WHO study group on tobacco product regulation

Report on the scientific basis of tobacco product regulation:

Seventh report of a WHO study group

World Health Organization

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World Health Organization

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5. A regulatory strategy for reducing exposure to toxicants in cigarette smoke

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7. Sugar content of tobacco products

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Minneapolis, Minnesota, United States of America, 5–7 December 2017

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Abbreviations

CI	confidence interval
CO	carbon monoxide
COP	Conference of the Parties of the WHO Framework Convention on Tobacco Control
EHCSS	electrically heated cigarette smoking system
EN&NNDS	electronic nicotine and non-nicotine delivery systems
ENDS	electronic nicotine delivery systems
ENNDS	electronic non-nicotine delivery systems
FDA	United States Food and Drug Administration
FTC	Federal Trade Commission (USA)
GC	gas chromatography
GRAS	generally recognized as safe
HCI	Health Canada intense
HPHC	harmful and potentially harmful constituent
HTP	heated tobacco product
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
LC	liquid chromatography
MRTP	modified risk tobacco product
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N´-nitrosonornicotine
NRT	nicotine replacement therapy
PAH	polycyclic aromatic hydrocarbon
PMI	Philip Morris International
PREP	potentially reduced exposure product
qPCR	quantitative polymerase chain reaction
SOP	standard operating procedure
THP	tobacco heating product
THS	tobacco heating system
TobLabNet	Tobacco Laboratory Network (WHO)
TobReg	WHO Study Group on Tobacco Product Regulation
TRP	transient receptor potential
TSNA	tobacco-specific N´-nitrosamine
VLNC	very low-nicotine content
VOC	volatile organic compound
WHO FCTC	WHO Framework Convention on Tobacco Control

Commentary

Scientific and regulatory approaches to reducing the harm associated with tobacco use have attracted much interest. This has fuelled in part the development of newer products, such as electronic nicotine delivery systems (ENDS) and heated tobacco products (HTPs), which have been promoted as less harmful or as aids to smoking cessation. The interest dates back several decades, well before the establishment of the WHO Study Group on Tobacco Product Regulation (TobReg) in 2003. The topic of product regulation has been addressed extensively in earlier WHO reports. During the past decade however, the market has developed extensively, with an ever-increasing range of products and industry activity and reports, as well as more research, country data and publications being made available.

This technical report, based largely on background papers prepared for a meeting of TobReg in December 2017, covers pertinent and critical issues in tobacco product regulation. While there was some increase in the number of publications relevant to sections 4–10 in the past year, there were marked increases in the number of scientific publications, data from national surveillance programmes/surveys and market developments with respect to ENDS and HTPs. For example, a bibliometric analysis of publications on electronic cigarettes (a subset of ENDS) published between 2003 and 2018 indicated an increase of nearly 24% between 2017 and 2018 *(1)*. The reports in this publication were written according to specific terms of reference and are based on the best evidence available at the time they were commissioned. WHO is aware of the unprecedented increase in the number of publications on ENDS and on market developments, recent trends in the use of these products by young people in some countries, including Canada, some countries in Europe and the United States of America, and regulatory approaches to curtailing use, which are not addressed in this report. The section on ENDS is limited to the clinical pharmacology of nicotine in these products and is based on evidence available up to December 2017, with some additions after review between March and December 2018. Only publications considered to be relevant to the topic were included.

WHO submitted two reports to the sixth (FCTC/COP/6/10 Rev. 1) and seventh (FCTC/COP/7/11) sessions of the Conference of the Parties to the WHO Framework Convention on Tobacco Control (WHO FCTC), in 2014 and 2016, respectively, on the health risks associated with ENDS, the efficacy of these products in helping smokers to quit smoking and reduce their nicotine dependence, interference with tobacco control and implementation of the WHO FCTC. These reports led to two decisions by the Conference of the Parties, in which Parties were invited to broadly pursue four regulatory objectives to prohibit or restrict the manufacture, importation, distribution, presentation, sale and use of these products, as appropriate to their national laws and public

health objectives and to consider prohibiting or regulating ENDS, including as tobacco products, medicinal products, consumer products, or other categories, as appropriate, taking into account a high level of protection for human health.

WHO and its technical groups, including TobReg, are monitoring market developments, research, publications, commentaries, industry activities and debate on ENDS (including e-cigarettes) and other novel products and are reviewing global surveillance mechanisms to ensure effective monitoring and evaluation of these products. WHO will address the increasing numbers of publications and new products, variants and flavours in its next comprehensive review of the evidence, which will update the 2016 report. The review will also include the mounting evidence on the effects of these products, marketing trends, concern about their use by young people and the engagement of the major tobacco companies, especially in relation to Article 5.3 of the WHO FCTC.

This report provides the best evidence available up to December 2017, with some additions after review between March and December 2018. It should be read in relation to the earlier evidence that guided the development of policies and regulations by WHO Member States on tobacco products up to that time.

Reference

1. Briganti M, Delnevo CD, Brown L, Hastings SE, Steinberg MB. Bibliometric analysis of electronic cigarette publications: 2003–2018. Int J Environ Res Public Health. 2019;16(3):320.

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 standards for product packaging and labelling. Tobacco product regulation is covered under Articles 9, 10 and 11 of the WHO Framework Convention on Tobacco Control (WHO FCTC) *(1)* and in the partial guidelines on implementation of Articles 9 and 10 *(2)*. Other WHO resources, including the basic handbook on tobacco product regulation *(3)* and the handbook on building laboratory testing capacity *(4)*, support Member States in this area.

The WHO Study Group on Tobacco Product Regulation (TobReg) was formally constituted by the Director-General of WHO in 2003 to address gaps in the regulation of tobacco products. 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, toxicology and laboratory analyses of tobacco product ingredients and emissions. The experts come 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, evaluated and reviewed by TobReg.

The ninth meeting of TobReg took place in Minneapolis, United States of America, on 5–7 December 2017, and was generously hosted by the Masonic Cancer Center, University of Minnesota, USA. The participants discussed priorities in the regulation of nicotine and novel and tobacco products and addressed requests from the WHO FCTC Conference of the Parties (COP) made at its seventh session, as outlined in documents FCTC/COP7(4), FCTC/COP7(9) and FCTC/COP7(14). The requests included the following.

- Continue to monitor and examine market developments and usage of novel and emerging tobacco products, such as heated tobacco products;
- Collect scientific information on the chemicals in the contents and emissions of smokeless tobacco products that contribute to their toxicity, addictiveness and attractiveness, on analytical methods for measuring them and on the levels in products on the market; and identify technical approaches for reducing toxicants in smokeless tobacco;
- Promote research on culturally relevant interventions to prevent the uptake of waterpipe tobacco smoking and to promote quitting (cessation); the epidemiology of use; acute and chronic health risks; cultural

practices; initiation and maintenance of use; the influence of flavourings on initiation, maintenance of use and increasing use; risk of dependence on low-nicotine tobacco products; and effective policies based on concepts such as information technology and communications.

In response to these requests, WHO commissioned the following background papers:

- Heated tobacco products (section 2);
- Clinical pharmacology of nicotine in electronic nicotine delivery systems (section 3);
- A global nicotine reduction strategy: state of the science (section 4);
- A regulatory strategy for reducing exposure to toxicants in cigarette smoke (section 5);
- The science of flavour in tobacco products (section 6);
- Sugar content of tobacco products (section 7);
- Updated priority list of toxicants in combusted tobacco products (section 8);
- Approaches to measuring and reducing toxicant concentrations in smokeless tobacco products (section 9);
- Waterpipe tobacco smoking: prevalence, health effects and interventions to reduce use (section 10).

1.1 References

1. WHO Framework Convention on Tobacco Control. Geneva: World Health Organization; 2003 (http://www.who.int/fctc/en/, accessed 14 May 2019).
2. Partial guidelines on implementation of Articles 9 and 10. Geneva: World Health Organization; 2012 (https://www.who.int/fctc/guidelines/Guideliness_Articles_9_10_rev_240613.pdf, accessed 14 January 2019).
3. Tobacco product regulation: basic handbook. Geneva: World Health Organization; 2018 (https://www.who.int/tobacco/publications/prod_regulation/basic-handbook/en/, accessed 14 January 2019).
4. Tobacco product regulation: building laboratory testing capacity. Geneva: World Health Organization; 2018 (https://www.who.int/tobacco/publications/prod_regulation/building-laboratory-testing-capacity/en/, accessed 14 January 2019).

2. Heated tobacco products

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"Nowadays, tobacco companies continue reassuring health concerned smokers by offering with their new products the illusion of safety." (World No Tobacco Day 2006)

Contents

2.1 Introduction

Heated tobacco products (HTPs), also sometimes referred to as "heat-not-burn" products, a term coined by the tobacco industry, are an emerging class of "potentially reduced exposure products" (PREPs) or "modified risk tobacco products" (MRTPs). The concept emerged in the 1980s from the tobacco companies Philip Morris and RJ Reynolds in the USA, which marketed Accord and Premier, the first generation of these products, respectively. Since then, these and conceptually similar products have continued to evolve and may now be poised to capture a significant market share. The introduction, aggressive marketing and growing popularity of electronic cigarettes may have facilitated the success of such products, partly by changing social norms and perceptions about conventional cigarette smoking and about the use of devices to deliver nicotine. There is substantial published literature on certain HTPs marketed in the 1990s and 2000s and emerging literature on newer products, although much of it comes from the tobacco industry. Further, there are few studies on prevalence and substitution in the marketplace, as many of these products were test-marketed rather than made broadly available. Still, laboratory and field research studies can provide information on the likelihood of such substitution.

    This review is based on the literature on HTPs available through October 2017, including their history, design, delivery of nicotine to the user and toxicants

and marketing (including online advertising and sales); HTP technology; manufacturer's claims of reduced toxicity, harm, risk and exposure; comparison with conventional cigarettes; consumer perceptions of these alternative products; and the implications of these products for regulatory, product and market policy. The review focuses on HTPs marketed by tobacco companies but also covers handheld or portable products for use as "dry herb" vaporizers (often for marijuana) that could be used with tobacco. Desktop "vaporizers" usually used to administer marijuana were excluded, as were hookah-, waterpipe- or narghile-type products. The review is based primarily on published literature and also on news reports and press releases, stockholder reports, scientific presentations and internet blogs, as necessary.

The PubMed search terms were: heat not burn; heat-not-burn; heated tobacco; tobacco heating; Accord; Eclipse; Heatbar; Premier; THS [tobacco heating system]; vaporizer; PREP; MRTP; THP [tobacco heating product]; iQOS; and glo.

2.2 The science of heated tobacco products

HTPs are based on the principle that most of the harm associated with tobacco smoking is due to the combustion process. In a conventional cigarette, the temperature of the burning cone can reach up to 900 °C, and the median temperature along the rod is 600 °C. This can result in combustion, pyrolysis, pyrosynthesis and myriad other reactions that result in the > 7000 compounds identified as components of tobacco smoke *(1)*. Polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines and some volatile organic compounds (VOCs) (e.g. benzene, 1,3-butadiene, acrolein, toluene) are formed primarily as a result of combustion. Tobacco-specific $N´$-nitrosamines (TSNAs) such as $N´$-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are present in cured tobacco and are partially transferred to smoke in a near-linear fashion at typical cigarette temperatures. Some TSNAs are formed during tobacco combustion. Toxic metals such as cadmium present in tobacco may also be transferred to smoke at typical tobacco combustion temperatures. Burning of tobacco is, however, ultimately unnecessary to "volatilize" nicotine (although it is efficient), and alternative means of liberating nicotine from tobacco in an inhalable form without combustion are preferable from the point of view of both toxicological risk and consumer acceptability. One way of extracting nicotine while maintaining something visually similar to smoking behaviour is to heat tobacco to a temperature that volatilizes nicotine but does not combust the plant material. Volatilized nicotine without combustion would, in principle, produce a less complex aerosol with fewer toxic constituents.

Nicotine is not efficiently delivered as a gas; to deliver nicotine to the user's lungs, an aerosol-forming agent must be included to suspend nicotine on aerosol

particles. This has been achieved by four main approaches through the decades. The first is in a cigarette-like device with an embedded heat source that can be used to aerosolize nicotine – this is the general principle underlying Premier and Eclipse and Philip Morris International (PMI)'s "Platform 2" product. The second approach is to use an external heat source to aerosolize nicotine from specially designed cigarettes (rolls of tobacco in paper). This is the basic design of Accord, Heatbar, iQOS and glo. The tobacco used in PMI's HTP is apparently not typical tobacco cut-filler but rather a reinforced web of cast-leaf tobacco (a type of reconstituted tobacco), which includes 5–30% by weight of compounds that form aerosols, such as polyols, glycol esters and fatty acids. The examples given include glycerine, erythritol, 1,3-butylene glycol, tetraethylene glycol, triethylene glycol, triethyl citrate, propylene carbonate, ethyl laurate, triactin, meso-erythritol, a diacetin mixture, a diethyl suberate, triethyl citrate, benzyl benzoate, benzyl phenyl acetate, ethyl vanillate, tributyrin, lauryl acetate, lauric acid, myristic acid and propylene glycol *(2)*. This composition is advantageous as an aerosol-forming substrate for use with a heating system. The sticks (45 mm long, 7 mm diameter) contain approximately 320 mg of tobacco material – much less than a conventional cigarette (~700 mg). In iQOS, the tobacco is heated by a blade in the heater device inserted into the end of the heat stick, so that the heat dissipates through the tobacco plug on a puff *(3)*. The aerosol then passes through a hollow acetate tube and a polymer film filter on the way to the mouth. The product is designed not to exceed 350 °C, at which point the energy supplied to the blade is cut off at a maximum of 14 puffs or 6 min *(3)*. British American Tobacco describes its glo product as a heating tube consisting of two separately controlled chambers, which are activated by a button on the device to reach the operating temperature (240 °C) within 30–40 s *(4)*. The 82-mm long, 5-mm diameter stick inserted into the heating chamber contains approximately 260 mg of reconstituted sheet tobacco with 14.5% glycerol as an aerosolizing agent. The vent holes on the stick are described as necessary to "…provide the right amount of drawing effort and to encourage the…vapour to coagulate and condense…." *(4)*. The stick consists of a tobacco rod, a tubular cooling section and a filter and mouthpiece.

A third approach is to use a heated sealed chamber to aerosolize nicotine from tobacco leaf directly – this is the principle underlying personal dry-herb vaporizers such as Pax; however, the prevalence of use of such devices for tobacco is unknown. A fourth approach is use of electronic nicotine delivery systems (ENDS) to derive flavour elements from small amounts of tobacco (see section 3 for further information). British American Tobacco's iFuse product appears to be a hybrid ENDS–tobacco product, in which the aerosol is passed over tobacco to pick up the flavour and is then inhaled by the user *(5)*. The vapour appears to lose a small amount of heat when it passes over the tobacco chamber (from

35 °C to 32 °C), indicating that some tobacco is heated. As the e-liquid contains nicotine at 1.86 mg/mL, however, with a machine delivery of 20–40 μg per puff, it is difficult to estimate the contribution, if any, of the tobacco in the device to delivery of nicotine. The delivery of toxicants under machine smoking is reported to be nearly identical to that of an ENDS without a tobacco chamber, implying a minimal contribution of tobacco *(5)*. The Japan Tobacco International Ploom TECH operates in a similar manner, except that the ENDS-like component appears not to contain nicotine.

2.3 A brief history of heated tobacco products

A historical perspective is important for understanding the current situation. Table 2.1 lists the HTPs known to have been introduced into at least a test market. The timeline in Fig. 2.1 places the introduction (and withdrawal) of various HTPs into context. Activity in this market space has been clustered, 2006–2008 and 2015–2017 being particularly active periods.

Table 2.1. Heated tobacco products by manufacturer

Company	Trade name®	Brief description	Current status
Philip Morris USA / Altria	Accord	THS consisting of small cigarettes placed in an external heating device. Available in tobacco and menthol flavours. Introduced in 1998 in test markets in Richmond, Virginia, USA, and Japan. Advertising focused on reduced second-hand smoke rather than reduced potential health risks.	Withdrawn in 2006. No longer sold.
Philip Morris International	Heatbar	THS, consists of small cigarettes (Heatsticks) placed in an external heating device with puff detection technology. Four blends available. Devices came in a range of colours. Introduced in Switzerland in 2006.	Withdrawn in 2008. No longer sold.
	iQOS	THS, consists of small cigarettes (HEETS) placed in an external heating device. Available in Marlboro brand. Launched in Japan in 2015.	Currently marketed in nearly 40 countries including Canada, Italy, Japan and the United Kingdom. Expanding to other markets. MRTP application to FDA filed in May 2017 and reviewed by the Tobacco Products Scientific Advisory Committee in January 2018.
	THS 2.2	THS described in a series of published papers in scientific journals.	Marketed as iQOS.
	Platform 2	Pressed carbon heat source (conceptually similar to Eclipse).	Unclear whether available on the market. Branded as TEEPS in several PMI presentations.

Japan Tobacco International	Ploom	Ploom began as independent company in 2007. Entered into marketing and commercialization agreement with Japan Tobacco International in 2011, which purchased patents and designs for device and pods (Ploom name and ModelTwo device) outright in 2015. Pods currently have the Mevius cigarette brand name. Former Ploom company became Pax, which produces dry herb vaporizers.	Currently marketed in Japan and Switzerland.
RJ Reynolds / Reynolds American	Premier	Cigarette-like device designed for heating and aerosolizing tobacco flavour. Cigarette column made of aluminium capsules containing tobacco pellets, with a carbon element at the tip to warm the tobacco column. Test-marketed in St Louis, Phoenix and Tucson, USA, in 1988. Subject of complaints to the FDA as a drug delivery device.	Withdrawn in 1989 because of poor sales. Not currently on the market.
	Eclipse	Cigarette-like device. Rod consists of reconstituted tobacco, with carbon element to heat and volatilize nicotine. Test-marketed in several versions between 1996 and 2000, when wider sales began, with accompanying advertisements and health claims. Subject of lawsuit over claims by the State of Vermont, USA.	Withdrawn from United States market in 2007; remained in limited distribution thereafter. Announced "substantial equivalence" filing with FDA in October 2017 to bring improved version to United States market.
	Revo	Eclipse-like product test-marketed in Wisconsin, USA, in 2014–2015.	Withdrawn in 2015.
British American Tobacco	glo	"glo™ heats proprietary Kent Neostiks™ to approximately 240 °C to provide a highly satisfying taste, similar to that of a cigarette, with around 90% less toxicants. glo™ also offers a number of added features, including: no burning or ash, less odour on your hands, hair, clothes and surroundings." Neostiks come in three flavours.	Available in Canada, Japan, Republic of Korea, Russian Federation and Switzerland.
	iFuse	Hybrid of e-cigarette and HTP. Cartridges contain e-liquid with flavourings and a chamber containing tobacco. Heating element aerosolizes the liquid, which passes through the tobacco chamber before reaching the user. Launched in 2015.	Test-marketed in Romania.
Dry herb vaporizers			
Pax	Pax 2	Rectangular device. "Magnetic oven" used to heat plant material, "thin film Kapton heater flex". Cannot be used with liquids, waxes or concentrates. Capacity, 1.6 mL. Comes in four colours.	Available online and from authorized retailers.
	Pax 3	Rectangular device. "Magnetic oven" used to heat plant material. Can also be used with concentrates with special insert. Oven starts when device senses lips on mouthpiece. 3500-mAh battery. Capacity, 0.17–0.35 g. Comes in four colours.	Available online and from authorized retailers.
V2	Pro Series 3	Cylindrical device. Three-in-one vaporizer, with different cartridges and mouthpieces for e-liquid, loose leaf or wax. Conduction oven. Pre-set temperature, 160–180 ºC. 650-mAh battery. Comes in three colours.	Available online.

	Pro Series 7	Rectangular device. Three-in-one vaporizer, with different cartridges and mouthpieces for e-liquid, loose leaf or wax. Larger capacity, more customization options. Higher-temperature conduction oven with three levels (200, 215, 225 ºC). 1800-mAh battery; 3.7–4.7 V. Comes in three colours.	Available online.
Vapor Fi	Orbit	Cylindrical device. 1.7-mL capacity cartridge for dry plant material. Temperature range, 182–216 ºC. 2200-mAh battery. Two colours (black, red).	Available online.
	Atom	Rectangular device. Temperature range, 182, 210, 240 ºC. Plant material packed into an external oven cartridge. 3000-mAh battery; 3.3–4.2 V. Motion sensing. Superficially similar to Pax device.	Available online.
Atmos	Jump	Cylindrical device. Maximum temperature, 200 ºC; not variable. 1300-mAh battery; 3.4 V. Available in four colours.	Available online.

FDA: United States Food and Drug Administration; HTP: heated tobacco product; MRTP: modified risk tobacco product; PMI: Philip Morris International; THS: tobacco heating system.

Fig. 2.1. Timeline of introduction of heated tobacco products, 1988–2016
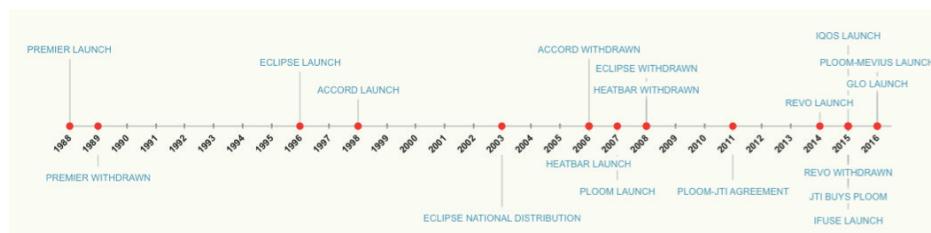
Eclipse

Eclipse, the successor of Premier, launched at the end of the 1980s, was available in various test markets in the USA between 1996 and 2007 and was extensively studied *(6–13)*. The product had a carbon-heating element embedded in the tip of a product resembling a cigarette to heat reconstituted tobacco and glycerol in the rod and generate a nicotine aerosol. To use it, the smoker would light the heating element and puff, much like a conventional cigarette. In two separate five-day trials, Breland and colleagues *(6, 7)* found that Eclipse reduced exposure to nicotine and NNK from that in the smoker's own brand but increased exposure to carbon monoxide (CO) and required a more intensive puffing pattern. Lee et al. *(10)* found a similar pattern of results for Eclipse in the laboratory. Fagerstrom and colleagues *(9)* showed that smokers' CO levels over two weeks of using Eclipse increased from 21.0 to 33.0 parts per million. In a subsequent eight-week study *(8)*, in which 10 participants were selected to continue using Eclipse, no difference in plasma nicotine levels was seen from baseline, but the exhaled CO levels were higher with Eclipse than with the smokers' own brand (32.5 versus 22.5 parts per million). Stewart and colleagues *(13)* reported that a small group

of smokers who used Eclipse for 4 weeks showed less alveolar epithelial injury but increased levels of carboxyhaemoglobin and other markers of oxidative stress. Pauly and colleagues *(14)* identified a potentially unique health risk of Eclipse due to contamination with glass fibre. They noted that the insulation surrounding the carbon-heating element could fray and become loose during handling of the packaging or the product itself and expose smokers to inhalation of these fibres. Previous research had shown evidence of inhalation of cellulosic and plastic fibres in 87% of 114 human lung specimens *(15)*, indicating that this was not simply a theoretical concern. A subsequent survey of consumers *(16)* showed that they viewed this as a health risk. RJ Reynolds vigorously objected to these observations and published several studies to refute them, arguing that the fibres were too large to be inhaled and that the number of loose fibres was not as large as reported *(17–20)*.

The first health claims were made for Eclipse when it was launched nationally in 2003, citing potentially reduced risks for cancer, bronchitis and emphysema *(21)*. The manufacturer's advertising claimed that Eclipse reduced emissions of harmful smoke constituents and suggested that, next to quitting, Eclipse was the smoker's "next best option". These claims resulted in a lawsuit by the Attorney-General of the State of Vermont, USA, in 2005, which resulted in the finding in 2010 that the advertising had violated consumer protection laws and the Master Settlement Agreement. This was followed by a judgement against RJ Reynolds for US$ 8 million in 2013. A repackaged version of Eclipse, called Revo, was briefly test-marketed in Wisconsin, USA, in 2015. In November 2017, RJ Reynolds announced that they would market an improved version of Eclipse in 2018; this had not been launched yet as of January 2019.

Accord

Accord was first marketed in 1998 in the USA and later in Japan. This product, a predecessor of iQOS, had an external battery-powered heating device, into which specifically designed "cigarettes" were inserted for smoking. Puffing on the cigarette activated the heating bars in the device and generated an aerosol. Accord was marketed as a "cleaner" cigarette, without ash or second-hand smoke; no health claims were made. Review of an early study indicated that Accord use resulted in less CO intake and tachycardia than conventional cigarettes *(22, 23)*. Accord was, however, associated with poor suppression of withdrawal *(22, 23)*. In a study of concurrent use of Accord and the smokers' own cigarettes, Accord suppressed cigarette smoking and exposure to CO dose-dependently after six weeks; i.e. the more often Accord was used, the less participants smoked their own cigarettes. Furthermore, the participants did not increase their puff intensity when they reduced the number of cigarettes per day, and smoke from Accord contained lower levels of CO *(24)*. Analysis of participant exit interviews showed

that study participants believed Accord to be a "safer cigarette". A study by Roethig et al. *(25)* of a second-generation electrically heated cigarette showed reductions in selected biomarkers of exposure (e.g. nicotine, 1-hydroxypyrene) of 43–85% relative to conventional cigarette smoking.

2.4 Recent products

2.4.1 Emissions

Table 2.2 summarizes the published literature on emissions of certain harmful and potentially harmful constituents (HPHCs) *(26)* under machine smoking conditions from the three HTPs for which there are the most published data – Eclipse (International Organization for Standardization (ISO) conditions), electrically heated cigarette smoking system (EHCSS; ISO conditions) and THS 2.2/iQOS (Health Canada Intense (HCI) conditions) – cognizant that the data were published by the manufacturers. PMI experiments showed that THS 2.2 aerosol did not contain solid carbon-derived particles, consistent with its claim of no combustion *(27)*. In general, the levels of constituents were lower than in a comparison reference cigarette (1R6F, which has certified levels of many of the emissions of concern *(28)*). Studies conducted within *(29)* and outside the industry *(30, 31)* largely replicated these findings, in particular the lower levels of nicotine in Eclipse and EHCSS than in a reference cigarette. British American Tobacco presented two posters on its HTP at the 2017 meeting of the Society for Research on Nicotine and Tobacco *(5, 32)* and in late 2017 had eight studies published in a supplement to a journal *(4, 33–40)*. The product, called THP1.0, is commercially marketed as glo. It heats tobacco (reconstituted sheet with 14.5% glycerol contained in a superslim cigarette) to a maximum of 250 °C *(32)*. Thermographic profiling suggested that the product releases moisture until 100 °C and then glycerol through 240 °C, followed by decomposition at 350 °C *(32)*. Data from machine smoking under modified HCI conditions generally showed lower emissions of TobReg priority toxicants *(41)* than in a reference cigarette (Table 2.2). THP1.0 was machine-smoked under HCI conditions except for the blocking of vent holes on the basis of a study that showed no vent blocking under actual smoking conditions (according to lip imprint measurements). A second poster *(42)* gave details of effects on indoor air and claimed that THP1.0 resulted in lower emissions than conventional cigarettes. In a laboratory comparison of British American Tobacco's iFuse with other HTPs (called cTHP in the paper; description fits iQOS) *(43)*, cTHP emitted higher levels of acetaldehyde (125 versus 35.9 µg per 10 puffs), while iFuse emitted more formaldehyde (< 4.18 versus 38.7 µg per 10 puffs). Both products emitted substantially less acetaldehyde than the 3R4F reference cigarette (> −88%), but iFuse emitted levels of formaldehyde comparable to those of a conventional cigarette (−8%). Bekki et al. *(31)* showed that the transfer rate of nicotine and nitrosamines from tobacco

filler to aerosol in iQOS was comparable to, if not slightly higher than, that in conventional cigarettes.

Table 2.2. Harmful and potentially harmful constituents emitted from reference cigarette 1R6F, Eclipse, an electrically heated cigarette smoking system (EHCSS), a tobacco heating system (THS) and a tobacco heating product (THP)

Constituent	1R6F (µg/ cigarette) ISO (28)	1R6F (µg/ciga- rette) HCI (28)	Eclipse (µg/ciga- rette) ISO (21)	ECHSS (µg/ciga- rette) ISO (44)	THS 2.2 (µg/ciga- rette) HCI (45)	THP1.0 (µg/ cigarette) HCI (32)
Acetaldehyde	522	1552	84.2	179	219	111
Acrolein	43	154	11.5	27.3	11.3	2.22
Acrylonitrile	7.0	24		0.44	0.258	< 0.032
4-Aminobiphenyl	1.2	2.3		0.058	< 0.051	< 0.005
2-Naphthol	8	14		0.123	0.046	< 0.012
Ammonia	9.0	30		4.37	14.2	4.01
Benzene	33	88		0.363	0.649	< 0.056
Benzo[a]pyrene	6.8	15	1.2	< 0.19	< 1.0	< 0.35
Carbon monoxide (mg)	10.1	28.0	7.5	0.465	0.531	< 0.223
Formaldehyde	27	104		12.9	5.53	3.29
Isoprene	320	881		34.3	2.35	< 0.135
Nicotine	0.721	1.90	0.18	0.313	1.32	0.462
NNK (ng)	71	187	31.8	6.18	6.7	6.61
NNN (ng)	85	212	26	19.8	17.2	24.7
Toluene	53	150		1.48	2.59	< 0.204
Tar (mg)	8.58	29.1	3.2	3.1	10.3	13.6

EHCSS: electrically heated cigarette smoking system; HCI: Health Canada intense smoking regimen; ISO: International Organization for Standardization smoking regimen; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: N´-nitrosonornicotine; THP: tobacco heating product; THS: tobacco heating system.

2.4.2   Biomarkers of exposure

In 2008, Philip Morris USA and PMI published a series of papers on its EHCSS, including studies of toxicology, emissions (also second-hand smoke) and short- and long-term clinical exposure, and randomized trials *(44, 46–48)*. A series of papers followed in 2012 *(49–56)*. In 2016, PMI published another series of studies, this time on THS 2.2 *(3, 45, 57–59)*, which reported the results of a broad range of product evaluations. The data on biomarkers after short-term exposure (~ 1 week, generally in residence) shown in Tables 2.3 and 2.4 were extracted from these papers *(47, 49, 51–54, 58, 59)*. In studies in several populations, the levels of key toxicants, except nicotine, were lower than with continued cigarette smoking. As the participants in these studies were generally confined for the duration of the study, however, the findings may not be generalizable to real conditions of use (e.g. pattern of product use, use with cigarettes or other combusted or non-combusted products).

Table 2.3A. Biomarker levels in short-term studies of electrically heated cigarette smoking systems, with end-of-study values for numbers of conventional cigarettes and heated tobacco products and for abstinence when available, Japan and USA

Biomarker	HPHC	Japan (6 days) (53)			USA (8 days) (47)			Japan (8 days) (52)			
		Cigarettes	EHCSS	Abstinence	Cigarettes	EHCSS	Abstinence	Cigarettes	EHCSS-K6	EHCSS-K3	Abstinence
Nicotine equivalent	Nicotine (mg/24 h)	7.2	3.4	0.6	16.1	8.2	0.2	9.6	5.1	3.9	0.2
Nicotine	Nicotine	NR	NR	NR	NR	NR	NR	19.0	10.1	8.1	0.5
Cotinine	Nicotine (ng/mL)	150.5	79.4	11.1	NR	NR	NR	177.9	111.2	79.5	7.4
NNAL	NNK (ng/24 h)	188.0	95.0	80.0	601.3	169.1	117.8	216.0	100.0	102.0	83.0
COHb	Carbon monoxide (%)	5.1	2.1	1.9	6.4	2.1	1.1	5.4	2.1	2.2	1.7
MHBMA	1,3-Butadiene (µg/24 h)	1.5	0.6	0.4	4.8	1.5	0.2	1.5	0.7	0.7	0.4
3-HPMA	Acrolein (mg/24 h)	1.2	0.8	0.5	1859.0	1056.7	302.3	1.4	1.1	1.0	0.5
S-PMA	Benzene (µg/24 h)	2.3	0.4	0.5	5.3	1.1	0.4	2.2	0.6	0.5	0.3
1-OHP	Benzo[a]pyrene (ng/24 h)	106.5	38.4	37.4	149.6	58.1	41.1	135.3	56.0	59.0	42.0
3-OH-Benzo[a]pyrene	Benzo[a]pyrene	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4-Aminobiphenyl	4-Aminobiphenyl (ng/24 h)	9.4	4.4	4.7	12.6	3.1	2.0	15.0	4.2	3.9	4.6
1-Naphthylamine	1-Naphthylamine	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2-Naphthylamine	2-Naphthylamine (ng/24 h)	4.6	4.4	4.7	20.0	4.2	2.2	27.2	6.9	6.1	5.9
o-Toluidine	Toluene (ng/24 h)	66.2	33.6	33.8	87.6	58.5	40.9	103.9	31.1	27.7	33.8
HMPMA	Croton-aldehyde (mg/24 h)	0.5	0.2	0.1	2320.7	750.4	299.0	1.3	0.6	0.6	0.5

COHb: carboxyhaemoglobin; EHCSS: electrically heated cigarette smoking system; HMPMA: hydroxy methyl propylmercapturic acid; HPHC: harmful and potentially harmful constituent; 3-HPMA: 3-hydroxypropyl mercapturic acid; MHBMA: monohydroxy-butenylmercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NR: not recorded; 1-OHP: 1-hydroxypyrene; S-PMA: S-phenylmercapturic acid. ᵃ K6 and K3 are two versions of the system tested.

Table 2.3B. Biomarker levels in short-term studies of electrically heated cigarette smoking systems, with end-of-study values for numbers of conventional cigarettes and heated tobacco products and for abstinence when available, United Kingdom and Republic of Korea

Biomarker	HPHC	Republic of Korea (8 days) *(51)*			United Kingdom (8 days) *(54)*		
		EHCSS-K3[a]	Absti-nence	Ciga-rette	EHCSS-K6[a]	EHCSS-K3[a]	Absti-nence
Nicotine equivalent	Nicotine (mg/24 h)	4.2	0.1	13.1	8.6	6.5	0.0
Nicotine	Nicotine	8.6	0.1	NR	NR	NR	NR
Cotinine	Nicotine (ng/mL)	85.8	0.1	234.3	158.3	125.0	0.0
NNAL	NNK (ng/24 h)	80.4	45.1	293.6	100.6	104.3	59.2
COHb	Carbon monoxide (%)	0.9	0.5	5.7	1.4	1.9	0.5
MHBMA	1,3-Butadiene (µg/24 h)	0.6	0.3	5.1	1.5	2.6	0.3
3-HPMA	Acrolein (mg/24 h)	2.4	1.7	1.8	1.3	1.2	0.5
S-PMA	Benzene (µg/24 h)	1.6	1.4	6.2	0.9	1.3	0.2
1-OHP	Benzo[a]pyrene (ng/24 h)	143.6	127.0	181.6	71.9	73.1	75.1
3-OH-Benzo[a]pyrene	Benzo[a]pyrene	NR	NR	NR	NR	NR	NR
4-Aminobiphenyl	4-Aminobiphenyl (ng/24 h)	5.5	5.5	NR	NR	NR	NR
1-Naphthylamine	1-Naphthylamine	NR	NR	NR	NR	NR	NR
2-Naphthylamine	2-Naphthylamine (ng/24 h)	5.4	5.5	NR	NR	NR	NR
o-Toluidine	Toluene (ng/24 h)	29.1	30.2	135.0	58.0	49.3	47.6
HMPMA	Crotonaldehyde (mg/24 h)	2.0	1.7	5.2	2.6	2.6	1.7

COHb: carboxyhaemoglobin; EHCSS: electrically heated cigarette smoking system; HMPMA: hydroxy methyl propylmercapturic acid; HPHC: harmful and potentially harmful constituent; 3-HPMA: 3-hydroxypropyl mercapturic acid; MHBMA: monohydroxy-butenylmercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NR: not reported; 1-OHP: 1-hydroxypyrene; S-PMA: S-phenylmercapturic acid. [a] K6 and K3 are two versions of the system tested.

Table 2.4. Levels of biomarkers reported in published short-term studies with tobacco heating system 2.2, with end-of-study values for conventional cigarettes, heated tobacco products and abstinence, when available

Biomarker	HPHC	Japan *(58, 60)*			Poland *(59)*		
		THS	Ciga-rettes	Absti-nence	THS	Ciga-rettes	Absti-nence
Nicotine equivalent	Nicotine (mg/g creatinine)	5.44	5.52	0.15	10.60	9.76	0.14
Nicotine	Nicotine	19.13	21.34	0.10	20.74	19.01	0.10
Cotinine	Nicotine	161.00	164.30	2.96	239.99	219.73	2.05
NNAL	NNK (pg/mg creatinine)	37.77	76.55	28.63	49.65	107.04	41.51
NNN	N´-nitrosonornicotine (pg/mg creatinine)	1.31	4.64	0.18	1.55	5.99	0.16
COHb	Carbon monoxide (%)	2.39	5.14	2.37	1.06	4.51	0.99
MHBMA	1,3-Butadiene (pg/mg creatinine)	107.39	450.19	92.18	192.93	2399.40	163.17
3-HPMA	Acrolein (ng/mg creatinine)	311.08	599.67	199.04	402.26	931.01	245.69
S-PMA	Benzene (pg/mg creatinine)	143.77	850.02	126.34	164.45	2922.81	143.70
1-OHP	Benzo[a]pyrene (pg/mg creatinine)	73.02	149.62	62.99	81.22	182.85	85.13
3-OH-benzo[a]pyrene	Benzo[a]pyrene (fg/mg creatinine)	29.52	96.42	24.47	37.07	130.29	33.64
4-Aminobiphenyl	4-Aminobiphenyl (pg/mg creatinine)	1.53	8.57	1.49	1.90	12.58	1.60
1-Naphthylamine	1-Naphthylamine (pg/mg creatinine)	2.47	57.08	2.45	3.30	89.37	2.56

2-Naphthylamine	2-Naphthylamine (pg/mg creatinine)	2.33	13.38	2.27	2.96	25.32	2.52
o-Toluidine	Toluene (pg/mg creatinine)	50.40	98.18	48.91	51.15	121.16	41.64
CEMA	Acrylonitrile (ng/mg creatinine)	10.61	54.19	9.04	13.18	99.48	12.60
HEMA	Ethylene oxide (pg/mg creatinine)	997.76	2099.41	806.29	1342.40	4504.00	1248.27
HMPMA	Crotonaldehyde (ng/mg creatinine)	59.51	157.83	47.84	86.65	376.78	63.25
S-BMA	Toluene (ng/mg creatinine)	2098.09	2354.17	2192.86	NR	NR	NR

CEMA: 2-chloroethylmethacrylate; COHb: carboxyhaemoglobin; HEMA: hydroxyethylmethacrylate; HMPMA: hydroxy methyl propylmercapturic acid; 3-HPMA: 3-hydroxypropyl mercapturic acid; MHBMA: monohydroxy-butenylmercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: N´-nitrosonornicotine; NR: not reported; 1-OHP: 1-hydroxypyrene; S-BMA: S-benzylmercapturic acid; S-PMA: S-phenylmercapturic acid; THS: tobacco heating system.

PMI also published the results of longer-term clinical studies of EHCSS (12 weeks) and THS 2.2 (90 days). After use of EHCSS for 12 weeks by 60 participants in the USA, the level of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, a biomarker of exposure to NNK) dropped by 63% (P <.0001), that of carboxyhaemoglobin (a biomarker of exposure to CO) by 23% (P <.0001) and that of S-phenylmercapturic acid (a biomarker of exposure to benzene) by 49% (P <.0001) (*48*). (The paper did not provide mean estimates at each time.) After 90 days of use of THS 2.2 by 76 participants in Japan, the level of NNAL dropped by 73%, that of carboxyhaemoglobin by 42% and that of S-phenylmercapturic acid by 86% (*61*). A number of other biomarkers of exposure were measured (Table 2.5), and participants were followed up for 90 days for continued smoking and abstinence. Exposure to certain constituents was reduced with use of the THS rather than conventional cigarettes, although exposure to a number of constituents, including nitrosamines and acrolein, remained substantially higher than during smoking abstinence.

