emission limits for the selected toxicants will not be permitted for sale. The allowable emission levels can be lowered regularly over time, and additional toxicants can be added, resulting in cigarettes with lower intrinsic toxicity.

This performance-based paradigm is particularly suitable for cigarettes, because, unlike most tobacco products, cigarettes are presented to the consumer ready to use. As a result, cigarettes have intrinsic emissions that can be measured reproducibly, generally within a variation of 15% relative to the standard deviation, by a given method *(32)*, and the responsibility for meeting regulatory performance standards rests on a clearly identifiable party: the manufacturer. These two characteristics are not applicable to the many tobacco products that are not standardized, such as bidis, roll-your-own cigarettes and waterpipes. While each component that is part of the final product is manufactured to clear specifications, the combination of components is controlled by the user or in a cottage industry under less rigid quality control. This combination of components for non-standard products involves selection and preparation of consumables and hardware. Waterpipe users select the hardware and accessories, the tobacco product, the charcoal and the aluminium foil, each of which is usually of a different origin and each of which can influence toxicant emissions, either as a source or by interacting with the other components. For example, while most of the carcinogenic PAH emissions in waterpipe smoke derive from the burning charcoal, PAHs survive the tortuous path through the waterpipe only by coalescing with particles emitted by the tobacco mixture. Thus, without

the particulate matter generated by the tobacco mixture, PAHs deposit on the interior surfaces of the waterpipe and do not exit the mouthpiece in significant quantities *(9)*. Other examples of interactions that influence toxicant emissions are the porosity of the waterpipe hose and the combustion conditions in the waterpipe head. Hose porosity, a property of the material of manufacture and construction quality, affects the amount of air passing through the charcoal and into the waterpipe head. The more porous the hose, the less air is drawn through the head, affecting both the combustion conditions in the charcoal and the heat transfer rate to the waterpipe tobacco preparation, which in turn affects both "tar" and CO emissions *(12)*. Therefore, waterpipe emissions are the net outcome of the combination of selections made by the consumer, and responsibility for meeting emission standards cannot readily be assigned to an entity that sells one or another product for use in waterpipe smoking.[7]

Furthermore, except for nicotine emissions, the smoke emitted from tobacco-free products, which are commonly advertised for health-conscious users, has essentially the same toxicant profile and biological activity as that of conventional tobacco-containing products *(33–35)*. In view of the lack of a demonstrated method for individually characterizing the emissions from the various consumables (charcoal, tobacco preparation, aluminium foil) and hardware options, setting product emission standards for regulating waterpipe products could be complicated.

Thus, a simpler approach – regulation of product contents – may be feasible, such as setting limits on ingredients that are known to result in high toxicant emissions and on harmful contaminants in waterpipe products that are not essential to their intended use (e.g. heavy metals in tobacco leaf). In accordance with the emissions standards paradigm advanced by TobReg, when systematic differences in contaminants are found in products available on the market, regulations can be promulgated to limit their concentrations to the minimum observed values. One component to which this approach could be applied immediately is waterpipe charcoal. The PAH content of waterpipe charcoal varies systematically by product type *(36)* and accounts for a significant fraction of the PAHs found in smoke. Similarly, the heavy metal content (e.g. lead, chromium, arsenic, nickel) varies systematically by tobacco preparation, so that it would be possible to require that their concentrations not exceed the lowest concentrations currently found in marketed products. Interestingly, emissions of furans and aldehydes have been found to be inversely related to the humectant content of tobacco preparations *(37, 38)*, probably because of the lower temperatures attained in the mixture when the humectant content is high.

[7] The scope of this report as commissioned by the COP does not include considering these complex interactions in developing an SOP. The scientific literature base is not yet sufficient to support development of an SOP that considers these factors

Thus, in the short term, regulation could be focused on the harmful contaminants that have been found in products marketed for waterpipe use – both tobacco and charcoal – such as inorganic metals and elements *(39, 40)*, nicotine *(41)*, TNSAs *(26)* and PAHs *(9, 36)*, as summarized in Table 5.2. In addition, the pH of the tobacco–humectant mixture may affect the fraction of total nicotine in mainstream smoke that is in the more biologically available unprotonated or "free-base" form *(14, 42)*.

Table 5.2. Candidate chemicals for regulation in tobacco and charcoal products marketed for waterpipe use

| Waterpipe sample matrix | Monitored chemical class | Target chemicals and metric |
|---|---|---|
| | Alkalinity | pH |
| | Humectants | Diethylene glycol, ethylene glycol, glycerol, propylene glycol |
| | Inorganic metals and elements | Arsenic, cadmium, chromium, cobalt, lead, mercury, nickel, selenium |
| | Nicotine | Nicotine |
| Tobacco | TSNAs | NNN, NNK, *N*-nitrosoanatabine, *N*-nitrosoanabasine |
| | Inorganic metals and elements | Arsenic, cadmium, chromium, cobalt, lead, mercury, nickel, selenium |
| Charcoal | PAHs | Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*+*k*] fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, dibenz[*a*,*h*]anthracene, indeno[1,2,3-*cd*]pyrene |

In the long term, as evidence and standardized measurement methods become available, the list of regulated waterpipe product constituents may be extended to include constituents that are found to contribute to the toxicant emissions, listed in Table 5.3.

Table 5.3. Chemicals recommended for measurement in mainstream smoke from waterpipe tobacco and charcoal brands

| Monitored chemical class | Target chemicals |
|---|---|
| Aldehydes | Acetaldehyde, acrolein, crotonaldehyde, formaldehyde |
| Aromatic amines | 1-Aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl |
| Flavours | Acetylpropionyl, diacetyl |
| Furans | 5-(Hydroxymethyl)-2-furaldehyde, 3-furan methanol, furfuryl alcohol, 2-furoic acid, 2-furaldehyde, 3-furaldehyde, 2-furyl methyl ketone, 5-methyl-2-furaldehyde, methyl-2-furoate |
| Humectants | Diethylene glycol, ethylene glycol, glycerol, propylene glycol |
| Inorganic metals and elements | Arsenic, cadmium, chromium, cobalt, lead, mercury, nickel, selenium |
| Nicotine | Nicotine |
| PAHs | Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*+*k*]fluoranthene, benzo[*a*] pyrene, benzo[*ghi*]perylene, dibenz[*a*,*h*]anthracene, indeno[1,2,3-*cd*]pyrene |
| Phenols | Catechol, *m*-cresol, *o*-cresol, *p*-cresol, phenol |
| TSNAs | NNN, NNK, *N*-nitrosoanatabine, *N*-nitrosoanabasine |
| VOCs | Acrylonitrile, benzene, 1,3-butadiene, CO, isoprene |

## 5.8 Conclusions and recommendations

The body of evidence on the toxicity, addictiveness and appeal of waterpipe tobacco smoke indicates the need for urgent public health intervention (*17*). Measurement of toxicant yields in mainstream waterpipe tobacco smoke is, however, in its infancy, and there are no standardized methods for waterpipe smoke analysis that could be used as a basis for regulating emissions. Given the complexity of the interactions among the waterpipe, accessories, tobacco, heat source and human puffing behaviour and the myriad products available, a product regulation approach that focuses on measuring and reporting the content of chemicals known to contribute to the toxicity, addictiveness and appeal of waterpipe tobacco smoking might be more effective than regulating emissions from various combinations of heating source, tobacco product, puff topography and waterpipe design.

The data reviewed in the previous sections lead to the following conclusions.

- Waterpipe puff topography is characterized by a much larger puff volume, flow rate and puff number than cigarette smoking.
- Machine-generated waterpipe toxicant emissions are sensitive to puff topography.
- Waterpipe-specific smoking machines are required to test emissions. One such machine is commercially available.
- Toxicant emissions do not depend only on a particular waterpipe, charcoal or tobacco product but rather on combinations of these variables and puff topography.
- Standard TobLabNet operating procedures for measuring the contents and emissions of cigarette tobacco products would have to be modified for use to test waterpipe products.
- Standard TobLabNet operating procedures are not suitable for measuring charcoal constituents.
- For research purposes, the Beirut method can be used to generate waterpipe smoke.

### 5.8.1 Recommendations for regulators

1. Regulations should focus primarily on the chemical composition of waterpipe tobacco products and charcoal.
2. Standard TobLabNet operating procedures should be adapted for the measurement of nicotine, TSNAs and humectants in the contents of waterpipe tobacco products.

3. Analytical methods should be adapted to determine the pH and the heavy metal content of waterpipe tobacco (and tobacco-free) products.
4. Analytical methods should be adapted for measuring metals and PAHs in emissions from waterpipes heated with charcoal products.
5. The priority of regulation should be to reduce the levels of TSNAs, PAHs and heavy metals in waterpipe products, in accordance with the approach recommended by TobReg *(43)*. The list of regulated constituents should evolve as knowledge becomes available on toxicant emissions and/or health effects.

### 5.8.2 Recommendation for researchers

1. The effects on toxicant emissions of waterpipe tobacco product composition, charcoal composition, puff regimen, waterpipe design and waterpipe use conditions should be elucidated to facilitate product regulation.