Table 2.5. End-of-study biomarker levels in participants in a 90-day study of random assignment to THS 2.2, continued cigarette smoking or abstinence, Japan

Biomarker	HPHC	THS	Ciga-rettes	Absti-nence	Differ-ence from cig-arettes (%)	Differ-ence from ab-stinence (%)
Nicotine equivalent	Nicotine (mg/g creatinine)	7	6	0	8	1751
NNAL	NNK (pg/mg creatinine)	23	95	14	−76	67
NNN	NNN (pg/mg creatinine)	1	4	0	−67	438
COHb	CO (%)	3	6	3	−48	−2
MHBMA	1,3–Butadiene (pg/mg creatinine)	142	785	137	−82	4
3-HPMA	Acrolein (ng/mg creatinine)	386	696	276	−44	40
S-PMA	Benzene (pg/mg creatinine)	146	1157	144	−87	1
1-OHP	Benzo[*a*]pyrene (pg/mg creatinine)	85	167	88	−49	−3
3-OH-Benzo[*a*]pyrene	Benzo[*a*]pyrene (fg/mg creatinine)	30	87	29	−65	4
4-Aminobiphenyl	4-Aminobiphenyl (pg/mg creatinine)	2	10	2	−78	−12
1-Naphthylamine	1-Naphthylamine (pg/mg creatinine)	4	55	4	−94	−16

2- Naphthylamine	2-Naphthylamine (pg/mg creatinine)	2	149	3	−98	−11
o-Toluidine	Toluene (pg/mg creatinine)	68	126	78	−46	−12
CEMA	Acrylonitrile (ng/mg creatinine)	8	84	8	−91	−6
HEMA	Ethylene oxide (pg/mg creatinine)	1742	3739	1633	−53	7
HMPMA	Crotonaldehyde (ng/mg creatinine)	154	299	159	−48	−3

CEMA: 2-chloroethylmethacrylate; CO: carbon monoxide; COHb: carboxyhaemoglobin; HEMA: hydroxyethylmethacrylate; HMPMA: hydroxy methyl propylmercapturic acid; HPHC: harmful and potentially harmful constituent; 3-HPMA: 3-hydroxypropyl mercapturic acid; MHBMA: monohydroxy-butenylmercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N´*-nitrosonornicotine; 1-OHP: 1-hydroxypyrene; S-PMA: S-phenylmercapturic acid.

When a compensation formula
$$1 - ((\ln(marker1)\text{-}\ln(marker0)) / (\ln(yield1)\text{–}\ln(yield0))$$
is used to compare changes in machine-measured emissions (ISO yields for EHCSS, HCI yields for THSs, each compared with 1R6F which has certified values for specific HPHCs in smoke) with levels of biomarkers of exposure, there is clearly potentially substantial compensation, including significant, near-total compensation for nicotine with the THS (Table 2.6). The ability to obtain increased levels of nicotine may partly explain differences in adoption of previous-generation HTPs and of iQOS.

Table 2.6. Potential compensation for selected harmful and potentially harmful constituents (HPHCs) in Philip Morris International heated tobacco products relative to a reference cigarette

HPHC	ECHSS (Japan), %	EHCSS (USA), %	THS (Japan), %	THS (Poland), %
Acrolein	79	67	75	68
Acrylonitrile	NR	NR	64	55
4-Aminobiphenyl	79	62	55	50
2-Naphthol	99	67	69	62
Benzene	66	71	64	41
Benzo[*a*]pyrene	77	78	74	70
Carbon monoxide (mg)	78	73	81	63
Nicotine	58	63	96	123
NNK (ng)	80	63	79	77
Toluene	85	91	84	79

ECHSS: electrically heated cigarette smoking system; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NR: not reported; THS: tobacco heating system.

No published reports of biomarkers of exposure to the THP1.0 product were identified, although a trial protocol for such a study has been published *(62)*. British American Tobacco has published one study of human use of the glo product to determine usage patterns *(38)*. Three groups took three products (glo-menthol, glo-tobacco, glo + iQOS) home for up to 14 days, with up to four laboratory visits, while a fourth group used glo in the laboratory only. The measures included puffing topography, mouth-level exposure and mouth insertion depth. Participants who took the products home completed daily diaries of product use.

Overall, the puff volume was about 60 mL with the glo product, with an average of 10–12 puffs per session, a duration of 1.8–2.0 s and a mean interval of 8 s. The volume per puff and the total volume were significantly higher than with comparison cigarettes but comparable to those for the iQOS product. At baseline, participants reported using 12–15 cigarettes per day and about 8–12 units of the glo and iQOS per day during the 4 days at home. The mouth-level exposure per product used was lower with glo than with conventional cigarettes, especially for nicotine. The average mouth insertion depth was 7.7 mm, and the company used this as an argument to omit the vent-blocking procedure from the HCI machine smoking regimen *(38)*.

Thus far, consumer use of personal vaporizers to heat tobacco has been examined in only one published study *(63)*, in which Pax (loaded with 1 g of roll-your-own cigarette tobacco) was compared with the smokers' own brand of cigarette and an e-GO-type e-cigarette among 15 participants in a laboratory paradigm with fixed puffing patterns. The vaporizer increased plasma nicotine to a lesser extent than the smokers' own brand (14.3 ng/mL vs 24.4 ng/mL) and was equivalent to an e-cigarette, with no substantial exposure to CO. No other biomarkers were reported.

2.5 Consumer perceptions of heated tobacco products

Population surveys and internal industry marketing studies reveal strong consumer demand for products that are claimed or implied to carry reduced health risks *(64–70)*. University students rated Eclipse less positively but also less negatively than Marlboro Lights after seeing a single advertisement for each product, although none had ever tried the product *(71)*. Shiffman and colleagues showed in two studies *(67, 69)* that smokers perceived Eclipse as reducing harm and that interest in using Eclipse was associated with reduced intention to quit smoking. Hamilton and colleagues *(64)* showed advertisements for several PREPs and conventional tobacco products to smokers and found that, even though the advertisements contained no health claims, PREPs were still perceived as less of a health risk and conveyed positive messages about health and safety. Data from Japan *(72)* suggested that a significant minority of consumers were aware of and interested in HTPs *(72)*. Although 48% of the 8240 respondents to the survey were aware of e-cigarettes and/or HTPs, actual use of any products was fairly low; 6.6% had used any of the products, and the vast majority had used e-cigarettes. These are not legally sold in Japan but may be imported by individuals for personal use. Only 0.51% of the respondents had ever used Ploom, and 0.55% had used iQOS (7.8% and 8.4%, respectively, among users of any HTP). A later study *(73)* showed a dramatic growth in use between 2016 and 2017, the prevalence of iQOS use rising from 0.6% in 2016 to 3.6% in 2017.

A common reason given for the failure of novel products is rejection by consumers because of their poor taste *(74,75)*. Sensory characteristics are an important part of cigarette design *(76, 77)*. A consistent finding of clinical studies of subjective effects of Eclipse and Accord is that the products are perceived negatively relative to their own brand, with generally low ratings of satisfaction and higher ratings of dislike *(6, 7, 10, 22, 23)*. Established smokers who took part in focus groups reported significant dislike of the PREP cigarettes that they tried, including Eclipse and Accord, and almost all reported that they would not recommend the products to other smokers *(78, 79)*. Caraballo and colleagues *(78)* found that established smokers who had tried Eclipse generally disliked the product and would not recommend it to other smokers. Most smokers who reported that they disliked Eclipse cigarettes considered them too mild and said that they did not deliver enough nicotine to satisfy their craving; many reported that they disliked the taste. Hughes and colleagues *(80)* reported that smokers who tried Eclipse generally did not like it, although they believed it to be safer than conventional cigarettes. A study of the Pax vaporizer *(63)* indicated that the vaporizer did partially suppress symptoms of abstinence but was considered significantly less satisfying and less tasty than the participants' own brand of cigarettes.

Studies published by PMI on its THS device include data on subjective responses to the product, which were not provided in reports of studies of EHCSS and previous-generation products. These data are often important for understanding why smokers use a product and how effective it might be as a substitute *(81, 82)*. The studies were conducted with the widely used modified cigarette evaluation questionnaire *(83)* and the brief questionnaire on smoking urges to measure craving *(84)*. The cigarette evaluation questionnaire consists of 12 items (seven-point scales) and resolves to five scales of: smoking satisfaction, aversion, craving reduction, enjoyment of respiratory tract sensation and psychological reward *(83)*. The questionnaire on smoking urges consists of 10 items and provides a single score on a seven-point scale *(84)*.

In a laboratory study of a THS in Japan, the mean satisfaction scores decreased more for the THS than for conventional cigarettes over the course of the study (mean = −0.69; 95% confidence interval (CI), −0.34, −1.04) *(58)*. Other scores in the cigarette evaluation questionnaire did not change or differ as substantially from those for cigarettes. No difference in the scores on the questionnaire on smoking urges was observed for the THS and conventional cigarettes, both of which were lower than that for smoking abstinence (as expected). In a similar study in Poland, the observed differences in subjective effects between a THS and cigarettes were substantially larger and statistically significant for satisfaction (mean = −1.26; 95% CI, −0.85, −1.68), craving reduction (mean = −1.12; 95% CI, −0.66, −1.58), sensation (mean = −1.00; 95% CI, −0.64, −1.36) and reward (mean = − 0.72; 95% CI, −0.39, −1.06). No

significant difference in craving scores was seen between the THS and cigarettes, and both were significantly lower than those for abstinence. These studies suggest that Polish smokers viewed the THS less positively than Japanese smokers; this may have implications for the generalization of observations from one market to another. An earlier study of THS 2.1 *(85)* showed a similar pattern of results, with satisfaction scores on day 5 an average of 1.4 points lower for the THS than for conventional cigarettes (P <.001). Significant differences were also seen on the reward, sensation and craving subscales, the THS scoring lower than cigarettes in all cases. A study on longer-term use in Japan *(61)* suggested that the difference in satisfaction fades with continued use over 90 days. These data suggest that scores on the questionnaire on smoking urges increase for the THS over time, as do scores for withdrawal (as measured on the Minnesota nicotine withdrawal scale *(86)*). This may suggest some dissatisfaction with the product as a longer-term substitute for smoking over time.

2.6   Uptake in selected markets where products are available

Three studies have been conducted of population uptake of HTPs in Italy and Japan. In Italy, 1% of "never smokers", 0.8% of former smokers and 3% of current cigarette smokers had tried iQOS *(87)*. Tabuchi and colleagues *(73)* showed a rising prevalence of current use of iQOS (from 0.3% to 3.6%) and Ploom (from 0.3% to 1.2%) after 2015, with comparable prevalence for use of glo in 2017 (0.8%). Predictors of use of HTPs were current cigarette smoking (stronger effect with intention to quit), living in a more deprived area and having seen television promotion of iQOS. Dual use was common (72%). Unlike for conventional cigarette smoking, there does not appear to be an inverse relation between education and HTP use in Japan *(88)*.

One reason that HTPs are gaining a market share in Japan is that nicotine-containing ENDS are not permitted for sale. Thus, HTPs may fill a market niche in a country with a relatively high smoking rate and relatively weak tobacco control laws. iQOS has become available in several markets in the European Union (which permit sale of ENDS) and in Canada (which did not permit such sales until 2018). In a presentation to the Consumer Analyst Group of New York in 2017 *(89)*, PMI reported that the attained market share of iQOS had reached 4.9% in Japan in the fourth quarter of 2016 but was substantially lower in marketing focus areas in Switzerland (1.7%), Portugal (0.7%), Romania (0.6%), Italy (0.4%) and the Russian Federation (0.3%), where the product had been available for at least one year. High rates of conversion to iQOS were claimed on the basis of their user panels, ranging from 54% in Switzerland to 72% in Japan.

2.7 Application by Philip Morris International for status as a "modified risk tobacco product" in the USA

In May 2017, PMI submitted an MRTP application for its THS/iQOS to the United States Food and Drug Administration (FDA) for review. The three health claims under consideration were:

- "Switching completely from cigarettes to the iQOS system can reduce the risks of tobacco-related diseases."
- "Switching completely to iQOS presents less risk of harm than continuing to smoke cigarettes."
- "Switching completely from cigarettes to the iQOS system significantly reduces your body's exposure to harmful and potentially harmful chemicals."

In compliance with the Tobacco Control Act, redacted[2] versions of the full application were made available for public review and comment on the website of the FDA (https://www.fda.gov/tobaccoproducts/labeling/marketingandadvertising/ucm546281.htm). Much of the technical information about the device has been redacted. The findings cited in publicly available parts of the application that are not discussed above (i.e. unpublished studies) are discussed below.

The online material includes an additional human trial with THS 2.2, conducted in the USA in 2013–2014 (NCT01989156). Of the 160 participants who were randomized, 88 completed the full 90-day study. As in the studies in Japan and Poland, key biomarkers of exposure were significantly reduced as compared with those for cigarette smoking after 5 and 90 days. Compliance with abstinence was, however, substantially lower in this study than in that in Japan during the ambulatory phase, suggesting that experience in Japan cannot necessarily be generalized to the USA.

The MRTP documents also report on a series of studies to test the specific claims requested for iQOS on the United States market. A series of qualitative and quantitative studies is described, concluding with three "assessment phase" studies on comprehension of claims and risk perception in a variety of contexts (package, brochure, direct mail). PMI developed a multi-factor risk perception scale for use in this study. The studies were conducted with three samples of approximately 2500 adult current smokers (with and without intention to quit), 2500 former smokers and 2500 nonsmokers. iQOS was rated as being of intermediate risk between cigarettes and quitting, comparable to the risk rating for e-cigarettes. Comprehension of claims was good, although there was evidence that about 25% of the participants extrapolated reductions in exposure to reduced harm

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2   Applications are redacted to remove information considered to be commercially confidential and/or trade secrets under United States law.

(which is potentially erroneous). It was not evident that the claims shifted risk perceptions of iQOS or led to increased intention to try iQOS among smokers.

A series of observational studies were reported on product-switching in Germany, Italy, Japan, the Republic of Korea, Switzerland and the USA. "Whole offer tests" were conducted with 2089 daily smokers in the first five countries. iQOS was made available for free for 4 weeks, and product use was recorded in electronic diaries. At the end of the 4-week trial, the rate of switching to a THS ranged from 10% in Germany to 37% in the Republic of Korea, and the rate of dual use ranged from 32% in Japan to 39% in the Republic of Korea. The study in the USA comprised 1106 current daily smokers who, after a 1-week baseline, were given free access to iQOS for 4 weeks. Product use (cigarettes and Heatsticks) was recorded in an electronic diary. At the end of the study, approximately 15% of subjects had switched to the THS, as defined in the study (> 70% of total consumption as Heatsticks), while 22% were dual users (30–70% of consumption as Heatsticks).

Limited post-marketing data were presented, primarily from Japan, drawing on PMI's registry of iQOS purchasers. They reported that the proportion of exclusive iQOS use (> 95% of total consumption) increased from 52% to 65% between January and July 2016. Markov modelling of transition in two cohorts of iQOS purchasers in Japan (September 2015 and May 2016) suggested that smokers who converted to exclusive iQOS use were unlikely to return to exclusive cigarette use (although scant details of the modelling are given in the executive summary).

At the meeting of the FDA Tobacco Products Scientific Advisory Committee in January 2018,[3] PMI presented evidence in support of iQOS, including much of the data contained in this report. The FDA presented its preliminary evaluation of the submission, and the Committee expressed concern about portions of the evidence base, in particular the definitions of "complete switching" and limitations to the studies that underlie consumer understanding of the claims. The Committee did not support the claims of risk modification, expressed support for the claim of exposure modification and expressed concern that the claims as worded would not be effective in communicating risk.[1] The Committee's recommendations to the FDA are nonbinding.

2.8   Implications for regulation and tobacco control policies

Predicting the uptake of unconventional tobacco products can be difficult. Analyses of trial testimony by the tobacco industry on older-generation PREPs showed that the industry sought to shift the focus from their responsibility to produce "safer" products to failure of smokers to adopt the "safer" products they

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3   See https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/TobaccoProductsScientificAdvisoryCommittee/UCM599235.pdf.

have been offered *(75)*. Nonetheless, this market segment appears to be growing rather than shrinking and is capturing a significant market share in at least one country, Japan, where PMI claims to have sold more than 1 million iQOS devices *(90)*. The human studies available (all performed by the manufacturer) indicated that iQOS deliver fewer toxicants than cigarettes and may serve as an effective short-term substitute for conventional cigarettes, as assessed by nicotine delivery and subjective effects measured on the cigarette evaluation questionnaire; one 90-day study suggested similar outcomes. It must be remembered, however, that the participants in these research studies were compensated, and the results may therefore not reflect real usage patterns, including concurrent use of multiple products. In December 2017, committees on the Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment evaluated two HTPs on the market in the United Kingdom (iQOS and iFuse) and concluded *(91)* that:

> "While there is a likely reduction in risk for smokers switching to 'heat-not-burn' tobacco products, there will be a residual risk and it would be more beneficial for smokers to quit smoking entirely. This should form part of any long-term strategy to minimize risk from tobacco use."

"Product drift" may be another important consideration. In their presentation to the Consumer Analyst Group of New York *(89)*, PMI described certain modifications made to the device from the original product pilot-tested in Japan in 2014–2017, including better aesthetics, blade self-cleaning, better user interface, faster charging, Bluetooth connectivity and an accompanying mobile application. Colours are being used to increase the appeal of the device. Thus, a product may be a "moving target" after its introduction. These practices are not unlike those of the tobacco industry of making minor adjustments to their cigarette products over time and by market *(92–94)*, such that a Marlboro cigarette in 2017 is not necessarily identical to one in 2010, and a Marlboro sold in France is not necessarily the same as one sold in the USA. In addition, data on a product from research studies may not fully reflect the product currently available to consumers. Thus, research to support a product is not necessarily conducted with the final marketed product but with a prototype or even a series of different prototypes. While this practice is not in itself nefarious, any differences in design, function or presentation between the studied and marketed product should be established, including their impact on consumer use. The FDA requires reporting of changes to an existing product for marketing authorization and may monitor them, with greater scrutiny and requirements for changes that impinge on public health (e.g. changes to delivery of HPHCs; substantial design changes). European Union Member States have similar provisions, in line with the Tobacco Products

Directive. Regulators in other countries should consider a similar requirement for notice and justification of changes to products.

Side-stream emissions and second-hand exposure are further concerns with the introduction of unconventional tobacco products. Some tobacco manufacturers claim that certain HTPs result in minimal exposure to side-stream smoke *(42, 46, 95, 96)*, whereas some studies indicate substantial levels *(97, 98)*. The emissions may depend in part on the design of the product. Side-stream emissions have health and regulatory implications, and further research is needed to characterize environmental exposure from HTPs. Depending on the wording of claims, HTPs may or may not be covered by smoke-free legislation, for example. Nevertheless, the precautionary principle would support covering such emissions in regulations.

2.9 Recommendations for research and policy

Independent scientific evidence is required to verify the claims of industry scientists for reduced exposure and risk. The studies published in the peer-reviewed literature up to October 2017, primarily by PMI on their THS 2.2 product, focused on a subset of HPHCs. Biomarkers of exposure to metals, such as cadmium, were not reported in these studies. Metals are a concern both as carcinogens and potentiators (cadmium, nickel, cobalt, arsenic) and as toxicants in their own right (lead, copper). While most of the studies indicated reduced exposure to HPHCs as compared with cigarette smoking, the studies did not address the possibility of novel exposure from the THS, either from the heating system itself or from the additives used in the tobacco. In its MRTP presentation to the FDA Tobacco Products Scientific Advisory Committee, when pressed on these issues, PMI acknowledged that 50 constituents in iQOS aerosol were present at higher levels than in conventional cigarette smoke, three of which are unique to iQOS; about 750 constituents occur at equal or lower levels in iQOS than in conventional cigarette smoke, and > 4000 are unique to cigarette smoke. Of the 50 constituents, four were identified as of toxicological concern (glycidol, 2-furanemethanol, 3-monochloro-1,2-propanediol and furfural).

Few published data are available on British American Tobacco's HTPs, although a study protocol for a randomized trial has been published *(62)*, suggesting ongoing work in this area by the company. No published studies on Japan Tobacco International's Ploom product were identified, and data on exposure to tobacco HPHCs from personal vaporizers are similarly lacking. Data on usage and sales of personal vaporizers for tobacco use are extremely difficult to locate, and comparisons with this market segment cannot be made. Use of such devices to administer cannabis appears to be increasing *(99–101)*.

Some suggestions for near- and long-term research priorities are:

- monitoring of product availability, sales and marketing with validated tools (e.g. *(102)*);
- monitoring of product use, including complete switching, dual (or poly) use, use as compared with ENDS and initiation by non-tobacco users, with a particular focus on low-risk young people who would not otherwise have smoked;
- verification of reported product contents and emissions of the 39 priority toxicants and/or the FDA HPHC list;
- evaluation of potential novel toxicants produced by heated tobacco products that are not covered by commonly accepted lists (e.g. Hoffman analytes, HPHCs);
- evaluation of aerosol particle size distribution;
- assessment of device function and safety (e.g. batteries);
- cross-market comparisons of products, e.g. whether iQOS is the same in all markets and differences in the characteristics, contents and emissions of products and how they have changed over time;
- independent clinical and biomarker analyses of users according to typical patterns of use, including dual or concurrent use of heat-not-burn products and cigarettes;
- public perceptions of products (awareness, intention to use, risk) among users and non-users of tobacco;
- information on who is purchasing a product, reasons for purchasing and use patterns;
- research on rates of conversion of smokers to HTPs and vaping (ENDS) products, to determine whether these products discourage smoking in a way that is acceptable to smokers;
- modelling of potential population-level effects of the introduction and use of products (e.g. SimSmoke; microsimulation);
- investigation of the influence of marketing strategies on user behaviour, including whether these products are marketed as complementary or alternative products;
- effect of heat treatment on components in products other than tobacco (e.g. flavours); and
- studies of exposure to second-hand emissions (including effects on children and pregnant women) and their contribution to background air quality.

2.10   References

1.   Rodgman A, Perfetti TA. The chemical components of tobacco and tobacco smoke. 2nd ed. Boca Raton (FL): CRC Press; 2013.

2.   Batista RN. Reinforced web of reconstituted tobacco. Google Patents WO2015193031A1; 2015 (https://patents.google.com/patent/WO2015193031A1/en, accessed 1 October 2018).

3.   Smith MR, Clark B, Ludicke F, Schaller P, Vanscheeuwijck P, Hoeing J et al. Evaluation of the tobacco heating system 2.2. Part 1: Description of the system and the scientific assessment program. Regul Toxicol Pharmacol. 2016;81(Suppl 2):S17–26.

4.   Eaton D, Jakaj B, Forster M, Nicol J, Mavropoulou E, Scott K et al. Assessment of tobacco heating product THP1.0. Part 2: Product design, operation and thermophysical characterisation. Regul Toxicol Pharmacol. 2018;93:4–13.

5.   Scott JK, Poynton S, Margham J, Forster M, Eaton D, Davis P et al. Controlled aerosol release to heat tobacco: product operation and aerosol chemistry assessment. Poster. Society for Research on Nicotine and Tobacco; 2–5 March 2016; Chicago (IL); 2016 (https://www.researchgate.net/publication/298793405_Controlled_aerosol_release_to_heat_tobacco_product_operation_and_aerosol_chemistry_assessment, accessed October 2018).

6.   Breland AB, Buchhalter AR, Evans SE, Eissenberg T. Evaluating acute effects of potential reduced-exposure products for smokers: clinical laboratory methodology. Nicotine Tob Res. 2002;4(Suppl 2):S131–40.

7.   Breland AB, Kleykamp BA, Eissenberg T. Clinical laboratory evaluation of potential reduced exposure products for smokers. Nicotine Tob Res. 2006;8(6):727–38.

8.   Fagerstrom KO, Hughes JR, Callas PW. Long-term effects of the Eclipse cigarette substitute and the nicotine inhaler in smokers not interested in quitting. Nicotine Tob Res. 2002;4(Suppl 2):S141–5.

9.   Fagerstrom KO, Hughes JR, Rasmussen T, Callas PW. Randomised trial investigating effect of a novel nicotine delivery device (Eclipse) and a nicotine oral inhaler on smoking behaviour, nicotine and carbon monoxide exposure, and motivation to quit. Tob Control. 2000;9(3):327–33.

10.   Lee EM, Malson JL, Moolchan ET, Pickworth WB. Quantitative comparisons between a nicotine delivery device (Eclipse) and conventional cigarette smoking. Nicotine Tob Res. 2004;6(1):95–102.

11.   Rennard SI, Umino T, Millatmal T, Daughton DM, Manouilova LS, Ullrich FA et al. Evaluation of subclinical respiratory tract inflammation in heavy smokers who switch to a cigarette-like nicotine delivery device that primarily heats tobacco. Nicotine Tob Res. 2002;4(4):467–76.

12.   Stapleton JA, Russell MA, Sutherland G, Feyerabend C. Nicotine availability from Eclipse tobacco-heating cigarette. Psychopharmacology. 1998;139(3):288–90.

13.   Stewart JC, Hyde RW, Boscia J, Chow MY, O'Mara RE, Perillo I et al. Changes in markers of epithelial permeability and inflammation in chronic smokers switching to a nonburning tobacco device (Eclipse). Nicotine Tob Res. 2006;8(6):773–83.

14.   Pauly JL, Lee HJ, Hurley EL, Cummings KM, Lesses JD, Streck RJ. Glass fiber contamination of cigarette filters: an additional health risk to the smoker? Cancer Epidemiol Biomarkers Prev. 1998;7(11):967–79.

15.   Pauly JL, Stegmeier SJ, Allaart HA, Cheney RJ, Zhang PJ, Mayer AG et al. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev. 1998;7(5):419–28.

16.   Cummings KM, Hastrup JL, Swedrock T, Hyland A, Perla J, Pauly JL. Consumer perception of risk associated with filters contaminated with glass fibers. Cancer Epidemiol Biomarkers Prev. 2000;9(9):977–9.

17.   Higuchi MA, Ayres PH, Swauger JE, Morgan WT, Mosberg AT. Quantitative analysis of potential transfer of continuous glass filament from eclipse prototype 9-014 cigarettes. Inhal Toxicol.

2000;12(11):1055–70.

18. Higuchi MA, Ayres PH, Swauger JE, Morgan WT, Mosberg AT. Analysis of potential transfer of continuous glass filament from Eclipse cigarettes. Inhal Toxicol. 2000;12(7):617–40.

19. Swauger JE, Foy JW. Safety assessment of continuous glass filaments used in Eclipse. Inhal Toxicol. 2000;12(11):1071–84.

20. Higuchi MA, Ayres PH, Swauger JE, Deal PA, Guy T, Morton M et al. Quantification of continuous glass filaments on eclipse cigarettes retrieved from the test market. Inhal Toxicol. 2003;15(7):715–25.

21. Slade J, Connolly GN, Lymperis D. Eclipse: does it live up to its health claims? Tob Control. 2002;11(Suppl 2):ii64–70.

22. Buchhalter AR, Eissenberg T. Preliminary evaluation of a novel smoking system: effects on subjective and physiological measures and on smoking behavior. Nicotine Tob Res. 2000;2(1):39–43.

23. Buchhalter AR, Schrinel L, Eissenberg T. Withdrawal-suppressing effects of a novel smoking system: comparison with own brand, not own brand, and de-nicotinized cigarettes. Nicotine Tob Res. 2001;3(2):111–8.

24. Hughes JR, Keely JP. The effect of a novel smoking system – Accord – on ongoing smoking and toxin exposure. Nicotine Tob Res. 2004;6(6):1021–7.

25. Roethig HJ, Zedler BK, Kinser RD, Feng S, Nelson BL, Liang Q. Short-term clinical exposure evaluation of a second-generation electrically heated cigarette smoking system. J Clin Pharmacol. 2007;47(4):518–30.

26. United States Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed Reg. 2012:20034–7.

27. Pratte P, Cosandey S, Goujon-Ginglinger C. Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette. Hum Exp Toxicol. 2017;36(11):1115–20.

28. Center for Tobacco Reference Products. Certificate of Analysis 1R6F Certified Reference Cigarette. Vol. 2016-002CTRP. Lexington (KY): University of Kentucky; 2016.

29. Li X, Luo Y, Jiang X, Zhang H, Zhu F, Hu S et al. Chemical analysis and simulated pyrolysis of tobacco heating system 2.2 compared to conventional cigarettes. Nicotine Tob Res. 2018. doi:10.1093/ntr/nty005.

30. Farsalinos KE, Yannovits N, Sarri T, Voudris V, Poulas K. Nicotine delivery to the aerosol of a heat-not-burn tobacco product: comparison with a tobacco cigarette and e-cigarettes. Nicotine Tob Res. 2018;27:e76–8.

31. Bekki K, Inaba Y, Uchuyama S, Kunugita N. Comparison of chemicals in mainstream smoke in heat-not-burn tobacco and combustion cigarettes. J Univ Occup Environ Health Japan. 2017;39(3):201–7.

32. Jakaj B, Eaton D, Forster M, Nicol T, Liu C, McAdam K et al. Characterizing key thermophysical processes in a novel tobacco heating product THP1.0(T). Poster. Society for Research on Nicotine and Tobacco; 7–11 March 2017, Florence, Italy; 2017 (http://www.bat-science.com/groupms/sites/BAT_9GVJXS.nsf/vwPagesWebLive/DOAKAK4V/$FILE/SRNT%202017%20poster%2036-Liu%20et%20al_JMcA%20KM.pdf?openelement, accessed 1 October 2018).

33. Forster M, Fiebelkorn S, Yurteri C, Mariner D, Liu C, Wright C et al. Assessment of novel tobacco heating product THP1.0. Part 3: Comprehensive chemical characterisation of harmful and potentially harmful aerosol emissions. Regul Toxicol Pharmacol. 2018;93:14–33.

34. Murphy J, Liu C, McAdam K, Gaça M, Prasad K, Camacko O et al. Assessment of tobacco heating product THP1.0. Part 9: The placement of a range of next-generation products on an emissions continuum relative to cigarettes via pre-clinical assessment studies. Regul Toxicol Pharmacol. 2018;93:92–104.

35. Taylor M, Thorne D, Carr T, Breheny D, Walker P, Proctor C et al. Assessment of novel tobacco heating product THP1.0. Part 6: A comparative in vitro study using contemporary screening approaches. Regul Toxicol Pharmacol. 2018;93:62–70.

36. Thorne D, Breheny D, Proctor C, Gaca M. Assessment of novel tobacco heating product THP1.0. Part 7: Comparative in vitro toxicological evaluation. Regul Toxicol Pharmacol. 2018;93:71–83.

37. Forster M, McAughey J, Prasad K, Mavropoulou E, Proctor C. Assessment of tobacco heating product THP1.0. Part 4: Characterisation of indoor air quality and odour. Regul Toxicol Pharmacol. 2018;93:34–51.

38. Gee J, Prasad K, Slayford S, Gray A, Nother K, Cunningham A et al. Assessment of tobacco heating product THP1.0. Part 8: Study to determine puffing topography, mouth level exposure and consumption among Japanese users. Regul Toxicol Pharmacol. 2018:93:84–91.

39. Jaunky T, Adamson J, Santopietro S, Terry A, Thorne D, Breheny D et al. Assessment of tobacco heating product THP1.0. Part 5: In vitro dosimetric and cytotoxic assessment. Regul Toxicol Pharmacol. 2018;93:52–61.

40. Proctor C. Assessment of tobacco heating product THP1.0. Part 1: Series introduction. Regul Toxicol Pharmacol. 2018;93:1–3.

41. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation. Seventh report of a WHO study group. Geneva: World Health Organization; 2015.

42. Forster M, McAughey J, Liu C, McAdam K, Murphy J, Proctor C. Characterization of a novel tobacco heating product THP1.0(T): Indoor air quality. In: Society for Research on Nicotine and Tobacco, Florence, Italy, 7–11 March 2017.

43. Breheny D, Adamson J, Azzopardi D, Baxter A, Bishop E, Carr T et al. A novel hybrid tobacco product that delivers a tobacco flavour note with vapour aerosol (Part 2): In vitro biological assessment and comparison with different tobacco-heating products. Food Chem Toxicol. 2017;106(Pt A):533–46.

44. Werley MS, Freelin SA, Wrenn SE, Gerstenberg B, Roemer E, Schramke H et al. Smoke chemistry, in vitro and in vivo toxicology evaluations of the electrically heated cigarette smoking system series K. Regul Toxicol Pharmacol. 2008;52(2):122–39.

45. Schaller JP, Keller D, Poget L, Pratte P, Kaelin E, McHugh D et al. Evaluation of the tobacco heating system 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Regul Toxicol Pharmacol. 2016;81(Suppl 2):S27–47.

46. Frost-Pineda K, Zedler BK, Liang Q, Roethig HJ. Environmental tobacco smoke (ETS) evaluation of a third-generation electrically heated cigarette smoking system (EHCSS). Regul Toxicol Pharmacol. 2008;52(2):118–21.

47. Frost-Pineda K, Zedler BK, Oliveri D, Feng S, Liang Q, Roethig HJ. Short-term clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul Toxicol Pharmacol. 2008;52(2):104–10.

48. Frost-Pineda K, Zedler BK, Oliveri D, Liang Q, Feng S, Roethig HJ. 12-week clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul Toxicol Pharmacol. 2008;52(2):111–7.

49. Martin Leroy C, Jarus-Dziedzic K, Ancerewicz J, Lindner D, Kulesza A, Magnette J. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 7: A one-month, randomized, ambulatory, controlled clinical study in Poland. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S74–84.

50. Schorp MK, Tricker AR, Dempsey R. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 1: Non-clinical and clinical insights. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S1–10.

51. Tricker AR, Jang IJ, Martin Leroy C, Lindner D, Dempsey R. Reduced exposure evaluation of

an electrically heated cigarette smoking system. Part 4: Eight-day randomized clinical trial in Korea. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S45–53.

52. Tricker AR, Kanada S, Takada K, Leroy CM, Lindner D, Schorp MK et al. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 5: 8-day randomized clinical trial in Japan. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S54–63.

53. Tricker AR, Kanada S, Takada K, Martin Leroy C, Lindner D, Schorp MK et al. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 6: 6-day randomized clinical trial of a menthol cigarette in Japan. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S64–73.

54. Tricker AR, Stewart AJ, Leroy CM, Lindner D, Schorp MK, Dempsey R. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 3: Eight-day randomized clinical trial in the UK. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S35–44.

55. Urban HJ, Tricker AR, Leyden DE, Forte N, Zenzen V, Feuersenger A et al. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 8: Nicotine bridging – estimating smoke constituent exposure by their relationships to both nicotine levels in mainstream cigarette smoke and in smokers. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S85–97.

56. Zenzen V, Diekmann J, Gerstenberg B, Weber S, Wittke S, Schorp MK. Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 2: Smoke chemistry and in vitro toxicological evaluation using smoking regimens reflecting human puffing behavior. Regul Toxicol Pharmacol. 2012;64(2 Suppl):S11–34.

57. Dayan AD. Investigating a toxic risk (self-inflicted) the example of conventional and advanced studies of a novel tobacco heating system. Regul Toxicol Pharmacol. 2016;81 Suppl 2:S15–6.

58. Haziza C, de La Bourdonnaye G, Merlet S, Benzimra M, Ancerewicz J, Donelli A et al. Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: a randomized controlled study in confinement. Regul Toxicol Pharmacol. 2016;81:489–99.

59. Haziza C, de La Bourdonnaye G, Skiada D, Ancerewicz J, Baker G, Picavet P et al. Evaluation of the tobacco heating system 2.2. Part 8: 5-day randomized reduced exposure clinical study in Poland. Regul Toxicol Pharmacol. 2016;81(Suppl 2):S139–50.

60. Haziza C, de La Bourdonnaye G, Skiada D, Ancerewicz J, Baker G, Picavet P et al. Biomarker of exposure level data set in smokers switching from conventional cigarettes to tobacco heating system 2.2, continuing smoking or abstaining from smoking for 5 days. Data Brief. 2017;10:283–93.

61. Lüdicke F, Picavet P, Baker G, Haziza C, Poux V, Lama N et al. Effects of switching to the menthol tobacco heating system 2.2, smoking abstinence, or continued cigarette smoking on clinically relevant risk markers: a randomized, controlled, open-Label, multicenter study in sequential confinement and ambulatory settings (Part 2). Nicotine Tob Res. 2018;20(2):173–82.

62. Gale N, McEwan M, Eldridge AC, Sherwood N, Bowen E, McDermott S et al. A randomised, controlled, two-centre open-label study in healthy Japanese subjects to evaluate the effect on biomarkers of exposure of switching from a conventional cigarette to a tobacco heating product. BMC Public Health. 2017;17(1):673.

63. Lopez AA, Hiler M, Maloney S, Eissenberg T, Breland AB. Expanding clinical laboratory tobacco product evaluation methods to loose-leaf tobacco vaporizers. Drug Alcohol Depend. 2016;169:33–40.

64. Hamilton WL, Norton G, Ouellette TK, Rhodes WM, Kling R, Connolly GN. Smokers' responses to advertisements for regular and light cigarettes and potential reduced-exposure tobacco products. Nicotine Tob Res. 2004;6(Suppl 3):S353–62.

65. O'Connor RJ, Hyland A, Giovino GA, Fong GT, Cummings KM. Smoker awareness of and beliefs about supposedly less-harmful tobacco products. Am J Prev Med. 2005;29(2):85–90.

66. Parascandola M, Augustson E, O'Connell ME, Marcus S. Consumer awareness and attitudes related to new potential reduced-exposure tobacco product brands. Nicotine Tob Res. 2009;11(7):886–95.

67. Shiffman S, Pillitteri JL, Burton SL, Di Marino ME. Smoker and ex-smoker reactions to cigarettes claiming reduced risk. Tob Control. 2004;13(1):78–84.

68. Hund LM, Farrelly MC, Allen JA, Chou RH, St Claire AW, Vallone DM et al. Findings and implications from a national study on potential reduced exposure products (PREPs). Nicotine Tob Res. 2006;8(6):791–7.

69. Shiffman S, Jarvis MJ, Pillitteri JL, Di Marino ME, Gitchell JG, Kemper KE. UK smokers' and ex-smokers' reactions to cigarettes promising reduced risk. Addiction. 2007;102(1):156–60.

70. Levy DT, Cummings KM, Villanti AC, Niaura R, Abrams DB, Fong GT et al. A framework for evaluating the public health impact of e-cigarettes and other vaporized nicotine products. Addiction. 2017;112(1):8–17.

71. O'Connor RJ, Ashare RL, Fix BV, Hawk LW, Cummings KM, Schmidt WC. College students' expectancies for light cigarettes and potential reduced exposure products. Am J Health Behav. 2007;31(4):402–10.

72. Tabuchi T, Kiyohara K, Hoshino T, Bekki K, Inaba Y, Kunugita N. Awareness and use of electronic cigarettes and heat-not-burn tobacco products in Japan. Addiction. 2016;111(4):706–13.

73. Tabuchi T, Gallus S, Shinozaki T, Nakaya T, Kunugita N, Colwell B. Heat-not-burn tobacco product use in Japan: its prevalence, predictors and perceived symptoms from exposure to secondhand heat-not-burn tobacco aerosol. Tob Control. 2018;27(e):e25–33.

74. Fairchild A, Colgrove J. Out of the ashes: the life, death, and rebirth of the "safer" cigarette in the United States. Am J Public Health. 2004;94(2):192–204.

75. Wayne GF. Potential reduced exposure products (PREPs) in industry trial testimony. Tob Control. 2006;15(Suppl 4):iv90–7.

76. Carpenter CM, Wayne GF, Connolly GN. The role of sensory perception in the development and targeting of tobacco products. Addiction. 2007;102(1):136–47.

77. Cook BL, Wayne GF, Keithly L, Connolly G. One size does not fit all: how the tobacco industry has altered cigarette design to target consumer groups with specific psychological and psychosocial needs. Addiction. 2003;98(11):1547–61.

78. Caraballo RS, Pederson LL, Gupta N. New tobacco products: do smokers like them? Tob Control. 2006;15(1):39–44.

79. O'Hegarty M, Richter P, Pederson LL. What do adult smokers think about ads and promotional materials for PREPs? Am J Health Behav. 2007;31(5):526–34.

80. Hughes JR, Keely JP, Callas PW. Ever users versus never users of a "less risky" cigarette. Psychol Addict Behav. 2005;19(4):439–42.