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# 6. Toxic contents and emissions of smokeless tobacco products

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**Contents**



## 6.1 Introduction

This report was prepared in response to the request made by the COP at its sixth session (Moscow, Russian Federation, 13–18 October 2014) to the Convention Secretariat, to invite WHO to prepare a report on the toxic contents and emissions of smokeless tobacco products.

Smokeless tobacco globally consists of a diverse array of manufactured products (moist snuff, dry snuff, dissolvables, *gutkha*, *khaini*, *snus*, chewing tobacco, *zarda*) and hand-made preparations (betel quid, *dohra*, *tombol*, *toombak*, *iq'mik*) (Table 6.1). Most smokeless tobaccos are used orally, although some drier products are used nasally. Oral smokeless tobacco products and preparations can be chewed, sucked, held against oral mucosa ("dipped") or applied to the teeth and gums. Addictive and toxic chemicals are liberated from the products during use, absorbed across the mucosa *(1)* and enter the bloodstream *(2, 3)*.

Smokeless tobacco use causes cancer *(4)*. The adverse health consequences of smokeless tobacco use were reviewed recently *(5)*.

Table 6.1. Types of smokeless tobacco products used globally

| Product | WHO region | | | | | |
|---|---|---|---|---|---|---|
| | African | Americas | Eastern Mediterranean | European | South-East Asian | Western Pacific |
| *Afzal* (Oman) | | | √ | | | |
| Betel quid (*paan*) | | | √ | | √ | √ |
| Caffeinated moist snuff | | √ | | | | |
| *Chimó* | | √ | | | | |
| Creamy snuff | | | | | √ | |
| Dissolvables | | √ | | | | √ |
| *Dohra* | | | | | √ | |
| Dry snuff | √ | √ | | √ | | |
| Ghana traditional snuff (*tawa*) | √ | | | | | |
| *Gudakhu* or *gudakha* | | | | | √ | |
| *Gul* | | | | | √ | |
| *Gundi* (*kadapan*) | | | | | √ | |
| *Gutka* | | | √ | | √ | |
| *Hnat hsey* | | | | | √ | |
| *Hogesoppu* (leaf tobacco) | | | | | √ | |
| *Iq'mik* | | √ | | | | |
| *Kadapan* | | | | | √ | |
| *Kaddipudi* | | | | | √ | |
| *Khaini* | | | | | √ | |
| *Kharra* | | | | | √ | |
| *Kiwam* (*qiwam*, *kimam*) | | | √ | | √ | |
| *Kuberi* | √ | | | | | |
| Loose leaf | | √ | | | | |
| *Mainpuri* (*kapoori*) | | | | | √ | |
| *Mawa* | | | | | √ | |
| *Mishri* (*masheri*, *misri*) | | | | | √ | |
| Moist snuff | √ | √ | | | | |
| *Nass* (*naswar*) | √ | | √ | √ | | |
| *Nasway* (*nasvay*) | | | √ | √ | √ | |
| *Neffa* | √ | | √ | √ | | |
| Nicotine chewing gum | | | | | | √ |
| Nigerian traditional snuff (*taaba*) | √ | | | | | |
| *NuNu* | | √ | | | | |
| *Pattiwalla* without lime | | | | | √ | |
| Plug (chewing tobacco) | | √ | | √ | | |
| *Rapé* | | √ | | | | |
| Red toothpowder (*lal dant manjan*) | | | | | √ | |
| *Sada pata* | | | | | √ | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Shammah* | √ | | | √ | | | |
| *Snus* | √ | √ | | | √ | | |
| *Surti* | | | | | | √ | |
| *Taaba* | √ | | | | | | |
| *Tapkeer* (*bajjar*, dry snuff) | | | | | | √ | |
| *Thinso* | √ | | | | | | |
| Tobacco leaf | √ | | | | | √ | |
| Tobacco water (*tuiber*) | | | | | | √ | |
| *Tombol* | | | | √ | | | |
| *Tombol* with *khat* | | | | √ | | | |
| *Toombak* | | | | √ | | | |
| Traditional South African snuff (*snuif*) | √ | | | | | | |
| *Tumbaco* | √ | | | | | | |
| Twist | | √ | | | | | |
| *Ugoro* | √ | | | | | | |
| *Zarda* | | | | √ | | √ | |

### 6.1.1 Global prevalence

It is estimated that more than 300 million people in the six WHO regions use some form of smokeless tobacco *(5)*. Adult use is highly prevalent in countries from Kazakhstan to the Lao People's Democratic Republic. The prevalence is also high in certain Pacific Islands, Norway, Sweden and other parts of western Europe, several African countries, Mongolia, South America and the USA *(6, 7)*. Globally, 89% of all use by adults is in South-East Asia (mainly Bangladesh and India), where 268 million adults use smokeless tobacco products *(5)*.

Smokeless tobacco use represents a substantial global health problem, with an estimated 1.7 million disability-adjusted life years (DALYs) lost due to cancers related to smokeless tobacco use *(8)*. In India, where the prevalence of smokeless tobacco use is high, an estimated 368 000 deaths are attributable to smokeless tobacco use among nonsmokers *(9)*. Globally, 652 494 deaths are estimated to be due to smokeless tobacco use *(10)*.

### 6.1.2 Diversity in the manufacture and physical properties of smokeless tobacco products

Smokeless tobacco products differ in appearance, scale of production, ingredients and formulation *(4, 5, 11, 12)*. They include products that are manufactured commercially and those that are made in traditional environments, such as homes, shops, market stalls and street vending sites. The products range from those containing only tobacco to elaborate hand-made preparations that consist of tobacco mixed with a wide spectrum of non-tobacco plant materials and chemicals. The products come in various forms, including entire tobacco leaves, finely cut tobacco, pulverized tobacco powder, pressed cakes, pellets, pastes, tars

and mixtures of tobacco with chemicals and plant materials *(4, 5, 11, 12)*. Ground, loose tobacco may be enclosed in teabag-like pouches for discreet, convenient use (e.g. *snus* and moist snuff). Products known as "dissolvables" consist of finely ground tobacco pressed into tablets, thin cylindrical rods (sticks) or thin wafers or strips that dissolve in the mouth when used *(13)*. Tobacco sticks are essentially dry snuff coated onto a toothpick that can be sucked to liberate the contents *(4, 11, 12, 14)*. A new product, Verve®, is a flavoured cellulose polymer disc impregnated with nicotine extracted from tobacco, which boosts blood nicotine concentrations when chewed, is physiologically active (i.e. raises heart rate and blood pressure) and reportedly satisfies the nicotine cravings of some users *(15)*.

## 6.2 Product composition

### 6.2.1 Tobacco

Tobacco (*Nicotiana* spp.) of one or more species is used in the manufacture of most smokeless tobaccos. Exceptions are products like Verve® that contain nicotine extracted from tobacco but no ground or loose tobacco. Although numerous *Nicotiana* species exist worldwide, *N. tabacum* is that most often used in commercially manufactured products, whereas *N. rustica,* which has higher concentrations of nicotine, minor alkaloids and TSNAs than *N. tabacum*, is commonly used in products in Africa, the Middle East, South America and South Asia *(4, 16)*. For example, in India, an estimated 35–40% of smokeless tobacco products contain *N. rustica (17, 18)*. Infrared analysis confirmed the presence of *N. rustica* in products sold in several countries, such as *gul* and some forms of *toombak*, *zarda* and *rapé (19, 20)*. *Toombak* and *gul* may also contain another tobacco species, *N. glauca (4, 21)*, which has no nicotine but contains a high level of *N*-nitrosoanabasine *(22)*. Despite the absence of nicotine, *N. glauca* is consider highly toxic, and ingestion of this species has been lethal in some cases *(17, 22)*. Use of high-nicotine tobacco (*N. rustica*) or a more toxic species (*N. glauca*) should be strongly discouraged.

### 6.2.2 Additives

In addition to tobacco, smokeless tobacco products often contain sweeteners, humectants, flavourings, salt and alkaline agents. In 1994, 10 manufacturers of smokeless tobacco products in the USA released a list of more than 560 additives used in the manufacture of their products *(4)*.

In products made by hand or in "cottage industries", it is common to mix tobacco with other plant materials. In South Asia, smokeless tobacco preparations such as *paan* (betel quid) and *dohra* contain tobacco, areca nut (*Areca catechu*), alkaline agents, catechu (*Acacia catechu*) and spices (e.g. ginger, clove, camphor, saffron) and may be wrapped in a betel leaf (*Piper betle*). Areca nut is also used

in *mainpuri*, *mawa*, *guthka*, *kharra* and some forms of *zarda* (in South Asia), *tombol* (in the Middle East) and *thinso* (in Africa) *(5, 10)*. In Yemen, some types of *tombol* are made by wrapping a mixture of tobacco and the psychoactive plant *khat* (*Catha edulis*) in a betel leaf *(5, 17)*. A smokeless tobacco product in South America, called *rapé*, can contain a considerable amount of tonka bean (*Dipteryx odorata*), which has high coumarin levels and is on the list of "harmful and potentially harmful constituents in tobacco products and tobacco smoke" of the Food and Drug Administration in the USA and is banned for use in food *(24)*. Other non-tobacco plant materials include coriander seeds, aniseed, musk, black pepper, vanilla, garlic, mustard, turmeric and ginseng *(5)*.

The sweeteners added include simple sugars, molasses, honey and xylitol. Commercial products like loose leaf tobacco and *gutkha* and cottage industry products such as *gul* are manufactured with sweeteners *(4)*. An early study of smokeless tobaccos sold in the USA *(25)* found that the sugar content of pouch and plug forms of tobacco (13.5–65.7%) was much higher than that of snuff (1.9%), and the sugar content of pouch and plug smokeless tobaccos was higher than that of pipe, cigarette or cigar tobacco.

Humectants, usually propylene glycol and glycerol, are added to maintain moisture. Research on loose-leaf chewing tobacco products at North Carolina State University in the USA revealed glycerol concentrations of 3.2% (CRP4) and 3.75% (STRP 1S1) and 3.0% propylene glycol (CRP1, *snus*) *(26)*. In *snus* manufactured to GothiaTek® standards (described in section 6.4), humectants are added at 1.5–3.5% *(27)* to reduce microbial growth in order to prevent the formation of TSNAs *(28)*.

Flavourings include individual flavour compounds, fruit juices, cocoa, rum, spice powders, extracts and more than 60 essential oils *(11, 29, 30)*. In a survey of the chemistry of smokeless tobacco products, methyl salicylate, ethyl salicylate, benzaldehyde, citronellol and menthol were the flavours found most frequently *(31)*. Other researchers have detected methyl salicylate, ethyl salicylate and menthol in moist snuff products with wintergreen and mint flavouring *(32)*. Further ingredients may include caffeine, coconut, liquorice, herbal medicines, vegetable dyes, colourings, edible oils, butter, soil, saltpetre (potassium nitrate) and flecks of silver metal. Dissolvable smokeless tobaccos may also contain adhesives, binders and whiteners *(5*, Appendix 1*)*.

Alkaline agents added to manufactured smokeless tobacco products include carbonates, bicarbonates and slaked lime (calcium hydroxide) *(5, 12, 29)*, whereas cottage industry products (*toombak*, *shammah*) and hand-made preparations (*iq'mik*, *nass*, betel quid) generally include slaked lime, sodium bicarbonate or ashes from certain plants or fungi *(4, 33, 34)*. *Iq'mik*, a product used by native populations of the North American Arctic contains tobacco in twist or leaf form mixed with fungus or ash *(35)*.

## 6.3 Emissions from smokeless tobacco products

### 6.3.1 Nicotine

Nicotine, the principal addictive chemical in tobacco, is present at a wide range of concentrations in smokeless tobacco products and plays a key role in repetitive use, resulting in continuous exposure to toxicants and carcinogens. Total nicotine – the entire amount of nicotine in a product, regardless of its ionic form – is an important consideration, but pH also plays a role in nicotine chemistry. In unprocessed tobacco, which is usually acidic (pH 5.0–6.5) *(36)*, very little nicotine is present in the un-ionized form (< 5%). Un-ionized nicotine, which is readily absorbed, is also called "un-protonated" or "free" nicotine. Oral absorption of nicotine usually requires added alkaline agents to raise the pH and convert a sufficient percentage of nicotine into free nicotine *(5)*.

Products with similar total nicotine content but different pH have widely different concentrations of free nicotine (*5*). Free nicotine, which increases as the pH rises, is readily released from tobacco and crosses biological membranes. Thus, alkaline agents play a key role in releasing nicotine, contributing (in conjunction with the total nicotine of a product) to higher blood nicotine concentrations, which are thought to contribute to the addictiveness of smokeless tobacco *(2–4, 37)*. Nicotine itself is toxic and has health effects, causing, e.g. cardiovascular diseases and diabetes; therefore, increasing its absorption by means of alkaline agents makes the products more addictive and potentially more toxic.

The pH values reported for smokeless tobacco products range from 4.6 to 11.8, which result in 0.02–99.9% of nicotine in the free form. *Iq'mik* and *nass*, which contain alkaline ash, have extremely high pH (11.0–11.8) *(38, 39)*. *Gul* powder, *naswar*, *khaini*, South African dry snuff *(19)* and *afzal* (in Oman) *(40)* also have high pH values (9–10.5). A survey of *zarda* products showed alkaline pH values of 8.1–9.0 (*41*). Other smokeless tobacco products, such as *toombak*, *chimó*, *rapé* and *snus*, range from acidic to very alkaline *(5, 19)*. Chewing tobaccos (twist, chew, plug and loose leaf) are generally acidic (pH < 7) *(42)*, and the pH of moist snuff generally ranges from 5.5 to 8.6 *(43, 44)*.

The total nicotine concentration (on a wet weight basis) in about 700 products ranged from 0.39 to 95 mg/g. The best-characterized smokeless tobacco product is moist snuff made in the USA (226 products). In these products, the total nicotine concentration ranged from 4.15 to 25.0 mg/g and that of free nicotine from 0.01 to 15.2 mg/g *(43, 44)*. The total nicotine concentration in less commonly used chewing tobaccos (twist, chew, plug and loose leaf) was 2.92–40.1 mg/g, but they contained less free nicotine (0.01–0.47 mg/g). The total nicotine concentrations in dry snuff products made in the USA, which are acidic to mildly basic, ranged from 0.30 to 28.0 mg/g and those of free nicotine from 0.05 to 3.12 mg/g *(42)*. Conversely, manufactured dry snuff in South Africa had lower

total nicotine concentrations (1.17–14.9 mg/g) but more free nicotine (1.16–13.8 mg/g) because of higher alkalinity *(19)*.

Although very limited data were available, the pH of Nigerian traditional and medicated snuff and South Africa traditional snuff was 9.0–9.5, and the concentrations of total nicotine (2.49–7.41 mg/g) and free nicotine (2.39–6.72 mg/g) were similar. One very alkaline product (pH 10.5) from Oman called *azfal* had very high total nicotine (48.8 mg/g) and free nicotine (48.6 mg/g) concentrations *(40)*. *Snus* products purchased in South Africa were mildly acidic and had moderate total nicotine concentrations (13.4–17.2 mg/g) but less free nicotine (0.47–1.19 mg/g) *(18)*.

Swedish *snus* products had a wide range of concentrations of total nicotine (6.83–20.6 mg/g) and free-base nicotine (0.71–15.5 mg/g) *(45)*, some of which were higher than those reported in moist snuff products in the USA *(44)*. Moderate concentrations of total nicotine (3.0–20.5 mg/g) and free nicotine (0.37–2.47 mg/g) were found in 124 dissolvable products *(46)*. A product similar to dissolvables, called Verve®, had low total nicotine (1.68 mg/g) and free nicotine (0.37 mg/g) concentrations *(15)*. Sudanese *toombak*, which contains the tobacco species *N. rustica*, had the highest reported concentration of total nicotine (95 mg/g) *(47)*.

Smokeless tobacco products in South Asia include red toothpowder, glycerine-based creamy snuff (both used as a dentifrice), *gutkha* and *zarda*. It is common in South-East Asia to mix tobacco with *supari* packets, which can include areca nut, spices, sweeteners and alkaline agents. Gupta and Sankar *(48)* found that red tooth powder is mildly acidic, with concentrations of total nicotine of 4.47–5.09 mg/g and of free nicotine of 0.03–0.23 mg/g, whereas creamy snuff, which is more alkaline, had higher concentrations of total nicotine (5.62–10.0 mg/g) and free nicotine (0.71–3.39 mg/g). They also found that *gutkha* is alkaline (pH 8.6–9.2), with a total nicotine concentration of 0.71–3.39 mg/g and free nicotine at 0.03–0.25 mg/g. *Zarda* products in India were slightly acidic and had total nicotine concentrations of 2.61–9.5 mg/g but very little free nicotine (0.01–0.02 mg/g). *Zarda* products in Pakistan were more alkaline and had higher total nicotine concentrations (7.35–26.7 mg/g) and free nicotine (5.52–21.4 mg/g) *(41)*.

As some products are intentionally combined with alkaline agents before use, the pH and free nicotine concentrations may be higher in the resulting smokeless tobacco preparation. Gupta and Sankar *(48)* reported that five mixtures of tobacco with *supari* were alkaline (pH 8.6–10.1) and had total nicotine concentrations of 1.77–4.96 mg/g and free nicotine of 1.56–4.06 mg/g. Alkaline agents may be added to hand-made preparations (e.g. betel quid) to suit the user's preference for a certain product "strength".

### 6.3.2 Toxic and carcinogenic agents

Because of the presence of cancer-causing agents in smokeless tobacco, it has been classified in IARC Group 1 (known human carcinogen) *(4)*. More than 40 compounds or agents that have been identified as carcinogens by working groups convened by the IARC *(4, 11)* have been found in smokeless tobacco products *(5)*, including reactive inorganic ions (nitrate and nitrite), TSNAs, *N*-nitrosamino acids, volatile *N*-nitrosamines, mycotoxins, PAHs, volatile aldehydes, metals and metalloids and areca nut. The most abundant carcinogens in smokeless tobacco are TSNAs, *N*-nitrosoamino acids, volatile *N*-nitrosamines and aldehydes *(4)*. The groups concluded that there is sufficient evidence that use of smokeless tobacco causes precancerous oral lesions and also oral, oesophageal and pancreatic cancers *(5)*.