81. Hanson K, O'Connor R, Hatsukami D. Measures for assessing subjective effects of potential reduced-exposure products. Cancer Epidemiol Biomarkers Prev. 2009;18(12):3209–24.

82. Rees VW, Kreslake JM, Cummings KM, O'Connor RJ, Hatsukami DK, Parascanola M et al. Assessing consumer responses to potential reduced-exposure tobacco products: a review of tobacco industry and independent research methods. Cancer Epidemiol Biomarkers Prev. 2009;18(12):3225–40.

83. Cappelleri JC, Bushmakin AG, Baker CL, Merikle E, Olufade AO, Gilbert DG. Confirmatory factor analyses and reliability of the modified cigarette evaluation questionnaire. Addict Behav. 2007;32(5):912–23.

84. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob Res. 2001;3(1):7–16.

85. Lüdicke F, Baker G, Magnette J, Picavet P, Weitkunat R. Reduced exposure to harmful and potentially harmful smoke constituents with the tobacco heating system 2.1. Nicotine Tob

Res. 2017;19(2):168–75.

86.   Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry. 1986;43(3):289–94.

87.   Liu X, Lugo A, Spizzichino L, Tabuchi T, Pacifici R, Gallus S. Heat-not-burn tobacco products: concerns from the Italian experience. Tob Control. 2018. doi: 10.1136/tobaccocontrol-2017-054054.

88.   Miyazaki Y, Tabuchi T. Educational gradients in the use of electronic cigarettes and heat-not-burn tobacco products in Japan. PLoS One. 2018;13(1):e0191008.

89.   Calentzopoulos A, Olczak J, King M. Consumer Analyst Group of New York (CAGNY) Conference. New York: Philip Morris International; 2017.

90.   Camilleri LC, Calantzopoulos A. Annual meeting of shareholders. Philip Morris International; 2016.

91.   Statement on the toxicological evaluation of novel heat-not-burn tobacco products (press release). London: Food Standards Agency; 2017 (https://cot.food.gov.uk/sites/default/files/heat_not_burn_tobacco_statement.pdf, accessed 1 October 2018).

92.   King B, Borland R, Abdul-Salaam S, Polzin G, Ashley D, Watson C et al. Divergence between strength indicators in packaging and cigarette engineering: a case study of Marlboro varieties in Australia and the USA. Tob Control. 2010;19(5):398–402.

93.   Wayne GF, Connolly GN. Regulatory assessment of brand changes in the commercial tobacco product market. Tob Control. 2009;18(4):302–9.

94.   Wu W, Zhang L, Jain RB, Ashley DL, Watson CH. Determination of carcinogenic tobacco-specific nitrosamines in mainstream smoke from US-brand and non-US-brand cigarettes from 14 countries. Nicotine Tob Res. 2005;7(3):443–51.

95.   Mitova MI, Campelos PB, Goujon-Ginglinger CG, Maeder S, Mottier N, Rouget EG et al. Comparison of the impact of the tobacco heating system 2.2 and a cigarette on indoor air quality. Regul Toxicol Pharmacol. 2016;80:91–101.

96.   Tricker AR, Schorp MK, Urban HJ, Leyden D, Hagedorn HW, Engl J et al. Comparison of environmental tobacco smoke (ETS) concentrations generated by an electrically heated cigarette smoking system and a conventional cigarette. Inhal Toxicol. 2009;21(1):62–77.

97.   Protano C, Manigrasso M, Avino P, Sernia S, Vitali M. Second-hand smoke exposure generated by new electronic devices (IQOS(R) and e-cigs) and traditional cigarettes: submicron particle behaviour in human respiratory system. Ann Ig. 2016;28(2):109–12.

98.   O'Connell G, Wilkinson P, Burseg KMM, Stotesbury SJ, Pritchard JD. Heated tobacco products create side-stream emissions: implications for regulation. J Environ Anal Chem. 2015;2(5):163.

99.   McDonald EA, Popova L, Ling PM. Traversing the triangulum: the intersection of tobacco, legalised marijuana and electronic vaporisers in Denver, Colorado. Tob Control. 2016;25(Suppl 1):i96–102.

100.   Lee DC, Crosier BS, Borodovsky JT, Sargent JD, Budney AJ. Online survey characterizing vaporizer use among cannabis users. Drug Alcohol Depend. 2016;159:227–33.

101.   Morean ME, Kong G, Camenga DR, Cavallo DA, Krishnan-Sarin S. High school students' use of electronic cigarettes to vaporize cannabis. Pediatrics. 2015;136(4):611–6.

102.   Henriksen L, Ribisl KM, Rogers T, Moreland-Russell S, Barker DM, Esquivel NS et al. Standardized tobacco assessment for retail settings (STARS): dissemination and implementation research. Tob Control. 2016;25(Suppl 1):i67–74.

3. Clinical pharmacology of nicotine in electronic nicotine delivery systems

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Contents

3.1 Introduction

Electronic nicotine delivery systems (ENDS) are a heterogeneous class of products in which an electrically powered coil is used to heat a liquid matrix, or e-liquid, that contains nicotine, solvents (e.g. propylene glycol, vegetable glycerine) and, usually, flavourings. The user inhales the resulting aerosol, which contains variable concentrations of nicotine *(1)*, a dependence-producing central nervous system stimulant. In many countries and certainly in the two largest markets – the European Union and the USA – ENDS are regulated either as generic consumer products or as tobacco products *(2)*.

Products such as ENDS that are marketed to the public and contain drugs that act on the central nervous system, such as nicotine, ideally should have little potential for abuse or dependence for public health reasons. This is true, unless some level of abuse potential is desirable to maintain compliance and support substitution in place of a substance of greater potential abuse and harm. ENDS fall into this category on the basis of claims of a potential role in smoking cessation and reduction.

The purpose of this background paper is to review the literature at the time of writing with some additions after review between March and December 2018 on the nicotine content and nicotine delivery of ENDS and to explore factors that influence the emissions of nicotine and non-nicotine toxicants. In addition,

we review the potential role of ENDS in smoking cessation and the prospective population health impact. We also identify some relevant research gaps and make recommendations for policy.

3.2   ENDS operations

Understanding how ENDS operate is useful. Fig. 3.1 is a schematic drawing of a common ENDS configuration. The heating coil is attached to an electrical power source (usually a battery, not shown in the figure) enclosed in a fabric wick that is in turn surrounded by the nicotine-containing e-liquid that saturates the wick. When power is flowing, the coil heats and thus vaporizes some of the e-liquid from the wick. As the user draws air from the mouth-end of the ENDS, the vapour is carried away and re-condenses to form an aerosol, which is inhaled by the user.

Fig. 3.1. Schematic drawing of ENDS operation
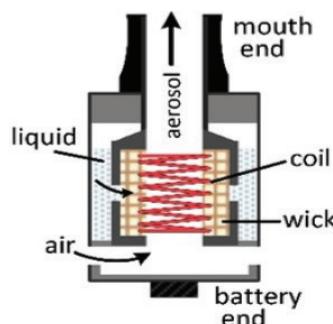
Source: Dr Alan Shihadeh, American University of Beirut, Lebanon.

Several factors influence the amount of nicotine carried by the aerosol, including the electrical power flowing through the ENDS, the inhalation behaviour (or "puff topography") of the user and the amount of nicotine in the e-liquid *(3)*. Electrical power (W) is a function of battery voltage (V) and coil resistance (Ω), such that $W = V^2/\Omega$. Early ENDS models were powered at ≤ 10 W, but the devices marketed currently are powered at ≥ 250 W *(4, 5)*. Higher power is often achieved with coils with low resistance (e.g. $< 1\ \Omega$), application of varying voltage to the coil or a combination.

Puff topography variables include puff number, duration and volume and the interval between puffs (inter-puff interval). User puff topography is highly individual. Experienced ENDS users, however, typically take longer puffs than ENDS-naive cigarette smokers *(6–9)* (see Fig. 3.2 and description below).

Fig. 3.2. Mean plasma nicotine concentrations before and after use of a combusted cigarette and of ENDS
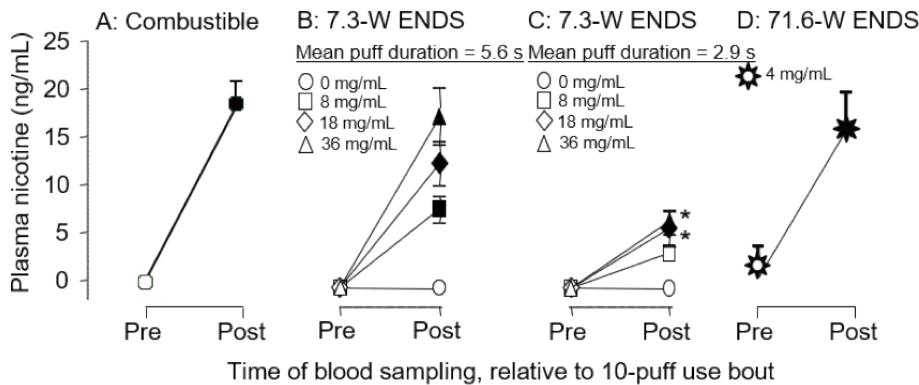
Panel A, N=32 *(8)*; Panel B, N=33 *(8)*; Panel C, N=31 *(8)*; Panel D, N=11 *(4)* (puff topography not available). *Source*: Figure adapted from one published previously *(1)* by adding puff duration data and updating Panel D.

3.3 Nicotine concentration in e-liquids

The nicotine-containing e-liquid used in ENDS comes in prefilled cartridges or refill bottles, depending on the type of device used. The concentration of nicotine in marketed e-liquid can reach 36 mg/mL or more *(1)*, and users can choose from a wide range of concentrations at the point of sale; some manufacturers provide labelling information relevant to the e-liquid. There has been no comprehensive study, however, of the extent to which manufacturers accurately inform consumers of the nicotine concentration in a representative sample of e-liquids, globally or by country. Existing studies give a partial picture based on convenience samples. The proportion of e-liquids that have clear label information on the nicotine content is unknown. Some studies indicate that such information is not always available *(10, 11)* or interpretable *(12)* from the manufacturer's label. Nevertheless, the concentration of nicotine is usually reported on the label as a percentage of total volume or as mg/mL. Table 3.1 lists studies in which the concentration of nicotine was analysed in e-liquids that allegedly contained nicotine and compared with the concentration reported on the manufacturer's label.

Table 3.1. Comparison of labelled and measured concentrations of nicotine in e-liquids with declared nicotine

First author and reference number	Type of e-liquid container	Number of samples		
		Analysed	> ±10% of labelled concentration	> ±25% of labelled concentration
Beauval (13)	Refill bottle	2	0	0
Buettner-Schmidt (14)	Refill bottle	70	36	NA
Cameron (15)	Prefilled cartridge and refill bottle	21	13	7
Cheah (10)	Cartridge	8[a]	8[b]	7[b]
Davis (16)	Refill bottle	81	36	21
El-Hellani (17)	Prefilled cartridge	4	4	4
Etter (18)	Refill bottle	35	4	0
Etter (19)	Refill bottle	34	10	0
Farsalinos (20)	Refill bottle	21	9	0
Goniewiscz (21)	Refill bottle	62	25	7
Kim (22)	Refill bottle	13	7	2
Kirschner (23)	Refill bottle	6	6	4
Kosmider (24)	Refill bottle	9	2	0
Lisko (25)	Refill bottle	29	15	7
Pagano (26)	Prefilled cartridge	4	3	2
Peace (27)	Refill bottle	27	16	7
Rahman (28)	Refill bottle	69	65	53
Raymond (29)	Refill bottle	35	22	22
Trehy (30)	Prefilled cartridge	22	22	19
Trehy (30)	Refill bottle	17	8	6

NA: not available. [a] Number of brands analysed; number of samples analysed not provided. [b] Number of brands in which at least one sample had a nicotine concentration per cartridge above the criterion.

The majority of the studies showed nicotine concentrations below those reported by the manufacturer, and all except one indicated that the nicotine concentrations in some samples were at least 10% below or above that reported on the label of the product, meeting a quality criterion recommended by a United States manufacturers' association (31). In a median of 53% of samples, the nicotine concentration was misreported on the label by at least 10%, and in a median of 26% of samples, the nicotine concentration was misreported by at least 25%.

We know of only three studies of the consistency of nicotine concentration in e-liquids in different batches of the same brand and model of e-liquid. The median variation among production batches was 0.5% in one (19) and 15% (16) and 16% (32) in the other two.

Other studies have shown that some products labelled as not containing nicotine do have measurable nicotine levels. Table 3.2 lists studies in which the concentration of nicotine in e-liquids was analysed and compared with a reported absence of nicotine on the label. Almost half the studies reported that small amounts of nicotine were present in some e-liquids advertised as not containing nicotine. Furthermore, in about 5% of samples of e-liquids allegedly without nicotine, the concentration of nicotine was significant.

Table 3.2. Labelled and measured nicotine concentrations in e-liquids with declared zero nicotine

First author and reference number	Analysed	Samples		Nicotine concentration in samples containing > 0.1 mg/mL
		Nicotine > 0.1 mg/mL	Nicotine > 10 mg/mL	
Beauval (13)	2	0	0	–
Cheah (10)	2	0	0	–
Davis (16)	10	0	0	–
Goniewiscz (21)	28	3	0	0.8–0.9
Kim (22)	20	0	0	–
Lisko (25)	5	0	0	–
Omaiye (33)	125	17	2	0.4–20.4
Raymond (29)	35	6	6	5.7–23.9
Trehy (30)	8	2	2	12.9–24.8/cartridge
Trehy (30)	5	2	2	12–21
Westenberger (34)	5	0	0	–

3.4 Nicotine delivery to ENDS users

The nicotine delivery profile of ENDS may be an important determinant of how effectively the product can substitute for a cigarette for a long-term smoker. Fig. 3.2 demonstrates the influence of the nicotine concentration in e-liquid, user behaviour and device power on the nicotine delivery profile of ENDS relative to a cigarette. Panel A *(9)* shows the nicotine delivery profile of a cigarette when smokers take 10 puffs with a 30-s inter-puff interval. Panel B shows the nicotine delivery profile of a 7.3-W ENDS loaded with 0, 8, 18 or 36 mg/mL nicotine e-liquid when users took 10 puffs of an average length of 3.6 s at a 30-s inter-puff interval. Clearly, the e-liquid nicotine concentration influences delivery of nicotine to the users' blood. When the 7.3-W ENDS is paired with 36 mg/mL nicotine e-liquid and when users take 10 ~5.6-s puffs, the pairing can match or exceed the nicotine delivery profile of a combusted cigarette *(8)*.

Puff duration is also a factor in ENDS nicotine delivery: Panel C *(8)* shows the same device and e-liquid nicotine concentration as in Panel B, but the study participants took shorter puffs (2.9 s on average). When the puff duration is shorter and all other device and e-liquid characteristics are constant, less nicotine is delivered. Panel D shows the nicotine delivery profile of higher-powered ENDS devices (mean power, 71.6 W) when users took 10 puffs at a 30-s inter-puff interval *(4)*. When these higher-powered devices were paired with 4 mg/mL nicotine liquid, they approximated the nicotine delivery profile of a combusted cigarette.

Overall, at least in some cases, these data suggest that some ENDS can deliver the same dose of nicotine, at the same rate as a cigarette, to venous blood. Unfortunately, few studies have been conducted to compare the ability of ENDS and cigarettes to deliver nicotine to arterial blood, an important indicator of exposure of the central nervous system to the drug *(35)*. In the only such comparison to date, 10 puffs (30-s inter-puff interval) from a 7.3-W ENDS

with 36 mg/mL liquid resulted in a lower mean arterial nicotine concentration (maximum, 12 ng/mL) than 10 puffs (30-s inter-puff interval) from a cigarette (maximum concentration, 27 ng/mL), although the time to peak concentration did not differ *(36)*. The sample was, however, small (four for ENDS; three for cigarettes), and puff duration was not measured. Under the controlled conditions of this study, positron emission tomography imaging showed that this ENDS effectively delivered nicotine to the central nervous system.

While the ENDS used to generate the data for Fig. 3.2 can deliver nicotine as effectively as a cigarette under some conditions, many ENDS cannot *(6, 9, 37–41)*. This heterogeneity in ENDS nicotine delivery is in contrast to regulated nicotine replacement products that deliver nicotine more reliably, although they often achieve lower plasma concentrations at a slower rate. For example, as shown in Panel A in Fig. 3.3 *(42)*, nicotine chewing-gum can take ≥ 30 min to achieve a peak plasma concentration, while Panel C shows that a nicotine patch can take > 2 h *(43, 44)*; other therapeutic products (e.g. nicotine lozenges) also deliver nicotine within this time frame *(43)*. Presumably, ENDS that deliver nicotine to the blood and brain as effectively as a cigarette are more likely to substitute for a cigarette, although this speculation has not been tested empirically, as the ENDS used in clinical trials on the question did not deliver nicotine effectively *(45)*.

Fig. 3.3. Plasma nicotine concentrations before, during and after administration of a single dose of nicotine in several therapeutic forms
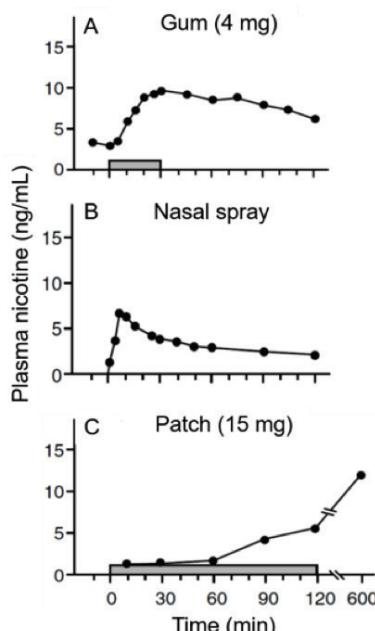
Note: the grey bar indicates duration of product use. *Source*: reference *42*. Reprinted with permission from the Massachusetts Medical Society.

3.5 Toxicant content of ENDS emissions

ENDS toxicant emissions are a function of a variety of factors, including device construction, device power, liquid constituents and user behaviour. We review below the literature on ENDS toxicant emissions, beginning with nicotine and then moving to non-nicotine toxicants (for reviews of older literature, see Breland et al. *(1)* and Department of Health and Human Services *(46)*).

3.5.1 Nicotine emissions

The "yield" of nicotine from ENDS is the amount (in mg) of nicotine in the aerosol produced by an ENDS under a specific puffing regimen. Knowing the yield of nicotine from ENDS has been considered important for understanding the pharmacokinetics of nicotine in ENDS users. One review of the literature *(47)* identified seven studies of nicotine yield *(30, 34, 48–52)*; since then, several other studies on this issue have been published *(3, 33, 53, 54)*.

The nicotine yields in these studies were highly variable, depending on the type of ENDS used, the nicotine concentration of the e-liquids and the puffing regime used to obtain the aerosol. Some methodological issues complicate the comparability of studies, including the fact that the ISO methods of machine-smoking ENDS fail to activate some ENDS models. Although the nicotine yields from ENDS in these studies are not fully comparable with those from machine-smoked cigarettes, they are usually much lower than those from cigarettes *(47)*. The literature is, however, limited, for two important reasons. First, nicotine yield does not capture the rate of nicotine emission, which is a measure not only of the amount but also of the speed at which nicotine is made available to the user. The rate of nicotine emission is almost certainly related to the rate of nicotine delivery, and the rate of nicotine delivery is probably a key factor in the capacity of a nicotine-containing product to substitute for cigarettes by providing nicotine that rapidly reaches peak levels in the bloodstream and enters the brain *(55)*. Secondly, ENDS and their e-liquids are so heterogeneous that the results of a study on a particular ENDS are probably not generalizable to another.

To address the first concern, there is growing interest in measuring nicotine "flux", the rate at which nicotine is emitted from ENDS *(56, 57)*. Nicotine flux can be measured (usually reported in μg/s) and can be compared among ENDS and with cigarettes. Those ENDS that mimic the flux of a cigarette may be more likely to substitute well for a cigarette than ENDS that do not. To address the second concern, a physics-based mathematical model has been developed to predict the nicotine flux of any ENDS *(58)* – even those that have not yet been constructed. The model accounts for the time it takes for the coil to heat up after electricity begins flowing and how much the coil cools down between puffs. It also accounts for the various ways in which heat can be transported away from the coil: by the air passing over it, by the latent heat of the e-liquid

as it evaporates, by conduction through the metal solder to the body of the device and by radiation to the surroundings. The inputs to the model are the length, diameter, electrical resistance and thermal capacitance of the heater coil; the composition and thermodynamic properties of the e-liquid (including nicotine concentration); puff velocity and duration and inter-puff interval; and the ambient air temperature. In a test of the model, the authors compared its predictions against actual nicotine flux measurements for 100 conditions in which power, puff topography, ENDS type (tank or cartomizer) and liquid composition were varied. The mathematically predicted nicotine flux was highly correlated to measured values ($r = 0.85$, $P < .0001$) *(58)*. In addition, the model accurately predicted the dependence of nicotine flux on device power and nicotine concentration (see Fig. 3.4), the ratio of propylene glycol and vegetable glycerine in the liquid and user puff duration. Fig. 3.4 shows that the higher the electrical power of the device, the lower the e-liquid nicotine concentration required to achieve a given flux. Cigarette flux is 100 µg/s, and the lines depict ENDS nicotine fluxes equivalent to twice, once and half that of a cigarette. Given the relation between ENDS power and liquid nicotine concentration shown in Fig. 3.4, a nicotine flux that is dramatically greater than that of a cigarette can be achieved by pairing a higher-powered ENDS with a higher concentration liquid. The figure does not show that some ENDS are powered well over 100 W *(4, 5)*.

Fig. 3.4. Relation between ENDS power and e-liquid nicotine concentration and effect on nicotine flux
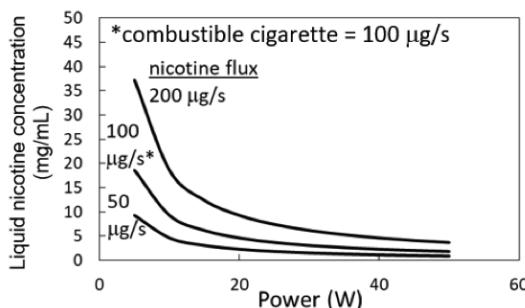
Source: reference *58*, reproduced with permission from Dr Alan Shihadeh, American University of Beirut, Lebanon.

Another important issue with regard to ENDS nicotine emissions is the amount of nicotine in e-liquids and aerosols that is present in its more bioavailable, free-base form, as opposed to the less bioavailable protonated form *(17)*. Some studies of nicotine emissions from e-cigarettes have reported nicotine yields without determining whether the methods used resulted in quantification of total nicotine or only one of its forms *(38, 58)*, so that the reported results are difficult to compare or to evaluate with regard to nicotine delivery to the user. In an evaluation of this issue, the free-base nicotine fraction in 19 commercial

liquids varied widely (10–90%), and, importantly, the differences were also seen in the aerosol *(17, 59)*, suggesting another factor that probably influences ENDS nicotine delivery to the user. Thus, in addition to measuring nicotine flux, the form of the nicotine in the aerosol should be determined. Overall, as for nicotine delivery to the user, there is considerable variation in nicotine emissions from ENDS, which can be explained and predicted by careful consideration of the many factors that influence it, especially ENDS power, liquid constituents and user behaviour.

3.5.2   Emissions of non-nicotine toxicants

Non-nicotine toxicants in ENDS aerosols are either present in the liquid or formed when the liquid is heated. Those present in the liquid before heating include propylene glycol and vegetable glycerine, which together make up 80–97% of the content of most e-liquids *(60)*, flavourings and other compounds added intentionally and contaminants not added intentionally. Aerosolized propylene glycol is a respiratory irritant *(61–64)* and, when administered intravenously at high doses, can cause potentially fatal lactic acidosis *(65)*. Preclinical work also indicates that vegetable glycerine may be toxic at high doses *(66, 67)*. The health effects of long-term, daily, chronic inhalation of aerosolized propylene glycol and/or vegetable glycerine are unknown. The flavourings used in e-liquids are usually compounds that are added to food, and their effects on the human lung after having been heated and aerosolized are unknown *(68)*. At least three flavourings that have been found in e-liquids and aerosols have raised health concerns: diacetyl (buttery flavour), which causes bronchiolitis obliterans *(69)*; benzaldehyde (fruity flavour), which is cytotoxic and genotoxic *(70)*; and cinnamaldehyde (cinnamon flavour), which is also cytotoxic and genotoxic *(71)* and can cause an inflammatory response in lung cells *(72)*. The contaminants include diethylene glycol, ethylene glycol and ethanol *(73, 74)*. Even if rigorous quality controls are imposed to ensure contaminant-free e-liquids, the uncertain effects of long-term, daily, frequent inhalation of aerosolized propylene glycol and vegetable glycerine and the many chemical flavourings that are often combined in a single liquid pose a potential health threat for ENDS users.

     The non-nicotine toxicants formed when the liquid is heated include metals, volatile aldehydes, furans and benzene. In one study of 11 "first-generation" ENDS brands (disposable ENDSs shaped like tobacco cigarettes), three of each brand were puffed for 4.3 s every 5 min for two series of 60 puffs, and the resulting aerosol was analysed for elements, including metals *(75)*. The results revealed substantial variation among brands, but many metals were found in the aerosol generated from most brands, "in some cases at concentrations that were significantly higher than in conventional cigarettes". The authors concluded that most of the elements and metals in ENDS aerosols probably originate from

components in the atomizer, such as the filament, solder joints, wick and sheath. These results show how ENDS construction can contribute to the non-nicotine toxicant profile of the aerosol.

In a study of an advanced-generation ENDS with a 1.5-Ω heating element and variable voltage battery (3.3–5.0 V), the aldehyde content of aerosols produced from a variety of liquids (all 6 mg/mL nicotine) was compared after 10 4-s puffs of 91 mL/puff *(76)*. Power was manipulated systematically from 9.1 to 16.6 W. Acetaldehyde, acetone, acrolein and formaldehyde were all present in ENDS aerosols, and aldehyde production increased proportionally as puff volume increased and dramatically when the power was > 11.7 W. The presence of aldehydes in ENDS aerosol is now well documented *(77–79)*, as is the role of device power in forming them: increasing ENDS power from 4.1 to 8.8 W approximately tripled volatile aldehyde emissions *(80–83)*. There also is some suggestion that flavourings contribute to non-nicotine toxicants formed during heating *(84–87)*. For example, heating sweeteners in e-liquids may expose users to furans, a toxic class of compounds. In one study *(88)*, a VaporFi platinum tank ENDS (2.3 Ω) was used to generate aerosol under various conditions, including power (4.2 and 10.8 W), puff duration (4 and 8 s) and sweetener (sorbitol, glucose and sucrose). The per-puff yield of some furans was comparable to values reported for combustible cigarettes, and, again, device power is a factor: increasing power from 4.3 to 10.8 W more than doubled furan emissions. With regard to benzene, increasing ENDS power from 6 to 13 W increased emissions of this carcinogen 100 times *(89)*, although the level remained far below those found in cigarette smoke. The fact that volatile aldehydes, furans and benzene are all formed by thermal degradation of the contents of e-liquids (e.g. propylene glycol, vegetable glycerine, sweeteners), coupled with the fact that increased device power increases the amount of these toxicants in ENDS aerosols, suggests that high-power ENDS are a particular public health concern. To date, most studies of the toxicant profile of ENDS aerosols have been limited to devices powered at 25 W or less (e.g. references *80, 83, 88, 90*), and much of the data reported here may not be relevant to the higher-powered devices common in some locations *(4, 5)*.

3.6 Potential role of ENDS in smoking cessation

Six narrative reviews *(91–96)* and six systematic reviews *(97)*, of which five were meta-analyses *(98–103)*, addressed the role of electronic nicotine and non-nicotine delivery systems (EN&NNDS) in smoking reduction and cessation. Two meta-analyses *(100, 102)* covered studies available up to January 2016.

All five systematic reviews of the quality of the evidence *(97, 98, 100, 102, 103)* concluded that the available studies provide evidence of low to very low certainty, due mainly to the limitations of the cross-sectional and cohort studies included in the reviews and the lack of detail in many of the published articles.

Given these limitations, El Dib et al. *(102)*, and Malas et al. *(97)* concluded that no credible inferences could be drawn from their reviews and that the evidence remains inconclusive. Similarly, a review of the systematic reviews concluded that "overall, there is limited evidence that e-cigarettes may be effective aids to promote smoking cessation" *(104)*. The other systematic reviews, however, came to a different conclusion. While Kalkhoran & Glantz *(101)* determined that "as currently used, e-cigarettes were associated with significantly less quitting among smokers", Hartmann-Boyce et al. *(100)* and Rahman et al. *(98)* concluded that use of e-cigarettes is associated with smoking cessation and reduction. Khoudigian et al. *(103)* included only randomized clinical trials. The striking disparity in the conclusions arises from differences in the criteria for selecting eligible studies and the availability of studies at the times at which the reviews were done. Table 3.3 summarizes the studies used in each review.

Table 3.3. Comparison of studies included in reviews of the effectiveness of electronic nicotine and non-nicotine delivery systems as quitting aids

Studies available for review	Review and cut-off date of literature review										
	Franck *(91)* Sep 2013	Harrell *(92)* Dec 2013	Rahman *(98)* May 2014	McRobbie *(99)* Aug 2014	Lam *(93)* Mar 2015	Ioakeimidis *(94)* Jun 2015	Kalkhoran *(101)* Jul 2015	Hartmann *(100)* Jan 2016	El Dib *(102)* Jan 2016	Malas *(97)* Feb 2016	Khoudigian *(103)* May 2016
Cohort studies											
Polosa, 2011	✓	✓		✓				✓			
Adkison, 2013		✓					✓			✓	
Caponnetto, 2013b	✓										
Ely, 2013				✓				✓			
Van Staden, 2013				✓				✓			
Vickerman, 2013 (*119*)		✓					✓		✓		
Borderud, 2014 (*123*)						✓	✓		✓		
Choi, 2014				✓			✓	✓			
Etter, 2014			✓	✓				✓			
Farsalinos, 2014 (*69*)						✓					
Grana, 2014				✓			✓	✓		✓	
Nides, 2014 (*39*)				✓				✓			
Pearson, 2014 (*122*)							✓				
Polosa, 2014	✓	✓	✓	✓		✓		✓		✓	
Prochaska, 2014							✓		✓		
Wagener, 2014		✓									
Al-Delaimy, 2015 (*120*)							✓		✓		
Biener, 2015						✓	✓		✓	✓	
Brose, 2015						✓			✓		
Harrington, 2015							✓		✓		
Hitchman, 2015 (*124*)							✓				
Manzoli, 2015						✓	✓		✓		
McRobbie, 2015				✓				✓			

Oncken, 2015							✓			
Pacifici, 2015							✓			
Pavlov, 2015						✓				
Polosa 2015							✓			
Shi, 2015						✓				
Sutfin, 2015						✓				
Cross-sectional studies										
Siegel, 2011		✓	✓							
Popova, 2013		✓								
Dawkins, 2013 (*37*)	✓								✓	
Goniewicz, 2013 (*32*)									✓	
Pokhrel, 2013		✓								
Brown, 2014			✓			✓			✓	
Christensen, 2014						✓			✓	
McQueen, 2015						✓				
Tackett, 2015									✓	
Randomized controlled trials with control group										
Bullen, 2010	✓				✓					✓
Bullen, 2013 (*45*)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Caponnetto, 2013a	✓	✓	✓	✓	✓		✓	✓		✓
Caponnetto, 2014		✓			✓		✓			
Adriaens, 2014					✓			✓	✓	
Randomized controlled trials without control group										
Hajek, 2015						✓		✓		
Unknown										
Humair, 2014				✓			✓			

The differences in the conclusions do not arise from the evidence provided by the randomized clinical trials. Meta-analysis of the few existing trials showed that ENDS use increases the likelihood of quitting smoking by a factor of two when compared with placebo. Two meta-analyses *(98, 99)* provided an estimated risk ratio of 2.29 (95% CI. 1.05, 4.96) in favour of quitting, one meta-analysis *(102)* gave an estimate of 2.03 (95% CI, 0.94, 4.38) and another *(103)* an estimate of 2.02 (95% CI, 0.97, 4.22). The differences are due to slight variations in the weight attributed to the two randomized clinical trials analysed and treatment of missing data. The different conclusions arise, more specifically, from the conflicting evidence presented by the longitudinal and cross-sectional studies reviewed. Below, we concentrate on the evidence from the longitudinal studies, because it is difficult to interpret the direction of possible associations in cross-sectional studies.

Since the last systematic review, seven new longitudinal studies have been published on the difference in quitting smoking between users and non-users of EN&NNDS *(105–110)*, including an update of a previous one with a longer follow-up *(111)*. Table 3.4 summarizes the findings of longitudinal studies according to sample attributes, characteristics of EN&NNDS products used by

participants, measures used to typify ENDS use, criteria for nicotine dependence and abstinence and a summary of the results. It summarizes the seven studies that found a statistically significant positive or negative association between EN&NNDS use and smoking abstinence in systematic reviews. It also summarizes all seven longitudinal studies that were not included in the reviews, for a total of 16 studies. To select the best longitudinal studies for assessing the evidence, we considered that the association between ENDS use and quitting smoking as the outcome of interest should be measured under at least three conditions to obtain valid results:

- Criterion 1: It should be known whether the e-liquid used contains nicotine and the type (electrical power) of device used. Ideally, devices should be classified on the basis of their tested capacity to deliver nicotine, but this might prove difficult in population studies without laboratory testing of the devices used by participants. Otherwise, it is difficult to assess whether the association is linked to the potential role of ENDS as a nicotine replacement aid. We know that some ENDS devices can deliver cigarette-like amounts of nicotine in some instances *(4, 8)*; however, use of ENNDS or ENDS that cannot deliver nicotine because of low power and other factors is still common in the USA *(112)* and many other countries.

- Criterion 2: The analysis must discriminate between people who use ENDS to quit smoking and those who do not. Many use ENDS for reasons other than to quit, including reducing their smoking *(113)*, use indoors when smoking is not allowed or for recreational purposes *(114, 115)*. Conflating ENDS users who do and do not do so for quitting may bias the association towards the null if, as expected, the real effects on smoking cessation are different or even opposite.

- Criterion 3: The measures of ENDS use must be accurate and refined in order to distinguish between established and transient, erratic use to assess the effects of ENDS on population health *(116, 117)*. As ENDS use is a relatively new population behaviour, many people may experiment briefly with EN&NNDS but not adopt an established pattern of use. Comparisons of "ever use" with "never use" of ENDS, for example, might classify as users people who have used an ENDS only once in their lives, while it has been standard practice to consider people smokers if they have smoked at least 100 cigarettes in their lifetime. Conflating experimenters with steadier users may result in the biases described in the previous paragraph.

Table 3.4. Characteristics of longitudinal studies that showed a statistically significant positive or negative association between use of electronic nicotine and non-nicotine delivery systems and smoking abstinence in existing systematic reviews and more recent studies not included in those reviews

Reference no.	Year data collected	Country and baseline	Age range (years)	No. at baseline	Follow-up	Retention rate at follow-up	EN&NNDS type	Nicotine in e-liquid	Frequency	Quantity	Duration	Reasons	Nicotine dependence	Criterion for smoking abstinence	Association between EN&NNDS use and quitting smoking
		Sample					**Product**		**EN&NNDS use** (Measures)				**Measures**		**Results**
118	2010	USA. Nationally representative sample of smokers in the general population	≥ 18	5255	1 year	63%	NM	NM	Ever used vs Never used	NM	NM	NM	M	SR 30 days at follow-up	Ever use of an e-cigarette to quit was associated with less success in quitting than those who had not, adjusted for use of pharmacological help in last attempt, age, sex, race, education, cigarettes smoked per day, nicotine dependence and early smoking initiation.
119	2011–2012	USA Smokers Quit-line callers[a]	≥ 18	2758	7 months	35%	NM	NM	Ever use vs use for ≥ 1 month vs use for < 1 month	NM	NM	N NU	M NU	SR 30 days at follow-up	Never ENDS users, 31.3% Use < 1 month, 16.6% Use > 1 month, 21.7% (P <.001). Not adjusted for use of NRT.
120	2011–2012	USA. Current smokers at baseline	18–59	1000	1 year	23%	NM	NM	EN&NNDS users classified as: ever used might use will never	NM	NM	NM	M	SR 1 month at follow-up	Smokers who had ever used e-cigarettes at baseline (not during follow-up) were significantly less likely to be abstinent at follow-up (AOR=0.41; 95% CI=0.186, 0.93) than smokers who reported at baseline they would never use e-cigarettes, adjusted for addiction (time to first cigarette in the morning), age, gender, education, ethnicity, desire to quit smoking, and smoking status. "Have used" and "will never use" include only those respondents with consistent responses at baseline and follow-up.

	Year	Setting / population	Age	N	Follow-up	%					E-cigarette use definition			Outcome	Results
121	2011–12	Representative sample of smokers in two United States metropolitan areas	18–65	1374	2 years	51%	NM	NM	NM	NM	Ever used an e-cigarette or not; if so, on how many of the past 30 days Currently used e-cigarettes every day, some days or not at all. If not at all, ever used e-cigarettes "fairly regularly" E-cigarette use: intensive (daily ≥ 1 month); intermittent (≥ once but not daily for ≥ 1 month); no use or at most once or twice.	M	M	SR 1 month at follow-up	Intensive e-cigarettes users were more than six times as likely to have quit smoking as those who had never used e-cigarettes or used them only once or twice, after adjustment for gender, age group, race/ethnicity and education level as well as baseline smoking level. Intermittent users were three times more likely not to quit than non-users or experimenters, although the association was not statistically significant.
122	2012	USA. Smokers on NRT recruited from a free, publicly available internet cessation programme. Randomized to social network integration or no social network × 2 (access to free NRT, no access)	30–52	3408	3 months	62%	NM	NM	NM	NM	Ever used during follow-up vs never used	NM	NM	SR 1 month at follow-up	The odds of abstinence were lower among smokers who used e-cigarettes to quit than those who did not use e-cigarettes to quit, after adjustment for (measured at baseline) gender, age, race/ethnicity, education, parent, study treatment allocation, Fagerstrom score, number of quit attempts in the past year, cigarettes per day, self-efficacy to quit and stage of change. After further adjustment for use of other quit methods in the past 3 months and number of quit attempts in the past 3 months, the association was not significant.

Ref	Year	Population	Age	N	Follow-up	%	Device	Definition				Outcome		Results
123	2012–2013	USA In-treatment cancer patients who were current smokers and had received cessation treatment	NR	781	1 year	53%	NM	NM	Selection: 1 < puff. Past 30 days at baseline, past 7 days use vs no use at follow-up	NM	NM	NM	SR 1 day at follow-up	M	After adjustment for nicotine dependence, number of past quit attempts and cancer diagnosis, current e-cigarette users were as likely to smoke non-users. Intention-to-treat analysis showed that non-users of e-cigarettes were twice as likely to abstain from smoking as e-cigarette users.
124	2012–2013	Great Britain. Current smokers	≥ 18	1759	1 year	44%	Cigalike tanks	Daily, less than daily but ≥ 1 weekly, less than weekly but ≥ 1 monthly, less than monthly, not at all	NM	NM	NM[a]	SR "I stopped smoking completely in the past year"	M	In comparison with no e-cigarette use at follow-up: non-daily cigalike users were less likely to quit (P <.001), daily cigalike or non-daily tank users were no more likely to quit (P =.4 and P =.4, respectively), and daily tank users were more likely to quit (P =.0012). Adjusted for age, sex, education level, income, motivation to quit and strength of urge to smoke.
111	2013–2015	Italy. Convenience sample of daily smokers for ≥ 6 months, EN&NNDS users for ≥ 6 months	30–75	932	2 years	69%	NM	EN&NNDS user: inhales ≥ 50 puffs/week	No. of months of continued EN&NNDS use	NM	NM	SR 30 days at follow-up with CO-tested subsample	NM[b]	Dual use did not improve the chances of smoking cessation at follow-up but reduced smoking.
110	2014	Canada. Smokers enrolled in cessation programme with access to free NRT and behavioural counselling	≥ 16	6526	6 months	–	NM	Any EN&NNDS use in the 3 months before follow-up	NM	NM	M NU	SR 7-day point prevalence of abstinence	M	Any EN&NNDS use was associated with less quitting at 6-month follow-up (AOR=0.50; 0.39, 0.64). Adjusted for heaviness of smoking at baseline and confidence in ability to quit.