#### 6.3.2.1 Tobacco-specific nitrosamines

TSNAs are formed during the curing, processing, fermentation and combustion of tobacco *(49, 50)*. In most tobaccos, the concentrations of NNN exceed those of NNK, except in bright tobacco, where those of NNK exceed those of NNN *(51)*. Consequently, the blend of the tobacco determines the amounts of NNN and NNK. Of the seven known TSNAs, NNN and NNK generally occur in larger quantities in tobacco products and are clearly the most carcinogenic *(52)*. NNN and NNK are classified as Group 1 human carcinogens *(4)* and are quantitatively the most prevalent "strong" carcinogens in smokeless tobacco *(53)*. NNN in particular is thought to play a role in oral cancer in smokeless tobacco users and has been found to occur at levels as high as 79 µg/g *(4, 53, 54)*. Table 6.2 provides a summary of the concentrations of TSNAs in commercial and hand-made smokeless tobacco products in various regions of the world.

Table 6.2. Concentrations of tobacco-specific nitrosamines in commercial and hand-made smokeless tobacco products

| | | Concentration (µg/g product wet weight) | | |
|---|---|---|---|---|
| **Product** | **Reference** | **NNK** | **NNN** | **All TSNAs** |
| *Toombak* | *47* | 578–7300 | 395–2860 | 1500–12 630 |
| *Toombak* | *19* | 147–516 | 115–368 | 295–992 |
| Snuff | | | | |
| Moist snuff | *44* | 0.38–9.95 | 2.20–42.6 | 5.11–90.0 |
| Dry snuff | *42* | 1.34–14.6 | 6.12–31.3 | 10.3–76.5 |
| Dry snuff (pouch) | *42* | 0.08–0.12 | 0.93–0.97 | 1.52–1.85 |
| Chewing tobacco | | | | |
| Plug | *42* | 0.34–0.94 | 2.92–4.64 | 4.09–7.75 |
| Loose leaf | *42* | 0.24–0.31 | 0.94–2.83 | 1.55–4.10 |
| Twist | *42* | 0.31–0.56 | 0.83–2.46 | 2.59–4.95 |
| *Snus* | *19, 42* | 0.084–1.34 | 0.27–5.57 | 0.60–5.85 |
| Dissolvables | *42, 46* | 0.31 | 0.06–0.26 | 0.31–0.74 |

| | | | | |
|---|---|---|---|---|
| Products in the Americas | | | | |
| *Iq'mik* | *39* | 0.19–0.54 | 1.99–4.00 | 5.64–8.84 |
| *Rapé* | *20* | 0.04–3.30 | 0.013–14.5 | 0.04–24.2 |
| *Chimó* | *19* | 0.31–2.60 | 0.32–4.62 | 0.95–9.39 |
| South Asian products | | | | |
| *Gul* | *19* | 5.19–8.02 | 1.33–1.37 | 13.4–17.1 |
| *Khaini* | *19* | 0.29–0.50 | 16.8–17.5 | 21.6–23.5 |
| *Zarda* | *19* | 0.46–3.84 | 2.91–28.6 | 5.49–53.7 |
| *Gutha* (handmade) | *19* | 0.007–0.38 | 0.21–18.6 | 0.26–23.9 |
| *Gutkha* | *19* | 0.057–0.46 | 0.17–1.28 | 0.37–2.25 |
| Central Asian products | | | | |
| *Naswar* | *19* | 0.029–0.31 | 0.36–0.54 | 0.48–1.38 |
| African products | | | | |
| Nigerian traditional snuff | *19* | 0.28 | 0.71 | 1.52 |
| Medicated dry snuff | *19* | 0.36 | 1.46 | 2.42 |
| Dry snuff | *19* | 0.13–0.35 | 0.89–3.40 | 1.71–4.67 |
| Traditional snuff | *19* | 1.61 | 5.57 | 20.5 |

Source: reference *4*

Smokeless products with higher TSNA concentration tend to be those with microbial contamination. The TSNA levels in products such as dissolvables (0.31–0.61 μg/g), which are solid, low-moisture products (46), and Swedish snus (0.60–5.85 μg/g), which is often pasteurized (19,42,55) are usually lower than typical products. Higher TSNA concentrations are found in fermented products such as Indian *zarda* (5.5–53.7 μg/g) *(19)*, moist snuff (5.11–90.0 μg/g) *(44)* and dry snuff made in the USA (10.3–76.5 μg/g) *(42)*. Traditional snuffs in Nigeria and South Africa had total TSNA concentrations of 1.52 and 20.5 μg/g, respectively *(19)*. Interestingly, dry snuff in Africa had lower TSNA concentrations (1.71–4.67 μg/g) than that made in the USA. Other products with high total TSNA concentrations include *khiani*, *naswar*, *iq'mik*, *rapé* and *chimó*. Chewing tobacco has very low TSNA concentrations (1.55–7.75 μg/g) (*42*).

In best-selling brands of moist snuff in the USA, the concentrations of NNN (2.2–42.6 μg/g) were higher than those of NNK (0.38–9.95 μg/g) (*44*). The TSNA concentrations in Sweden-made *snus* decreased by approximately 85% between 1983 and 2002, to very low average concentrations of NNN (0.49 μg/g) and NNK (0.19 μg/g) in 27 products in 2002 (*56*, *57*), which are among the lowest reported in commercial smokeless tobacco products. A product known as *chaini khaini,* labelled and marketed in India as "snus", had very high levels of NNN ((22.9 ± 4.9 μg/g) and NNK (2.6 ± 1.0 μg/g) (*58*).

In a study of 117 "spit-free" and dissolvable smokeless tobacco products, the concentration of total TSNA (the sum of NNN, NNK, *N*-nitrosoanatabine and *N*-nitrosoanabasine) was slightly lower in Camel Strips (0.53 μg/g) than in Camel Snus (1.19 μg/g) *(46)*. In a study of 53 products from nine countries *(19)*, the highest NNK concentrations were found in *toombak* from Sudan and dry

*zarda* from Bangladesh, whereas the highest NNN concentrations were found in *toombak*, dry *zarda* and *khaini* from India. Handmade *gutkha* and *mawa* from Pakistan had the lowest NNK concentrations among these products.

The highest TSNA concentrations ever reported in smokeless tobacco products were in Sudanese *toombak*, a highly fermented product, with total TSNA concentrations reaching 12 600 µg/g, perhaps due to the extremely high concentrations of alkaloids, which are important reactants in TSNA formation. The NNN concentrations in *toombak* were as high as 2860 µg/g and those of NNK were up to 7300 µg/g *(47)*. TSNAs were also found at extremely high concentrations in saliva from *toombak* users *(47, 59, 60)*. Over 50% of oral cancers in Sudanese men are attributed to use of *toombak* or other oral tobacco products, probably due to the high concentrations and carcinogenicity of TSNAs *(10, 60, 61)*.

#### 6.3.2.2 Volatile nitrosamines

Accumulation of nitrite is thought to lead to formation of carcinogenic volatile *N*-nitrosamines during curing through the same microbial reactions that lead to formation of TSNAs *(5)*. Analysis of Swedish snuff and chewing tobacco in the early 1980s demonstrated the presence of volatile *N*-nitrosamines (*N*-nitrosdimethylamine, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine and *N*-nitrosomorpholine) at levels ranging from 0.5 to 145.9 µg/kg wet weight *(56)*. A reduction in the use of the agricultural chemical maleic hydrazide diethanolamine and of the manufacturing chemical morpholine have reduced the levels of *N*-nitrosodiethanolamine and *N*-nitrosmorpholine in commercial tobacco products *(62)*. *Nass* (also called *nasswar*), a mixture of tobacco, alkaline agents and cotton oil used in Afghanistan, India, the Islamic Republic of Iran, Pakistan, the Russian Federation and Central Asia *(63)* was also found to contain volatile *N*-nitrosamines but at lower levels than in chewing tobacco or snuff. The difference in levels of volatile *N*-nitrosamine has been attributed to shorter ageing in *nass* manufacture *(64)*.

#### 6.3.2.3 Volatile aldehydes

Carcinogenic aldehydes (formaldehyde, acrolein, crotonaldehyde, acetaldehyde) have been shown to be present at levels of parts per million in smokeless tobaccos, including *snus* products. The levels tend to be higher in fire-cured tobacco than in air-cured tobacco *(5, 55)*.

#### 6.3.2.4 Polycyclic aromatic hydrocarbons

PAHs may be present in smokeless tobaccos that contain tobacco cured with wood and sawdust burnt during fire-curing, and the concentrations are higher

in fire-cured than air-cured tobacco *(5)*. Moist snuff produced with fire-cured tobacco has a higher concentration of PAHs (including IARC Group 1 and 2 carcinogens) than *snus*, which does not contain fire-cured tobacco *(55, 65)*. Ten PAHs in IARC groups 1 (benzo[*a*]pyrene), 2A (dibenz[*a*,*h*]anthracene) and 2B (benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenzo[*a*,*i*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, naphthalene and benz[*a*]anthracene) *(66)* have been found in smokeless products *(65)*.

The total concentration of PAHs in 23 products made in the USA ranged from 921 to 9070 ng/g in moist snuff and 660 to 1100 ng/g in *snus*. The concentrations of benzo[*a*]pyrene in moist snuff (9.7–44.6 ng/g) were higher than those in *snus* (3.0–12.3 ng/g), and about 40% of the *snus* brands analysed had levels below the detectable limit (1.6 ng/g). The concentrations of naphthalene in moist snuff (409–1110 ng/g) were similar to those in *snus* (636–1065 ng/g). When the values for naphthalene were excluded from the total PAH concentration, those of the remaining PAHs in moist snuff (145–8120 ng/g) exceeded those in *snus* (21–213 ng/g). One brand, often viewed as a "starter", contained only 145 ng/g of PAHs other than naphthalene (776 ng/g). Marlboro *snus* products contained seven PAHs at detectable levels of 1.1–13.5 ng/g individually; when naphthalene was excluded, the summed concentration of PAHs was 20–70 ng/g. Camel *snus* brands contained detectable levels of 14 PAHs at 3.1–79.4 ng/g and 110–320 ng/g when naphthalene was excluded *(65)*. Very low PAH concentrations can be attained when fire-cured tobacco content is decreased or eliminated from smokeless tobaccos.

#### 6.3.2.5 Areca nut

Unripe areca nuts have extremely high alkaloid levels, and they are preferred in certain cultures because they "generate a better buzz" *(5)*. IARC working groups have placed areca nut in Group 1 *(66)*. Arecoline is thought to be the most important alkaloid. Extracts of areca nut are highly cytotoxic and genotoxic, including to human oral mucosal cells and fibroblasts. Betel quid alone, not mixed with tobacco, has also been shown to be genotoxic *(5)* and carcinogenic *(11)*.

#### 6.3.2.6 Metals

Metals and metalloids may accumulate in tobacco plants or on leaf surfaces, depending on the soil composition, pH and environmental contamination *(67)*. Metals found in various smokeless tobacco products include some in IARC Group 1 (human) carcinogens (arsenic, beryllium, chromium VI, cadmium, polonium-210) and also Group 2A probable carcinogens (nickel compounds) and Group 2B possible carcinogens (lead, cobalt). Arsenic, which is technically a metalloid, is a Group 1 human carcinogen. Mercury and aluminium have also been detected. Detectable concentrations of arsenic (0.1–14.0 μg/g), beryllium

(0.01–0.038 μg/g), chromium (0.71–54.0 μg/g), cadmium (0.25–9.2 μg/g), nickel (0.84–64.8 μg/g), lead (0.23–111 μg/g) and cobalt (0.056–1.22 μg/g) have been found in smokeless tobacco products from Canada, Ghana, India, Pakistan and the USA *(67)*. In a study of smokeless tobacco products from India (*zarda*, creamy snuff, *khaini*, *gutkha*), higher concentrations of copper were found in four *gutka* products (237–656 μg/g) than in the other products (0.012–36.1 μg/g) *(68)*. Arsenic, cadmium and lead were found in components such as slaked lime, betel leaves and flavoured tobacco (*zarda*) used to make betel quid *(69)*.

#### 6.3.2.7 Nitrate and nitrite

Plants take up fertilizer-derived nitrate from soil, which is used by plant cells. When tobacco dries during curing, the cells rupture, releasing nitrate *(70–72)*. Microbes are present as endophytes in plants *(73)*. If viable nitrate-reducing microorganisms are present, nitrite is produced and released. Several genera of bacteria and fungi identified in tobacco and tobacco products *(71, 74, 75)* can convert nitrate to nitrite. Nitrite released by microbes can react with tobacco alkaloids to form TSNAs and can also contribute to the formation of volatile nitrosamines and nitrosamino acids *(70, 76)*. Nitrite and TSNA concentrations increase during tobacco fermentation *(71, 72)* and tobacco storage, especially at elevated temperature and moisture *(77)*. If nitrate-reducing microorganisms are not eliminated during processing, they can affect the chemistry of tobacco products *(70–72)*.

### 6.3.3 Microbes and their constituents

Microorganisms such as bacteria and fungi are often present in tobacco and tobacco products (*71, 75, 78, 79*). In studies with microbial DNA sequencing methods *(75, 80)*, 33 bacterial families were identified in various smokeless tobacco products. Genes for respiratory nitrate reductases and, to a lesser extent, periplasmic nitrate reductases were predicted to be involved in the production and extracellular release of nitrite. Some bacterial families include known anaerobes, which use nitrate as an electron acceptor instead of oxygen *(81)*, which may account for the accumulation of extracellular nitrite in the conditions of low oxygen that are likely to occur in processes such as fermentation, ageing and storage of tobacco *(71, 72, 77)*. Bacterial genera that contain genes for respiratory nitrate reductases include *Corynebacterium*, *Lactobacillus* and *Staphylococcus* species and certain bacteria in the *Enterobacteriaceae* family *(75)*.

Bacteria and fungi may proliferate more rapidly and form harmful or reactive by-products during tobacco fermentation *(70–72)*. Accordingly, the concentrations of both nitrite and TSNAs are higher in fermented products such as *khaini (82),* dry snuff *(82)*, moist snuff *(65)* and Sudanese *toombak (19, 47)* than in products such as *snus*, which is pasteurized *(19, 46)*.

A few fungal species (e.g. *Fusarium*, *Alternaria* and *Candida*) have also been identified in tobacco and tobacco products *(71, 78, 79, 83)*. Aflatoxin B1, a mycotoxin produced by *Aspergillus* fungi, was reported in six dry snuff products made in the USA (0.01–0.27 µg/g) but not in 16 moist snuff or 3 *snus* products *(84)*.

## 6.4 Reducing the concentrations of toxicants in smokeless tobacco products

Reduction of the concentrations of toxicants in tobacco products requires understanding of the agricultural practices and manufacturing processes that result in their formation and accumulation. Table 6.3 lists the toxic and carcinogenic substances found in tobacco and their potential sources during tobacco processing.

Table 6.3. Possible sources of IARC carcinogens, toxicants and biologically active compounds in smokeless tobacco products

| Agent class | IARC carcinogens (groups 1, 2A, 2B), toxicants or biologically active compounds | Possible source |
|---|---|---|
| Metals and metalloids | Group 1: Arsenic, beryllium, cadmium, nickel compounds, polonium-210<br>Group 2A: Inorganic lead compounds<br>Group 2B: Cobalt sensitization: aluminum, chromium, cobalt, nickel<br>Dermal irritants: barium, mercury<br>May contribute to oral submucosal fibrosis: copper (in areca nut) | Soil absorption or present in soil particles deposited on tobacco; potentially present in other ingredients (betel leaf, areca nut, slaked lime, etc.) used in conjunction with tobacco |
| Nitrosation agents | Group 2B: Nitrate<br>Group 2B: Nitrite | Soil absorption<br>Generated by microorganisms |
| Mycotoxins | Group 1: Aflatoxins (mixtures of)<br>Group 2B: Aflatoxin M1, ochratoxin A | Formed by fungi (*Aspergillus)* |
| Nitrosamines<br>TSNAs | Group 1: NNN, NNK, NNAL | Formed by nitrosation during curing, fermentation and ageing (nitrite reacts with alkaloids) |
| Volatile *N*′-nitrosoamines | Group 2A: *N*-Nitrosodimethylamine<br>Group 2B: *N*-Nitrosopyrrolidine, *N*-nitrosopiperidine, *N*-nitrosomorpholine, *N*-nitrosodiethanolamine | Formed by nitrosation during curing, fermentation and ageing (nitrite reacts with secondary and tertiary amines) |
| Nitrosoacids | Group 2B: *N*-Nitrososarcosine | |
| Carbamates | Group 2A: Ethyl carbamate | Formed during fermentation (reaction of urea and ethanol) |
| PAHs | Group 1: Benzo[*a*]pyrene<br>Group 2A: Dibenz[*a*,*h*]anthracene<br>Group 2B: Benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*i*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, naphthalene | Deposited on tobacco during fire-curing |
| Volatile aldehydes | Group 1: Formaldehyde<br>Group 2B: Acetaldehyde | Deposited on tobacco during fire curing |
| Non-tobacco plant materials | Group 1: Areca nut<br>Liver toxicant: Tonka bean<br>Stimulant: *Khat* | Additives |

Source: reference 85

During cultivation, plants such as tobacco absorb metals, metalloids and dissolved ions (e.g. nitrate and ammonium) from the soil *(86)*, and soil particles (including metals), agricultural chemicals and microorganisms in the soil and other constituents of the environment can deposit and remain on tobacco leaves. The levels of metals in tobacco are affected by soil pH, soil composition and environmental contaminants *(67)*. Because deposited materials may remain on the leaf throughout processing, removing soil and microbes, including those that produce nitrite, from tobacco could help to decrease the formation of TSNAs and other nitrosamines and lower the levels of metals and agrochemicals deposited on the leaves.