No.	Year	Setting/population	Age	N	Follow-up	%							Outcome measure	Results
106	2014–2015	Hong Kong. Young current smokers from Quit line free counselling service not on other cessation programmes	18–25	190	6 months	99%	NM	NM	Ever tried	NM	NM	M	7 days at 6-month follow-up Validated salivary cotinine	EN&NNDS ever users had nonsignificantly lower odds of quitting (AOR, 0.56; 95% CI, 0.24, 1.35) than non-users after adjustment for sex, age, smoking friends, smoking family members, baseline quit attempts and nicotine dependence level. They tried quitting more frequently than non-users.
105	2014–2015	France. Exclusive smokers and dual users	15–85	2057	6 months	695	NM	NM	Use of e-cigarettes: regular use in past 30 days. No use: used e-cigarettes sometimes, rarely or never	NM	NM	NM	SR 7 days at 6-month follow-up	Baseline dual users were not more likely than exclusive tobacco smokers to have quit smoking at follow-up (12.5% versus 9.5%, P =.18, AOR, 1.2; 95% CI, 0.8, 1.9) after adjustment for age, sex, intention to quit smoking in the next 6 months, attempt to quit for at least 24 h in the previous 30 days at baseline and heaviness of smoking. They were more likely to reduce their smoking by half and tried to quit.
107	2014	USA. Smokers seeking treatment enrolled in a quitline programme offering counselling and NRT	≥18	5672	NR	NR	NR	NR	Number of days used EN&NNDS in past 30 days Classified as 0, none 1–5 6–20 30, daily	1 month before follow-up	NM	NM	Self-reported 30-day complete abstinence at follow-up	Quit rate of daily EN&NNDS users no different from that of non-users. Quit rate of non-daily users lower than that of non-users. Results were adjusted for age, sex, education level, tobacco use, type of quitline programme and counselling with NRT use.
108	2013–2015	USA. Current cigarette smokers with no current use of EN&NNDS at baseline	≥25	5124	1 year	83%	NM	Reported use of cartridges, refills and tanks	Number of days used EN&NNDS in past 30 days	NM	NM	NM	Time to first cigarette	Daily EN&NNDS users were eight time more likely to quit than non-users when using non-cartridge, refillable tanks. Experimenters were half as likely as non-users to quit. Non-daily users tended to quit less than non-users, but the association was statistically nonsignificant. Less quitting seen mainly among users of cartridge, non-refillable devices.

	Years	Country / description	Age	N	Follow-up	Retention	ENDS type	Intervention	Outcome 1	Outcome 2	Outcome 3	Outcome 4	Outcome 5	Results
109	2012–2015	USA. Hospital-discharged daily smokers who had received inpatient counselling plus encouragement to use NRT and who planned to stop smoking after discharge	≥ 18	Controls: 677, Intervention: 680	6 months	75% for controls 77% for intervention	NM	NM	Any use of EN&NNDS in past 7 and 30 days	NM	NM	All participants planned to quit smoking	No. of cigarettes per day and time to first cigarette	Cotinine-validated 7-day point prevalence of tobacco abstinence	Proportion of daily smokers motivated to quit who actually quit was slightly higher among EN&NNDS non-users than among EN&NNDS users in the 7 days before follow-up, but the difference was statistically nonsignificant.
125	2014–2016	USA. Smokers of ≥5 cigarettes/day for ≥ 1 year, ENDS-naive, not seeking treatment to quit	≥ 18	Control= 22; arm 1 = 25; arm 2= 21	3 months	Control= 73%; arm 1=76%; arm 2=71%. EMA compliance 58%	Ciga-like	Arm 1= 3-week provision of 16 mg/mL nicotine Arm 2= 24 mg/mL nicotine Control = no provision of ENDS. No ENNDs provided but nor precluded from buying them	Number of days used EN&NNDS per week	Number of puffing sessions/ day	From baseline to follow-up		CO-verified 7-day abstinence at follow-up	9.5% of users of 24 mg/mL nicotine e-liquid and 4.0% of 16 mg/mL users quit smoking vs 4.6% in controls. Difference was not statistically significant. No analysis by frequency of use of ENDS presented. Motivation to quit interacted, varying by group and over time within groups.

Italy. Smokers of ≥ 10 cigarettes/day for ≥ 10 years highly motivated to quit but not quitting or using NRT at baseline. ENDS-naive	2015–2016	126	≥ 55	70 per arm	Arm 1 = ENDS plus support Placebo = ENNDS plus support Control = no EN&NNDS	eGO 3.3–4.2 V working voltage	Arm 1 = ENDS plus support Placebo = ENNDS plus support Control = no EN&NNDS	Quitting rate higher among EN&NNDS users than in control group; however, there was no difference between ENDS and ENNDS users. Authors indicate that ENDS users had an e-liquid that delivered very little nicotine in the aerosol (0.1 mg/puff).

AOR: adjusted odds ratio; CI: confidence interval; CO: carbon monoxide; EN&NNDS: electronic nicotine and non-nicotine delivery systems; M: measured; NM: not measured; NRT: nicotine replacement therapy; NU: not used in analysis; SR: self-reported; U, used in analysis. [a]The authors did not report whether a caller was abstinent when enrolled. Some of the participants attended multiple calls, and the authors do not indicate whether they were "new" callers. [a] Instead, they measured desire to quit. [b] Instead, they measured strength of urge to smoke.

With these criteria in mind, we find that, of the 14 studies examined,

- only two characterized the type of device used (criterion 1);
- 12 studies did not restrict by or analyse the reasons for use of EN&NNDS, although two included adjustment for or analysis of some variables that could be used as proxies for using EN&NNDS (criterion 2); and
- seven studies compared cessation only between ever and never users of EN&NNDS, three used a crude measure of current use, and six used a more elaborated measure of frequency (criterion 3).

Seven longitudinal studies met at least one of the three criteria; none met all three. The combined evidence from the seven studies suggests that their samples consisted of different subgroups that experienced different or opposing effects of EN&NNDS use on cigarette cessation. Consequently, it could be hypothesized that **some** smokers may successfully quit tobacco use by using **some** types of ENDS frequently or intensively, while others experience no difference or are even prevented from quitting. The findings of these studies are shown in Table 3.5.

Table 3.5. Results of seven longitudinal studies of the efficacy of use of EN&NNDS for quitting tobacco use

Reference	Results by criterion		
	Frequency of use	**Type of device used**	**Use for quitting smoking**
	More daily users of EN&NNDS quit smoking than users, especially if they used refillable tank devices; however, the quit rate of experimenters with EN&NNDS and non-daily users of non-refillable cartridge devices was lower than that of non-users.		

EN&NNDS use	**AOR[a]**	**95% CI**	**P**
Non-users	Reference		
Experimenters	0.5	0.26, 1.00	0.05
Current users (not daily)	0.5	0.17, 1.47	0.21
Current users (daily)	7.9	4.45, 13.95	< 0.001

EN&NNDS use by type of device			
Non-users			
Daily users of:			
non-cartridge	10.1	5.4, 10.1	< 0.001
refillable	9.1	4.9, 17.0	< 0.001
tank	10.2	5.4, 19.4	< 0.001
non-tank	3.6	1.04, 12.2	0.04
Experimental or not daily users of:			
cartridge	0.3	0.1, 0.9	0.03
non-refillable	0.25	0.1, 0.9	0.04
non-tank	0.34	0.1, 0.8	0.02

[a] Adjusted for age, sex, race, region, income, frequency, intensity of cigarette use, nicotine dependence and previous quit attempts.

Berry (108)

51

Hitchman (124)

Smokers who were daily tanks users were more likely to quit smoking than non-users of EN&NNDS, while non-daily users of cigalikes were less likely to quit.

EN&NNDS use	AOR*	95% CI	P
Non-users	Reference		
Non-daily cigalike	0.35	0.20, 0.60	0.002
Daily cigalike	0.74	0.39, 1.42	0.36
Non-daily tank	0.7	0.29, 1.68	0.42
Daily tank	2.7	1.48, 4.89	0.001

*Adjusted for gender, age, education, income, motivation to stop smoking, strength of urge to smoke.

Although motivation to quit was measured, no information was collected on whether ENNDS were used specifically to quit.

Biener (121)

Smokers who used EN&NNDS daily were more likely to quit smoking than those who never used EN&NNDS. Non-daily users, however, were less likely to quit than non-users, although the association was not statistically significant.

EN&NNDS USE	AOR*	95% CI
Non-users	Reference	
Current non-daily	0.31	0.04, 2.80
Current daily	6.07	1.15, 24.4

*Adjusted for gender, age, race, education and baseline level of smoking

Motivation to quit with EN&NNDS was measured as two variables: expectation to be smoking in 1 year and plans to quit within 6 months. Smokers who were not daily EN&NNDS users were six times more likely to expect to continue smoking in 1 year than smokers who were non-users. The expectation to continue smoking was similar for daily users and non-users. Smokers who did not use EN&NNDS were less like to have plans to quit smoking than smokers who were EN&NNDS users, but the differences were not statistically significant.

Manzoli (111)

Smokers who used ENDS or ENNDS regularly (inhaled ≥ 50 puffs/week) quit at the same rate as smokers who did not use EN&NNDS (AOR = 1.25; 95% CI, 0.85, 1.84.) Adjustment for baseline age, gender, body mass index, marital status, educational level, occupation, alcohol use, hypertension, hypercholesterolaemia, diabetes, self-reported health, years of tobacco smoking (former smoking for e-cigarette users), number of tobacco cigarettes smoked per day (or puffs per day for smokers of e-cigarettes only).

Nowariak (107)	The quitting behaviour of daily and non-daily users of EN&NNDs was different from that of non-users. While the rate of quitting was no different for daily users and non-users, non-daily users quit at a lower rate than non-users.

EN&NNDS use in past 30 days	AOR	95% CI	P
Non-users	Reference		
Infrequent (1–5 days)	0.35	0.20, 0.59	< 0.001
Intermediate (6–29 days)	0.50	0.32, 0.80	0.004
Daily (30 days)	1.16	0.71, 1.70	0.453

Adjusted for age, gender, education, tobacco type, advice from health professional, state programme and medication use.

Rigotti (109)	The quitting rate of smokers was 13.7% among those who had used EN&NNDS in the past 7 days and 17.9% among those who had not used them. The difference in risk was −4.3% (95% CI, −13.6, 5.1), which was not statistically significant. Quitting was therefore slightly but not statistically significantly lower among ENNDS users.

Zawartalio (110)	At the 6-month follow-up, the quit rate of EN&NNDS users (measured crudely as at least once in the past 3 months) who did not use them for smoking cessation was similar to that of non-users (37.6% vs 42.0%; P = 0.43), while the quit rate of EN&NNDS users who reported using them to help them quit smoking cigarettes was significantly lower than that of non-users.

AOR: adjusted odds ratio; CI: confidence interval; ENDS: electronic nicotine delivery systems; ENNDS: electronic non-nicotine delivery systems; EN&NNDS: electronic nicotine and non-nicotine delivery systems.

A cross-sectional study by Giovenco et al. *(127)* of current and former smokers who had quit since 2010, as reported in the 2014–2015 National Health Interview in the USA, lends some support to this hypothesis. The prevalence of quitting smoking tripled among daily ENDS users as compared with those who had never used ENDS, in line with the findings of Zhu et al. *(128)*. Interestingly, Giovenco et al. found the opposite effect among non-daily ENDS users and former experimenters, with a prevalence of quitting smoking of 2.6 and 1.5 times less than those who had never used ENDS, respectively. Success or failure in quitting in different subgroups may be influenced by:

- motivation to use EN&NNDS, including for quitting smoking;
- patterns of quantity, frequency and duration of ENDS use;
- technology used, including type of devices and e-liquids;
- type of smoker, including level of nicotine dependence and history of previous successful and unsuccessful quit attempts; and
- the regulatory environment for ENDS and tobacco use *(131-133)*.

Further support for the possibility that some smokers may successfully quit smoking by using ENDS includes the fact that ENDS may be economic substitutes for cigarettes *(134–136)* and the absence of a reversal in the decreasing rate of smoking rate in the two major EN&NNDS markets. Current cigarette smoking among adults in the USA decreased from 20.9% in 2005 to 15.1% in 2015, a 27.7% decrease (*P* for trend, < 0.05) *(137)*. The decrease includes a significant 1-year drop between 2014 and 2015 of 1.7 percentage points, which coincided with a notable increase in the cessation rate in 2014–2015, attributed by the authors partly to use of EN&NNDS. The results were adjusted for other changes to the policy environment that might affect quit attempts, such as tax increases and the "Tips from former smokers" media campaign of the Centers for Disease Control and Prevention in the USA.

In the United Kingdom, the proportion of current adult (≥ 18 years) smokers in 2016 was 15.8%, the lowest prevalence recorded since the start of the Annual Population Survey in 2010 *(138)*. At the same time, the increase in the use of EN&NNDS in England has been associated with the increasing success of quit attempts *(139)*.

These data in themselves do not prove that use of EN&NNDS by the population is an effective quitting aid. They do show, however, that use of EN&NNDS is at least not changing the trend to a decreasing prevalence of smoking in the United Kingdom.

3.7   Potential health impact of ENDS

As some ENDS may help some smokers to quit, what is their potential health benefit for the population? The overall impact of using ENDS on population

health depends primarily on two factors. One is the capacity of ENDS to help prevent smoking, and the other is the relative risk associated with their use in comparison with a defined alternative, such as smoking *(140)*.

3.7.1   Behavioural trajectories associated with use of ENDS

If ENDS prevent smoking, they do not entice nonsmokers into smoking but instead lure smokers into quitting smoking and, ideally, abstaining from nicotine. In other words, whatever the initial status of a person – never, current or former smoker – behavioural paths or trajectories associated with ENDS use must lead away from smoking and ultimately from nicotine dependence. Fig. 3.5 presents the 27 possible paths from an initial state of never, current or former smoker into one of four possible final states: exclusive smoker, exclusive ENDS user, dual user or dual abstainer. The web of trajectories in Fig. 3.5 represents only the behavioural paths between two nicotine products. In reality, it may be complicated by competition among more than two products, be they pharmaceutical, tobacco or consumer products.

Fig. 3.5. Web of trajectories associated with ENDS use
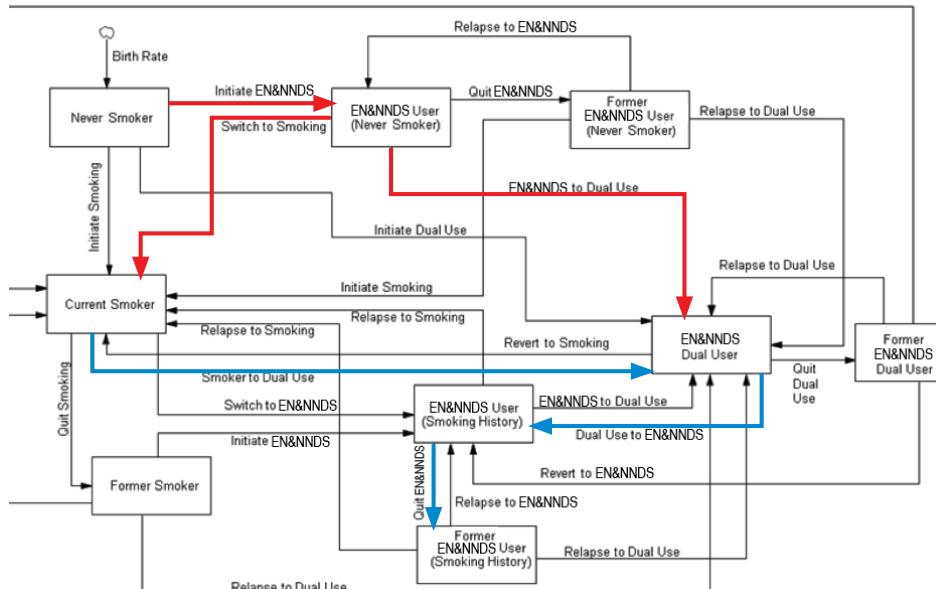
EN&NNDS, electronic nicotine and non-nicotine delivery systems. *Source*: Modified from reference *141*.

The first step in understanding the effect on population health of using ENDS is, therefore, to estimate the probability that people in each initial state will end over time in one of the four final states. The probabilities are context sensitive

and therefore cannot be transferred among different cultural and regulatory environments for EN&NNDS and tobacco. Estimating the probabilities is complex, especially in light of the scant empirical evidence for characterizing them. The discussion has focused on the two most relevant combinations of trajectories in which EN&NNDS can play a role for or against health. One is the combination that leads smokers to quit smoking (blue lines in the figure), and the other is that which leads never smokers to smoke (red lines in the figure).

Trajectories that lead smokers to quit smoking

We discussed above the evidence for the role of EN&NNDS in quitting smoking. Contrary to the polarized discussion on whether ENNDS support or dissuade quitting, we concluded that the effects of EN&NNDS use on smoking cessation might depend on individual patterns of use and smoking, attitudes and behaviour, technology and the regulatory environment. The overall usefulness of ENDS for quitting might depend on the predominance of the subgroups for whom ENDS use might have an effect. For example, Giovenco et al. *(127)* showed that daily ENDS users quit smoking 3.2 times more often than never users; however, daily users represented only 5.1% of the sample. Non-daily ENDS users and former attempters, who represented 9.8% and 33.1% of the sample, respectively, however, quit smoking 2.6 and 1.5 times less often than those who had never used ENDS. Overall, the adjusted percentage of the total sample that quit is 26.5% with EN&NNDS and 28.2% without (Table 3.6). Given the predominance of non-daily EN&NNDS users and former experimenters in the population, preventing quitting predominated over promoting quitting among daily users.

Table 3.6. Theoretical impact on the prevalence of population quitting among smokers who use and do not use electronic nicotine and non-nicotine delivery systems (EN&NNDS) by type of user

Type of EN&NNDS user	Prevalence of EN&NNDS use (%)	Rate attributable to EN&NNDS use (%)	Adjusted[a] prevalence of quitting attributable to EN&NNDS use (%)	Prevalence in the absence[b] of EN&NNDS use (%)
Daily	5.1	52.2	4.6	1.4
Non-daily	9.8	12.1	1.1	2.8
Former	33.1	20.2	6.3	9.3
Non-user	51.9	28.2	14.7	14.7
Total	100	--	26.5	28.2

[a] Quit rate adjusted for a prevalence rate for daily and non-daily users, former experimenters and non-users of 3.18, 0.38, 0.67 and 1, respectively. [b] If the whole population were non-users at a quit rate of 28.2%.

Trajectories of never smokers to smoking

Young never smokers who experiment with ENDS are more likely to experiment with smoking later. A meta-analysis *(142)* of three longitudinal studies in the

USA *(143–145)* showed that young people who had used ENDS even once in their lives at baseline were twice as likely to experiment later with smoking than those who had never used ENDS. A more recent meta-analysis *(146)* that included the three previously mentioned studies and six additional ones *(147)* concluded that the likelihood of subsequent smoking initiation by young people who had ever used ENDS was about 3.5 times higher than that of never ENDS users. The authors also reported that using ENDS during the previous 30 days increased the chance of smoking at least once in the next 30 days by four. Two longitudinal studies in the United Kingdom *(148, 149)* showed a similar association between experimental use of ENDS and subsequent experimental smoking. The data available so far do not, however, prove that this evident association is causal or due mostly to ENDS use.

This association is difficult to understand, for several reasons *(150, 151)*. In most of the longitudinal studies, use of these products was measured as at least once in either a lifetime or in the previous 30 days. These recall periods cover a mixture of behaviour in the formative years of young people, including more frequent experimental use of ENDS and smoking, which is tentative and volatile, and also less prevalent established behaviour. It can be assumed that established ENDS use patterns better define the likelihood of future smoking than volatile, tentative ENDS use, such as having a puff once in a while.

Furthermore, there are three theoretical explanations for the association. The first is the "common liability conjecture". According to this theory, ENDS use and smoking are initiated independently of each other because they are the result of a common latent propensity to risky behaviour. Thus, it has been suggested that a large proportion of the young people who try ENDS and then smoke would have tried smoking regardless of the existence of ENDS. The fact that ENDS are used before smoking and not the other way around is due to several factors, including the novelty of ENDS. The second theory is the "renormalization" hypothesis, by which ENDS use is widespread and frequent among young people, and the devices and mannerisms of its use remind them of smoking. The similarity between ENDS use and smoking facilitates the trajectory from one product to the other within a social learning framework. The third theory is the "catalyst" theory, which comprises six hypotheses for initiation of ENDS: flavour, health, price, role model, concealment and acceptance. Another three hypotheses are proposed to explain the transition to smoking: addiction, accessibility and experience *(152)*. Proving any of these theories will face critical methodological challenges *(153)*. In some longitudinal studies, adjustment has been made for variables to measure common susceptibility traits; however, residual confounding always muddles the association between ENDS use and smoking, and no one has proven beyond doubt which hypothesis or combination best explains the transition from never using nicotine to ENDS use and later to smoking.

The fact that some "never smokers" who experiment with ENDS end up smoking must be reconciled with the fact that the prevalence of current smoking among young people in the two countries with the most prominent ENDS markets continues to decrease. One review *(142)* shows that the prevalence of use of ENDS at least once a month increased quickly in some countries like the USA *(154)* (probably EN&NNDS), while in others such as the United Kingdom the rate among nonsmokers has been stable at very low levels.

3.7.2 Harm from ENDS and electronic non-nicotine delivery systems

Although EN&NNDS may route the population through trajectories in and out of smoking, the overall health impact of use of ENDS depends on the health risks associated with their use. The long-term health effects of EN&NNDS use are still unknown, and determination of such effects with some degree of certainty will require investigations of the health outcomes of large cohorts of well-characterized users who are followed for many years. In the meantime, conclusions about the toxicity of EN&NNDS are based mainly on empirical evidence from chemical and toxicological studies and, to a lesser degree, clinical studies. Reviews of these studies have led various authors to conclude, with more or fewer caveats, that EN&NNDS are not harmless but are generally less dangerous than cigarettes *(155–160)*, especially with regard to death from diseases associated with cigarette use. Efforts have been made to specify and characterize the health risks of EN&NNDS use by type of health condition.

Cancer risk

Ideal combinations of EN&NNDS device power settings, liquid formulation and use should produce an aerosol containing carcinogenic chemicals at a potency < 1% that of tobacco smoke and two orders of magnitude higher than that of a medicinal nicotine inhaler. As shown in Fig. 3.6, however, some products and circumstances can increase the cancer risk of EN&NNDS aerosol considerably, sometimes close to that of tobacco smoke *(161)*. Aerosols with higher carcinogenic potency appear to be formed when the user applies excessive power to the atomizer coil *(76)*. It has been argued that this occurs only under "dry puff" conditions *(162)* – brief situations that are readily detectable by EN&NNDS users. There is no empirical evidence, however, that this is due only to dry puff conditions or, if so, how often such conditions occur.

Fig. 3.6. Carcinogenic potency of formaldehyde and acetaldehyde in aerosol from electronic nicotine and non-nicotine delivery systems and in tobacco smoke, heat-not-burn devices, a nicotine inhaler and ambient air
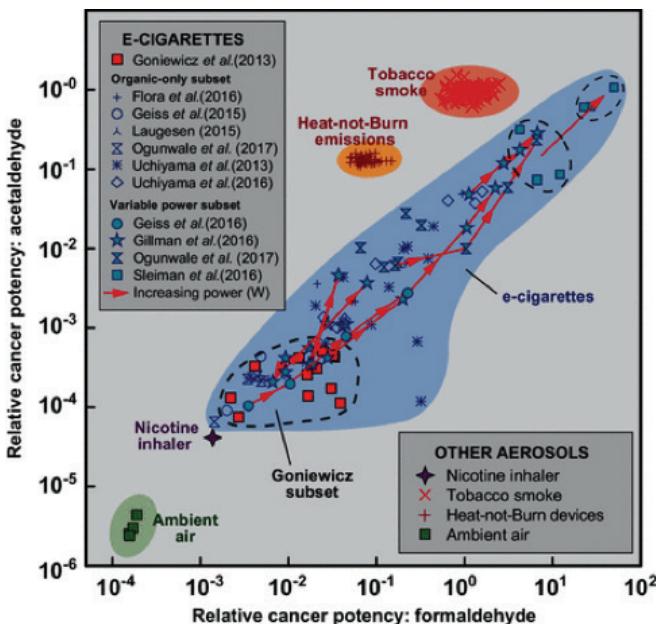
Source: reproduced from reference *161* with permission from BMJ Publishing Group Ltd.

Cardiovascular risk

There is controversy about whether the risk for cardiovascular events associated with use of EN&NNDS is as low as its carcinogenic potential. Some consider that the main cardiovascular risk of ENDS aerosol is due to the toxicity of nicotine, which appears to pose a low short-term cardiovascular risk in healthy users *(163)*. A review of clinical and cell culture studies conducted in 2015–2017 addressed the relation between ENDS use and indicators of risk for cardiovascular disease, including heart rate, blood pressure, and vagal tone; platelet aggregation and adhesion; aortic stiffness and endothelial function; expression of genes for antioxidant defence and immune system function; and indices of oxidative stress. Of the six studies reviewed that showed significant adverse cardiovascular effects, three found that ENDS had less effect on physiological cardiovascular risk indicators than cigarettes, and the other three found that ENDS had the same effect as cigarette smoking. Some studies indicated that these adverse cardiovascular effects are independent of nicotine, although adding nicotine may enhance them *(164)*.

Pulmonary risk

While EN&NNDS aerosol is probably less toxic than tobacco smoke and causes less mortality than cigarettes, the reduction in toxicity in the lung remains unknown for both long-term users who quit smoking and dual users. The authors of a review on the topic concluded that the induction of inflammation by EN&NNDS might differentially affect the risks for lung cancer and chronic obstructive pulmonary disease *(165)*. Thus, the most recent empirical evidence suggests that EN&NNDS aerosol is less toxic than cigarette smoke; however, there are no empirical data to quantify the relative risks of exposure to EN&NNDS aerosol and tobacco smoke.

Several efforts have been made to model the potential population impact of EN&NNDS *(166–168)*; however, the results are only as good as the data put into the model. Given the paucity of data, it is unclear which should be included in calculating the benefits of ENDS in worst- and best-case scenarios *(169, 170)*, especially for variables such as the efficacy of ENDS in helping people quit smoking and their safety relative to cigarettes.

Quantifying the effects of ENDS use on the health of the population is highly complex, as many variables must be taken into account. The available evidence indicates a possible positive effect of ENDS on population health, particularly if appropriate ENDS regulation is enacted to maximize their benefits and minimize their risks.

3.8 Summary of evidence, research gaps and policy issues derived from the evidence

ENDS are a heterogeneous class of products, with various profiles of nicotine and non-nicotine toxicants, which depend on factors including their construction, power, liquid constituents, nicotine concentration and user behaviour. The amount of nicotine delivered can range from none to doses that exceed those delivered by tobacco cigarettes in the same number of puffs. Nicotine from ENDS reaches users' blood faster than from most types of nicotine replacement therapy (NRT), and, at least with some ENDS, at higher concentrations. ENDS could be effective in cessation for some smokers under some circumstances, while, for other smokers, in different circumstances, it might have the opposite effect. Whether an ENDS has beneficial or detrimental effects on smoking cessation appears to depend on the technology, the motivation and consumer behaviour of the ENDS user, the type of smoker who seeks ENDS use and the regulatory environment for ENDS and tobacco use.

Translating the evidence into a potential role of EN&NNDS in smoking cessation is difficult. The evidence does not allow a blanket policy recommendation for or against general use of ENDS and ENNDS as cessation aids. Nevertheless, it points to four areas for regulatory consideration by policy-makers.

The concept of nicotine flux in ENDS regulation: regulators who wish to maximize the potential of the ENDS technology for nicotine substitution should

consider the rate at which nicotine is emitted (i.e. nicotine flux) as a primary factor in their decision. In practical terms, factors that influence nicotine flux should not be regulated in isolation. ENDS nicotine flux can be modelled mathematically for product standards for regulatory purposes, although such standards should also be based on a clinical evaluation (i.e. effects in humans who are and are not ENDS users).

The relation between nicotine flux and toxicant profile: a corollary to the above is that the conditions under which different nicotine fluxes are obtained may affect the toxicant profile, because some of the same factors that increase the nicotine flux, such as power, also increase the concentrations of some toxicants in the aerosol, such as aldehydes. Therefore, regulators might consider how the manufacturers and the government should inform users of the balance between creating an adequate nicotine flux and the associated toxicant delivery.

Nicotine e-liquid concentration: despite some industry guidelines on labelling nicotine concentrations, the labels on many e-liquids do not indicate the concentration, are difficult to interpret or, most often, do not provide accurate information. Depriving ENDS users of accurate information on the nicotine concentration in e-liquids denies them important information for controlling their self-administration of nicotine.

Labelling and quality control for ENDS devices and e-liquids: the labels on all e-liquids should display the total amount of nicotine per receptacle, the ratio of free-base to protonated nicotine and the liquid concentration in mg/mL, visibly and understandably; otherwise, they should indicate that the e-liquids do not contain nicotine at a concentration above, for example, 0.1 mg/mL. Quality control must be used to ensure the veracity of labelling information and conformity to production standards.

Although the topic is not reviewed in this paper, there is conclusive evidence that exposure to nicotine in e-liquids other than through aerosol inhalation can harm health, sometimes fatally *(171)*. In order to avoid accidental exposure to nicotine, regulators should consider requiring child-resistant containers for all e-liquid receptacles.

The development of adequate policies and regulations on the ENDS issues described in this paper would benefit from disclosure requirements for manufacturers and effective, organized, systematic national surveillance. Key disclosure data to be requested from manufacturers include the voltage, resistance and power of marketed devices and the e-liquid constituents. In addition, monitoring should be conducted to determine consumer behaviour towards ENDS, such as who uses them, for what purpose, what and how products are used and the frequency of use.

Table 3.7 summarizes the evidence on the delivery of nicotine by ENDS, their effect on smoking cessation and their prospective impact on population health. The table also lists gaps in research and policy issues for each element of the evidence.

Table 3.7. Summary of evidence, research gaps and policy issues

Topic	Evidence	Research gaps	Policy and regulatory issues
Effective nicotine delivery	**Technology** A nicotine flux similar to that of cigarettes with regard to the levels and speed of nicotine delivery can be produced. The flux is influenced by: • the voltage applied to a coil of a given resistance: the higher the power, the higher the concentration of nicotine in the aerosol in a range of values between a threshold and a ceiling; • the concentration of nicotine in e-liquid: the higher the concentration in the e-liquid the higher concentration in aerosol at a given power value; and • the puffing behaviour of the user: the longer the puff, the more nicotine is delivered.	ENDS differ widely in device technology and e-liquid components. Although the technological characteristics of ENDS that govern the delivery of nicotine are known, further research is needed to generalize the findings to the whole class of ENDS, beyond individual products. Programmatic and transdisciplinary research is required, combining aerosol research, analytical chemistry and clinical laboratory methods to complete the mathematical model for predicting nicotine flux and to begin to describe similar models for predicting non-nicotine toxicants to the extent possible. The relation between nicotine flux and smoking cessation must be characterized at population level. Characterizing the relation between nicotine flux and potential abuse might help to explain and prevent initiation of cigarette smoking by non-tobacco users via ENDS.	The concept of nicotine flux in ENDS regulation As nicotine flux is the primary determinant of the capacity of ENDS to substitute for nicotine from cigarettes, regulators should consider this factor in endeavours to maximize the nicotine substitution potential of ENDS technology. Regulation of only one of the factors that influence nicotine flux (e.g. concentration in e-liquids) might result in changes to other factors by ENDS users (e.g. increased power). Such changes may or may not increase nicotine flux but may increase health risks (e.g. by increasing volatile aldehyde emissions at higher power values). ENDS that cannot deliver nicotine at a speed and concentration similar to those of cigarettes should, at a minimum, bear a warning that they cannot assist in smoking cessation.
	Relation between toxicant profile of ENDS emissions and nicotine flux Some of the factors that increase the nicotine flux, such as power, also increase the concentrations of some toxicants in the aerosol, such as aldehydes.	Further research is needed to characterize the association between the toxicant profile of ENDS emissions and the factors that influence nicotine flux, under a variety of conditions, such as: e-liquid composition: types of solvents and flavourings. Many flavourings used in ENDS liquids are intended to be consumed orally and have not been tested for safety after heating and inhalation. device construction: including general design, metals used, wicking materials.	Toxicant profile information Regulators might consider how to make users aware of the factors that influence the balance between creating an adequate nicotine flux and the associated toxicant delivery.

Concentration of nicotine in e-liquid

Despite some industry guidelines on labelling of e-liquids for nicotine concentration, many do not carry such a label, it is difficult to interpret or, most often, does not provide accurate information.

Depriving ENDS users of accurate information on the nicotine concentration in e-liquids denies them adequate information for deciding whether to self-administer nicotine.

A major research gap is how to communicate this information in a manner that is useful to the consumer and increases the chance that ENDS will be used to increase cessation rates. The same applies to ENDS power. Some ENDS users may not realize that they are using an ENDS that cannot deliver nicotine effectively, no matter what strength of nicotine liquid it contains.

Range of nicotine concentration in e-liquids

Regulation of a minimum and a maximum amount and concentration of nicotine in e-liquids should ensure a balance between:

ensuring a sufficient concentration to reach an adequate flux for nicotine replacement; and

the risk of accidental exposure to e-liquid containing nicotine.

The total amount of nicotine per e-liquid container should be small enough to avoid the risk of lethal or serious cases of accidental nicotine poisoning if packaging safeguards fail.

The nicotine concentration should be high enough to provide an adequate nicotine flux and to limit production of toxicants when the coil is heated too much to obtain more nicotine aerosol.

Labelling and quality control of ENDS devices and e-liquids

Consumers must have accurate, reliable information on product design characteristics and e-liquid ingredients, including:

the ingredients listed comprise all liquid constituents (i.e. no contaminants); and

all e-liquid containers are labelled to provide accurate information on the amount of nicotine, the ratio of free-base to protonated nicotine and the concentration.

E-liquid contaminants known to pose a severe risk should be banned (e.g., diethylene glycol, diacetyl).

Quality control must be used ensure the veracity of labelling information and implementation of production standards.

Effectiveness of ENDS as a smoking cessation aid	ENDS can be effective for cessation for some smokers under some circumstances. It may have the opposite effect for other smokers under different circumstances.	Better understanding is needed of the circumstances in which ENDS use can promote or be detrimental to smoking cessation, including:	Regulators should be aware that ENDS use may have opposite effects on smoking cessation. Without further research, however, it is not possible to recommend policies to maximize their potential to help quit smoking and to minimize their detrimental effects on cessation.
		features of ENDS that facilitate cessation:	
		the nicotine delivery profiles most strongly associated with quitting;	
		whether flavours are necessary and, if so, which and how many will maximize quitting; and	
		which user behaviour and use frequencies are most strongly associated with quitting and avoiding relapse to cigarettes;	
		the subpopulations of smokers in whom ENDS are likely to be effective for cessation and those in whom they are not;	
		the features of ENDS that maintain dual cigarette and ENDS use and how they could be manipulated to encourage cigarette cessation; and the features of ENDS that maintain dual cigarette and ENDS use and how they could be manipulated to encourage cigarette cessation; and	
		how to help long-term ENDS users to cease ENDS use, should they desire that outcome.	
	The conditions that appear to affect the potential of ENDS as a smoking cessation aid include:		
	the appropriate combination of ENDS device and e-liquid to deliver nicotine at levels and speed similar to those of cigarettes;		
	use of ENDS for quitting smoking with a minimal pattern of quantity, frequency and duration of use;		
	the type of smoker, including level of nicotine dependence and history of previous successful and unsuccessful attempts to quit; and		
	the regulatory environment of ENDS and tobacco use.		

| Potential health impact of ENDS | The overall population health effects of using ENDS depends primarily on:

the capacity of ENDS to lead smokers away from smoking, while dissuading never smokers from starting to smoke and ex-smokers from relapsing; and

the health risks associated with their use relative to defined alternatives such as never using ENDS or smoking. | The gaps in research on the effectiveness of ENDS as smoking cessation aid are described above.

Further research is needed on the long-term health consequences of ENDS use in comparison with non-use and with cigarette smoking in: smokers, including adults, children and pregnant women; and nonsmokers.

In some cases, in-vitro (e.g. cell preparations) or in-vivo (e.g. animal models) methods may be appropriate for addressing these questions or to guide subsequent clinical investigation. For human studies, any diseases associated with ENDS use may not necessarily be those associated with cigarette smoking. Thus, particular attention must be paid to disease states and biomarkers of disease that might be associated with ENDS use, with an initial focus on known ENDS emissions that include propylene glycol, vegetable glycerine, flavourings, sweeteners and by-products of these liquid constituents that are produced when they are heated. | Development of adequate policies and regulations on the ENDS issues described in this paper would benefit from effective, organized national and global surveillance of the types of ENDS marketed and their use. More specifically, information is required on who is using them, for what purpose (including to deliver other drugs of abuse) and the products being used (including measures of voltage, resistance, power, liquid constituents, use frequency). |

3.9   **References**

1.   Breland A, Soule E, Lopez A, Ramôa C, El-Hellani A, Eissenberg T et al. Electronic cigarettes: what are they and what do they do? Ann N Y Acad Sci. 2016;1394:5–30.

2.   Kennedy RD, Awopegba A, De León E, Cohen JE. Global approaches to regulating electronic cigarettes. Tob Control 2016;26:440–5.

3.   Talih S, Balhas Z, Eissenberg T, Salman R, Karaoghlanian N, El Hellani H et al. Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: measurements and model predictions. Nicotine Tob Res. 2014;17:150–7.

4.   Wagener T, Floyd E, Stepanov I, Driskill LM, Frank SG, Meier E et al. Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users. Tob Control. 2016;26:e23–8.

5.   Rudy A, Leventhal A, Goldenson NI, Eissenberg T. Assessing electronic cigarette effects and regulatory impact: challenges with user self-reported device power. Drug Alcohol Depend. 2017;179:337–40.

6.   Farsalinos K, Spyrou A, Stefopoulos C, Tsimopoulou K, Kourkoveli P, Tsiapris D et al. Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naïve users (smokers). Sci Rep. 2015;5:11269.

7.   Hua M, Yip H, Talbot P. Mining data on usage of electronic nicotine delivery systems (ENDS) from YouTube videos. Tob Control. 2011;22:103–6.

8.   Hiler M, Breland A, Spindle T, Maloney S, Lipato T, Karaoghlanian N et al. Electronic cigarette user plasma nicotine concentration, puff topography, heart rate, and subjective effects: the influence of liquid nicotine concentration and user experience. Exp Clin Psychopharmacol. 2017;25(5):380–92.

9.   Vansickel A, Cobb C, Weaver M, Eissenberg T. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and subjective effects. Cancer Epidemiol Biomarkers Prev. 2010;19:1945–53.

10.   Cheah N, Chong N, Tan J, Morsed FA, Yee SK. Electronic nicotine delivery systems: regulatory and safety challenges: Singapore perspective. Tob Control. 2012;23:119–25.

11.   Chaudhry IW, Leigh NJ, Smith DM, O'Connor RJ, Goniewicz ML. Labeling information on electronic nicotine delivery systems. Tob Regul Sci. 2017;3:3–9.

12.   Buonocore F, Marques Gomes ACN, Nabhani-Gebara S, Barton SJ, Calabrese G. Labelling of electronic cigarettes: regulations and current practice. Tob Control. 2016;26:46–52.

13.   Beauval N, Antherieu S, Soyez M, Gengler N, Grova N, Howsam M et al. Chemical evaluation of electronic cigarettes: multicomponent analysis of liquid refills and their corresponding aerosols. J Anal Toxicol. 2017;41:670–8.

14.   Buettner-Schmidt K, Miller DR, Balasubramanian N. Electronic cigarette refill liquids: child-resistant packaging, nicotine content, and sales to minors. J Pediatric Nurs. 2016;31:373–9.

15.   Cameron JM, Howell DN, White JR, Andrenyak DM, Layton ME, Roll JM. Variable and potentially fatal amounts of nicotine in e-cigarette nicotine solutions: Table 1. Tob Control 2013;23:77–8.

16.   Davis B, Dang M, Kim J, Talbot P. Nicotine concentrations in electronic cigarette refill and do-it-yourself fluids. Nicotine Tob Res. 2014;17:134–41.