Nitrate, commonly found in soils and certain fertilizers, increases plant biomass but remains in tobacco after harvesting *(5)*. When microbes capable of converting nitrate to nitrite are present, nitrite can be generated. Nitrite expelled from microbial cells can react with tobacco alkaloids to form TSNAs. TSNA production can be minimized by washing tobacco at harvest *(88)*, heat treatment in a closed system (pasteurization) *(28)*, cleaning of fermentation equipment and addition of non-nitrite-producing microbes during fermentation *(75)*. Refrigerated storage can also slow the growth of microbial populations and reduce formation of nitrosamine compounds; at least one manufacturer encourages retailers to refrigerate products to prevent formation of TSNAs during storage *(4)*. Eliminating or reducing the use of nitrate-containing fertilizers or employing other strategies (e.g., using urea or other non-nitrate fertilizers late in the growing season) could also limit the formation of nitrosamines by decreasing the accumulation of nitrate present at harvest *(5)*. Use of air-cured rather than fire-cured tobacco could reduce the levels of PAHs and volatile aldehydes.

In Sweden, the GothiaTek® standard established maximum levels for contents of public health concern in *snus*, which are nitrite, NNN, NNK, *N*-nitrosodimethylamine, benzo[*a*]pyrene, aflatoxin, cadmium, lead, arsenic, nickel, chromium and agrochemicals. The constituents of the starting materials must be carefully controlled to minimize their levels in the final *snus* product. In addition, the flavour additives used in these products must comply with the Swedish Food Act *(28)*. The results of adherence to these standards suggest that integrated agrochemical policies, specification of raw material and process controls can result in lower concentrations of targeted toxicants in the *snus* (moist snuff) variety of smokeless tobacco. Such rigorous attention to the constituents of products might decrease the levels of harmful constituents in other tobacco product types.

WHO has recommended *(88)* that, when feasible, the upper limit of TSNAs in smokeless tobacco be reduced to 2 μg/g; when this is not immediately feasible, the level should be gradually reduced to 2 μg/g.

## 6.5 Conclusions and recommendations

Smokeless tobaccos include a wide range of products, ranging from those that contain only tobacco to those consisting of tobacco combined with chemicals and non-tobacco plant materials. The products differ in appearance, production methods, contents and ingredients, and the ways in which the products are used. Many of the harmful chemicals present in these products and preparations result from organic, inorganic and microbiologic components and the interactions among them as tobacco is processed into the final product. Plant materials and other additives used with tobacco can effect product appeal (taste or appearance), absorption of nicotine, addictive potential, toxicity and, most notably, their cancer- and disease-causing properties *(4, 5, 11, 12)*. As 89% of all smokeless tobacco users are in South Asia, ingredients unique to South Asian products, particularly areca nut, should be given priority in assessing the health risks associated with smokeless tobacco products. Areca nut is an IARC Group 1 carcinogen *(66)* and is used both with and without tobacco by an estimated 600 million people worldwide *(89)*. Areca nut use is a global health concern because of its carcinogenicity, addictiveness and prevalent global use *(89)* and its continuing spread in some form *(90)*.

Some of the concerns associated with use of smokeless tobacco products worldwide are:

- inclusion of high-nicotine (N. rustica) or toxic (N. glauca) tobacco species;
- presence of toxic metals in tobacco due to soil uptake or leaf surface deposition from contaminated soil;
- soil fertilization practices that result in elevated levels of nitrate in tobacco at harvest;
- presence of harmful agricultural chemical residues remaining on the tobacco at harvest;
- presence of microbial contamination on tobacco leaves that promotes the formation of nitrosamines, particularly TSNAs;
- fermentation or ageing, which provides an anaerobic environment that contributes to rapid nitrite and TSNA formation;
- fire-curing, which can introduce chemicals from smoke, such as PAHs and volatile aldehydes;
- alkaline agents that raise the pH and increase the free nicotine concentration; and
- presence of areca nut (IARC Group 1 human carcinogen) and other additives with recognized toxicity.

Worldwide, only GothiaTek® *snus* products manufactured by Swedish Match are tested for certain pesticides, metals and nitrosamines and also for nitrite and benzo[*a*]pyrene (a PAH) to ensure that the concentrations do not exceed certain thresholds. Although the testing does not result in a risk-free product, maintenance of these concentrations shows that they can be decreased and maintained for some toxicants *(28)*.

Manufacturers of smokeless tobacco products can control a number of factors, including the type of and quality of the tobacco used, processes and ingredients used or omitted from their products. Unfortunately, although techniques are available to reduce the levels of carcinogens and other toxicants, manufacturers use the techniques selectively. Newer products often have lower levels of TSNAs, while older and traditional products that continue to be sold have higher levels of TSNAs *(91)*. Regulators have the opportunity to monitor and regulate pH and the nicotine, metal, PAH, TSNA and nitrite contents. An integrated process consisting of specifications for raw materials and process controls could reduce the levels of toxicants, especially those attributed to the curing of tobacco and microbial reactions responsible for the formation of TSNAs and volatile *N*-nitrosamines. The technology required to test pH (pH paper, pH probe), nitrate/nitrite (indicators, handheld probe) and microbial contamination (culture plates) is not expensive and could be implemented in most countries. Hand-held infrared scanners could be used to identify harmful tobacco species (*N. rustica*, *N. glauca*), non-tobacco plant materials (areca nut, tonka bean, *khat*), and alkaline agents (magnesium carbonate, slake lime). Regulators should also consider requiring better storage conditions, such as refrigerating product before sale, affixing the date of manufacture and regulating packaging material. Manufacturers should also be required to inform retailers about the effect of storage conditions on smokeless tobacco products.

The information summarized in this section supports the WHO TobReg recommendation that smokeless tobacco should be subjected to comprehensive regulatory control by an independent, scientific government agency *(92)*. In view of the diversity of the composition and concentrations of toxicants in smokeless tobacco products, the serious adverse health outcomes and the extremely high prevalence of use in regions of the world with disproportionately high rates of oral cancer and other health effects *(92)*, it may not be appropriate to considering these products as a homogeneous class of tobacco products in a generalized policy or regulatory decision. Use of the term "*snus*," which connotes a Swedish moist snuff product, to denote products manufactured by different processes and with different characteristics *(93)* is an example of marketing that can create confusion among consumers and others. Careful review of the design, composition and content of smokeless tobacco products and process controls is warranted for regulation to reduce the harm due to their use throughout the world.

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# 7. Applicability or adaptability of standard operating procedures for nicotine, tobacco-specific *N*-nitrosamines and benzo[*a*]pyrene in cigarette contents and emissions to tobacco products other than cigarettes, particularly smokeless tobacco products

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**Contents**



## 7.1 Introduction

The COP to the WHO FCTC at its fifth session *(1)* asked WHO to identify options to regulate chemicals in smokeless tobacco products. At its sixth session, the COP asked the Secretariat to invite WHO to assess, within two years, whether the SOPs for nicotine, TSNAs and benzo[*a*]pyrene in cigarette contents and emissions are applicable or adaptable, as appropriate, to tobacco products other than cigarettes, including smokeless tobacco. The China National Tobacco Quality Supervision and Test Centre, the CDC (USA) and the Health Sciences Authority (Singapore) agreed to undertake the task, to determine whether the WHO SOPs for nicotine in tobacco filler, TSNAs in mainstream tobacco smoke and benzo[*a*]pyrene in

mainstream tobacco smoke could be adapted for use in analysing smokeless tobaccos. Commercial and research smokeless tobaccos representing *snus*, moist snuff, dry snuff and loose leaf chewing tobacco were selected for testing. To meet WHO's deadline, the testing laboratories agreed to use test materials that had been characterized chemically to some extent, represented common forms of smokeless tobacco and differed in physical and chemical properties. The assessment of the applicability and adaptability of validated WHO SOPs to smokeless tobacco products and the recommended approach are presented in this section.

## 7.2 Nicotine, tobacco-specific *N*-nitrosamines and benzo[*a*]pyrene in smokeless tobacco products

### 7.2.1 Nicotine

As discussed in section 6, nicotine is considered to be the primary addictive agent in smokeless tobaccos. It is present in an ionized or an un-ionized (also referred to as unprotonated or free) state. The un-ionized form is of particular public health and regulatory interest because it is the form in which nicotine is absorbed most rapidly across the mucous membranes of the mouth *(2)*. Products may have similar levels of total nicotine yet provide different amounts of un-ionized nicotine according to their pH. The total and the percentage of un-ionized nicotine can be calculated from the measured pH and total nicotine content, from the pKa of nicotine and Henderson-Hasselbalch equations *(2)*. Consequently, measurement of nicotine levels and pH is important for informing policy and regulation. Table 7.1 lists nicotine levels reported in the published literature.

Table 7.1. Concentrations of nicotine, un-ionized nicotine, pH, moisture, tobacco-specific *N*-nitrosamines and benzo[*a*]pyrene in various smokeless tobacco products

| Type | Total nicotine, wet weight (mg/g) | Calculated un-ionized nicotine (mg/g) | pH | Moisture (%) | Total TSNAs (µg/g) | Benzo[*a*]pyrene (ng/g) |
|---|---|---|---|---|---|---|
| *Gul* powder, tobacco leaf, *zarda* | 9.55–65.0 | 0.05–31.0 | 5.22–9.22 | | 7.47–25.23 (wet weight) | 3–38.2 |
| *Khaini* and *gutkha* | 0.16–21.3 | 0.12–4.68 | 7.43–9.65 | | 0.14–127.93 (dry weight) | |
| *Mawa*, *mainpuri*, *naswar*, *toombak* | 0.16–40.6 | 0.11–13.2 | 7.38–11.0 | 6–60 | 0.10–7870 | |
| Moist snuff (*snus*) | 7.76–26.92 (dry weight) | < 0.01–13.8 | 5.54–10.1 | 35–60 | 2.0–7870 | ≤ 940 |
| Dry snuff | < 0.01–71.4 | | | 6–7 | ≤ 1219 | > 0.1–90 |

From references *3–12*

### 7.2.2 Tobacco-specific *N*-nitrosamines

TSNAs are strong carcinogens *(13)* formed from tobacco alkaloids and nitrosating agents during curing, fermentation, ageing and storage at high temperature and high relative humidity *(3)*. The TSNA concentrations in smokeless tobaccos are 500-fold higher than in mainstream cigarette smoke (Table 7.2), although they vary widely by product and country *(6, 14)*. The highest concentration of total TSNAs (992 000 ng/g) was reported in *toombak*, a smokeless tobacco used in Sudan *(5)*.

**Table 7.2.** Concentrations of nicotine, tobacco-specific *N*-nitrosamines and benzo[*a*]pyrene in smokeless tobacco and cigarette tobacco and emissions

| Analyte | Smokeless tobacco product | Cigarette tobacco filler | Cigarette mainstream smoke (ng/cigarette) | Fold difference between concentration in smokeless tobacco and in cigarettes |
|---|---|---|---|---|
| Nicotine | ≤ 71.4 mg/g | 23.18 mg/g | – | > 2.5 |
| TSNAs | ≤ 992 000 ng/g | – | 1068.8 | |
| Benzo[*a*]pyrene | ≤ 940 ng/g | – | 29.93 | |

From references *3*, *15*, *16*

### 7.2.3 Benzo[*a*]pyrene

Benzo[*a*]pyrene emitted in the mainstream smoke of cigarettes is the result of tobacco combustion, while that in smokeless tobacco is due to use of fire-cured tobacco, which contains detectable levels of PAHs *(17)*. Benzo[*a*]pyrene was present in smokeless tobaccos that contain fire-cured tobacco, at levels from not detected to 940 ng/g, which is significantly higher than the yields from mainstream cigarette tobacco (Table 7.2). Benzo[*a*]pyrene is an IARC Group I human carcinogen. It is frequently measured as a surrogate for exposure to PAHs *(18)*.

## 7.3 Evaluation of applicability of WHO standard operating procedures for analysis of smokeless tobacco products

### 7.3.1 Analytical considerations

Numerous methods have been published for the analysis of nicotine, including determination of pH and moisture content. Techniques based on GC-FID *(2)* are the most widely used; they have been adopted by the Commonwealth of Massachusetts in the USA *(19)* and validated by TobLabNet for the analysis of cigarette tobacco filler. Other published procedures include use of MS for detection *(4, 5)*. GC coupled with a thermal energy analyser (*20*) or MS (*7*) are commonly used in the determination of TSNAs in smokeless tobaccos. A modification of a GC–MS method (*21*) for analysis of PAHs in cigarette mainstream smoke *(20)* was adapted for their analysis in smokeless tobaccos.

### 7.3.2 Determination of nicotine

Determination of nicotine in smokeless tobaccos can be based on WHO SOP-04 *(22)*. The values of both total and un-ionized nicotine are important for evaluating the addiction potential of smokeless tobaccos *(20, 23)*. In SOP-04, nicotine is extracted from cigarette filler with an aqueous solution of sodium hydroxide and hexane, during which, all the nicotine is transferred to hexane. The extract is analysed by GC–FID, which is commonly used for analysis of nicotine in mainstream cigarette smoke and in e-liquid. The equipment is generally available in analytical laboratories.

The concentrations of nicotine in smokeless tobacco products are comparable to or slightly higher than those reported in cigarette tobacco filler (Table 7.2). pH and moisture content (up to 50% in moist snuff) should also be measured so that the results can be reported on both a dry and a wet weight basis. Measurements of moisture and pH are not included in TobLabNet SOP-04. Gravimetric methods for measuring volatile compounds in smokeless tobacco and the pH of a mixture of tobacco and water have been described *(2, 5, 20, 24)*. These additional measurements are not complex but require equipment for processing tobacco samples (e.g. grinding samples that contain large pieces of tobacco leaf, such as loose-leaf tobacco) and a drying oven capable of maintaining a temperature of 99–100 °C for several hours. One method for measuring the moisture content of smokeless tobacco is a modification of AOAC Method 966.02 *(25)*, referred to as "total moisture determination", for determining water and tobacco constituents that are volatile at 99 ± 1.0 °C *(2)*.

The pH of smokeless tobacco should be determined with a standard pH meter. Usually, 2 g of smokeless tobacco are mixed with 20 mL of analytical-grade water to create a slurry, and the pH is measured with a calibrated pH meter within 60 min of shaking or stirring at room temperature (20–25 °C). The pH meter is calibrated with certified standard buffers. It is important to confirm that there is no systematic drift in pH values *(2)*. Depending on the type of sample, an additional 10 mL of water are added to dilute the mixture to facilitate measurement.

The total and un-ionized nicotine content of smokeless tobacco are measured from the pH and total nicotine, with the Henderson-Hasselbalch equation based on total measured nicotine, pH and a pKa value of 8.02 *(2, 20)*.

### 7.3.3 Determination of tobacco-specific *N*-nitrosamines

The current CDC methods for determining TSNAs in smoke emissions and tobacco are similar to WHO SOP-03 *(26)*, with a few exceptions.

The sample preparation and analytical sections of WHO SOP-03 could be adapted for smokeless tobacco, and extension of the TobLabNet method for determining TSNAs in cigarette filler to analysis of smokeless tobacco should be

relatively straightforward. The NNN and NNK contents of smokeless tobacco can vary from 20 to 10 000 ng/g, whereas those in mainstream tobacco smoke (Table 7.2) are comparable at the lower calibration end. Thus, the upper calibration range would have to be extended to cover smokeless tobacco products with concentrations of NNN and NNK anticipated to be higher. This should not be problematic for linearity or detector saturation.

Adaptations to SOP-03 should be based on a comparison with the current CDC method for TSNAs in mainstream smoke emissions and tobacco content *(15)*. Specifically, as noted above, the calibration curve for smokeless tobaccos should be extended (and remain linear), and smokeless tobacco samples might have to be ground and filtered so that tobacco "fines" do not clog the injection system. Sample preparation should be identical to those in the WHO TobLabNet method and the current CDC method, including extraction procedures. The sample size for extraction will have to be optimized, and other modifications, such as grinding tobacco to improve extraction efficiency, should be considered. Thus, the TobLabNet method for measuring TSNAs in cigarette emissions, with appropriate modifications, could be used for measuring NNN and NNK in smokeless tobacco.

### 7.3.4 Determination of benzo[*a*]pyrene

Determination of benzo[a]pyrene in smokeless tobaccos could be based on WHO SOP-05 for the determination of benzo[*a*]pyrene in mainstream cigarette smoke *(27)*. In the WHO method, mainstream cigarette smoke is trapped on a CFP made of 1-µm glass fibre. After smoking, the filter pad is extracted with a cyclohexane solution containing an isotopically labelled internal standard, deuterated benzo[*a*]pyrene-$D^{12}$.

The cyclohexane extract is eluted through a silica solid-phase extraction cartridge, and the eluent is collected and analysed by GC–MS in electron ionization mode. Samples of 0.2–1.0 g of smokeless tobacco product (amount to be optimized during verification) should be extracted with cyclohexane (10 mL at room temperature) and shaken for 1 h and the extract centrifuged at 200 rpm for 60–80 min. A 5-mL aliquot of the extract should be spiked with benzo[*a*]pyrene-$D^{12}$ internal standard and mixed well. Sample clean-up indicated in SOP-05 includes solid-phase extraction on a silica cartridge, followed by rotary evaporation. Laboratories should investigate whether rotary evaporation is required.

For sample clean-up with solid-phase extraction, the mixture should be loaded onto a pre-cleaned cartridge (Sep-pak Vac silica cartridge from Waters or equivalent), which will be washed and eluted with cyclohexane. The eluent from both the load and the wash should be combined and dried. The residue will then be reconstituted with 1 mL cyclohexane and a reconstituted aliquot used for GC–MS analysis. Optional steps, which should be investigated during method

verification, include sample clean-up with solid-phase extraction, followed by rotary evaporation, as specified in reference *27*. The aliquot should be analysed by GC-MS.

Seven smokeless tobacco products (*snus*, moist snuff, dry snuff and loose leaf) were selected for this study by CDC (Table 7.3). Four were reference products obtained from CORESTA, and three were obtained from a commercial vendor (Lab Depot, Atlanta, GA, USA). CDC shipped the seven smokeless products to the China National Tobacco Quality Supervision and Test Centre and the Health Sciences Authority in Singapore for method verification.