17.   El-Hellani A, El-Hage R, Baalbaki R, Salman R, Talih S, Shihadeh A et al. Free-base and protonated nicotine in electronic cigarette liquids and aerosols. Chem Res Toxicol. 2015;28:1532–7.

18.   Etter J, Zäther E, Svensson S. Analysis of refill liquids for electronic cigarettes. Addiction. 2013;108:1671–9.

19.   Etter J, Bugey A. E-cigarette liquids: constancy of content across batches and accuracy of labeling. Addictive Behav. 2017;73:137–43.

20.   Farsalinos KE, Gillman IG, Melvin MS, Paolantonio AR, Gardow WJ, Humphries KE et al. Nicotine

levels and presence of selected tobacco-derived toxins in tobacco flavoured electronic cigarette refill liquids. Int J Environ Res Public Health. 2015;12:3439–52.

21. Goniewicz ML, Gupta R, Lee YH, Reinhardt S, Kim S, Kim B et al. Nicotine levels in electronic cigarette refill solutions: a comparative analysis of products from the US, Korea, and Poland. Int J Drug Policy. 2015;26:583–8.

22. Kim S, Goniewicz M, Yu S, Kim B, Gupta R. Variations in label information and nicotine levels in electronic cigarette refill liquids in South Korea: regulation challenges. Int J Environ Res Public Health. 2015;12:4859–68.

23. Kirschner RI, Gerona R, Jacobitz KL. Nicotine content of liquid for electronic cigarettes. Clin Toxicol. 2013;51:684–4.

24. Kosmider L, Sobczak A, Szołtysek-Bołdys I, Prokopowicz A, Skórka A, Abdulafeez O et al. Assessment of nicotine concentration in electronic nicotine delivery system (ENDS) liquids and precision of dosing to aerosol. Przegl Lek. 2015;72:500–4.

25. Lisko JG, Tran H, Stanfill SB, Blount BC, Watson CH. Chemical composition and evaluation of nicotine, tobacco alkaloids, pH, and selected flavors in e-cigarette cartridges and refill solutions. Nicotine Tob Res. 2015;17:1270–8.

26. Pagano T, DiFrancesco AG, Smith SB, George J, Wink G, Rahman I et al. Determination of nicotine content and delivery in disposable electronic cigarettes available in the United States by gas chromatography–mass spectrometry. Nicotine Tob Res. 2015;18:700–7.

27. Peace MR, Baird TR, Smith N, Wolf CE, Poklis JL, Poklis A. Concentration of nicotine and glycols in 27 electronic cigarette formulations. J Anal Toxicol. 2016;40:403–7.

28. Rahman A, Nik Mohamed MH, Mahmood S. Nicotine estimations in electronic cigarette e-liquids among Malaysian marketed samples. Anal Chem Lett. 2018;8:54–62.

29. Raymond BH, Collette-Merrill K, Harrison RG, Jarvis S, Rasmussen RJ. The nicotine content of a sample of e-cigarette liquid manufactured in the United States. J Addict Med. 2018;12:127–31.

30. Trehy ML, Ye W, Hadwiger ME, Moore TW, Allgire JF, Woodruff JT et al. Analysis of electronic cigarette cartridges, refill solutions, and smoke for nicotine and nicotine related impurities. J Liquid Chromatogr Relat Technol. 2011;34:1442–58.

31. E-liquid manufacturing standards 2017 version 2.3.2. Mooresville (NC): American E-liquid Manufacturing Standards Association; 2017 (http://www.aemsa.org/standards/, accessed 19 September 2017).

32. Goniewicz ML, Hajek P, McRobbie H. Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. Addiction. 2013;109:500–7.

33. Omaiye E, Cordova I, Davis B, Talbot P. Counterfeit electronic cigarette products with mislabeled nicotine concentrations. Tob Regul Sci. 2017;3:347–57.

34. Westenberger B. Evaluation of e-cigarettes. St Louis (MO): Center for Drug Evaluation and Research, Office of Pharmaceutical Science, Office of Testing and Research; 2009 (https://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm088761.htm, accessed 1 October 2018).

35. Henningfield J, London E, Benowitz N. Arterial–venous differences in plasma concentrations of nicotine after cigarette smoking. JAMA. 1990;263:2049–50.

36. Baldassarri SR, Hillmer AT, Anderson JM, Jatlow P, Nabulsi N, Labaree D et al. Use of electronic cigarettes leads to significant beta2-nicotinic acetylcholine receptor occupancy: evidence from a PET imaging study. Nicotine Tob Res. 2018;20(4):425–33.

37. Dawkins L, Corcoran O. Acute electronic cigarette use: nicotine delivery and subjective effects in regular users. Psychopharmacology. 2013;231:401–7.

38. Hajek P, Goniewicz ML, Phillips A, Myers Smith K, West O, McRobbie H. Nicotine intake from electronic cigarettes on initial use and after 4 weeks of regular use. Nicotine Tob Res. 2014;17:175–9.

39. Nides MA, Leischow SJ, Bhatter M, Simmons M. Nicotine blood levels and short-term smoking

reduction with an electronic nicotine delivery system. Am J Health Behav. 2014;38:265–74.

40. St Helen G, Havel C, Dempsey DA, Jacob P 3rd, Benowitz NL. Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes. Addiction. 2015;111:535–44.

41. Yan XS, D'Ruiz C. Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. Regul Toxicol Pharmacol 2015;71:24–34.

42. Henningfield JE. Nicotine medications for smoking cessation. N Engl J Med. 1995;333:1196–203.

43. Choi JH, Dresler CM, Norton MR, Strahs KR. Pharmacokinetics of a nicotine polacrilex lozenge. Nicotine Tob Res. 2003;5:635–44.

44. Evans SE, Blank M, Sams C, Weaver MF, Eissenberg T. Transdermal nicotine-induced tobacco abstinence symptom suppression: nicotine dose and smokers' gender. Exp Clin Psychopharmacol. 2006;14:121–35.

45. Bullen C, Howe C, Laugesen M, McRobbie H, Parag V, Williman J et al. Electronic cigarettes for smoking cessation: a randomised controlled trial. Lancet 2013;382:1629–37.

46. E-Cigarette use among youth and young adults. A report of the Surgeon General. Atlanta (GA): Department of Health and Human Services, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2016.

47. Schroeder MJ, Hoffman AC. Electronic cigarettes and nicotine clinical pharmacology. Tob Control 2014;23:ii30–5.

48. Cobb NK, Byron MJ, Abrams DB, Shields PG. Novel nicotine delivery systems and public health: the rise of the "e-cigarette". Am J Public Health. 2010;100:2340–2.

49. McAuley TR, Hopke PK, Zhao J, Babaian S. Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. Inhal Toxicol. 2012;24:850–7.

50. Pellegrino RM, Tinghino B, Mangiaracina G, Marani A, Vitali M, Protano C et al. Electronic cigarettes: an evaluation of exposure to chemicals and fine particulate matter (PM). Ann Ig. 2012;24:79–88.

51. Goniewicz ML, Kuma T, Gawron M, Knysak J, Kosmider L. Nicotine levels in electronic cigarettes. Nicotine Tob Res. 2012;15:158–66.

52. Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Voudris V. Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: implications for research protocol standards definition and for public health authorities' regulation. Int J Environ Res Public Health 2013;10:2500–14.

53. Laugesen M. Nicotine and toxicant yield ratings of electronic cigarette brands in New Zealand. NZ Med J. 2015;128:77–82.

54. Tayyarah R, Long GA. Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. Regul Toxicol Pharmacol. 2014;70:704–10.

55. Henningfield JE, Keenan RM. Nicotine delivery kinetics and abuse liability. J Consult Clin Psychol 1993;61:743–50.

56. Shihadeh A, Eissenberg T. Electronic cigarette effectiveness and abuse liability: predicting and regulating nicotine flux. Nicotine Tob Res. 2014;17:158–62.

57. Eissenberg T, Shihadeh A. Nicotine flux: a potentially important tool for regulating electronic cigarettes. Nicotine Tob Res. 2014;17:165–7.

58. Talih S, Balhas Z, Salman R, El-Hage R, Karaoghlanian N, El-Hellani A et al. Transport phenomena governing nicotine emissions from electronic cigarettes: model formulation and experimental investigation. Aerosol Sci Technol. 2016;51:1–11.

59. Stepanov I, Fujioka N. Bringing attention to e-cigarette pH as an important element for research and regulation. Tob Control. 2014;24:413–4.

60. Han S, Chen H, Zhang X, Liu T, Fu Y. Levels of selected groups of compounds in refill solutions for electronic cigarettes. Nicotine Tob Res. 2015;18:708–14.

61. Burr GA, Van Gilder TJ, Trout BD, Wilcox TC, Driscoll R. Health hazard evaluation report HETA

90-0355-2449, Actors' Equity Association/The League of American Theatres and Producers, Inc. Washington (DC): National Institute for Occupational Safety and Health, Hazard Evaluations and Technical Assistance Branch Division of Surveillance, Hazard Evaluations, and Field Studies; 1994 (http://www.cdc.gov/niosh/hhe/reports/pdfs/1990-0355-2449.pdf, accessed 1 October 2018).

62. Moline JM, Golden AL, Highland JH, Wilmarth KR, Kao AS. Health effects evaluation of theatrical smoke, haze, and pyrotechnics. Report to Equity-League Pension and Health Trust Funds, 2000.

63. Varughese S, Teschke K, Brauer M, Chow Y, van Netten C, Kennedy SM. Effects of theatrical smokes and fogs on respiratory health in the entertainment industry. Am J Ind Med. 2005;47:411–8.

64. Wieslander G. Experimental exposure to propylene glycol mist in aviation emergency training: acute ocular and respiratory effects. Occup Environ Med. 2001;58:649–55.

65. Neale BW, Mesler EL, Young M, Rebuck JA, Weise WJ. Propylene glycol-induced lactic acidosis in a patient with normal renal function: a proposed mechanism and monitoring recommendations. Ann Pharmacother. 2005;39:1732–6.

66. Deichmann W. Glycerol:–effects upon rabbits and rats–. Am Ind Hyg Assoc Q 1941;2:5–6.

67. Hine CH, Anderson HH, Moon HO, Dunlop MK, Morse MS. Comparative toxicity of synthetic and natural glycerin. Arch Ind Hyg Occup Med. 1953;7:282–91.

68. Safety assessment and regulatory authority to use flavors: focus on electronic nicotine delivery systems and flavored tobacco products. Washington (DC): Federal Emergency Management Agency; 2013 (https://www.femaflavor.org/safety-assessment-and-regulatory-authority-use-flavors-focus-electronic-nicotine-delivery-systems, accessed 18 Oct 2017).

69. Farsalinos KE, Kistler KA, Gillman G, Voudris V. Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Nicotine Tob Res. 2014;17:168–74.

70. Kosmider L, Sobczak A, Prokopowicz A, Kurek J, Zaciera M, Knysak J et al. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. Thorax. 2016;71:376–7.

71. Behar RZ, Luo W, Lin SC, Wang Y, Valle J, Pankow JE et al. Distribution, quantification and toxicity of cinnamaldehyde in electronic cigarette refill fluids and aerosols. Tob Control 2016;25:ii94–102.

72. Gerloff J, Sundar IK, Freter R, Sekera ER, Friedman AE, Robinson R et al. Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography–mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts. Appl In Vitro Toxicol. 2017;3:28–40.

73. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J, Luch A. Chemical hazards present in liquids and vapors of electronic cigarettes. Arch Toxicol. 2014;88:1295–308.

74. Varlet V, Farsalinos K, Augsburger M, Thomas A, Etter JF. Toxicity assessment of refill liquids for electronic cigarettes. Int J Environ Res Public Health. 2015;12:4796–815.

75. Williams M, Bozhilov K, Ghai S, Talbot P. Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. PLoS One. 2017;12:e0175430.

76. Ogunwale MA, Li M, Ramakrishnam Raju MV, Chen Y, Nantz MH, Conklin DJ et al. Aldehyde detection in electronic cigarette aerosols. ACS Omega 2017;2:1207–14.

77. Herrington JS, Myers C. Electronic cigarette solutions and resultant aerosol profiles. J Chromatogr A 2015;1418:192–9.

78. Talih S, Balhas Z, Salman R, Karaoghlanian N, Shihadeh A. "Direct dripping": a high-temperature, high-formaldehyde emission electronic cigarette use method. Nicotine Tob Res. 2015;18:453–9.

79. Bekki K, Uchiyama S, Ohta K, Inaba Y, Nakagome H, Kunugita N. Carbonyl compounds generated from electronic cigarettes. Int J Environ Res Public Health. 2014;11:11192–200.

80. Sleiman M, Logue JM, Montesinos VN, Russell ML, Litter MI, Gundel LA et al. Emissions from electronic cigarettes: key parameters affecting the release of harmful chemicals. Environ Sci Technol. 2016;50:9644–51.

81. Wang P, Chen W, Liao J, Matsuo T, Ito K, Fowles J et al. A device-independent evaluation of carbonyl emissions from heated electronic cigarette solvents. PLoS One. 2017;12:e0169811.

82. Havel CM, Benowitz NL, Jacob P 3rd, St Helen G. An electronic cigarette vaping machine for the characterization of aerosol delivery and composition. Nicotine Tob Res. 2017;19(10):1224–31.

83. Geiss O, Bianchi I, Barrero-Moreno J. Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapours. Int J Hyg Environ Health. 2016;219:268–77.

84. Khlystov A, Samburova V. Flavoring compounds dominate toxic aldehyde production during e-cigarette vaping. Environ Sci Technol. 2016;50:13080–5.

85. Farsalinos K, Gillman G, Kistler K, Yannovits N. Comment on "Flavoring compounds dominate toxic aldehyde production during e-cigarette vaping". Environ Sci Technol. 2017;51:2491–2.

86. Khlystov A, Samburova V. Response to comment on "Flavoring compounds dominate toxic aldehyde production during e-cigarette vaping". Environ Sci Technol. 2017;51:2493–4.

87. Tierney P, Karpinski C, Brown J, Luo W, Pankow JF. Flavour chemicals in electronic cigarette fluids. Tob Control. 2015;25:e10–5.

88. Soussy S, EL-Hellani A, Baalbaki R, Salman R, Shihadeh A, Saliba NA. Detection of 5-hydroxymethylfurfural and furfural in the aerosol of electronic cigarettes. Tob Control. 2016;25:ii88–93.

89. Pankow JF, Kim K, McWhirter KJ, Luo W, Escobedo JO, Strongin RM et al. Benzene formation in electronic cigarettes. PLoS One. 2017;12:e0173055.

90. Gillman I, Kistler K, Stewart E, Paolantonio AR. Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. Regul Toxicol Pharmacol. 2016;75:58–65.

91. Franck C, Budlovsky T, Windle S, Filion KB, Eisenberg MJ. Electronic cigarettes in North America: history, use, and implications for smoking cessation. Circulation. 2014;129:1945–52.

92. Harrell PT, Simmons VN, Correa JB, Padhya TA, Brandon TH. Electronic nicotine delivery systems ("e-cigarettes"): review of safety and smoking cessation efficacy. Otolaryngol Head Neck Surg. 2014;151:381–93.

93. Lam C, West A. Are electronic nicotine delivery systems an effective smoking cessation tool? Can J Resp Ther. 2015;51:93–8.

94. Ioakeimidis N, Vlachopoulos C, Tousoulis D. Efficacy and safety of electronic cigarettes for smoking cessation: a critical approach. Hell J Cardiol. 2016;57:1–6.

95. Waghel RC, Battise DM, Ducker ML. Effectiveness of electronic cigarettes as a tool for smoking cessation or reduction. J Pharm Technol. 2014;31:8–12.

96. Orr KK, Asal NJ. Efficacy of electronic cigarettes for smoking cessation. Ann Pharmacother 2014;48:1502–6.

97. Malas M, van der Tempel J, Schwartz R, Mimichiello A, Lightfoot C, Noormohamed A et al. Electronic cigarettes for smoking cessation: a systematic review. Nicotine Tob Res. 2016;18:1926–36.

98. Rahman MA, Hann N, Wilson A, Mnatzganian G, Worrell-Carter L. E-cigarettes and smoking cessation: evidence from a systematic review and meta-analysis. PLoS One. 2015;10:e0122544.

99. McRobbie H, Bullen C, Hartmann-Boyce J, Hajek P. Electronic cigarettes for smoking cessation and reduction. Cochrane Database Syst Rev. 2014;(12):CD010216.

100. Hartmann-Boyce J, McRobbie H, Bullen C, Begh R, Stead LF, Hajek P. Electronic cigarettes for smoking cessation. Cochrane Database Syst Rev. 2016;9:CD010216.

101. Kalkhoran S, Glantz SA. E-cigarettes and smoking cessation in real-world and clinical settings: a systematic review and meta-analysis. Lancet Resp Med. 2016;4:116–28.

102. El Dib R, Suzumura EA, Akl EA, Gomaa H, Agarwal A, Chang Y et al. Electronic nicotine delivery

systems and/or electronic non-nicotine delivery systems for tobacco smoking cessation or reduction: a systematic review and meta-analysis. BMJ Open. 2017;7:e012680.

103. Khoudigian S, Devji T, Lytvyn L, Campbell K, Hopkins R, O'Reilly D. The efficacy and short-term effects of electronic cigarettes as a method for smoking cessation: a systematic review and a meta-analysis. Int J Public Health. 2016;61:257–67.

104. Stratton K, Kwan LY, Eaton DL, editors. Smoking cessation among adults. Chapter 17. In: Public health consequences of e-cigarettes. Washington (DC): National Academies Press;2018;541–88 (http://nationalacademies.org/hmd/Reports/2018/public-health-consequences-of-e-ciga-rettes.aspx, accessed 20 April 2018).

105. Pasquereau A, Guignard R, Andler R, Nguyen-Thanh V. Electronic cigarettes, quit attempts and smoking cessation: a 6-month follow-up. Addiction. 2017;112:1620–8.

106. Wang M, Li W, Wu Y, Lam TH, Chan SS. Electronic cigarette use is not associated with quitting of conventional cigarettes in youth smokers. Pediatric Res. 2017;82:14–8.

107. Subialka Nowariak EN, Lien RK, Boyle RG, Amato MS, Beebe LA. E-cigarette use among treatment-seeking smokers: moderation of abstinence by use frequency. Addict Behav 2018;77:137–42.

108. Berry KM, Reynolds LM, Collins JM, Siegel MB, Fetterman JL, Hamburg NM et al. E-cigarette initiation and associated changes in smoking cessation and reduction: the Population Assessment of Tobacco and Health Study, 2013–2015. Tob Control 2018; doi:10.1136/tobaccocontrol-2017-054108.

109. Rigotti NA, Chang Y, Tindle HA, Kalkhoran SM, Levy DE, Regan S et al. Association of e-cigarette use with smoking cessation among smokers who plan to quit after a hospitalization. Ann Internal Med. 2018;168(9):613–20..

110. Zawertailo L, Pavlov D, Ivanova A, Ng G, Baliunas D, Selby P. Concurrent e-cigarette use during tobacco dependence treatment in primary care settings: association with smoking cessation at three and six months. Nicotine Tob Res. 2016;19:183–9.

111. Manzoli L, Flacco ME, Ferrante M, La Vecchia C, Siliquini R, Ricciardi W et al. Cohort study of electronic cigarette use: effectiveness and safety at 24 months. Tob Control 2017;26:284–92.

112. Barrington-Trimis JL, Gibson LA, Halpern-Felsher B, Harrell MB, Kong G, Krishnan-Sarin S et al. Type of e-cigarette device used among adolescents and young adults: findings from a pooled analysis of 8 studies of 2,166 vapers. Nicotine Tob Res. 2018;20(2):271–4.

113. Hummel K, Hoving C, Nagelhout GE, de Vries H, van den Putte B, Candel MJJM et al. Prevalence and reasons for use of electronic cigarettes among smokers: findings from the International Tobacco Control (ITC) Netherlands Survey. Int J Drug Policy. 2014;26(6):601–8.

114. Attitudes of Europeans towards tobacco and electronic cigarettes (Special Eurobarometer 458). Brussels: European Union; 2017.

115. Ayers JW, Leas EC, Allem JP, Benton A, Dredze M, Althouse BM et al. Why do people use electronic nicotine delivery systems (electronic cigarettes)? A content analysis of Twitter, 2012–2015. PLoS One. 2017;12(3):e0170702.

116. Pearson JL, Hitchman SC, Brose LS, Bauld L, Glasser AM, Vilanti AC et al. Recommended core items to assess e-cigarette use in population-based surveys. Tob Control. 2018;27(3):341–6.

117. Amato MS, Boyle RG, Levy D. How to define e-cigarette prevalence? Finding clues in the use frequency distribution. Tob Control. 2015;25:e24–9.

118. Shi Y, Pierce J, White M, Vijayaraghavan M, Compton W, Conway K et al. E-cigarette use and smoking reduction or cessation in the 2010/2011 TUS-CPS longitudinal cohort. BMC Public Health. 2016;16(1):1105.

119. Vickerman K, Carpenter K, Altman T, Nash CM, Zbikowski SM. Use of electronic cigarettes among state tobacco cessation quitline callers. Nicotine Tobacco Res. 2013;15:1787–791.

120. Al-Delaimy W, Myers M, Leas E, Strong DR, Hofstetter CR. E-Cigarette use in the past and quit-

ting behavior in the future: a population-based study. Am J Public Health. 2015;105:1213–9.

121. Biener L, Hargraves JL. A longitudinal study of electronic cigarette use among a population-based sample of adult smokers: association with smoking cessation and motivation to quit. Nicotine Tob Res. 2014;17:127–33.

122. Pearson J, Stanton C, Cha S, Niaura RS, Luta G, Graham AL. E-Cigarettes and smoking cessation: insights and cautions from a secondary analysis of data from a study of online treatment-seeking smokers. Nicotine Tob Res. 2014;17:1219–27.

123. Borderud S, Li Y, Burkhalter J, Sheffer CE, Ostroff JS. Electronic cigarette use among patients with cancer: Characteristics of electronic cigarette users and their smoking cessation outcomes. Cancer. 2014;120:3527–35.

124. Hitchman SC, Brose LS, Brown J, Robson D, McNeill A. Associations between e-cigarette type, frequency of use, and quitting smoking: findings from a longitudinal online panel survey in Great Britain. Nicotine Tob Res. 2015;17:1187–94.

125. Carpenter M, Heckman B, Wahlquist A, Wagener TL, Goniewicz ML, Gray KM et al. A naturalistic, randomized pilot trial of e-cigarettes: uptake, exposure, and behavioral effects. Cancer Epidemiol Biomarkers Prev. 2017;26:1795–803.

126. Masiero M, Lucchiari C, Mazzocco K, Veronesi G, Maisonneuve P, Jemos C et al. E-Cigarettes may support smokers with high smoking-related risk awareness to stop smoking in the short run: preliminary results by randomized controlled trial. Nicotine Tob Res. 2018. doi:10.1093/ntr/nty175.

127. Giovenco DP, Delnevo CD. Prevalence of population smoking cessation by electronic cigarette use status in a national sample of recent smokers. Addict Behav. 2017;76:129–34.

128. Zhu S, Zhuang Y, Wong S, Cummins SE, Tedeschi GJ. E-cigarette use and associated changes in population smoking cessation: evidence from US current population surveys. BMJ. 2017;358:j3262.

129. Cho HJ, Dutra LM, Glantz SA. Differences in adolescent e-cigarette and cigarette prevalence in two policy environments: South Korea and the United States. Nicotine Tob Res. 2018;20(8):949–53.

130. Benmarhnia T, Leas E, Hendrickson E, Trinidad DR, Strong DR, Pierce JP. The potential influence of regulatory environment for e-cigarettes on the effectiveness of e-cigarettes for smoking cessation: different reasons to temper the conclusions from inadequate data. Nicotine Tob Res. 2018;20(5):659.

131. Yong HH, Hitchman S, Cummings K, Borland R, Gravely SML, McNeill A et al. Does the regulatory environment for e-cigarettes influence the effectiveness of e-cigarettes for smoking cessation? Longitudinal findings from the ITC four country survey. Nicotine Tob Res. 2017;19(11):1268–76.

132. Yong HH, Borland R, Balmford J, McNeill A, Hitchman S, Driezen P et al. Trends in e-cigarette awareness, trial, and use under the different regulatory environments of Australia and the United Kingdom. Nicotine Tob Res. 2015;17:1203–11.

133. Yong HH, Borland R, Hitchman SCB, Cummings KM, Gravely SML, McNeill AD et al. Response to letter to the Editor by Benmarhnia T, Leas E, Hendrickson E, Trinidad D, Strong D, Pierce J. The potential influence of regulatory environment for e-cigarettes on the effectiveness of e-cigarettes for smoking cessation: different reasons to temper the conclusions from inadequate data. Nicotine Tob Res. 2017;20(5):660–1.

134. Grace RC, Kivell BM, Laugesen M. Estimating cross-price elasticity of e-cigarettes using a simulated demand procedure. Nicotine Tob Res. 2014;17:592–8.

135. Johnson MW, Johnson PS, Rass O, Pacek LR. Behavioral economic substitutability of e-cigarettes, tobacco cigarettes, and nicotine gum. J Psychopharmacol. 2017;31:851–60.

136. Snider SE, Cummings KM, Bickel WK. Behavioral economic substitution between conventional cigarettes and e-cigarettes differs as a function of the frequency of e-cigarette use. Drug Al-

cohol Depend. 2017;177:14–22.

137. Jamal A, King BA, Neff LJ, Whitmill J, Babb SD, Graffunder CM. Current cigarette smoking among adults – United States, 2005–2015. Morbid Mortal Wkly Rep. 2016;65:1205–11.

138. Adult smoking habits in the UK: 2016. Cigarette smoking among adults including the proportion of people who smoke, their demographic breakdowns, changes over time, and e-cigarettes. Lonson: Office for National Statistics; 2017 (https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandlifeexpectancies/bulletins/adultsmokinghabitsingreatbritain/2016#toc, accessed October 2018).

139. Beard E, West R, Michie S, Brown J. Association between electronic cigarette use and changes in quit attempts, success of quit attempts, use of smoking cessation pharmacotherapy, and use of stop smoking services in England: time series analysis of population trends. BMJ 2016;354:i4645.

140. McRobbie H. Modelling the population health effects of e-cigarettes use: current data can help guide future policy decisions. Nicotine Tob Res. 2016;19:131–2.

141. Hill A, Camacho OM. A system dynamics modelling approach to assess the impact of launching a new nicotine product on population health outcomes. Regul Toxicol Pharmacol 2017;86:265–78.

142. Yoong S, Tzelepis F, Wiggers JH, Oldmeadow C, Chai K, Paul CL et al. Prevalence of smoking-proxy electronic inhaling system (SEIS) use and its association with tobacco initiation in youths: a systematic review. Geneva: World Health Organization; 2015 (http://who.int/tobacco/industry/product_regulation/BackgroundPapersENDS2_4November.pdf?ua=1, accessed 3 October 2017).

143. Leventhal AM, Strong DR, Kirkpatrick MG, Unger JB, Sussman S, Riggs NR et al. Association of electronic cigarette use with initiation of combustible tobacco product smoking in early adolescence. JAMA. 2015;314:700.

144. Primack B, Soneji S, Stoolmiller M, Fine MJ, Sargent JD. Progression to traditional cigarette smoking after electronic cigarette use among US adolescents and young adults. JAMA Pediatrics. 2015;169:1018–23.

145. Wills T, Knight R, Sargent J, Gibbons FX, Pagano I, Williams RJ. Longitudinal study of e-cigarette use and onset of cigarette smoking among high school students in Hawaii. Tob Control 2016;26:34–9.

146. Soneji S, Barrington-Trimis J, Wills T, Leventhal AM, Unger JB, Gibson LA et al. Association between initial use of e-cigarettes and subsequent cigarette smoking among adolescents and young adults. JAMA Pediatrics. 2017;171:788.

147. Miech R, Patrick M, O'Malley P et al. E-cigarette use as a predictor of cigarette smoking: results from a 1-year follow-up of a national sample of 12th grade students. Tobacco Control 2017. doi:10.1136/tobaccocontrol-2016-053291.

148. Best C, Haseen F, Currie D, Ozakinci G, MacKintosh AM, Stead M et al. Relationship between trying an electronic cigarette and subsequent cigarette experimentation in Scottish adolescents: a cohort study. Tob Control. 2017. doi:10.1136/tobaccocontrol-2017-053691.

149. Conner M, Grogan S, Simms-Ellis R, Flett K, Sykes-Muskett B, Cowap L et al. Do electronic cigarettes increase cigarette smoking in UK adolescents? Evidence from a 12-month prospective study. Tob Control. 2017. doi:10.1136/tobaccocontrol-2016-053539.

150. Kozlowski LT, Warner KE. Adolescents and e-cigarettes: Objects of concern may appear larger than they are. Drug Alcohol Depend. 2017;174:209–14.

151. Gartner CE. E-cigarettes and youth smoking: be alert but not alarmed. Tob Control 2017. doi:10.1136/tobaccocontrol-2017-054002.

152. Schneider S, Diehl K. Vaping as a catalyst for smoking? An initial model on the initiation of electronic cigarette use and the transition to tobacco smoking among adolescents. Nicotine Tob Res 2015;18:647–53.

153. Lee PN. Appropriate and inappropriate methods for investigating the "gateway" hypothesis, with a review of the evidence linking prior snus use to later cigarette smoking. Harm Reduction J. 2015;12:8.

154. Jamal A, Gentzke A, Hu SS, Cullen KA, Apelberg BJ, Homa DM et al. Tobacco use among middle and high school students – United States, 2011–2016. Morb Mortal Wkly Rep. 2017;66:597–603.

155. Dinakar C, O'Connor GT. The health effects of electronic cigarettes. N Engl J Med. 2016;375:1372–81.

156. Hua M, Talbot P. Potential health effects of electronic cigarettes: a systematic review of case reports. Prev Med Rep. 2016;4:169–78.

157. Farsalinos KE, Polosa R. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. Ther Adv Drug Saf. 2014;5:67–86.

158. Burstyn I. Peering through the mist: systematic review of what the chemistry of contaminants in electronic cigarettes tells us about health risks. BMC Public Health. 2014;14:18.

159. Pisinger C. A systematic review of health effects of electronic cigarettes. Geneva: World Health Organization; 2017 (http://www.who.int/tobacco/industry/product_regulation/Background PapersENDS3_4November-.pdf, accessed 30 September 2017).

160. Glasser AM, Collins L, Pearson JL, Abudayyeh H, Niaura RS, Abrams DB et al. Overview of electronic nicotine delivery systems: a systematic review. Am J Prev Med. 2017;52:e33–66.

161. Stephens WE. Comparing the cancer potencies of emissions from vapourised nicotine products including e-cigarettes with those of tobacco smoke. Tob Control. 2017. doi:10.1136/tobaccocontrol-2017-053808.

162. Farsalinos KE, Voudris V, Poulas K. E-cigarettes generate high levels of aldehydes only in "dry puff" conditions. Addiction. 2015;110:1352–6.

163. Benowitz NL, Burbank AD. Cardiovascular toxicity of nicotine: implications for electronic cigarette use. Trends Cardiovasc Med. 2016;26:515–23.

164. Shields PG, Berman M, Brasky TM, Freudenheim JL, Mathe E, McElroy JP et al. A review of pulmonary toxicity of electronic cigarettes in the context of smoking: a focus on inflammation. Cancer Epidemiol Biomarkers Prev. 2017;26:1175–91.

165. Levy DT, Borland R, Villanti AC, Niaura R, Yuan Z, Zhang Y et al. The application of a decision–theoretic model to estimate the public health impact of vaporized nicotine product initiation in the United States. Nicotine Tob Res 2016;19:149–59.

166. Kalkhoran S, Glantz SA. Modeling the health effects of expanding e-cigarette sales in the United States and United Kingdom. JAMA Internal Med. 2015;175:1671.

167. Levy D, Borland R, Lindblom E, Goniewicz ML, Meza R, Holford TR et al. Potential deaths averted in USA by replacing cigarettes with e-cigarettes. Tob Control 2017. doi:10.1136/tobaccocontrol-2017-053759.

168. Soneji S, Sung H, Primack B, Pierce JP, Sargent J. Problematic assessment of the impact of vaporized nicotine product initiation in the United States. Nicotine Tob Res 2016;19:264–5.

169. Glantz SA. Need for examination of broader range of risks when predicting the effects of new tobacco products. Nicotine Tob Res. 2016;19:266–7.

170. Levy DT, Borland R, Fong GT, Vilanti AC, Niaura R, Meza R et al. Developing consistent and transparent models of e-cigarette use: reply to Glantz and Soneji et al. Nicotine Tob Res. 2016;19:268–70.

171. Stratton K, Kwan LY, Eaton DL, editors. Injuries and poisonings. Chapter 14. In: Public health consequences of e-cigarettes. Washington (DC): National Academies Press;2018;541–88 (http://nationalacademies.org/hmd/Reports/2018/public-health-consequences-of-e-cigarettes.aspx, accessed 20 April 2018).

4. A global nicotine reduction strategy: state of the science

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Contents

4.1 Background

In 2015, TobReg published an advisory note in support of policy to reduce the nicotine content of the tobacco used in cigarettes to a level below that necessary to develop or maintain addiction *(1)*. Policy to reduce nicotine could have a significant effect on public health by minimizing progression from experimental cigarette use to dependence, reducing duration of use, facilitating quitting, reducing the prevalence of smoking among addicted smokers and encouraging a transition to less harmful products for those who want or need nicotine from other sources *(1, 2)*. About one billion people in the world smoke, and global consumption of cigarettes

and other combusted tobacco products (including cigarillos and roll-your-own tobacco) continues to rise *(3)*. Article 9 of the WHO FCTC authorizes Parties to regulate the content and emissions of tobacco products, including nicotine *(4, 5)*. There is clear evidence that reducing the nicotine content of cigarettes to a very low level can reduce dependence on cigarettes *(1, 6, 7)*.

The WHO advisory note described the potential health outcomes of a nicotine reduction policy, while observing that many research projects, including clinical trials of very low nicotine content (VLNC) cigarettes, were still under way. The advisory note identified a number of open questions on possible health outcomes, including:

- the use and effects of VLNC cigarettes in non-smoking adolescents and adults and non-dependent smokers;
- the use and effects of VLNC cigarettes in vulnerable populations such as those with moderate or severe mental illness and pregnant women;
- the use and effects of VLNC cigarettes with other forms of nicotine or other drugs; and
- the effects of long-term use of VLNC cigarettes.

Critical discussion since publication of the advisory note focused on the open questions listed above *(8–10)*, the difficulty of drawing conclusions about the outcomes of restricted clinical trials for the open market *(8, 9, 11)* and potential unintended effects, such as the belief that VLNC products are less toxic than regular cigarettes *(11–13)*. The advisory note did not provide a detailed proposal of how a nicotine reduction policy would be enacted, and critical discussion has highlighted practical challenges to implementation of a nicotine reduction policy *(9, 14)*.

This section updates the fast-growing science on nicotine reduction and use of VLNC products and addresses the questions about a nicotine reduction policy raised in both the 2015 advisory note and the critical discussion that followed. More than 100 relevant studies have been published since the background paper on nicotine reduction was presented to TobReg at its seventh meeting in December 2013, when discussions on nicotine reduction began. In line with decision FCTC/COP7(14) of the seventh session of the COP to the WHO FCTC, the Convention Secretariat and WHO convened a face-to-face meeting of experts in many disciplines on measures for reducing the addictiveness of tobacco (15–16 May 2018, Berlin, Germany). Various clinical trials remained under way as of September 2017, at the time this section was written (19 September 2017; see Table 4.1), and the scope and relevant preliminary outcomes of these trials are discussed below. The plan of the FDA to pursue a nicotine reduction strategy *(15)* is also discussed.

Table 4.1. Clinical trials of very-low-nicotine content (VLNC) cigarettes under way or still being analysed at 19 September 2017

Principal investigator(s)	Clinical trial number	Purpose	Key feature	Sample size	Estimated completion date
Cassidy	NCT02587312	To determine how reducing the level of nicotine in cigarettes affects adolescent smoking behaviour.	Adolescents	90	May 2019
Cinciripini	NCT02964182	To assess the effects of different nicotine levels in cigarettes and electronic cigarettes.	Combination with e-cigarettes	480	November 2020
Colby	NCT03194256	To determine how the nicotine content of cigarettes and the nicotine concentration and flavours in e-liquids influence responses to and use of these products by adolescent smokers.	Laboratory-based; adolescents; e-cigarettes as alternative	120	February 2022
Donny	NCT02301325	To evaluate the impact of low-nicotine cigarettes with and without transdermal nicotine.	Transdermal nicotine	240	April 2017
Donny	NCT03185546	To evaluate the effects of VLNC cigarettes, e-cigarettes with different nicotine contents and e-cigarette flavourings on smoking.	Access to e-cigarettes with varying nicotine content and flavours	480	September 2021
Drobes	NCT02796391	To determine the impact of gradual vs immediate reduction of nicotine content in cigarettes, in combination with targeted behavioural treatment, on smoking cessation and intermediate outcomes.	Cessation	220	April 2021
Foulds, Evins	NCT01928758	To evaluate the effect of progressive nicotine reduction in cigarettes on smoking behaviour, exposure to toxins and psychiatric symptoms in smokers with comorbid mood and/or anxiety disorders.	Affective disorders	280	October 2018
Ganz	NCT01964807	To test products that provide a wide range of concentrations of nicotine, particles and other cardiovascular toxins to determine how the components associated with tobacco use adversely affect cardiovascular risk.	Laboratory-based; cardiovascular effects	90	September 2018
Hatsukami	NCT02139930	To compare two approaches to reducing levels of nicotine in cigarettes: an immediate vs a gradual reduction.	Gradual vs immediate	1250	March 2017
Hatsukami	NCT03272685	To determine the effect of VLNC cigarettes in the complex tobacco and nicotine product marketplace.	Access to wide range of alternative products	700	December 2022
Higgins, Heil	NCT02250534	To determine the effect of extended exposure to cigarettes with various nicotine contents in disadvantaged women.	Disadvantaged women	282	October 2019
Higgins, Sigmon	NCT02250664	To determine the effect of extended exposure to cigarettes with various nicotine contents in opioid abusers.	Opioid abusers	282	October 2019
Klemperer	NCT03060083	To examine two strategies by randomizing smokers to (i) switch to VLNC cigarettes or (ii) reduce the number of cigarettes smoked per day. All smokers use a nicotine patch to help them reduce their nicotine intake.	Compare nicotine reduction to cigarette reduction	74	November 2017

Koffarnus, Bickel	NCT02951143	To assess how smokers purchase and consume reduced-nicotine cigarettes.	Laboratory-based; cost	232	July 2020
Kollins, McClernon	NCT02599571	To investigate the effects of different nicotine levels in cigarettes in individuals with attention deficit hyperactivity disorder.	Attention deficit hyperactivity disorder	350	June 2020
McClernon	NCT02989038	To evaluate reactions and choices to self-administer cigarette smoke with various nicotine contents among low-frequency, non-dependent smokers aged 18–25 years.	Young adult light smokers	90	April 2019
Muscat, Horn	NCT01928719	To determine whether progressively lowering the nicotine content of cigarettes reduces or eliminates nicotine dependence in smokers of low socioeconomic status.	Low socioeconomic status	400	October 2018
Oncken	NCT02048852	To determine the effect of reducing the nicotine or menthol content of cigarettes or both in women of reproductive age.	Menthol; women of reproductive age	320	December 2018
Oncken, Dornelas	NCT02592772	To observe the effect of reducing the nicotine or menthol content of cigarettes or both in men.	Menthol; men; gender differences	57	December 2018
Peters	NCT02990455	To determine whether smokers with and without current alcohol use disorder respond to reduced-nicotine cigarettes by increasing their alcohol consumption or exposure to smoke.	Alcohol use	90	November 2019
Richie	NCT02415270	To determine the short-term effects of switching to tobacco products that deliver low levels of nicotine or reactive oxygen or nitrogen species on smoking behaviour and biomarkers of exposure to tobacco smoke and oxidative stress.	Biomarkers of exposure and harm	70	May 2017
Rohsenow	NCT01989507	To determine the effect of VLNC cigarettes in smokers with substance use disorders currently or in the past year.	Substance abuse	250	May 2019
Rose	NCT02870218	To assess use of cigarettes with 0.4, 1.4, 2.5, 5.6 or 16.9 mg nicotine in heavy, long-time smokers and light smokers with a shorter smoking history (< 10 cigarettes/day for < 10 years). Participants have free access to nicotine-containing e-cigarettes (15 mg/ mL) throughout the 12-week study.	Dose-effect relation; heavy and light smokers; access to e-cigarettes	320	June 2019
Shiffman	NCT02228824	To investigate the effects of cigarettes with different nicotine levels in non-daily smokers.	Non-daily smokers	312	July 2017
Strasser	NCT01898507	To examine the effects of smoking low-nicotine cigarettes in groups of smokers with different nicotine metabolism.	Nicotine metabolism	210	July 2018
Tidey	NCT02019459	To examine whether reducing the nicotine content of cigarettes to non-addictive levels reduces smoking in smokers with schizophrenia.	Schizophrenia	80	August 2018
Tidey	NCT02232737	To determine the effect of extended exposure to cigarettes with various nicotine contents in people with current affective disorders.	Affective disorders	282	October 2019

Source: Data from clinicaltrials.gov and personal communication (EC Donny). Studies found at Clinicaltrials.gov on or before 19 September 2017 were identified with the search terms "low nicotine" and "reduced nicotine" and listed as not yet recruiting, recruiting, enrolling by invitation and active not recruiting. Studies with fewer than 50 participants or designs that did not address the impact of nicotine reduction relative to usual brand or normal nicotine content controls were omitted. A previous version of the table was published by Donny et al. (*16*). Listed by estimated date of completion.

4.2   Individual outcomes of nicotine reduction

Most estimates of the impact of nicotine reduction on individuals are from randomized clinical trials in which participants are given normal or VLNC cigarettes for an extended time, usually under double-blind conditions (see Table 4.1). Laboratory assessments that allow relatively rapid assessment of issues such as abuse liability and compensatory smoking have provided useful complementary information about nicotine reduction. In both cases, the cigarettes used contain less nicotine in the filler, generally as a result of genetic engineering (e.g. Quest brands; Spectrum cigarettes made available by the United States National Institute on Drug Abuse). Although nicotine can be reduced by other methods (e.g. nicotine extraction, as in Philip Morris' Next cigarette in the 1990s), relatively little clinical research has been done on cigarettes made by these methods because of practical issues such as cost, taste and availability. Furthermore, to our knowledge, no studies have been conducted to assess the effects of nicotine reduction in other forms of combusted tobacco (e.g. roll-your-own products, little cigars, cigarillos). The results of research to date cannot predict the full effects of a policy of nicotine reduction in the real world, although this is important. Hence, post-marketing surveillance (see e.g. International Tobacco Control project: http://www.itcproject.org/) would be an important component of any regulatory action on nicotine reduction.

4.2.1   Behavioural compensation and exposure to toxicants

The toxicants emitted from VLNC cigarettes are generally similar to those from regular cigarettes *(17)*; consequently, their effect on health depends largely on changes in smoking behaviour. Randomized clinical trials confirm earlier suggestions *(1, 18, 19)* that nicotine reduction reduces the number of cigarettes smoked per day from the number of both the usual brand and control cigarettes with a normal nicotine content *(6, 20)*.[4] Within 6 weeks of use, participants typically reported smoking 25–40% fewer cigarettes than controls *(6, 19–21)*.[1]

     The common concern that smokers will compensate for the lack of nicotine by smoking more intensely is not supported by the evidence. Compensatory smoking is often observed when the nicotine content of the filler is only modestly reduced *(20, 22)*[1] and when the nicotine yield of cigarettes is reduced by ventilation but the filler remains unchanged (often called "light" cigarettes) *(23)*. Compensatory smoking does not, however, appear to be induced by cigarettes with ≤b2.4 mg nicotine per g of tobacco (as compared with a typical range of 10–20 mg/g) *(6, 18, 19, 22)*, possibly because the noxious components of smoke limit extreme compensatory behaviour, and more modest compensatory behaviour is ineffective in maintaining exposure to nicotine, given the large reduction in nicotine content. Instead, many studies found a reduction in exposure to the

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4   EC Donny, unpublished observation.

toxicants that result from smoking. For example, participants who switched to VLNC cigarettes had similar or lower expired CO, total puff volume per cigarette, mouth-level exposure to smoke constituents *(24)* and biomarkers of exposure to toxicants *(6, 18, 19, 21, 22, 25–27)*.

4.2.2   Threshold for establishing or maintaining nicotine addiction

An important question raised in the 2015 WHO TobReg advisory note is the threshold for establishing or maintaining nicotine addiction. It is not known whether there is a single threshold for different measures of addiction and different populations of smokers; however, data suggest that nicotine should be reduced to a maximum of 2.4 mg/g of tobacco and that decreasing the content to 0.4 mg/g of tobacco may have additional benefits. Although most smokers cannot discriminate between VLNC cigarettes with 2.4 and 0.4 mg/g, some can, suggesting that reducing the nicotine content below 2.4 mg/g might affect more smokers *(28)*. Furthermore, Donny et al. *(6)* found that, although the number of cigarettes smoked per day and self-reported craving after abstinence were significantly reduced at ≤f2.4 mg/g, composite measures of nicotine dependence were significantly reduced only at 1.3 mg/g (one measure) and 0.4 mg/g (multiple measures), and the number of quit attempts during the follow-up period was significantly increased only at 0.4 mg/g. Interestingly, research on rats also suggested that a reduction of 85–90% or more (relative to doses that reinforce dependence in most rats) is required to decrease intravenous self-administration of nicotine reliably *(29)*.

It is not known whether reducing nicotine to ≤t0.4 mg/g would also limit the development of addiction in adolescent smokers. Although some studies suggested greater reinforcing effects of nicotine in adolescent rats *(30, 31)*, other research suggests that the threshold dose required for animals to learn to self-administer nicotine is similar to or higher than the dose required to maintain behaviour *(32–34)*. The latter studies indicate that a product standard based on evidence for adult smokers would be expected also to limit smoking in tobacco-naive young people. Furthermore, a secondary analysis of the data of Donny et al. *(6)* showed that nicotine reduction resulted in more rapid decreases in indices of abuse liability (smoking satisfaction, psychological reward and enjoyment of respiratory tract sensations) in 18–24-year-old smokers than in adults aged ≥ 25 years *(35)*. A study of adolescent smokers' responses to use of single cigarettes with different nicotine contents *(36)* found that reduced-nicotine cigarettes had fewer positive subjective effects but no significant effect on withdrawal or exposure. Additional studies of adolescent and young adult smokers were under way as of September 2017 (see Table 4.1). Nevertheless, the possibility that adolescents (and other populations) are more sensitive to nicotine is one reason for considering product standards with a nicotine concentration well below 2.4 mg/g.

4.2.3 Tobacco cessation after use of VLNC cigarettes

Whether reductions in the number of cigarettes per day, exposure to nicotine and nicotine dependence increase cessation is less clear. Research with participants who were intending to quit smoking suggest that use of VLNC cigarettes can increase the rate of cessation *(19, 37, 38)*, whereas studies with participants who were not currently interested in quitting found persistence of smoking after weeks or even months of VLNC cigarette use *(6, 22, 26, 39)*. Most of the studies, however, were not designed to address cessation, were not powerful enough to determine cessation outcomes, assessed behaviour for only a relatively short time, did not include giving participants alternative sources of nicotine and were conducted with commercially available cigarettes with a normal nicotine content. Two studies are important in this regard. In a 10-site clinical trial of 1250 smokers who were not intending to quit, participants who were randomized to receive VLNC cigarettes for 20 weeks self-reported more days of abstinence (10.99) than those assigned to cigarettes with a normal nicotine content (3.1; P <.0001) and were more likely to be abstinent (biochemically confirmed) at the end of the trial *(20)*. In an exploratory trial, Hatsukami et al. *(40)* found that VLNC cigarettes increased the use of alternative nicotine products, including ENDS, cigars, cigarillos and NRT and that the more non-combusted products were used, the greater the number of days of abstinence from smoking. The potential interactive effect of nicotine reduction and alternative products has been discussed by several researchers *(41, 42)*, was the topic of an announcement by the FDA *(15)* (discussed further below) and was the objective of several clinical trials that were under way in September 2017 (see Table 4.1).

4.2.4 Non-compliance in clinical trials

Since the 2015 WHO TobReg advisory note, it has become clear that participants in most studies of VLNC cigarettes continue to use non-study cigarettes, despite instructions to the contrary and the provision of free study cigarettes. For example, 75–80% of participants randomized to VLNC study cigarettes in the study by Donny et al. *(6)* were at least partially non-compliant *(43)*. Smokers who were young and heavily dependent and reported that VLNC cigarettes were dissatisfying were more likely to be non-compliant. Most smokers had clearly reduced exposure to nicotine, suggesting that they had replaced many of their daily cigarettes with VLNC cigarettes but still used some regular-nicotine cigarettes. The most commonly reported context for non-compliance is the first cigarette of the day, underscoring the fact that non-compliance is due to nicotine dependence.[5] To address this limitation, statistical approaches have been used in which data are weighted according to the probability of compliance. This

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5   EC Donny, unpublished observation.

indicated similar effects on the number of cigarettes per day and dependence as non-weighted data *(44)*.[6] Other studies of incentivized compliance resulted in somewhat less non-compliance (40–50%) (based on urinary biomarkers) and replicated the reported effects of VLNC cigarettes on smoking behaviour and dependence, with little evidence of negative consequences beyond mild, transient withdrawal symptoms.[1,2] Nevertheless, non-compliance may attenuate both positive (e.g. abstinence; see *45*) and negative effects (e.g. withdrawal symptoms) of nicotine reduction. The extent of non-compliance despite the provision of free VLNC cigarettes and incentives for compliance indicates that some smokers are likely to seek alternative sources of nicotine, whether on the black market, by hoarding, by product tampering or in non-cigarette products.

4.2.5   **Adverse health effects and vulnerable populations**

Analyses of the potential unintended consequences of nicotine reduction have shown few effects on the health of participants. VLNC cigarette use is associated with either no change in or less expired CO *(6, 18, 19, 21)*.[7] VLNC cigarette use may temporarily increase the occurrence of non-serious adverse events related to withdrawal symptoms *(19, 46)*[1] and attention deficit *(47, 48)*, although few deficits are observed after the first week or so.[8] Smoking of regular cigarettes suppresses body weight gain, raising the possibility that nicotine reduction could have unique adverse effects on obese smokers *(49, 50)*. It is important to note that unintended consequences could be masked by the use of regular commercial cigarettes, as described above. Thus, for example, an association between VLNC cigarettes use and weight gain in a clinical trial was obscured by participant non-compliance *(49)*.

Most clinical trials of VLNC cigarettes have involved relatively healthy daily smokers, generally with the exclusion of individuals such as those with serious mental illness or those who did not smoke every day. The positive and/or negative effects of nicotine reduction might vary among subpopulations, and several clinical trials were under way or were not published as of September 2017 on subpopulations who might be at risk (see Table 4.1). To date, the best data on the potential effects on different subpopulations of smokers are from secondary analyses of previous trials (commonly *6*) and from laboratory studies. Bandiera et al. *(51)* reported greater increases in the number of cigarettes smoked per day in a relatively small subgroup of 24 highly dependent smokers in the trial of gradual nicotine reduction by Benowitz *(52)*; however, analysis of highly dependent smokers in the trial of abrupt reduction by Donny et al. *(6)* indicated no effect of dependence on the

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6   DK Hatsukami, personal communication.

7   DK Hatsukami, personal communication.

8   Ribisl KM, Hatsukami D, Johnson, Huang J, Williams R, Donny E. Strategies to prevent illicit trade for very low nicotine cigarettes (unpublished information).

number of cigarettes smoked per day or other measures of possible compensatory smoking after randomization to VLNC cigarettes.[2] In addition, highly dependent smokers showed the greatest reduction in dependence as a consequence of nicotine reduction.[2] As noted above, studies often exclude non-daily smokers; however, a completed trial with non-daily smokers showed a significant (> 50%) decrease in the number of cigarettes per day as a consequence of nicotine reduction *(53)*.

Participants with a history of using alcohol *(54)* or cannabis *(55)* had reductions in smoking similar to that of smokers without such a history when randomized to VLNC cigarettes, with no increase in alcohol or cannabis use, although nicotine deprivation might increase the motivation of some individuals to drink *(45, 56)*. Tidey et al. *(57)* found that having depressive symptoms at baseline did not moderate the effects of nicotine reduction on the number of cigarettes smoked or measures of nicotine dependence and that randomization to VLNC cigarettes of smokers with severe depressive symptoms at baseline resulted in lower levels of depression than controls by the end of the trial. These findings are consistent with the results of a broader review of the potential impact of nicotine reduction on smokers with affective disorders *(58)*. An intensive laboratory study of smokers in three vulnerable populations (with affective disorders, with opioid dependence and socioeconomically disadvantaged women of childbearing age) found that reducing nicotine levels in a dose-dependent fashion decreased the reinforcing effects of cigarettes similarly in these populations *(59)*. Other trials under way as of September 2017 may provide further information about the potential unintended consequences of extended use of VLNC cigarettes in vulnerable populations; however, any negative effects must be weighed against the potential benefits, given the disproportionate harm of smoking to these populations *(60–64)*.

4.2.6 **Summary**

- Use of VLNC cigarettes in place of regular cigarettes reduces the number of cigarettes smoked per day and results in similar or less exposure to toxicants in clinical trials.

- Compensatory smoking is observed when the nicotine content of tobacco filler is modestly reduced but not with VLNC cigarettes.

- Less cigarette use is observed at a nicotine content of ≤ 2.4 mg/g. Less nicotine dependence and more quit attempts have been most reliably observed at levels of 0.4 mg/g, although this observation must be confirmed in long-term studies. Reducing nicotine to ≤ 0.4 mg/g may benefit the broadest population of current and potential smokers.

- Most studies were not designed to address cessation; however, most of the evidence suggests that use of VLNC cigarettes is likely to increase abstinence, even among smokers who are not intending to quit.

- Although the data are limited, reducing the nicotine content of cigarettes is likely to reduce smoking among adolescents.

- Use of VLNC cigarettes increases the use of alternative nicotine products such as ENDS, cigars, cigarillos and NRT in place of regular cigarettes. This conclusion warrants consideration of how nicotine reduction should be applied to nicotine products other than regular cigarettes.

- Significant non-compliance in clinical trials indicates that some smokers experiencing nicotine reduction are likely to seek alternative sources of nicotine.

- No significant adverse health effects have been identified with use of VLNC cigarettes in place of regular cigarettes.

- Reduced nicotine levels decrease the reinforcing effects of regular cigarettes similarly in a number of potentially vulnerable populations, including smokers with affective disorders, those who use alcohol and other substances and women of childbearing age of low socioeconomic status.

4.3 Population impact of nicotine reduction

Although clinical trials provide mounting evidence on the effects of VLNC cigarettes on individual behaviour and health when they are used instead of regular cigarettes, estimation of the public health impact of a nicotine reduction policy requires projection of these findings to the broader population *(9)* under market conditions, which may differ significantly from clinical trial settings, and will be affected by industry promotional activity *(9, 11)*. For example, observational studies indicate a smaller reduction in cigarette consumption with use of NRT than would be predicted by clinical trials, possibly because of underuse of the products in real situations *(65)*. Compliance with medication is probably better in a clinical setting than in real situations, whereas the opposite may be true in the case of clinical trials with VLNC cigarettes because of the commercial availability of regular nicotine tobacco products outside the trial *(44)*.

In the absence of population-based evidence on nicotine reduction, the WHO advisory note concluded that data from clinical studies and studies in experimental animals strongly suggest reduced risks at population level *(1, 2)*. Questions remained, however, about the potential role of illicit sales, potential product manipulation by either tobacco companies or smokers, the effects of a nicotine reduction policy on health beliefs, experimentation and potential use of other highly toxic combusted tobacco products (e.g. cigarillos or roll-your-own tobacco), either as substitutes for or in combination with VLNC cigarettes in the

absence of policy measures to discourage their use *(1)* and the impacts of tobacco industry marketing that promotes use of VLNC cigarettes as part of a programme to counter further constraints on their other (regular) products. In this section, we review the current evidence on the probable population response and health impact of nicotine reduction.

4.3.1 VLNC cigarettes as replacements for regular cigarettes

The popularity of regular cigarettes is supported by their efficacy as a behavioural reinforcer *(66)*. Nicotine is absorbed more rapidly from regular cigarettes than from other nicotine-containing products, even when systemic exposure to nicotine is similar (although the technology used in alternative products such as ENDS is developing rapidly) *(66, 67)*. Cigarettes also provide a rich network of behavioural and sensory stimuli associated with nicotine delivery that further contributes to the strong conditioned rewarding effects of smoking *(1, 68, 69)* and to the ability of VLNC cigarettes to reinforce behaviour *(1, 70, 71)*. Smokers' consumption of regular cigarettes is correspondingly less sensitive to increases in price *(72–75)*, indicating greater abuse liability than with other forms of tobacco or nicotine, including *snus (76, 77)*, little cigars *(75, 78)*, large cigars *(75)*, loose smoking tobacco *(75, 79)*, nicotine chewing-gum *(77, 80)* and e-cigarettes *(80–83)*.

Despite the high abuse liability of regular nicotine cigarettes, extensive research shows that increased cost can promote quitting by current users, deter initiation by potential users and reduce tobacco use by continuing users *(72, 74, 84, 85)*. Sensitivity to cigarette price is greatest among low-income smokers *(86, 87)* and is influenced by environmental factors that include tobacco control and public education campaigns *(88)*. Smokers of high-equity or "premium" cigarette brands are less likely to quit, indicating the importance of marketing and brand-consumer relations *(89)*. Subjective characteristics, such as flavour and acceptability, also play a role in product use *(90–92)*, and perceived negative characteristics (e.g. of *snus* or chewing-gum) outweigh potential reinforcing effects, resulting in limited demand for some tobacco substitutes regardless of price *(77)*.

VLNC cigarettes can substitute at least partly for regular cigarettes *(1, 93–95)*, and laboratory studies suggest that they are a more reinforcing alternative than nicotine chewing-gum *(96)*. Use of VLNC cigarettes in place of regular cigarettes decreases cigarette demand *(6, 94)*. As the price breakpoint (the point at which purchase is no longer sustained) is higher for regular cigarettes than for VLNC cigarettes, smokers would quit at relatively lower prices if VLNC cigarettes replaced regular ones, and a subset of smokers have no demand for VLNC cigarettes, regardless of price *(95)*. This is consistent with self-reports from as many as half of participants who use VLNC cigarettes that they would stop smoking within 1 year if these were the only cigarettes available for purchase *(94)*. Smokers of mentholated cigarettes reported a similar intention to quit when

confronted with a potential ban on menthol *(97)*. Such sensitivity to cost suggests that a nicotine reduction policy could increase the effectiveness of other tobacco control approaches such as raised price, smoke-free environments and media campaigns. Conversely, marketing and other social approaches could be used to support continued demand if the nicotine content of cigarettes were reduced.

As indicated in clinical trials (section 4.2.4), many smokers of VLNC cigarettes supplement their cigarette consumption outside of the study parameters. In a small pilot trial in New Zealand, a large price differential based on nicotine content prompted smokers to reduce the number of but not to eliminate their usual cigarettes and to replace them with VLNC cigarettes *(93)*. This suggests that, in a market in which both VLNC and regular cigarettes are available, most smokers who continue to smoke are unlikely to use VLNC cigarettes exclusively, even with a significant differential in price *(94, 95)*.

Few data are available on young and tobacco-naive users, although clinical trials with adolescents were under way as of September 2017. Meanwhile, the implications of studies of price and other abuse liability is that VLNC cigarettes are less likely than regular nicotine cigarettes to encourage the transition from experimentation to regular use. A possible, although not yet demonstrated outcome of nicotine reduction is that it may prevent some (possibly significant) proportion of children and adolescents who experiment with the most toxic (i.e. combusted) forms of nicotine from becoming long-term users of those products. In contrast, promotion may directly or indirectly encourage young people to take up VLNC products by convincing them that they are acceptable from a health perspective.

4.3.2   Substitution of VLNC cigarettes with alternative tobacco products

Tobacco products differ widely in terms of price, availability, marketing, social acceptability and perceived risk, as well as nicotine delivery and sensory and non-pharmacological characteristics. The balance of these environmental and product factors can result in significant population changes in tobacco product use. For example, studies in many countries have found a shift from manufactured to roll-your-own cigarettes, for reasons that include price and lower perceived risk *(98– 100)*, even though roll-your-own products are inferior on subjective measures *(79)*. Concurrent use of several tobacco products is increasingly common *(101, 102)*, including the use of tobacco with e-cigarettes and other ENDS such as HTPs *(103, 104)*.

Although the prices of other combusted tobacco products are more sensitive than those of cigarettes, most are strong substitutes for regular cigarettes *(75)*. Some cigarette smokers maintain their tobacco consumption when cigarette prices rise by changing from regular cigarettes to little cigars *(78, 105)*, and interest is increasing in other substitutes (ENDS, *snus*, large cigars,

roll-your-own) *(106)*. Large proportions of current smokers do not accurately differentiate regular cigarettes, little cigars, cigarillos, roll-your-own tobacco and cigars when presented with images of these products and their packaging, indicating the possibility of substitutions among relatively similar products *(107)*. The perceptions may be exaggerated by manufacturing and/or marketing by the tobacco industry, which may mimic or emphasize similarities in appearance or function among combusted or alternative products (e.g. ENDS and HTPs).

The presence of product substitutes can determine interactions among multiple products. For example, in a behavioural study in 2016, *snus* became a significant substitute for cigarettes once cigarillos had been removed as an option, and participants were less likely to break the study protocol by purchasing other products when cigarillos were available *(108)*. Findings on the substitution patterns of users of smokeless tobacco (moist snuff, *snus*, chewing tobacco) and cigarettes are mixed *(75, 92, 109–112)*: smokers, particularly in the USA, showed poor acceptance of smokeless products *(90, 92, 113–115)*. In Sweden, although lower taxes on *snus* contributed to the switch of many male cigarette smokers to *snus*, women did not switch to the same extent, illustrating the significant role of social and demographic factors *(114, 116)*.

ENDS are potential substitutes for regular cigarettes *(75, 108, 117–120)*, and, although ENDS are a heterogeneous class of products (see section 3), at least some are more effective in decreasing regular cigarette use than NRT *(119, 121–123)*, partly because of similar subjective and sensorimotor characteristics *(69)*. It has been reported ENDS are better accepted, provide more satisfaction and can better reduce craving, negative affect and stress in smokers than NRT *(122, 124)*, although they are rated as less satisfying than regular cigarettes *(42)*. ENDS remain highly sensitive to price *(81, 118)* and were found to be more expensive than combusted products in 45 countries *(125)*. Users of both cigarettes and e-cigarettes more successfully maintain reduced cigarette consumption in the short term than those who smoke only cigarettes *(126)*. ENDS are generally perceived to pose a lower risk than regular cigarettes, although it is a misperception that ENDS pose an equal or greater health risk than cigarettes *(127)*.

Use of ENDS by adults is concentrated primarily among current and former smokers *(104, 128–137)*. Studies on young people have had mixed results, some indicating a high level of interest and/or experimentation by never smokers *(133, 138, 139)* and others reporting concurrent use of ENDS and other tobacco products by current smokers *(130–132, 133, 140–145)*. Some countries have reported dramatic increases in ENDS use among young people; current use exceeded 20% among high-school-aged young people in the USA in 2018 *(146)*. The association between ENDS use and cigarette smoking has led to concern that ENDS might act as a gateway to the use of other tobacco products, specifically regular cigarettes, particularly among adolescents and young adults *(137, 147–*

154), although confounding factors make it difficult to interpret the study results *(155–159)*. Further substantial concern has, however, been expressed recently in the USA about the "epidemic of youth e-cigarette use" *(160)* and the possible involvement of e-cigarettes in encouraging young people to smoke conventional products *(161)*. ENDS are less likely to displace cigarette smoking among people who want or need to quit as long as regular nicotine cigarettes are widely available. A reduction of the nicotine level in combusted and other tobacco products might, however, increase substitution with ENDS *(40)*.

4.3.3 The black market

If the only combusted tobacco products legally available contained very low nicotine levels, there would potentially be illicit trade in cigarettes with regular nicotine levels. As no jurisdiction has yet restricted cigarettes with regular nicotine, the information about illicit trade pertains to products that have been banned or on which taxes have been raised, and consumers have sought cheaper, lower-tax products.

Illicit trade can be conducted at retail locations, by street sellers and via the internet. Banning of some tobacco products in some jurisdictions (e.g. menthol and other flavoured cigarettes in Canada and clove and other flavoured cigarettes in the USA) has not created a major black market, and most illicit trade is conducted to avoid cigarette excise taxes. The National Research Council in the USA estimated that illicit sales accounted for 8.5–21% of the total cigarette market globally and for 11.6% of cigarette consumption in 84 countries in 2007 *(162)*. The volume of illicit trade depends on factors that include the magnitude of the price (or tax) differential, the ease of accessing products and the efficiency of tax administration.[9] In jurisdictions with strong tax administration, illicit trade is modest. The practices of these jurisdictions include strong "track-and-trace" systems to follow products from the manufacturer to the distributor and the retailer, strong customs control and rigorous tax stamping. Although thermal onion-skin stamps are used in many countries, encrypted tax stamps are used in California and Massachusetts, USA, as they are more durable and can include encoded information about the supply chain *(163)*. Many countries do not have strong track-and-trace systems to minimize illicit trade, and these should be instituted to minimize illicit trade in cigarettes containing normal nicotine levels.

Another means of reducing illicit trade is to implement strong policies, including a penalty structure, the likelihood of detection and provisions for adequate staff for surveillance and enforcement. To reduce the likelihood of a black market, policies could address the manufacture, sales, purchase, use and/or possession of contraband products with a normal nicotine level.

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9   Ribisl KM, Hatsukami D, Johnson, Huang J, Williams R, Donny E. Strategies to prevent illicit trade for very low nicotine cigarettes (unpublished information).

Manufacturers should be prohibited from making regular nicotine cigarettes for domestic distribution, and importers should be prohibited from importing such products. As for many other illicit products, the strongest penalties and the most enforcement should be imposed on the manufacturers and sellers of the products, such as retailers and internet vendors, rather than the smokers who possess them.

In the same way that taxation agencies check compliance by visiting manufacturing plants and retailers, an inspections programme should exist that includes testing of purchases to ensure that products with regular nicotine are not circulated through the retail supply chain. Policies to restrict sales from internet vendors are also required. In the USA, high rates of cigarette sales to minors and violation of tax policies led to new restrictions on payment options (e.g. Visa, Mastercard) and shipping options (UPS, FedEx), which drastically reduced the number of internet cigarette vendors and traffic to these websites *(164)*. Similar policies should be enacted to restrict sales into countries that have low-nicotine products. Australia, for instance, has some of the highest tobacco excise taxes in the world, yet has successfully blocked nearly all websites selling regular cigarettes. In addition, enforcement agencies should make routine test purchases to ensure that internet vendors do not sell regular nicotine products in countries in which they are restricted.

Illicit trade can also be reduced by the widespread availability of substitute products that appeal to adult consumers. As discussed earlier, these products may include ENDS or *snus* containing nicotine. If these products are banned or restricted, as they are in some countries, there is likely to be greater demand for cigarettes on the black market.

4.3.4 Manipulation of VLNC cigarettes

Research on VLNC cigarettes has been conducted with experimental cigarettes that have chemical and physical properties sufficiently similar to those of commercial cigarettes that they are considered acceptable for use in studies of behaviour *(17)*. Experimental products do not necessarily mimic the complex product designs and are not subject to the marketing tactics used to increase the appeal of commercial cigarettes and other tobacco products *(89, 165)*. In an open market, branding may alter expectations or perceptions of VLNC cigarettes, with subsequent behavioural effects *(89)*. Companies are likely to use heavy promotion both to use VLNC cigarettes to deflect attention from evidence-based measures to reduce smoking and as a means of maintaining use of current products. The physical or chemical parameters of cigarette construction could also be manipulated to alter the formulation of VLNC cigarettes *(1)*.

A number of studies have addressed the behavioural effects of non-nicotine constituents present in tobacco to determine whether they add to or interact with the abuse liability of nicotine. Some studies of self-administration

by experimental animals indicated greater reinforcing effects than nicotine alone *(166–169)*, while others found no additive or synergistic effects *(170–172)*. The role of specific tobacco compounds and their interaction with nicotine remains poorly understood *(1, 7, 173)*. Nornicotine, norharman and acetaldehyde have been shown to support self-administration independently of tobacco *(1, 174–176)* but only at doses substantially higher than those in cigarette smoke *(7)*. Monoamine inhibitors increase self-administration of low doses of nicotine by rats, although reinforcement is minimal if nicotine is further reduced *(177)*. Menthol appears to strengthen the reinforcing mechanism of nicotine and thus promote nicotine consumption and tobacco smoking, even at reduced levels of nicotine *(178, 179)*, although this has not been demonstrated at the levels produced by VLNC cigarettes. Other research has been conducted on the development of nicotine analogues and their potential as substitutes *(180)*. These and other non-nicotine constituents may play a greater role as determinants of behavioural reinforcement in the context of the low doses of nicotine available in VLNC cigarettes *(171, 181)*.

Another possible response to a nicotine reduction policy is manipulation of products by cigarette smokers *(2)*. In Malaysia, banning of the sale of nicotine-containing e-liquid in ENDS shops resulted in not only a black-market supply of nicotine-containing e-liquid but also a significant increase in home-made nicotine-containing e-liquid *(182)*. It is not known whether adding nicotine to VLNC cigarettes or other direct consumer manipulation of products would yield an appealing, acceptable alternative. Further types of commercial product manipulation that have not yet been studied include changes to the protonation of nicotine or the size distribution of aerosol particles, which determine the deposition and absorption of nicotine and other constituents *(1)*.

4.3.5   Beliefs and attitudes regarding VLNC cigarettes and nicotine reduction

Many cigarette smokers have inaccurate beliefs about nicotine. Most participants in many studies misattributed nicotine as the primary cause of cancers and smoking-related morbidity *(1, 12, 183)*. Beliefs about use of VLNC cigarettes reflect these misconceptions, as smokers perceive them as significantly less harmful than regular cigarettes *(184, 185)*, potentially reinforcing interest in and use of these cigarettes to a greater extent than would be expected on the basis of subjective ratings *(185, 186)*. Although nicotine is a precursor of carcinogenic TSNAs, and reduction of nicotine might decrease the levels of TSNAs in cigarette smoke, there is no evidence that VLNC cigarettes are less toxic. Health communication strategies are necessary to educate smokers about their relative toxicity and harm. Inaccurate beliefs about risk are not limited to VLNC cigarettes, as substantial proportions of smokers are unable to accurately assess the risks associated with a range of tobacco product categories *(187, 188)*. A policy targeting nicotine might add further confusion about its relative harm, which could result in more negative

perceptions of NRT, ENDS or other alternative nicotine delivery systems than of more toxic tobacco products. Clear communication that the purpose of a nicotine reduction policy is to reduce dependence on the most toxic (combusted) tobacco products and education about the role of nicotine in addiction are therefore critical. The announcement by the FDA *(15),* which identified a continuum of risk across tobacco products, is an example of a health message that is sensitive to the role of nicotine in use versus harm.

Expectations about nicotine content influence smokers' subjective response to regular and VLNC cigarettes *(189, 190)*, including responses of the insula to craving and learning, as measured by functional magnetic resonance imaging *(191, 192)*. Sensory responses to VLNC cigarettes, specifically taste and strength ratings, moderate associations between beliefs and daily cigarette consumption, with higher subjective ratings (on scales of strength, taste, harshness and mildness), and beliefs about reduced harm support increased consumption *(185)*. Expectations may also influence brand choice and smoking behaviour; for example, positive expectations of ENDS users are associated with a greater likelihood of quitting smoking but less likelihood of intention to quit ENDS use *(193)*.

The WHO advisory note in 2015 reported strong public support for nicotine reduction *(1, 194)*, and support has been demonstrated in population-based surveys in New Zealand and the USA *(186, 195)*, which may reflect beliefs about the disease risk associated with nicotine *(186)*. Both surveys found about 80% support, mainly among smokers but also among nonsmokers and various ethnic minority populations *(186, 195)*. Consistently less support is shown for a ban on menthol than for regulation of nicotine *(179, 186)*. Trial surveys differ from population surveys by providing an experiential basis for expectations. In an interview study in which New Zealand smokers were first given VLNC cigarettes to smoke, smokers were less interested in mandated nicotine reduction but supported their sale at a much cheaper price concurrently with regular cigarettes *(196)*. In a trial in the USA, however, smokers who used VLNC cigarettes for 6 weeks and who judged that their experimental cigarettes contained low or very low nicotine were approximately twice as likely to support as to oppose a regulated reduction in nicotine content *(197)*.

4.3.6 Summary

- VLNC cigarettes are a partial but incomplete substitute for regular cigarettes. A subset of current smokers would probably give up smoking rather than switch to VLNC cigarettes if current high-nicotine cigarettes were no longer legally marketed. Most smokers who continue to smoke are unlikely to use VLNC cigarettes exclusively.

- Countries should put mechanisms in place to ensure strong tax administration and consider other enforcement measures to limit illicit trade, restrict sales of regular nicotine content cigarettes into countries requiring low-nicotine content cigarettes and restrict websites selling regular nicotine content cigarettes.

- Research was under way as of September 2017 on adolescents' response to low-nicotine products. It is anticipated that nicotine reduction will prevent a significant portion of children and adolescents who experiment with combusted tobacco products from becoming long-term users of those products.

- Substitution among combusted tobacco products is common. A nicotine reduction policy might have to be applied to all combusted tobacco products rather than only to regular cigarettes to ensure a shift in population use from the most toxic and addictive products.

- Reducing the nicotine content of combusted tobacco products might increase substitution by ENDS and possibly HTPs, which are marketed and promoted by manufacturers as reduced risk products and safer alternatives to regular cigarettes. Independent research to substantiate industry research and claims, appropriate health communication, effective surveillance and regulatory oversight of these products are necessary.

- Strong policies and enforcement systems can help reduce the supply of illicit cigarettes. Policies to reduce demand (e.g. treatment services, substitute non-combusted products) may also reduce illicit trade, although the evidence is limited.

- Smokers perceive VLNC cigarettes as less harmful than regular cigarettes, which might support greater interest in and use of VLNC cigarettes than would be expected from subjective ratings. Health communication can address misperceptions of risk.

- Physical or chemical parameters of cigarette construction could be manipulated by manufacturers or smokers to alter the formulation and reinforcing effects of VLNC cigarettes, and the effects might not readily be anticipated.

- Support for nicotine reduction is reported from both population-based and trial surveys.

- Overall, the research suggests that a nicotine reduction policy, as part of a comprehensive approach and implemented with appropriate safeguards, could be an effective method for reducing the population prevalence of smoking.

4.4   Regulatory approaches to nicotine reduction

Much of the concern about a nicotine reduction policy is based not on evidence of the individual effects of use of VLNC cigarettes but on the difficulties in successful implementation of such a policy *(14)* and potential unanticipated consequences of implementation, such as continued availability of toxic forms of nicotine on the black market and/or failure to adequately support less toxic forms of nicotine as alternatives to cigarettes *(8, 10, 11, 13)*. These real challenges are difficult to model in advance of regulatory action *(11, 14)*. One proposed solution is to monitor the impact of implementation in one or more communities that adopted reduced nicotine early on *(11, 198)*. New Zealand has been discussed as a potentially ideal site for nicotine reduction, partly because of strong public support *(194)*, continuing discussion of a policy of nicotine reduction *(95, 198, 199)* and a geographical situation that limits the likelihood of illicit sales *(198)*. In July 2017, the FDA announced that nicotine reduction was part of a comprehensive multi-year plan to reduce the health effects of tobacco use *(15)*. In this section, we consider the feasibility of nicotine reduction, various regulatory approaches, potential challenges to implementation and broader philosophical arguments against nicotine reduction.

4.4.1   Feasibility of nicotine reduction and potential challenges

Commercial production of VLNC cigarettes that meet the product standard of om0.4 mg of nicotine per gram of tobacco without measurably increasing the toxicity of the product has been clearly demonstrated *(1, 17, 198)*. The probable substitution for VLNC cigarettes of other combusted tobacco products (such as little cigars and roll-your-own tobacco) that approximate regular cigarettes in appearance and nicotine content indicates that reduced nicotine product standards should be extended to all combusted tobacco products. As noted in section 4.3.4, changes to VLNC products by manufacturers in response to a nicotine reduction policy could result in products with higher levels of pharmacologically relevant compounds that might impact behaviour. Products must be monitored to ensure successful policy implementation *(1, 93)* and to verify that non-nicotine compounds such as monoamine oxidase inhibitors and nicotine analogues do not change the reinforcement threshold of VLNC cigarettes *(177)*.

As noted in both the WHO advisory note *(1)* and in subsequent discussions *(8, 10)*, a nicotine reduction policy is unlikely to be successful without support from smokers of regular cigarettes, particularly those who are dependent on nicotine. While strong public acceptance has been found in population surveys (section 4.3.5), at least some smokers will consider the subjective trade-offs and policy unacceptable *(200)*. Informing the public that nicotine reduction is part of a long-term strategy to phase out addiction to the most highly toxic tobacco products and to support the availability of less toxic alternatives, including easy

access to smoking cessation medication and behavioural treatment, will be necessary to maintain support among smokers and to provide time for smokers to switch from regular cigarettes before the policy is enacted *(1, 198)*.

Political and legal challenges to nicotine reduction will be substantial, given that nicotine is a central feature of all successful tobacco products, as will tobacco industry efforts to undermine and misuse such policy *(9, 14)*. One strategy that should be considered would be to prohibit the manufacture and sale of cigarettes with regular nicotine without criminalizing possession or use. This would shift the burden of compliance from individuals, and continued consumer demand could be moderated by education, treatment services and the availability of legal alternatives. Although countries that are considering nicotine reduction will recognize the potential threat to tax revenue, significant savings in health care would be expected. Ultimately, the net economic impact will be specific to each country and should be evaluated, as it will be an important determinant of the viability of a policy, even if the public health impact is clear.

4.4.2 Prerequisites for successful implementation of a nicotine reduction policy

The WHO advisory note *(1)* listed a number of preconditions that were considered necessary for a successful nicotine reduction policy:

- comprehensive regulation of all nicotine- and tobacco-containing products;
- other comprehensive tobacco control (increased taxes, smoking bans, graphic warning labels, plain packaging);
- continuing communication of the health risks of smoking to both the general population and health professionals;
- availability of affordable treatment and alternative forms of nicotine to reduce withdrawal symptoms in dependent smokers; and
- capacity for market surveillance and product testing.

The inaccurate belief that nicotine is the main harmful constituent of cigarettes could decrease the sense of urgency of current smokers to quit *(12, 183)*. Although this unintended consequence is unlikely to outweigh the potential benefits of nicotine reduction, it should be addressed by clear communication *(185, 198)*. Further research and continuous evaluation will be required to ensure appropriate messages (e.g. accounting for differences among populations) without denying the harmful effects of nicotine *(201)*. It must also be recognized that effective communication may not be possible in an environment where governments underfund evidence-based communication about the harms of smoking and where the bulk of communication still comes from tobacco interests and their

allies. Ultimately, subjective effects rather than the perceived harmfulness of cigarettes may best predict long-term smoking behaviour *(184)*.

It has been speculated that regulatory environments in which principles of harm reduction are included with other elements of tobacco control would provide more favourable conditions for a nicotine reduction strategy *(1, 10, 11, 198)*. This might entail readily available, consumer-acceptable non-combusted forms of nicotine that could be used to manage withdrawal symptoms and provide smokers with a viable alternative to regular cigarettes, and it would demonstrate that reduction of the nicotine in combusted tobacco is not prohibition of nicotine *(41)*. This will require further consideration in a real-world context in which there is already concern about the use and promotion of some alternative nicotine products.

While a nicotine reduction approach could substantially improve public health, it also offers considerable scope for tobacco companies to mislead consumers, both directly and indirectly. For example, they might use lower-nicotine products to promote similarly branded products, to imply the safety of both new and established products and to present themselves as policy advisers. Given the industry's long history of abusing health policy initiatives for promotional purposes and public relations, any enacted regulations should preclude inappropriate or misleading promotion by tobacco companies, directly or indirectly, with strong monitoring and enforcement capacity. Further, regulations should ensure that information and advice on the health aspects of VLNC cigarettes or other tobacco products come from health authorities, not tobacco companies or their agents. It is recommended that an international approach be found for regulation of communication strategies and promotion of VLNC, with commitments from all relevant Parties.