Table 7.3. Smokeless tobacco test materials selected for method verification

| Smokeless tobacco product | Type | Reference or commercial | Total nicotine | pH | Moisture (%) | TSNAs | Benzo[*a*] pyrene |
|---|---|---|---|---|---|---|---|
| CRP1 | *Snus* | Reference | 0.8% (wet weight) | 8.5 | 52 | ~1.46 ppm | To be determined |
| CRP2 | Moist snuff | Reference | 1.2% (wet weight) | 7.7 | 54.6 | ~4.40 ppm | To be determined |
| CRP3 | Dry snuff | Reference | 1.2% (wet weight) | 7.7 | 54.6 | 18–19 ppm | To be determined |
| CRP4 | Loose leaf | Reference | 1.9% (wet weight) | 6.9 | 8.0 | ~3.70 | To be determined |
| Silvercreek Wintergreen *(7)* | Moist snuff | Commercial | 8.2 to 11.96 mg/g (wet weight) | 6.29–7.08 | 51.9–52.6 | 15.86 μg/g (wet weight) | To be determined |
| Skoal Original *(14, 28)* | Moist snuff | Commercial | 11.4 mg/g (dry weight) | 7.27 | 59 | ? | To be determined |
| Red Seal Wintergreen | Moist snuff | Commercial | 14.9 mg/g (wet weight) | 7.55 | 53.3 | 4.87–5.27 μg/g (wet weight) | To be determined |

ppm, parts per million

## 7.4 Discussion and recommendations

The objective of this section is to recommend quantitative analytical procedures for adapting and applying existing TobLabNet-validated methods for cigarettes to the analysis of smokeless tobaccos. Numerous methods have been published for the analysis of nicotine and TSNA, including pH determination and moisture content. GC–FID is the method of choice, as the equipment is commonly available in analytical laboratories globally.

The conclusion of this review of the TobLabNet SOPs for nicotine, benzo[*a*] pyrene and TSNAs by knowledgeable experts is that these methods should be applicable for smokeless tobacco products. Cross-matrix studies will have to be performed on representative samples for confirmation. Although a variety of research and commercial smokeless tobacco test materials were selected in order to cover a range of physical and chemical properties (Table 7.3), this sample

does not cover all the varieties of this diverse type of tobacco product. Limited method optimization will be required for sample preparation, and, for NNN and NNK, the calibration range will have to be extended to cover the higher contents typically present in smokeless tobacco (Table 7.1). In addition, the methods for determining pH and moisture should be discussed and consensus reached. We recommend that cross-matrix validation be conducted for nicotine, pH, benzo[*a*] pyrene, NNN and NNK in smokeless tobacco products with adapted versions of the TobLabNet SOPs.

## Conclusions

- TobLabNet methods for TSNAs and nicotine could be applied or adapted for determination of smokeless tobacco products.
- The applicability of the TobLabNet method for determining benzo[*a*] pyrene should be validated, as the matrix is different from that specified in the SOP.
- The specific, selective TobLabNet methods, with clean up steps, should allow extraction of toxicants.
- Extension of the calibration range or dilution of samples should be considered to cover the higher values found in smokeless tobacco products.

## Recommendations

- Require manufacturers to disclose the pH of products and the levels of the toxicants TSNAs, benzo[*a*]pyrene and nicotine, measured with WHO-verified methods or country's official methods, by an independent laboratory
- Compliance can be tested in any analytical laboratory designated by a government authority

## Further work

- Analyse metals, humectants and aldehydes in smokeless tobacco products by published methods for tobacco, food, plants and environmental matrices with available laboratory resources.

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# 8. Overall recommendations

The WHO Study Group on Tobacco Product Regulation (TobReg) publishes a series of reports to provide a scientific foundation for tobacco product regulation. In line with Articles 9 and 10 of the WHO Framework Convention on Tobacco Control (WHO FCTC),[1] these reports identify evidence-based approaches to the regulation of tobacco products.

The eighth meeting focused on issues critical to advancing the regulation of tobacco products, particularly as outlined at the sixth session of the Conference of the Parties to the WHO FCTC.[2] The topics discussed included: (1) cigarette characteristics and design features; (2) toxicants in waterpipe tobacco and smokeless tobacco; and (3) applicability of WHO Tobacco Laboratory Network (TobLabNet) standard operating procedures (SOPs) of measuring selected content and emission chemicals in cigarette tobacco products to ENDS, waterpipe tobacco and smokeless tobacco products.

## Main recommendations

1. This report provides relevant guidance regarding specific cigarette design features, as well as testing and disclosure of the contents and emissions of a wide array of smokeless tobacco products, waterpipe tobacco products, and other devices like ENDS.

– ***Design features:*** Member States should require that manufacturers and importers of tobacco products disclose information on design features listed in Appendix 2 of the Partial Guidelines of the WHO FCTC to governmental authorities at specified intervals, including the results of tests conducted by the tobacco industry. Member States should also consider restricting or prohibiting other design features that may increase the attractiveness of tobacco products such as flavours and capsules. Lastly, should there be any change to the design features of a particular brand of tobacco product, Member States should require that manufacturers notify governmental authorities of the change and provide the updated information when the change is made.
– ***Smokeless tobacco:*** Manufacturers could be required to disclose the levels of tobacco-specific nitrosamines (TSNAs), benzo[*a*]pyrene (B[*a*]P) and nicotine, as well as pH levels in SLT products, as

[1] For more information, see: http://apps.who.int/iris/bitstream/10665/42811/1/9241591013.pdf?ua=1 (accessed 20 September 2016)

[2] For more information on the Conference of the Parties of the WHO Framework Convention on Tobacco Control, see decision FCTC/COP6(10), paragraph 2(a) and decision FCTC/COP6(12) paragraph 2(b) at http://apps.who.int/gb/fctc/E/E_cop6.htm (accessed 20 September 2016).

the WHO TobLabNet methods can be adapted or applied to these specific toxicants. Furthermore, since there are existing technologies which can reduce levels of SLT carcinogens, manufacturers should be required to use these in order to reduce the toxicity of these products. Regulators should also consider requiring improved storage conditions such as refrigerating product before sale, affixing date of manufacture, and regulating packaging material. Lastly, manufacturers should also be required to educate retailers on the effect of storage conditions on the SLT product.

- ***Waterpipe tobacco:*** Waterpipe smoking normally utilizes burning charcoal as the heat source, thus, waterpipe smoke includes toxicants emitted from the charcoal in addition to those from the tobacco product itself. Because of this complexity, regulators should consider an approach which focuses initially on measuring and reporting the chemical contents in the waterpipe tobacco products which are known to contribute to their toxicity, addictiveness and appeal, and expand this to selected chemicals and toxicants in emissions as the assessment and analytical methods are validated.
- ***ENDS:*** Sufficient data exist to support extension of existing and pending WHO SOPs for nicotine, humectants (solvents), carbonyls, B[*a*]P and TSNAs in ENDS liquid and aerosol. It is recommended to measure the pH of the liquid to establish the range of pH across ENDS liquids, as this will assist with investigations into the addictive potential of the nicotine delivered to the user. Metals should be examined to determine if there is the potential for associated health risk.

## Significance for public health policies

2. One of the challenges in developing a comprehensive and effective tobacco control policy is the wide range and heterogeneity of commercially available tobacco products. TobReg's report provides helpful guidance in understanding the contents, emissions and design features of selected products such as cigarettes, smokeless tobacco, and waterpipes. The report highlights the impact of their toxicants or features on public health. In addition, the report expounds on how the WHO TobLabNet SOPs can serve as reliable methods by which to test these products. The current state of knowledge dictates the need to keep active surveys of use of the diverse tobacco products and also monitoring novel new tobacco products.

## Significance for the Organization's programmes

3. This report fulfils TobReg's mandate to provide the WHO Director-General with scientifically sound, evidence-based recommendations for Member States about tobacco product regulation. In line with the provisions of Articles 9 and 10 of the WHO FCTC, TobReg has identified evidence-based approaches to regulating the vast array of tobacco products which concern Member States. TobReg's report also lists areas for future research which will expand the knowledge base with respect to tobacco product regulation.

## WHO study group on tobacco product regulation

This report presents the conclusions reached and recommendations made by the members of the WHO Study Group on Tobacco Product Regulation at its eighth meeting, where the group reviewed background papers specially commissioned for the meeting and considered the following topics:

1. Cigarette characteristics and design features
2. Possible application of WHO Tobacco Laboratory Network standard operating procedures to evaluation of electronic nicotine delivery systems
3. Waterpipe toxicant content and emissions
4. Possible application of WHO Tobacco Laboratory Network standard operating procedures for cigarettes to waterpipe tobacco
5. Toxic contents and emissions of smokeless tobacco products
6. Possible application or adaptation of standard operating procedures for nicotine, tobacco-specific *N*-nitro-samines and benzo[*a*]pyrene in cigarette contents and emissions to tobacco products other than cigarettes, particularly smokeless tobacco products

The Study Group's recommendations in relation to each theme are set out at the end of the relevant chapter, and overall recommendations are summarized in the final chapter of the report.

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