The WHO advisory note *(1)* raised the issue of the potential health risks introduced by alternative forms of tobacco or nicotine, which have also been discussed critically elsewhere (see section 4.3.2). Many of the concerns about the promotion of ENDS and other non-combusted tobacco product alternatives are related to the possibility that they would support cigarette initiation or use; however, these concerns should become less relevant in the context of cigarettes with low addiction potential *(41)*. Likewise, concurrent use of two or more products, which could potentially support continued use of VLNC cigarettes, would be less of a problem, because these cigarettes would be less desirable and alternative products would be more satisfying in comparison *(41)*. Evaluation and monitoring of the use and health effects of other products, including less toxic alternatives, and patterns of concurrent use of several products would nonetheless be a critical component of a nicotine reduction policy *(1, 2, 9)*. Constraints on marketing and on the availability of ENDS and other alternative nicotine products would be appropriate to limit experimentation by nonsmokers, especially young people *(10, 41)*. Health agencies should clearly and accurately communicate to

the public the potential health risks of alternative products, including ENDS and HTPs, and should be careful not to encourage addiction to alternative products.

A nicotine reduction strategy should be part of a comprehensive tobacco control strategy that complements other evidence-based policies, such as increased taxes, sustained and adequately funded public education campaigns, restrictions on smoking in public, further product regulation as required, bans on tobacco industry marketing and public relations, the availability of low-cost NRT and behavioural cessation treatment (e.g. telephone counselling lines) and special programmes for disadvantaged groups *(95)*. The regulatory considerations reported by the FDA in its announcement of nicotine reduction *(15)* were continuing regulation of combusted cigarettes and the characteristics related to toxicity and the attractiveness of combusted products; the use, availability and health effects of ENDS and other alternatives to cigarettes, such as medicinal nicotine products; and regulation of health claims and other forms of communication of risk.

4.4.3 Strategies for implementation of a nicotine reduction policy

The WHO advisory note *(1)* addressed the question of a gradual versus an immediate reduction in nicotine content, noting that an immediate reduction is preferable insofar as it is less likely to introduce such unintended consequences as a short-term increase in compensatory smoking by dependent smokers *(93)*. A 10-site, double-blind study showed that immediate, not gradual, reductions in nicotine content decreased the number of cigarettes smoked per day and biomarkers of exposure in a 20-week intervention, and measures of craving and dependence were significantly lower at week 20 in the group that experienced an immediate reduction *(20)*. Although these data suggest potential benefits of an immediate reduction, it is important to note that, consistent with other reports *(202)*, more participants drop out of studies when the reduction is immediate, suggesting that immediate reductions may make it more difficult for smokers to adjust, particularly in the absence of alternative sources of nicotine or smoking cessation treatment. As smokers will continue to have access to regular cigarettes until the supplies have been exhausted, more gradual introduction of VLNC cigarettes is the probable practical outcome, even in an immediate reduction policy *(40)*. Concern about the potential discomfort of dependent smokers of regular cigarettes faced with immediate reduction emphasizes the need for readily available alternative forms of nicotine and/or medication *(40, 198)*.

Population responses to nicotine reduction will partly reflect contextual differences. For example, reducing nicotine in Sweden would be likely to result in the adoption of *snus*, whereas *snus* is banned in most other countries in the European Union *(11)*. Thus, strategies for reducing nicotine must be sensitive to differences in context, including other regulation and population preferences.

Some countries have restricted or banned the sale and importation of ENDS (e.g. Brazil, Singapore, Thailand, Uruguay and Venezuela). Such a ban would not necessarily preclude a nicotine reduction policy for combusted tobacco products; however, it might raise additional challenges. Care must be taken to ensure the availability of alternative sources of nicotine, such as medicinal nicotine, access to tobacco dependence treatment and the capacity or infrastructure necessary to address a potentially stronger illicit market.

4.4.4   Philosophical objections to nicotine reduction

Some discussions of nicotine reduction represent the policy as a de-facto prohibition of cigarettes *(2, 10, 13, 14)*. In this viewpoint, nicotine reduction differs significantly from other tobacco control measures such as plain packaging, graphic warnings or flavour bans, in that it fundamentally alters the primary function of the product, which is to deliver nicotine *(14)*. Nevertheless, smokers use VLNC cigarettes even when no other products are available, although they may be less likely to persist in their use over time *(196)*. Further, unlike in a prohibition strategy, smokers would still have access to nicotine but in potentially less harmful delivery systems. The availability of other forms of nicotine would also minimize the efforts of dependent smokers to seek nicotine through illegal means *(198)*.

A second objection to nicotine reduction is that it is fundamentally a "top–down" or paternalistic means for reducing the health risks of tobacco *(9, 10)*. Limiting the sale of cigarettes to those with a VLNC intrudes on personal choice. Some argue, however, that support for ENDS and other alternative sources of nicotine could attract smokers away from cigarettes without imposing invasive regulations *(11, 13)*. The advantages of avoiding a nicotine reduction policy include the elimination of legal or political challenges to policy, a potential black market and opposition from smokers *(9)*. Questions remain about the long-term health consequences of alternative products, including ENDS and HTPs, the use, function and emissions of which continue to evolve rapidly. Ultimately, some combination of policies may be necessary to reduce the desirability of combusted products relative to alternatives; this might include increased communication of relative risk, increased availability of lower-risk products, an increased price differential between low- and high-risk products or nicotine product standards that reduce dependence on the most toxic products.

4.4.5   Summary

- The feasibility of producing commercial VLNC cigarettes with sub-jective characteristics similar to those of regular cigarettes but with reduced potential for dependence has now been reasonably estab-lished. The toxicity of these cigarettes remains high, however, due to combustion and inhalation of tobacco.

- The physical characteristics and construction of VLNC cigarettes used in clinical trials are relatively uniform; in a commercial market of VLNC cigarettes, product characteristics other than nicotine might diverge significantly. Some differences, such as the levels of non-nicotine compounds with pharmacological effects, might have to be carefully monitored, or restrictions might have to be imposed to limit product changes.

- Public education campaigns should communicate nicotine reduction as part of a long-term strategy, including a comprehensive tobacco control programme, to phase out addiction to the most highly toxic tobacco products. If VLNC cigarettes are sold concurrently with regular cigarettes, without substantive policy interventions or education campaigns, their use is likely to remain low.

- The preconditions for a successful nicotine reduction strategy identified in the WHO advisory note remain valid, namely: comprehensive regulation of all tobacco- and nicotine-containing products, capacity for surveillance and product testing, commitment to continuing public education and the availability of and access to tried and tested forms of treatment, as approved by national authorities. Inappropriate or misleading promotion by tobacco product manufacturers and manufacturers of alternative products should be prohibited.

- An immediate reduction would have clear advantages over a gradual reduction in nicotine content but might require a comprehensive approach in order to reduce unintended consequences.

- The intent of reducing the nicotine content of regular cigarettes is not to prohibit their sale and use but to reduce their addictiveness, so that people can choose whether or not to continue using an extremely toxic product.

4.5 Research questions

- Research to date has focused exclusively on reduced-nicotine cigarettes. Future studies should address the potential effects of reduced nicotine in other combusted tobacco products (e.g. roll-your-own tobacco, little cigars, cigarillos, waterpipe tobacco) and the benefits and drawbacks of reducing nicotine in non-combusted tobacco products.

- The positive and negative impacts of VLNC cigarettes on vulnerable populations, including women of reproductive age, should continue to be assessed. Research on the potential uptake and use of VLNC cigarettes by adolescents and tobacco-naive users would be particu-

larly informative; this may have to be assessed primarily after the implementation of product standards through surveillance.

- Further research should be conducted on concomitant use of VLNC cigarettes, ENDS and other alternative tobacco and nicotine products, by both dependent and non-dependent smokers and tobacco-naive users.

- Studies conducted to date do not show that use of VLNC cigarettes increases the use of alcohol or dependence on other substances (such as cannabis), but continued assessment is warranted.

- VLNC products with altered physical or chemical characteristics designed to replace or enhance the pharmacological effects of nicotine have not been developed or tested to date.

- Research on the impact of specific public communication strategies would help to maximize the benefit of a policy, minimize misunderstanding of the impact of the change in cigarette addictiveness on its toxicity and inform those who are dependent on nicotine about appropriate alternatives.

- Research on the nature of tobacco industry abuse of nicotine reduction strategies for continued promotion of current products and for undermining evidence-based tobacco control policies should be conducted. Research is also needed on means of regulating such activity nationally and internationally.

- Few data are available for estimating the extent of illicit sales or of product tampering and related factors that could mitigate the effects of nicotine reduction.

4.6 Policy recommendations

- A nicotine reduction policy has considerable promise as a means to accelerate a reduction in use of the most toxic tobacco products and encourage smoking cessation. Such a policy should be part of a comprehensive approach and might be most effective when coordinated with other relevant policies designed to support cessation and reduce smoking.

- Substitution among combusted tobacco products is common, and the availability of high-nicotine combusted products that are similar in use and appearance to regular cigarettes is likely to mitigate the potential public health benefit of nicotine reduction. Therefore, a nicotine reduction policy might have to be applied to all combusted

products rather than only to regular cigarettes to support a shift in population use from the most toxic and addictive products.

- In view of the reduced exposure to nicotine and decreased tobacco consumption demonstrated in many clinical and behavioural studies, there is insufficient evidence that a significant population of non-smoking adolescents or adults who would not otherwise have smoked will adopt and maintain use of VLNC cigarettes. To minimize any unintended consequences in these populations, the health risks of both VLNC cigarettes and alternative forms of nicotine must be clearly communicated, including the fact that VLNC cigarettes are not safer than regular cigarettes.

- As indicated in the 2015 WHO advisory note, a nicotine reduction policy should not be considered to be supplanting a comprehensive tobacco control policy, which should include increased taxes, sustained, adequately funded public education, smoking bans, graphic warning labels, plain packaging and other forms of product regulation. A coordinated policy for all tobacco- and nicotine-containing products is essential, as is access to treatment for tobacco dependence.

- Capacity to conduct continuous population surveillance, monitoring and testing of products and enforcement of product standards is critical to support a nicotine reduction policy. Monitoring should also include industry promotional and other activities as well as product availability and sales.

4.7   References

1. WHO Study Group on Tobacco Product Regulation. Advisory note: global nicotine reduction strategy (WHO Technical Report Series No. 989). Geneva: World Health Organization; 2015 (http://apps.who.int/iris/bitstream/10665/189651/1/9789241509329_eng.pdf, accessed 15 May 2019).

2. Hatsukami DK, Zaatari G, Donny E. The case for the WHO advisory note, global nicotine reduction strategy. Tob Control. 2017;26(e1):e29–30.

3. Eriksen M, Mackay J, Schluger N, Gomeshtapeh FI, Drope J, editors. The tobacco atlas, 5th edition, Atlanta (GA), American Cancer Society; 2015.

4. WHO Framework Convention on Tobacco Control. Geneva: World Health Organization; 2003 (http://www.who.int/fctc/en/, accessed 15 May 2019).

5. Report on the scientific basis of tobacco product regulation: fourth report of a WHO study group (WHO Technical Report Series No. 967). Geneva: World Health Organization; 2012 (http://www.who.int/tobacco/publications/prod_regulation/trs_967/en/index.html, accessed 15 May 2019).

6. Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG et al. Randomized trial of reduced-nicotine standards for cigarettes. N Engl J Med. 2015;373(14):1340–9.

7. Smith TT, Rupprecht LE, Denlinger-Apte RL, Weeks JJ, Panas RS, Donny EC et al. Animal research

on nicotine reduction: current evidence and research gaps. Nicotine Tob Res. 2017;19(9):1005–15.

8. Kozlowski LT. Commentary on Nardone et al. (2016): satisfaction, dissatisfaction and complicating the nicotine-reduction strategy with more nicotine. Addiction. 2016;111(12):2217–8.

9. Kozlowski LT. Cigarette prohibition and the need for more prior testing of the WHO TobReg's global nicotine-reduction strategy. Tob Control. 2017;26(e1):e31–4.

10. Borland R. Paying more attention to the "elephant in the room". Tob Control. 2017;26(e1):e35–6.

11. Kozlowski LT. Let actual markets help assess the worth of optional very-low-nicotine cigarettes before deciding on mandatory regulations. Addiction. 2017;112(1):3–5.

12. Pacek LR, Rass O, Johnson MW. Knowledge about nicotine among HIV-positive smokers: implications for tobacco regulatory science policy. Addict Behav. 2017;65:81–6.

13. Kozlowski LT, Abrams DB. Obsolete tobacco control themes can be hazardous to public health: the need for updating views on absolute product risks and harm reduction. BMC Public Health. 2016;16:432.

14. Kozlowski LT. Prospects for a nicotine-reduction strategy in the cigarette endgame: alternative tobacco harm reduction scenarios. Int J Drug Policy. 2015;26(6):543–7.

15. Gottlieb S, Zeller M. A nicotine-focused framework for public health. N Engl J Med. 2017;377(12):1111–4.

16. Donny EC, Walker N, Hatsukami DK, Bullen C. Reducing the nicotine content of combusted tobacco products sold in New Zealand. Tob Control. 2017;26:e37–42.

17. Richter P, Steven PR, Bravo R, Lisko JG, Damian M, Gonzalez-Jimenez N et al. Characterization of Spectrum variable nicotine research cigarettes. Tob Regul Sci. 2016;2(2):94–105.

18. Donny EC, Houtsmuller E, Stitzer ML. Smoking in the absence of nicotine: behavioral, subjective and physiological effects over 11 days. Addiction. 2007;102(2):324–34.

19. Hatsukami DK, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen JA et al. Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. Addiction. 2010;105(2):343–55.

20. Hatsukami DK, Luo X, Jensen JA, al'Absi M, Allen SS, Carmella SG et al. Effect of immediate vs gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: a randomized clinical trial. JAMA. 2018;320(9):880–91.

21. Hatsukami DK, Hertsgaard LA, Vogel RI, Jensen JA, Murphy SE, Hecht SS et al. Reduced nicotine content cigarettes and nicotine patch. Cancer Epidemiol Biomarkers Prev. 2013;22(6):1015–24.

22. Hammond D, O'Connor RJ. Reduced nicotine cigarettes: smoking behavior and biomarkers of exposure among smokers not intending to quit. Cancer Epidemiol Biomarkers Prev. 2014;23(10):2032–40.

23. Kozlowski LT, O'Connor RJ. Cigarette filter ventilation is a defective design because of misleading taste, bigger puffs, and blocked vents. Tob Control. 2002;11(Suppl 1):I40–50.

24. Ding YS, Ward J, Hammond D, Watson CH. Mouth-level intake of benzo[a]pyrene from reduced nicotine cigarettes. Int J Environ Res Public Health. 2014;11(11):11898–914.

25. Benowitz NL, Jacob P 3rd, Herrera B. Nicotine intake and dose response when smoking reduced-nicotine content cigarettes. Clin Pharmacol Ther. 2006;80(6):703–14.

26. Benowitz NL, Nardone N, Dains KM, Hall SM, Stewart S, Dempsey D et al. Effect of reducing the nicotine content of cigarettes on cigarette smoking behavior and tobacco smoke toxicant exposure: 2-year follow up. Addiction. 2015;110(10):1667–75.

27. Mercincavage M, Souprountchouk V, Tang KZ, Dumont RL, Wileyto EP, Carmella SG et al. A randomized controlled trial of progressively reduced nicotine content cigarettes on smoking behaviors, biomarkers of exposure, and subjective ratings. Cancer Epidemiol Biomarkers Prev. 2016;25(7):1125–33.

28. Perkins KA, Kunkle N, Karelitz JL, Perkins KA, Kunkle N, Karelitz JL. Preliminary test of cigarette nicotine discrimination threshold in non-dependent versus dependent smokers. Drug Alcohol

Depend. 2017;175:36–41.

29. Smith TT, Levin ME, Schassburger RL, Buffalari DM, Sved AF, Donny EC. Gradual and immediate nicotine reduction result in similar low-dose nicotine self-administration. Nicotine Tob Res. 2013;15(11):1918–25.

30. Chen H, Matta SG, Sharp BM. Acquisition of nicotine self-administration in adolescent rats given prolonged access to the drug. Neuropsychopharmacology. 2007;32(3):700–9.

31. Levin ED, Lawrence SS, Petro A, Horton K, Rezvani AH, Seidler FJ et al. Adolescent vs. adult-onset nicotine self-administration in male rats: duration of effect and differential nicotinic receptor correlates. Neurotoxicol Teratol. 2007;29(4):458–65.

32. Donny EC, Hatsukami DK, Benowitz NL, Sved AF, Tidey JW, Cassidy RN. Reduced nicotine product standards for combustible tobacco: building an empirical basis for effective regulation. Prev Med. 2014;68:17–22.

33. Smith TT, Schassburger RL, Buffalari DM, Sved AF, Donny EC. Low-dose nicotine self-administration is reduced in adult male rats naive to high doses of nicotine: implications for nicotine product standards. Exp Clin Psychopharmacol. 2014;22(5):453–9.

34. Schassburger RL, Pitzer EM, Smith TT, Rupprecht LE, Thiels E, Donny EC et al. Adolescent rats self-administer less nicotine than adults at low doses. Nicotine Tob Res. 2016;18(9):1861–8.

35. Cassidy RN, Tidey JW, Cao Q, Colby SM, McClernon FJ, Koopmeiners JS et al. Age moderates smokers' subjective response to very low nicotine content cigarettes: evidence from a randomized controlled trial. Nicotine Tob Res. 2018; doi:10.1093/ntr/nty079.

36. Jackson KM, Cioe PA, Krishnan-Sarin S, Hatsukami D. Adolescent smokers' response to reducing the nicotine content of cigarettes: acute effects on withdrawal symptoms and subjective evaluations. Drug Alcohol Depend. 2018;188:153–60.

37. Walker N, Howe C, Bullen C, Grigg M, Glover M, McRobbie H et al. The combined effect of very low nicotine content cigarettes, used as an adjunct to usual Quitline care (nicotine replacement therapy and behavioural support), on smoking cessation: a randomized controlled trial. Addiction. 2012;107(10):1857–67.

38. McRobbie H, Przulj D, Smith KM, Cornwall D. Complementing the standard multicomponent treatment for smokers with denicotinized cigarettes: a randomized trial. Nicotine Tob Res. 2016;18(5):1134–41.

39. Donny EC, Jones M. Prolonged exposure to denicotinized cigarettes with or without transdermal nicotine. Drug Alcohol Depend. 2009;104(1–2):23–33.

40. Hatsukami DK, Luo X, Dick L, Kangkum M, Allen SS, Murphy SE et al. Reduced nicotine content cigarettes and use of alternative nicotine products: exploratory trial. Addiction. 2017;112(1):156–67.

41. Benowitz NL, Donny EC, Hatsukami DK. Reduced nicotine content cigarettes, e-cigarettes and the cigarette end game. Addiction. 2017;112(1):6–7.

42. Pechacek TF, Nayak P, Gregory KR, Weaver SR, Eriksen MP. The potential that electronic nicotine delivery systems can be a disruptive technology: results from a national survey. Nicotine Tob Res. 2016;18(10):1989–97.

43. Nardone N, Donny EC, Hatsukami DK, Koopmeiners JS, Murphy SE, Strasser AA et al. Estimations and predictors of non-compliance in switchers to reduced nicotine content cigarettes. Addiction. 2016;111(12):2208–16.

44. Boatman JA, Vock DM, Koopmeiners JS, Donny EC. Estimating causal effects from a randomized clinical trial when noncompliance is measured with error. Biostatistics. 2018;19(1):103–18.

45. Dermody SS, Donny EC, Hertsgaard LA, Hatsukami DK. Greater reductions in nicotine exposure while smoking very low nicotine content cigarettes predict smoking cessation. Tob Control. 2015;24(6):536–9.

46. Dermody SS, McClernon FJ, Benowitz N, Luo X, Tidey JW, Smith TT et al. Effects of reduced nicotine content cigarettes on individual withdrawal symptoms over time and during abstinence.

Exp Clin Psychopharmacol. 2018;26(3):223–32.

47. Faulkner P, Ghahremani DG, Tyndale RF, Cox CM, Kazanjian AS, Paterson N et al. Reduced-nicotine cigarettes in young smokers: impact of nicotine metabolism on nicotine dose effects. Neuropsychopharmacology. 2017;42(8):1610–8.

48. Keith DR, Kurti AN, Davis DR, Zvorsky IA, Higgins ST. A review of the effects of very low nicotine content cigarettes on behavioral and cognitive performance. Prev Med. 2017;104:100–16.

49. Rupprecht LE, Koopmeiners JS, Dermody SS, Oliver JA, al'Absi M, Benowitz NL et al. Reducing nicotine exposure results in weight gain in smokers randomised to very low nicotine content cigarettes. Tob Control. 2017;26(e1):e43–8.

50. Rupprecht LE, Donny EC, Sved AF. Obese smokers as a potential subpopulation of risk in tobacco reduction policy. Yale J Biol Med. 2015;88(3):289–94.

51. Bandiera FC, Ross KC, Taghavi S, Delucchi K, Tyndale RF, Benowitz NL. Nicotine dependence, nicotine metabolism, and the extent of compensation in response to reduced nicotine content cigarettes. Nicotine Tob Res. 2015;17(9):1167–72.

52. Benowitz NL, Dains KM, Hall SM, Stewart S, Wilson M, Dempsey D et al. Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. Cancer Epidemiol Biomarkers Prev. 2012;21(5):761–9.

53. Shiffman S, Kurland BF, Scholl SM, Mao JM. Nondaily smokers' changes in cigarette consumption with very low-nicotine-content cigarettes: a randomized double-blind clinical trial. JAMA Psychiatry. 2018;75(10):995–1002.

54. Dermody SS, Tidey JW, Denlinger RL, Pacek LR, al'Absi M, Drobes DJ. The impact of smoking very low nicotine content cigarettes on alcohol use. Alcohol Clin Exp Res. 2016;40(3):606–15.

55. Pacek LR, Vandrey R, Dermody SS, Denlinger-Apte RL, Lemieux A, Tidey JW et al. Evaluation of a reduced nicotine product standard: moderating effects of and impact on cannabis use. Drug Alcohol Depend. 2016;167:228–32.

56. Cohn A, Ehlke S, Cobb CO. Relationship of nicotine deprivation and indices of alcohol use behavior to implicit alcohol and cigarette approach cognitions in smokers. Addict Behav. 2017;67:58–65.

57. Tidey JW, Pacek LR, Koopmeiners JS, Vandrey R, Nardone N, Drobes DJ et al. Effects of 6-week use of reduced-nicotine content cigarettes in smokers with and without elevated depressive symptoms. Nicotine Tob Res. 2017;19(1):59–67.

58. Gaalema DE, Miller ME, Tidey JW. Predicted impact of nicotine reduction on smokers with affective disorders. Tob Regul Sci. 2015;1(2):154–65.

59. Higgins ST, Heil SH, Sigmon SC, Tidey JW, Gaalema DE, Stitzer ML et al. Response to varying the nicotine content of cigarettes in vulnerable populations: an initial experimental examination of acute effects. Psychopharmacology. 2017;234(1):89–98.

60. Lawrence D, Mitrou F, Zubrick SR. Smoking and mental illness: results from population surveys in Australia and the United States. BMC Public Health. 2009;9:285.

61. Lasser K1, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: a population-based prevalence study. JAMA. 2000;284(20):2606–10.

62. Smith PH, Mazure CM, McKee SA. Smoking and mental illness in the US population. Tob Control. 2014;23(e2):e147–53.

63. Adler NE, Glymour MM, Fielding J. Addressing social determinants of health and health inequalities. JAMA. 2016;316(16):1641–2.

64. Das S, Prochaska JJ. Innovative approaches to support smoking cessation for individuals with mental illness and co-occurring substance use disorders. Expert Rev Respir Med. 2017;11(10):841–50.

65. Beard E, Bruguera C, McNeill A, Brown J, West R. Association of amount and duration of NRT use in smokers with cigarette consumption and motivation to stop smoking: a national survey of smokers in England. Addict Behav. 2015;40:33–8.

66. Benowitz NL, Hukkanen J, Jacob P III. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol. 2009;192:29–60.

67. Digard H, Proctor C, Kulasekaran A, Malmqvist U, Richter A. Determination of nicotine absorption from multiple tobacco products and nicotine gum. Nicotine Tob Res. 2013;15(1):255–61.

68. Caggiula AR, Donny EC, Palmatier MI, Liu X, Chaudhri N, Sved AF. The role of nicotine in smoking: a dual-reinforcement model. Nebr Symp Motiv. 2009;55:91–109.

69. Van Heel M, Van Gucht D, Vanbrabant K, Baeyens F. The importance of conditioned stimuli in cigarette and e-cigarette craving reduction by e-cigarettes. Int J Environ Res Public Health. 2017;14(2):193.

70. Rose JE. Nicotine and nonnicotine factors in cigarette addiction. Psychopharmacology. 2006;184(3–4):274–85.

71. Perkins K, Sayette M, Conklin C, Caggiula A. Placebo effects of tobacco smoking and other nicotine intake. Nicotine Tob Res. 2003;5(5):695–709.

72. Chaloupka FJ, Warner KE. The economics of smoking. In: Culyer AJ, Newhouse JP, editors. Handbook of health economics. Oxford: Elsevier Science; 2000:1539–627.

73. MacKillop J, Murphy JG, Ray LA, Eisenberg DT, Lisman SA, Lum JK et al. Further validation of a cigarette purchase task for assessing the relative reinforcing efficacy of nicotine in college smokers. Exp Clin Psychopharmacol. 2008;16(1):57–65.

74. Guindon GE, Paraje GR, Chaloupka FJ. The impact of prices and taxes on the use of tobacco products in Latin America and the Caribbean. Am J Public Health. 2015;105(3):e9–19.

75. Zheng Y, Zhen C, Dench D, Nonnemaker JM. US demand for tobacco products in a system framework. Health Econ. 2017;26(8):1067–86.

76. O'Connor RJ, June KM, Bansal-Travers M, Rousu MC, Thrasher JF, Hyland A et al. Estimating demand for alternatives to cigarettes with online purchase tasks. Am J Health Behav. 2014;38(1):103–13.

77. Stein JS, Wilson AG, Koffarnus MN, Judd MC, Bickel WK. Naturalistic assessment of demand for cigarettes, snus, and nicotine gum. Psychopharmacology. 2017;234(2):245–54.

78. Gammon DG, Loomis BR, Dench DL, King BA, Fulmer EB, Rogers T. Effect of price changes in little cigars and cigarettes on little cigar sales: USA, Q4 2011–Q4 2013. Tob Control. 2016;25(5):538–44.

79. Tait P, Rutherford P, Saunders C. Do consumers of manufactured cigarettes respond differently to price changes compared with their roll-your-own counterparts? Evidence from New Zealand. Tob Control. 2015;24(3):285–9.

80. Etter JF, Eissenberg T. Dependence levels in users of electronic cigarettes, nicotine gums and tobacco cigarettes. Drug Alcohol Depend. 2015;147:68–75.

81. Huang J, Tauras J, Chaloupka FJ. The impact of price and tobacco control policies on the demand for electronic nicotine delivery systems. Tob Control. 2014;23(Suppl 3):iii41–7.

82. McPherson S, Howell D, Lewis J, Barbosa-Leiker C, Bertotti Metoyer P, Roll J. Self-reported smoking effects and comparative value between cigarettes and high dose e-cigarettes in nicotine-dependent cigarette smokers. Behav Pharmacol. 2016;27(2–3 Spec Issue):301–7.

83. Liu G, Wasserman E, Kong L, Foulds J. A comparison of nicotine dependence among exclusive e-cigarette and cigarette users in the PATH study. Prev Med. 2017;104:86–91.

84. National Cancer Institute, World Health Organization. The economics of tobacco and tobacco control (NCI Tobacco Control Monograph 21). Bethesda (MD): Department of Health and Human Services; Geneva: World Health Organization; 2016.

85. Chaloupka FJ, Yurekli A, Fong GT. Tobacco taxes as a tobacco control strategy. Tob Control. 2012;21(2):172–80.

86. Choi SE. Are lower income smokers more price sensitive? The evidence from Korean cigarette tax increases. Tob Control. 2016;25(2):141–6.

87. Koffarnus MN, Wilson AG, Bickel WK. Effects of experimental income on demand for potentially

real cigarettes. Nicotine Tob Res. 2015;17(3):292–8.

88. Yang T, Peng S, Yu L, Jiang S, Stroub WB, Cottrell RR et al. Chinese smokers' behavioral response toward cigarette price: individual and regional correlates. Tob Induc Dis. 2016;14:13.

89. Lewis M, Wang Y, Cahn Z, Berg CJ. An exploratory analysis of cigarette price premium, market share and consumer loyalty in relation to continued consumption versus cessation in a national US panel. BMJ Open. 2015;5(11):e008796.

90. Biener L, Roman AM, McInerney SA, Bolcic-Jankovic D, Hatsukami DK, Loukas A et al. Snus use and rejection in the USA. Tob Control. 2016;25(4):386–92.

91. Nonnemaker J, Kim AE, Lee YO, MacMonegle A. Quantifying how smokers value attributes of electronic cigarettes. Tob Control. 2016;25(e1):e37–43.

92. Meier E, Burris JL, Wahlquist A, Garrett-Mayer E, Gray KM, Alberg AJ et al. Perceptions of snus among US adult smokers given free product. Nicotine Tob Res. 2017;20(1):22–9.

93. Walker N, Fraser T, Howe C, Laugesen M, Truman P, Parag V et al. Abrupt nicotine reduction as an endgame policy: a randomised trial. Tob Control. 2015;24(e4):e251–7.

94. Smith TT, Cassidy RN, Tidey JW, Luo X, Le CT, Hatsukami DK et al. Impact of smoking reduced nicotine content cigarettes on sensitivity to cigarette price: further results from a multi-site clinical trial. Addiction. 2017;112(2):349–59.

95. Tucker MR, Laugesen M, Grace RC. Estimating demand and cross-price elasticity for very low nicotine content (VLNC) cigarettes using a simulated demand task. Nicotine Tob Res. 2018;20(4):528.

96. Johnson MW, Bickel WK, Kirshenbaum AP. Substitutes for tobacco smoking: a behavioral economic analysis of nicotine gum, denicotinized cigarettes, and nicotine-containing cigarettes. Drug Alcohol Depend. 2004;74(3):253–64.

97. O'Connor RJ, Bansal-Travers M, Carter LP, Cummings KM. What would menthol smokers do if menthol in cigarettes were banned? Behavioral intentions and simulated demand. Addiction. 2012;107(7):1330–8.

98. Sureda X, Fu M, Martínez-Sánchez JM, Martínez C, Ballbé M, Pérez-Ortuño R et al. Manufactured and roll-your-own cigarettes: a changing pattern of smoking in Barcelona, Spain. Environ Res. 2017;155:167–74.

99. Brown AK, Nagelhout GE, van den Putte B, Willemsen MC, Mons U, Guignard R et al. Trends and socioeconomic differences in roll-your-own tobacco use: findings from the ITC Europe surveys. Tob Control. 2015;24(Suppl 3):iii11–6.

100. Ayo-Yusuf OA, Olutola BG. "Roll-your-own" cigarette smoking in South Africa between 2007 and 2010. BMC Public Health. 2013;13:597.

101. Agaku IT, Filippidis FT, Vardavas CI, Odukoya OO, Awopegba AJ, Ayo-Yusuf OA et al. Poly-tobacco use among adults in 44 countries during 2008–2012: evidence for an integrative and comprehensive approach in tobacco control. Drug Alcohol Depend. 2014;139:60–70.

102. Lee YO, Hebert CJ, Nonnemaker JM, Kim AE. Multiple tobacco product use among adults in the United States: cigarettes, cigars, electronic cigarettes, hookah, smokeless tobacco, and snus. Prev Med. 2014;62:14–9.

103. Gravely S, Fong GT, Cummings KM, Yan M, Quah AC, Borland R et al. Awareness, trial, and current use of electronic cigarettes in 10 countries: findings from the ITC project. Int J Environ Res Public Health. 2014;11(11):11691–704.

104. Palipudi KM, Mbulo L, Morton J, Mbulo L, Bunnell R, Blutcher-Nelson G et al. Awareness and current use of electronic cigarettes in Indonesia, Malaysia, Qatar, and Greece: findings from 2011–2013 global adult tobacco surveys. Nicotine Tob Res. 2016;18(4):501–7.

105. Delnevo CD, Giovenco DP, Miller Lo EJ. Changes in the mass-merchandise cigar market since the Tobacco Control Act. Tob Regul Sci. 2017;3(2 Suppl 1):S8–16.

106. Jo CL, Williams RS, Ribisl KM. Tobacco products sold by Internet vendors following restrictions on flavors and light descriptors. Nicotine Tob Res. 2015;17(3):344–9.

107. Casseus M, Garmon J, Hrywna M, Delnevo CD. Cigarette smokers' classification of tobacco products. Tob Control. 2016;25(6):628–30.

108. Quisenberry AJ, Koffarnus MN, Hatz LE, Epstein LH, Bickel WK. The experimental tobacco marketplace I: Substitutability as a function of the price of conventional cigarettes. Nicotine Tob Res. 2016;18(7):1642–8.

109. Dave D, Saffer H. Demand for smokeless tobacco: role of advertising. J Health Econ. 2013;32(4):682–97.

110. Adams S, Cotti C, Fuhrmann D. Smokeless tobacco use following smoking bans in bars. South Econ J. 2013;80(1):162–80.

111. Tam J, Day HR, Rostron BL, Apelberg BJ. A systematic review of transitions between cigarette and smokeless tobacco product use in the United States. BMC Public Health. 2015;15:258.

112. Lund KE, Vedøy TF, Bauld L. Do never smokers make up an increasing share of snus users as cigarette smoking declines? Changes in smoking status among male snus users in Norway 2003–15. Addiction. 2017;112(2):340–8.

113. Hatsukami DK, Severson H, Anderson A, Vogel RI, Jensen J, Broadbent B et al. Randomised clinical trial of snus versus medicinal nicotine among smokers interested in product switching. Tob Control. 2016;25(3):267–74.

114. Allen A, Vogel RI, Meier E, Anderson A, Jensen J, Severson HH et al. Gender differences in snus versus nicotine gum for cigarette avoidance among a sample of US smokers. Drug Alcohol Depend. 2016;168:8–12.

115. Berman ML, Bickel WK, Harris AC, LeSage MG, O'Connor RJ, Stepanov I et al. Consortium on methods evaluating tobacco: research tools to inform FDA regulation of snus. Nicotine Tob Res. 2018;20(11):1292–300.

116. Chaloupka FJ, Sweanor D, Warner KE. Differential taxes for differential risks – toward reduced harm from nicotine-yielding products. N Engl J Med. 2015;373(7):594–7.

117. Grace RC, Kivell BM, Laugesen M. Estimating cross-price elasticity of e-cigarettes using a simulated demand procedure. Nicotine Tob Res. 2015;17(5):592–8.

118. Stoklosa M, Drope J, Chaloupka FJ. Prices and e-cigarette demand: evidence from the European Union. Nicotine Tob Res. 2016;18(10):1973–80.

119. Johnson MW, Johnson PS, Rass O, Pacek LR. Behavioral economic substitutability of e-cigarettes, tobacco cigarettes, and nicotine gum. J Psychopharmacol. 201731(7);851–60.

120. Snider SE, Cummings KM, Bickel WK. Behavioral economic substitution between conventional cigarettes and e-cigarettes differs as a function of the frequency of e-cigarette use. Drug Alcohol Depend. 2017;177:14–22.

121. Beard E, West R, Michie S, Brown J. Association between electronic cigarette use and changes in quit attempts, success of quit attempts, use of smoking cessation pharmacotherapy, and use of stop smoking services in England: time series analysis of population trends. BMJ. 2016;354:i4645.

122. O'Brien B, Knight-West O, Walker N, Parag V, Bullen C. E-cigarettes versus NRT for smoking reduction or cessation in people with mental illness: secondary analysis of data from the ASCEND trial. Tob Induc Dis. 2015;13(1):5.

123. Brown J, Beard E, Kotz D, Michie S, West R. Real-world effectiveness of e-cigarettes when used to aid smoking cessation: a cross-sectional population study. Addiction. 2014;109(9):1531–40.

124. Harrell PT, Marquinez NS, Correa JB, Meltzer LR, Unrod M, Sutton SK et al. Expectancies for cigarettes, e-cigarettes, and nicotine replacement therapies among e-cigarette users (aka vapers). Nicotine Tob Res. 2015;17(2):193–200.

125. Liber AC, Drope JM, Stoklosa M. Combustible cigarettes cost less to use than e-cigarettes: global evidence and tax policy implications. Tob Control. 2017;26(2):158–63.

126. Jorenby DE, Smith SS, Fiore MC, Baker TB. Nicotine levels, withdrawal symptoms, and smoking

reduction success in real world use: a comparison of cigarette smokers and dual users of both cigarettes and E-cigarettes. Drug Alcohol Depend. 2017;170:93–101.

127. Majeed BA, Weaver SR, Gregory KR, Whitney CF, Slovic P, Pechacek TF et al. Changing perceptions of harm of e-cigarettes among U.S. adults, 2012–2015. Am J Prev Med. 2017;52(3):331–8.

128. Chou SP, Saha TD, Zhang H, Ruan WJ, Huang B, Grant BF et al. Prevalence, correlates, comorbidity and treatment of electronic nicotine delivery system use in the United States. Drug Alcohol Depend. 2017;178:296–301.

129. Lee JA, Kim SH, Cho HJ. Electronic cigarette use among Korean adults. Int J Public Health. 2016;61(2):151–7.

130. Wang M, Wang JW, Cao SS, Wang HQ, Hu RY. Cigarette smoking and electronic cigarettes use: a meta-analysis. Int J Environ Res Public Health. 2016;13(1):120.

131. Carroll Chapman SL, Wu LT. E-cigarette prevalence and correlates of use among adolescents versus adults: a review and comparison. J Psychiatr Res. 2014;54:43–54.

132. Shiplo S, Czoli CD, Hammond D. E-cigarette use in Canada: prevalence and patterns of use in a regulated market. BMJ Open. 2015;5(8):e007971.

133. Reid JL, Rynard VL, Czoli CD, Hammond D. Who is using e-cigarettes in Canada? Nationally representative data on the prevalence of e-cigarette use among Canadians. Prev Med. 2015;81:180–3.

134. Kilibarda B, Mravcik V, Martens MS. E-cigarette use among Serbian adults: prevalence and user characteristics. Int J Public Health. 2016;61(2):167–75.

135. Vardavas CI, Filippidis FT, Agaku IT. Determinants and prevalence of e-cigarette use throughout the European Union: a secondary analysis of 26 566 youth and adults from 27 Countries. Tob Control. 2015;24(5):442–8.

136. Farsalinos KE, Poulas K, Voudris V, Le Houezec J. Electronic cigarette use in the European Union: analysis of a representative sample of 27 460 Europeans from 28 countries. Addiction. 2016;111(11):2032–40.

137. E-cigarette use among youth and young adults: a report of the Surgeon General. Washington (DC): Department of Health and Human Services; 2016 (http://www.surgeongeneral.gov/library/2016ecigarettes/index.html#execsumm, accessed 15 May 2019).

138. Rennie LJ, Bazillier-Bruneau C, Rouëssé J. Harm reduction or harm introduction? Prevalence and correlates of E-cigarette use among French adolescents. J Adolesc Health. 2016;58(4):440–5.

139. Mays D, Smith C, Johnson AC, Tercyak KP, Niaura RS. An experimental study of the effects of electronic cigarette warnings on young adult nonsmokers' perceptions and behavioral intentions. Tob Induc Dis. 2016;14:17.

140. Jiang N, Wang MP, Ho SY, Leung LT, Lam TH. Electronic cigarette use among adolescents: a cross-sectional study in Hong Kong. BMC Public Health. 2016;16:202.

141. Lee JA, Lee S, Cho HJ. The relation between frequency of e-cigarette use and frequency and intensity of cigarette smoking among South Korean adolescents. Int J Environ Res Public Health. 2017;14(3):305.

142. Chang HC, Tsai YW, Shiu MN, Wang YT, Chang PY. Elucidating challenges that electronic cigarettes pose to tobacco control in Asia: a population-based national survey in Taiwan. BMJ Open. 2017;7(3):e014263.

143. White J, Li J, Newcombe R, Walton D. Tripling use of electronic cigarettes among New Zealand adolescents between 2012 and 2014. J Adolesc Health. 2015;56(5):522–8.

144. Chaffee BW, Couch ET, Gansky SA. Trends in characteristics and multi-product use among adolescents who use electronic cigarettes, United States 2011–2015. PLoS One. 2017;12(5):e0177073.

145. Fotiou A, Kanavou E, Stavrou M, Richardson C, Kokkevi A. Prevalence and correlates of electronic cigarette use among adolescents in Greece: a preliminary cross-sectional analysis of nationwide survey data. Addict Behav. 2015;51:88–92.

146. Cullen KA, Ambrose BK, Gentzke AS, Apelberg BJ, Jamal A, King BA. Notes from the field: use of electronic cigarettes and any tobacco product among middle and high school students – United States, 2011–2018. Morb Mortal Wkly Rep. 2018;67:1276–7.

147. Chatterjee K, Alzghoul B, Innabi A, Meena N. Is vaping a gateway to smoking: a review of the longitudinal studies. Int J Adolesc Med Health. 2016;30(3). doi: 10.1515/ijamh-2016-0033.

148. Lanza ST, Russell MA, Braymiller JL. Emergence of electronic cigarette use in US adolescents and the link to traditional cigarette use. Addict Behav. 2017;67:38–43.

149. Schneider S, Diehl K. Vaping as a catalyst for smoking? An initial model on the initiation of electronic cigarette use and the transition to tobacco smoking among adolescents. Nicotine Tob Res. 2016;18(5):647–53.

150. Zhong J, Cao S, Gong W, Fei F, Wang M. Electronic cigarettes use and intention to cigarette smoking among never-smoking adolescents and young adults: a meta-analysis. Int J Environ Res Public Health. 2016;13(5):465.

151. Barrington-Trimis JL, Urman R, Berhane K, Unger JB, Cruz TB, Pentz MA et al. E-cigarettes and future cigarette use. Pediatrics. 2016;138(1). doi: 10.1542/peds.2016-0379.

152. Wills TA, Sargent JD, Knight R, Pagano I, Gibbons FX. E-cigarette use and willingness to smoke: a sample of adolescent non-smokers. Tob Control. 2016;25(e1):e52–9.

153. Conner M, Grogan S, Simms-Ellis R, Flett K, Sykes-Muskett B, Cowap L et al. Do electronic cigarettes increase cigarette smoking in UK adolescents? Evidence from a 12-month prospective study. Tob Control. 2017;27(4). doi: 10.1136/tobaccocontrol-2016-053539.

154. Soneji S, Barrington-Trimis JL, Wills TA, Leventhal AM, Unger JB, Gibson LA et al. Association between initial use of e-cigarettes and subsequent cigarette smoking among adolescents and young adults: a systematic review and meta-analysis. JAMA Pediatr. 2017;171(8):788–97.

155. Etter JF. Gateway effects and electronic cigarettes. Addiction. 2018;113(10):1776–83.

156. Franck C, Filion KB, Kimmelman J, Grad R, Eisenberg MJ. Ethical considerations of e-cigarette use for tobacco harm reduction. Respir Res. 2016;17(1):53.

157. Meier EM, Tackett AP, Miller MB, Grant DM, Wagener TL. Which nicotine products are gateways to regular use? First-tried tobacco and current use in college students. Am J Prev Med. 2015;48(1 Suppl 1):S86–93.

158. Polosa R, Russell C, Nitzkin J, Farsalinos KE. A critique of the US Surgeon General's conclusions regarding e-cigarette use among youth and young adults in the United States of America. Harm Reduct J. 2017;14(1):61.

159. Kozlowski LT, Warner KE. Adolescents and e-cigarettes: objects of concern may appear larger than they are. Drug Alcohol Depend. 2017;174:209–14.

160. Surgeon General's advisory on e-cigarette use among youth. Atlanta (GA): Office of the Surgeon General, Office on Smoking and Health; 2018 (https://e-cigarettes.surgeongeneral.gov/documents/surgeon-generals-advisory-on-e-cigarette-use-among-youth-2018.pdf, accessed 1 January 2019).

161. Azar AM, Gottlieb S. Opinion. We cannot let e-cigarettes become an on-ramp for teenage addiction. The Washington Post, 11 October 2018 (https://www.washingtonpost.com/opinions/we-cannot-let-e-cigarettes-become-an-on-ramp-for-teenage-addiction/2018/10/11/55ce424e-ccc6-11e8-a360-85875bac0b1f_story.html?utm_term=.3b243a1a45f5, accessed 1 January 2019).

162. National Research Council. Understanding the US illicit tobacco market: characteristics, policy context, and lessons from international experiences. Washington (DC): National Academies Press; 2015 (https://doi.org/10.17226/19016).

163. Lee JGL, Golden SD, Ribisl KM. Limited indications of tax stamp discordance and counterfeiting on cigarette packs purchased in tobacco retailers, 97 counties, USA, 2012. Prev Med Rep. 2017;8:148–52.

164. Ribisl KM, Williams R, Gizlice Z, Herring AH. Effectiveness of state and federal government

agreements with major credit card and shipping companies to block illegal Internet cigarette sales. PLoS One. 2011;6(2):e16745.

165. Hatsukami DK, Heishman SJ, Vogel RI, Denlinger RL, Roper-Batker AN, Mackowick KM et al. Dose–response effects of Spectrum research cigarettes. Nicotine Tob Res. 2013;15(6):1113–21.

166. Wiley JL, Marusich JA, Thomas BF, Jackson KJ. Determination of behaviorally effective tobacco constituent doses in rats. Nicotine Tob Res. 2015;17(3):368–71.

167. Grizzell JA, Echeverria V. New insights into the mechanisms of action of cotinine and its distinctive effects from nicotine. Neurochem Res. 2015;40(10):2032–46.

168. Brennan KA, Laugesen M, Truman P. Whole tobacco smoke extracts to model tobacco dependence in animals. Neurosci Biobehav Rev. 2014;47:53–69.

169. Costello MR, Reynaga DD, Mojica CY, Zaveri NT, Belluzzi JD, Leslie FM. Comparison of the reinforcing properties of nicotine and cigarette smoke extract in rats. Neuropsychopharmacology. 2014;39(8):1843–51.

170. Gellner CA, Belluzzi JD, Leslie FM. Self-administration of nicotine and cigarette smoke extract in adolescent and adult rats. Neuropharmacology. 2016;109:247–53.

171. Smith TT, Schaff MB, Rupprecht LE, Schassburger RL, Buffalari DM, Murphy SE et al. Effects of MAO inhibition and a combination of minor alkaloids, β-carbolines, and acetaldehyde on nicotine self-administration in adult male rats. Drug Alcohol Depend. 2015;155:243–52.

172. Harris AC, Tally L, Schmidt CE, Muelken P, Stepanov I, Saha S et al. Animal models to assess the abuse liability of tobacco products: effects of smokeless tobacco extracts on intracranial self-stimulation. Drug Alcohol Depend. 2015;147:60–7.

173. Truman P, Grounds P, Brennan KA. Monoamine oxidase inhibitory activity in tobacco particulate matter: Are harman and norharman the only physiologically relevant inhibitors? Neurotoxicology. 2017;59:22–6.

174. Arnold MM, Loughlin SE, Belluzzi JD, Leslie FM. Reinforcing and neural activating effects of norharmane, a non-nicotine tobacco constituent, alone and in combination with nicotine. Neuropharmacology. 2014;85:293–304.

175. Caine SB, Collins GT, Thomsen M, Wright C, Lanier RK, Mello NK. Nicotine-like behavioral effects of the minor tobacco alkaloids nornicotine, anabasine, and anatabine in male rodents. Exp Clin Psychopharmacol. 2014;22(1):9–22.

176. Plescia F, Brancato A, Venniro M, Maniaci G, Cannizzaro E, Sutera FM et al. Acetaldehyde self-administration by a two-bottle choice paradigm: consequences on emotional reactivity, spatial learning, and memory. Alcohol. 2015;49(2):139–48.

177. Smith TT, Rupprecht LE, Cwalina SN, Onimus MJ, Murphy SE, Donny EC et al. Effects of monoamine oxidase inhibition on the reinforcing properties of low-dose nicotine. Neuropsychopharmacology. 2016;41(9):2335–43.

178. Biswas L, Harrison E, Gong Y, Avusula R, Lee J, Zhang M et al. Enhancing effect of menthol on nicotine self-administration in rats. Psychopharmacology. 2016;233(18):3417–27.

179. Advisory note: banning menthol in tobacco products. WHO Study Group on Tobacco Product Regulation. Geneva: World Health Organization; 2016 (http://apps.who.int/iris/bitstream/10665/205928/1/9789241510332_eng.pdf, accessed 15 May 2019).

180. Vagg R, Chapman S. Nicotine analogues: a review of tobacco industry research interests. Addiction. 2015;100:701–12.

181. Desai RI, Doyle MR, Withey SL, Bergman J. Nicotinic effects of tobacco smoke constituents in nonhuman primates. Psychopharmacology. 2016;233(10):1779–89.

182. Wong LP, Alias H, Agha Mohammadi N, Ghadimi A, Hoe VCW. E-cigarette users' attitudes on the banning of sales of nicotine e-liquid, its implication on e-cigarette use behaviours and alternative sources of nicotine e-liquid. J Community Health. 2017;42(6):1225–32.

183. O'Brien EK, Nguyen AB, Persoskie A, Hoffman AC. US adults' addiction and harm beliefs about

nicotine and low nicotine cigarettes. Prev Med. 2017;96:94–100.

184. Denlinger-Apte RL, Joel DL, Strasser AA, Donny EC. Low nicotine content descriptors reduce perceived health risks and positive cigarette ratings in participants using very low nicotine content cigarettes. Nicotine Tob Res. 2017;19(10):1149–54.

185. Mercincavage M, Saddleson ML, Gup E, Halstead A, Mays D, Strasser AA. Reduced nicotine content cigarette advertising: how false beliefs and subjective ratings affect smoking behavior. Drug Alcohol Depend. 2017;173:99–106.

186. Bolcic-Jankovic D, Biener L. Public opinion about FDA regulation of menthol and nicotine. Tob Control. 2015;24(e4):e241–5.

187. Kiviniemi MT, Kozlowski LT. Deficiencies in public understanding about tobacco harm reduction: results from a United States national survey. Harm Reduct J. 2015;12:21.

188. Czoli CD, Fong GT, Mays D, Hammond D. How do consumers perceive differences in risk across nicotine products? A review of relative risk perceptions across smokeless tobacco, e-cigarettes, nicotine replacement therapy and combustible cigarettes. Tob Control. 2017;26(e1):e49–58.

189. Darredeau C, Stewart SH, Barrett SP. The effects of nicotine content information on subjective and behavioural responses to nicotine-containing and denicotinized cigarettes. Behav Pharmacol. 2013;24(4):291–7.

190. Mercincavage M, Smyth JM, Strasser AA, Branstetter SA. Reduced nicotine content expectancies affect initial responses to smoking. Tob Regul Sci. 2016;2(4):309–16.

191. Harrell PT, Juliano LM. A direct test of the influence of nicotine response expectancies on the subjective and cognitive effects of smoking. Exp Clin Psychopharmacol. 2012;20(4):278–86.

192. Gu X, Lohrenz T, Salas R, Baldwin PR, Soltani A, Kirk U et al. Belief about nicotine modulates subjective craving and insula activity in deprived smokers. Front Psychiatry. 2016;7:126.

193. Harrell PT, Simmons VN, Piñeiro B, Correa JB, Menzie NS, Meltzer LR et al. E-cigarettes and expectancies: why do some users keep smoking? Addiction. 2015;110(11):1833–43.

194. Pearson JL, Abrams DB, Niaura RS, Richardson A, Vallone DM. Public support for mandated nicotine reduction in cigarettes. Am J Public Health. 2013;103(3):562–7.

195. Li J, Newcombe R, Walton D. Responses towards additional tobacco control measures: data from a population-based survey of New Zealand adults. N Z Med J 2016;129:87–92.

196. Fraser T, Kira A. Perspectives of key stakeholders and smokers on a very low nicotine content cigarette-only policy: qualitative study. N Z Med J. 2017;130:36–45.

197. Denlinger-Apte RL, Tidey JW, Koopmeiners JS, Hatsukami DK, Smith TT, Pacek LR et al. Correlates of support for a nicotine-reduction policy in smokers with 6-week exposure to very low nicotine cigarettes. Tob Control. 2018. pii: tobaccocontrol-2018-054622.

198. Donny EC, Walker N, Hatsukami D, Bullen C. Reducing the nicotine content of combusted tobacco products sold in New Zealand. Tob Control. 2017;26(e1):e37–42.

199. Laugesen M. Modelling a two-tier tobacco excise tax policy to reduce smoking by focusing on the addictive component (nicotine) more than the tobacco weight. N Z Med J. 2012;125:35–48.

200. Yuke K, Ford P, Foley W, Mutch A, Fitzgerald L, Gartner C. Australian urban Indigenous smokers' perspectives on nicotine products and tobacco harm reduction. Drug Alcohol Rev. 2018;42(6):1225–32.

201. Danielle LJ, Hatsukami DK, Hertsgaard L, Dermody SS, Donny EC. Gender differences in the perceptions of very low nicotine content cigarettes (abstract). Annual meeting of the College on Problems of Drug Dependence. Drug Alcohol Depend. 2014;140:e97.

202. Mercincavage M, Wileyto EP, Saddleson ML, Lochbuehler K, Donny EC, Strasser AA. Attrition during a randomized controlled trial of reduced nicotine content cigarettes as a proxy for understanding acceptability of nicotine product standards. Addiction. 2017;112(6):1095–103.

5. A regulatory strategy for reducing exposure to toxicants in cigarette smoke

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Contents

5.1   Introduction

The report *Scientific Basis of Tobacco Product Regulation* (WHO Technical Report Series No. 951) *(1)*, published in 2008, specifies an approach to lowering the levels of selected toxicants in mainstream cigarette smoke. We review these recommendations and summarize several papers published by the tobacco industry that discuss aspects of the proposed regulatory strategy. We also discuss the significant progress in evaluating biomarkers of tobacco smoke toxicants and carcinogens that has been made since the 2008 report and the relation of these biomarkers to smoke constituent levels and, in some cases, to disease incidence. We consider the use of mandated toxicant levels in a product regulatory strategy and present recommendations for an updated regulatory strategy based on nicotine levels in cigarettes.

5.2   Regulation of cigarette smoke constituents described in WHO Technical Report Series No. 951

The report *Scientific Basis of Tobacco Product Regulation* (WHO Technical Report Series No. 951) recognizes that regulatory strategies based on machine-measured tar, nicotine and CO yields **per cigarette** under the ISO smoking machine regimen were causing harm by misleading smokers to believe that so-called "low-yield cigarettes" were less harmful. Thus, the report recommended establishing limits for certain smoke constituents expressed **per mg nicotine** rather than per cigarette, as people smoke cigarettes to obtain adequate nicotine to satisfy their physiological and behavioural needs, and most toxicants in smoke are delivered in proportion to nicotine. This shifts the emphasis towards the potential toxicity of smoke generated under standardized conditions. The report also recommended

prohibiting any communications to consumers that were based on machine measurements, as these can be easily misunderstood.

The report concluded that it was premature to consider biomarkers in a regulatory strategy, because, while biomarkers of exposure existed at the time, there were no validated biomarkers of harm. As discussed below, significant progress has been made in the application of biomarkers to evaluating the potential toxicity and carcinogenicity of cigarette smoke. There is presently no doubt that the average levels of certain biomarkers of exposure reflect the measured amounts of their parent compounds in cigarette smoke.

In WHO Technical Report Series No. 951, TobReg selected toxicants for regulation on the basis of a number of factors, including established cardiovascular and pulmonary toxicity and carcinogenicity, the feasibility of lowering their concentrations with available technology and variation in chemical classes and brands. Consideration was also given to the inclusion of compounds in both the gas and the particulate phases of cigarette smoke. Of these characteristics, the most important was evidence of toxicity.

Data from Health Canada and a paper on constituent levels in Philip Morris brands in both the USA and elsewhere *(2)* were available to assess differences in the levels of the selected toxicants in cigarettes on the market in Canada and the USA. These data were used to select the initial levels of the regulated constituents as a first step in an overall strategy to eliminate brands with higher levels from the market, thus lowering the overall mean values of the toxicants in the remaining brands. A rolling mean was envisioned, which would eventually drive down levels of the selected toxicants in all brands left on the market and prevent introduction onto the market of brands with toxicant levels higher than the mean.

The report recommended that the regulatory strategy be implemented in phases, beginning with required annual reporting of selected toxicant levels, followed by promulgation of the levels for toxicants above which brands could not be sold and, finally, enforcement of the established levels. Some progress has been made towards achieving these goals, but it is unclear whether there has been sufficient momentum.

The toxicants for which mandatory lowering is recommended and the initially recommended levels are summarized in columns 1–3 of Table 5.1. The modified intense machine smoking regimen used by Health Canada (HCI) was selected for measuring toxicants. This regimen is modified from the ISO method by increasing the puff volume from 35 mL to 55 mL and decreasing the puff interval from 60 s to 30 s. In addition, all ventilation holes are blocked. This method is referred to in the report as the "modified intense smoking" regimen. The levels in Table 5.1 for "international brands" (i.e. all those other than United States brands) are derived from a sample of brands of United-States-style cigarettes with

a blend of tobaccos *(2)*. The levels in "Canadian brands" are derived from reports to Health Canada and are for cigarettes made mainly with Bright (flue-cured) tobacco. These were selected as examples because of the availability of data and the different types of tobacco and were expected roughly to represent the markets to be regulated, depending on the type of product prevalent in that market. In addition to the compounds listed in Table 5.1, priority toxicants recommended for reporting were acrylonitrile, 4-aminobiphenyl, 2-aminonapthalene, cadmium, catechol, crotonaldehyde, hydrogen cyanide, hydroquinone and nitrogen oxides *(1)*. An additional group of 20 compounds to be considered for reporting was added in 2013 (see section 8).

Table 5.1. Levels of toxicants recommended for mandated lowering (μg/mg nicotine under the Canadian intense smoking regimen)

Toxicant	Levels recommended in WHO Technical Report Series No. 951 *(1)*		Levels in some published surveys			Criterion for selecting recommended levels
	Interna-tional brands *(2)*	Canadian brands[a]	*(3)*[b]	*(4)*[c]	*(5)*[d]	
NNK	0.072	0.047	0.080	0.050–0.072	0.0273	Median value
NNN	0.114	0.027	0.134	0.077–0.142	0.0916	Median value
Acetaldehyde	860	670	676	468–626	684.1	125% of median value
Acrolein	83	97	85.4	48.8–64.3	64.5	125% of median value
Benzene	48	50	43.1	40.8–53.7	66.1	125% of median value
Benzo[*a*]pyrene	0.011	0.011	0.0096	0.0074–0.0084	0.0085	125% of median value
1,3-Butadiene	67	53	50.5	38.1–52.1	71.9	125% of median value
Carbon monoxide	18 400	15 400	15 700	11 500–14 800	16 100	125% of median value
Formaldehyde	47	97	47.3	28.2–43.7	81.4	125% of median value
Nicotine (per cigarette)	–	–	2.13 mg	1.56–2.33 mg	1.6 mg	–

NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N´*-nitrosonornicotine. [a] Data from Health Canada. [b] 61 United States brand styles, mean. [c] Range of means of three international brands. [d] 20 brands on the Chinese market, mean.

The list of compounds in Table 5.1 is still highly relevant to regulation, as all have well documented toxic and/or carcinogenic activity. The FDA has published a list of "harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke" that includes all the compounds in Table 5.1 as well as those recommended for reporting *(6)*. The FDA list of HPHCs, which focuses on chemicals linked to cancer, cardiovascular disease, reproductive problems, addiction and respiratory effects, is longer than that presented here, with 93 constituents, because it includes every identified toxic or carcinogenic compound in each class rather than representatives (e.g. NNK and NNN for *N*-nitrosamines and benzo[*a*]pyrene for PAHs in Table 5.1).

Table 5.1 also presents the levels of the selected toxicants per mg of nicotine as reported in three industry studies published subsequent to WHO Technical Report Series No. 951 *(3–5)*. Generally, the means and ranges of constituent concentrations were similar to those in the WHO report. One industry study, not shown in Table 5.1, reported levels of NNN and NNK in smoke and concluded that they were decreasing *(7)*.

WHO Technical Report Series No. 951 also addressed questions on variations in measurement of constituent levels and among brands and reported the coefficient of variation (standard deviation of a series of measurements divided by the mean of that series) for the levels of constituents in various brands. This was divided by the coefficient of variation of multiple measurements of a given constituent (e.g. variation in the analytical method) to produce a ratio. Constituents with high ratios, e.g. with wide variation in levels, as determined by analytical methods with little variation, would be the most appropriate targets for regulation. The report concluded that the ratios for individual toxicants in the Philip Morris International brands and the Canadian brands indicated that there was sufficient variation in the levels of most toxicants among brands that mandated reductions would have a substantial effect on the levels in brands remaining on the market.

5.3 Industry response to WHO Technical Report Series No. 951

Bodnar et al. *(3)* conducted a survey of selected mainstream smoke constituents from commercially marketed United States cigarettes, segmenting the market into 13 strata according to "tar" category and cigarette design parameters. The HCI smoking regimen was used, and the yields of mainstream smoke constituent per mg nicotine were estimated. Normalization per mg nicotine gave an inverse ranking of constituent yields from those expressed per cigarette according to the ISO/FTC Cambridge filter method "tar" categories, i.e. "Full flavour" > "Lights" > "Ultra-lights" – designations that are prohibited by the WHO FCTC.

Purkis et al. *(8)* reviewed measurements of smoke constituents and aspects of variability that can affect regulatory standards. They discussed method development, harmonization and standardization and reviewed such issues as variation among products, machine-smoking regimens and laboratory measurements and their potential consequences, such as misinterpretation of data. They called for internationally agreed testing standards and measurement tolerances.

Haussmann *(9)* provided a detailed discussion of the use of hazard indices for evaluating cigarette smoke composition, including the advantages and limitations of the approach described in WHO Technical Report Series No. 951. Haussmann concluded that application of the suggested approach would result in banning 39 of the 50 brands in the data set of Counts et al. (referred to as "International brands" in Table 5.1). He demonstrated that excluding one brand

because the yield of one constituent exceeded the recommended ceiling could mean that the yields of the other eight constituents that were present below their respective ceilings were not accounted for. The result would be to increase rather than decrease the average yield of those constituents in the remaining brands. Thus, the recommended levels of various constituents should be assessed carefully.

Piade et al. *(10)* determined the levels of the 18 priority constituents (expressed per mg nicotine) identified in WHO Technical Report Series No. 951 in 262 commercial brands, including various blends from 13 countries. Principal component analysis was used to identify inverse correlations and other patterns. Three principal components explained about 75% of the data variation. The first was sensitive to the relative levels of gas- and particle-phase compounds, while the other two components grouped American and Virginia blends, revealing inverse correlations. For example, the levels of nitrogen oxides and amino- or nitroso-aromatic compounds were inversely correlated with either formaldehyde, acrolein, benzo[*a*]pyrene or dihydroxybenzenes. They concluded that regulatory approaches might be confounded by such inverse correlations.

Belushkin et al. *(11)* studied the variation in yields of smoke constituents for a Kentucky reference cigarette and one commercial brand analysed on several occasions over 7 years. They showed that statistically significant differences in the yields of some smoke constituents did not necessarily correspond to differences between products and that tolerances should be defined. They concluded that use of two approaches – minimal detectable differences and statistical equivalence – was more meaningful for comparisons than the statistical Student *t* test.

Eldridge et al. *(4)* measured toxicant levels in the tobacco and smoke of reference cigarettes and three commercial products, monthly for 10 months. The monthly variation was < 15% for most analytes but increased somewhat when reported as a ratio to nicotine level. They concluded that measurement of emissions from commercial cigarettes was subject to considerable variation, particularly for toxicants present at low levels.

Deng et al. *(5)* studied the influence of measurement uncertainty on the recommended regulation of the nine constituents listed in Table 5.1. Uncertainty was evaluated in collaborative studies conducted in 2012–2016 of the compliance of 20 representative cigarette samples. They concluded that measurement uncertainty would strongly influence implementation of the proposed regulations.

Collectively, these studies indicate some cautionary and practical notes for application of the proposed regulation of nine constituents per mg nicotine. The uncertainty in analytical chemistry measurements is well known but in most cases is relatively minor, particularly for constituents that occur at high levels. Validated methods are available for measuring all the compounds proposed for measurement (*12–18*; see also http://www.who.int/tobacco/industry/product_regulation/toblabnet/en/). Thus, further studies on standardization of

methods, including time-consuming ring trials that may last years, are probably not required. Furthermore, the inverse correlations among constituent levels in certain brands may be problematic, as lowering the level of one constituent may increase those of others. This has been known for decades *(19, 20)*, and it is industry's responsibility to address these levels in products that they wish to maintain on the market. In conclusion, the main goal of these industry studies was to provide reasons to challenge and delay implementation of product standards for cigarette smoke emissions, and there are no valid scientific reasons that implementation cannot proceed.

5.4 Relation between smoke constituent levels and biomarkers

Biomarkers of carcinogens and toxicants in tobacco, most of which are urinary metabolites of tobacco smoke constituents, are robust, validated, reliable indicators of human exposure to tobacco product constituents *(21)*. Several of these biomarkers are directly relevant to the toxicants recommended for mandated lowering listed in Table 5.1. For NNK, total NNAL (the sum of free NNAL and its glucuronides) is an accepted biomarker of exposure *(22)*. For acrolein, the urinary metabolite 3-hydroxypropyl mercapturic acid has been widely used *(23, 24)*. Similarly, for benzene and 1,3-butadiene, the urinary mercapturic acids, *S*-phenyl mercapturic acid and monohydroxybutyl mercapturic acid, respectively, have been quantified *(25–27)*. The urinary metabolite of pyrene, 1-hydroxypyrene, has been used extensively as a biomarker of uptake of PAH, the class of compounds that includes benzo[*a*]pyrene *(21, 28)*. Exhaled CO is an accepted biomarker of CO exposure in cigarette smokers *(21)*. Total nicotine equivalents – the sum of nicotine, cotinine and 3´-hydroxycotinine and their glucuronides in urine – represent a high percentage of the nicotine dose received by a tobacco user and are an excellent biomarker of nicotine dose *(29)*.

Most of these biomarkers have been quantified in large studies, such as the Health Measures Study in Canada, the National Health and Nutrition Examination Survey in the USA and the industry-sponsored Total Exposure Study, as well as in epidemiological and clinical studies *(26, 30–42)*. The levels of all the biomarkers decrease significantly when people stop smoking, and all the urinary biomarkers correlate with total nicotine equivalents *(24, 26–28, 43, 44)*. The levels of constituents in smoke and the levels of acrolein, NNK, nicotine and pyrene in the mouth are clearly related to the respective urinary biomarkers *(33, 34, 38, 41)*. Collectively, these studies demonstrate that evaluation of biomarkers can buttress analyses of smoke constituents in regulations by providing a definitive link between the levels of smoke constituents and actual human exposure. Furthermore, nicotine biomarkers and total NNAL have been positively related to lung cancer in prospective epidemiological studies *(45–47)* and can thus be considered biomarkers of potential harm.

5.5   Use of mandated lowering of toxicant levels in a product regulatory strategy

The public health impact of mandated lowering of toxicant levels of cigarettes is virtually unknown. In order to improve public health by decreasing the disease and death due to tobacco product use, evaluation of a product standard must include population effects. According to the Family Smoking Prevention and Tobacco Control Act in the USA, which gives the FDA regulatory authority over tobacco products in the country, tobacco product standards can be adopted if the standard is "appropriate for the protection of the public health." The scientific evidence that is considered in evaluating the public health impact of a product standard includes:

- the risks and benefits to the population as a whole, including users and non-users of tobacco products, of the proposed standard;
- the increased or decreased likelihood that existing users of tobacco products will stop using such products; and
- the increased or decreased likelihood that those who do not use tobacco products will start using such products.

To date, a cross-sectional study has shown that urinary levels of NNAL are lower in countries in which cigarettes have lower levels of NNK and NNN *(48)*, but it is not known whether these differences are related to the country-specific incidence of cancer. A dose–response relation has, however, been found between biomarkers of exposure to NNK and NNN and risks for cancers of the lung *(46, 47, 49)* and the oesophagus *(50)*, respectively.

To avoid replicating the negative public health impact of the low-tar and low-nicotine-yield cigarette experience *(51)* before implementation of mandated standards, their potential effects on smoking behaviour, exposure to toxicants and potential harm, consumer perceptions and misperceptions of the regulated product and uptake and continued use of the product *(52–54)* should be determined and appropriate action taken as necessary. One constituent that was not targeted in WHO Technical Report Series No. 951 but was targeted in a subsequent advisory note *(55)* is nicotine. Regulation of nicotine, as discussed in the previous section, might result in the greatest public health benefit (see below). Reducing nicotine to minimally addictive levels would prevent the development of dependence on cigarettes and facilitate abstinence among smokers, thereby reducing the prevalence of smoking.

It is notable that, since the WHO Technical Report was issued, products such as ENDS, with significantly lower toxicant levels and toxicity than conventional cigarettes *(56–58)*, have been introduced in some markets in full force. If products that are demonstrated to be substantially less toxic replace

cigarettes among smokers and are used exclusively by smokers, the public health impact might be substantial provided that there are no adverse consequences such as inappropriate promotion or use by children and/or young people or continuing dual use with conventional cigarettes or other tobacco products.

5.6 Toxicants recommended for mandated lowering and recommended limits

Donny et al. *(40)* obtained highly relevant results that could change the regulatory landscape. They conducted a double-blind, parallel, randomized clinical trial of cigarettes with nicotine contents varying from 15.8 mg/g of tobacco, which is typical of commercial brands in the USA, to 0.4 mg/g. Regular smokers were assigned to smoke either low-nicotine cigarettes or their usual brand of cigarettes for 6 weeks. At week 6, the average number of cigarettes smoked per day was significantly lower for the participants randomly assigned to cigarettes containing 2.4 mg, 1.3 mg or 0.4 mg of nicotine per gram of tobacco (16.5, 16.3 and 14.9 cigarettes/day, respectively) than for participants who were randomly assigned to their usual brand or to cigarettes containing 15.8 mg/g tobacco (22.2 and 21.3 cigarettes/day, respectively). The cigarettes with a lower nicotine content reduced exposure to and dependence on nicotine and also craving during abstinence from smoking, without increasing the expired CO level or total puff volume, which suggests that there was minimal compensation. These results are consistent with those of several smaller trials *(30, 59–63)* and of a trial with vulnerable adults *(64)*.

In the trial by Donny et al. *(40)*, participants assigned to cigarettes containing ≤ 2.4 mg of nicotine per gram of tobacco smoked 23–30% fewer cigarettes per day at week 6 than did participants assigned to cigarettes containing 15.8 mg/g of tobacco. The results showed a clear, significant breakpoint in the number of cigarettes smoked per day and total nicotine equivalents in urine between the cigarettes containing 5.2 mg/g nicotine and those with 2.4 mg/g. Furthermore, and most significantly, dependence, as assessed in the Wisconsin inventory of smoking dependence motives, was significantly lower at week 6 among participants who smoked cigarettes with 0.4 mg/g nicotine than among those who smoked cigarettes with 15.8 mg/g nicotine ($P = 0.001$). The cigarettes containing 0.4 mg/g nicotine delivered 0.04 mg nicotine in their smoke under HCI conditions *(65)*.

These results suggest that a product standard could have a significant public health benefit for countries with the appropriate resources (see reference 55 for more details). The amount of nicotine in cigarette tobacco filler should be regulated such that the level of nicotine in mainstream smoke does not exceed 0.04 mg per cigarette under HCI conditions (39–58 times lower than in typical current brands, Table 5.1). With respect to other constituents, our new recommended levels are the median values per mg of nicotine of the constituents

listed in Table 5.1, according to the latest market survey information, which would be obtained from major tobacco companies and Health Canada data on the brands with compliant nicotine levels, e.g. that deliver 0.04 mg nicotine per cigarette under HCI smoking conditions. This recommendation is stronger than the "125% of the median value" previously established by TobReg. We also retain the requirement for reporting levels of acrylonitrile, 4-aminobiphenyl, 2-aminonaphthalene, cadmium, catechol, crotonaldehyde, hydrogen cyanide, hydroquinone and nitrogen oxides. These toxicants satisfy several criteria for inclusion: sufficient evidence of carcinogenicity, known respiratory or cardiotoxicity, differences in levels of toxicants in different brands in different countries, measurability and potential for decreased yields in a product *(1)*.

We note that, when expressed per mg nicotine, the mandated toxicant levels would be higher than the current mandated levels for the toxicants listed in Table 5.2, based on data for the Spectrum cigarettes used in the clinical study described above *(65)*. This is obviously a consequence of the lower recommended nicotine level of 0.04 mg per cigarette in mainstream smoke as compared with current levels, which range from 1.5 to 2 mg/cigarette. Measures of biomarkers, however, clearly show that smokers of these low-nicotine cigarettes excrete significantly lower levels of total nicotine equivalents and total NNAL in their urine and thus have less uptake of toxic and carcinogenic compounds *(40)*.

Table 5.2. Levels of selected toxicants in mainstream smoke of Spectrum cigarettes (NRC 102, 0.04 mg nicotine per cigarette; Health Canada intense smoking regimen)

Toxicant	µg/mg nicotine
Acetaldehyde	41 500
Acrolein	2070
Benzo[*a*]pyrene	0.220
Carbon monoxide	720 000
Formaldehyde	258
NNK	0.798
NNN	2.88

NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N´*-nitrosonornicotine. *Source*: reference *65*.

5.7   Implementation of mandated lower levels of toxicants

In order for regulators to effectively mandate lower levels of toxicants and nicotine in cigarettes, appropriate procedures and sufficient infrastructure and resources are required. These include requiring the disclosure of levels of harmful constituents in cigarettes and an independent laboratory to validate and monitor these levels routinely. In the previous WHO report *(1)*, analysis of the constituent yields of cigarettes was considered to be the first step towards a mandate to reduce toxicants. Standardized testing procedures should be instituted that include not only laboratory methods (e.g. machine-determined yields that reflect a range of

human exposures and validated analytical chemistry methods) but also the way in which the cigarettes to be tested are systematically procured (e.g. different geographical locations, from several retail outlets within a geographical location, directly from manufacturers, in different months). Tracking cigarettes that are licit and illicit should also be considered, as well as a plan to reduce the entry of illicit cigarettes to the market. Furthermore, education campaigns and messages should be designed to minimize any misperception of the harm associated with smoking cigarettes. Thus, the message that combusted products are hazardous to health, regardless of a mandate to lower toxicant levels, must be communicated explicitly and clearly. Finally, a surveillance system should be in place to monitor any unintended consequences, and methods for correction should be considered.

5.8   Conclusions and recommendations

The regulatory strategy recommended here retains the principle of the previous recommendations by TobReg, revising and strengthening it such that brands on the market cannot exceed the median values of the constituents listed in Table 5.1. We also retain the requirement for reporting of several other constituents. Furthermore, we recommend considering a regulatory strategy in which the nicotine level in tobacco does not exceed 0.4 mg/g of tobacco (0.04 mg nicotine per cigarette in mainstream smoke under HCI smoking conditions). The new regulation based on nicotine is the result of decades of research that has demonstrated that nicotine is the main chemical that drives people to smoke *(66, 67)* or otherwise use tobacco and the results of clinical trials that demonstrate that low-nicotine cigarettes reduce exposure to and dependence on nicotine and the number of cigarettes smoked. Thus, we recommend that the previous strategy of limiting specific toxicants and the present new recommendation of limiting nicotine be pursued aggressively in parallel. The first, critical step in the toxicant reduction strategy is collection of data on current products by methods established by WHO Tobacco Laboratory Network (TobLabNet).

We are aware that challenges may exist in implementing product standards for cigarettes and that unintended consequences are possible (e.g. illegal markets). Therefore, it will be necessary to enforce regulations such that cigarettes with levels of toxicants and nicotine above the established means are removed from the market. A reliable system of monitoring regulated constituents in tobacco and smoke will have to be established. Representative analyses of cigarettes on the international market will have to be performed, so that the mean levels of the mandated toxicants in their smoke and possibly nicotine in tobacco are reported. Vigilant monitoring for evidence that no harm results from the new regulations will be necessary.

5.9   References

1.   The scientific basis of tobacco product regulation. Second report of a WHO study group (WHO Technical Report Series, No. 951). Geneva: World Health Organization; 2008: 45–277.
2.   Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. Regul Toxicol Pharmacol. 2005;41(3):185–227.
3.   Bodnar JA, Morgan WT, Murphy PA, Ogden MW. Mainstream smoke chemistry analysis of samples from the 2009 US cigarette market. Regul Toxicol Pharmacol. 2012;64(1):35–42.
4.   Eldridge A, Betson TR, Gama MV, McAdam K. Variation in tobacco and mainstream smoke toxicant yields from selected commercial cigarette products. Regul Toxicol Pharmacol. 2015;71(3):409–27.
5.   Deng H, Li Z, Bian Z, Yang F, Liu S, Fan Z et al. Influence of measurement uncertainty on the World Health Organization recommended regulation for mainstream cigarette smoke constituents. Regul Toxicol Pharmacol. 2017;86:231–40.
6.   Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed Reg. 2012;77:20034–7.
7.   Gunduz I, Kondylis A, Jaccard G, Renaud JM, Hofer R, Ruffieux L et al. Tobacco-specific N-nitrosamines NNN and NNK levels in cigarette brands between 2000 and 2014. Regul Toxicol Pharmacol. 2016;76:113–20.
8.   Purkis SW, Meger M, Wuttke R. A review of current smoke constituent measurement activities and aspects of yield variability. Regul Toxicol Pharmacol. 2012;62(1):202–13.
9.   Haussmann HJ. Use of hazard indices for a theoretical evaluation of cigarette smoke composition. Chem Res Toxicol. 2012;25(4):794–810.
10. Piade JJ, Wajrock S, Jaccard G, Janeke G. Formation of mainstream cigarette smoke constituents prioritized by the World Health Organization – yield patterns observed in market surveys, clustering and inverse correlations. Food Chem Toxicol. 2013;55:329–347.
11. Belushkin M, Jaccard G, Kondylis A. Considerations for comparative tobacco product assessments based on smoke constituent yields. Regul Toxicol Pharmacol. 2015;73(1):105–13.
12. Eschner MS, Selmani I, Groger TM, Zimmermann R. Online comprehensive two-dimensional characterization of puff-by-puff resolved cigarette smoke by hyphenation of fast gas chromatography to single-photon ionization time-of-flight mass spectrometry: quantification of hazardous volatile organic compounds. Anal Chem. 2011;83(17):6619–27.
13. Pang X, Lewis AC. Carbonyl compounds in gas and particle phases of mainstream cigarette smoke. Sci Total Environ. 2011;409(23):5000–9.
14. Pappas RS, Fresquez MR, Martone N, Watson CH. Toxic metal concentrations in mainstream smoke from cigarettes available in the USA. J Anal Toxicol. 2014;38(4):204–11.
15. Vu AT, Taylor KM, Holman MR, Ding YS, Hearn B, Watson CH. Polycyclic aromatic hydrocarbons in the mainstream smoke of popular US cigarettes. Chem Res Toxicol. 2015;28(8):1616–26.
16. Ding YS, Yan X, Wong J, Chan M, Watson CH. In situ derivatization and quantification of seven carbonyls in cigarette mainstream smoke. Chem Res Toxicol. 2016;29(1):125–31.
17. Cecil TL, Brewer TM, Young M, Holman MR. Acrolein yields in mainstream smoke from commercial cigarette and little cigar tobacco products. Nicotine Tob Res. 2017;19(7):865–70.
18. Edwards SH, Rossiter LM, Taylor KM, Holman MR, Zhang L, Ding YS et al. Tobacco-specific nitrosamines in the tobacco and mainstream smoke of U.S. commercial cigarettes. Chem Res Toxicol. 2017;30(2):540–51.
19. Adams JD, Lee SJ, Hoffmann D. Carcinogenic agents in cigarette smoke and the influence of nitrate on their formation. Carcinogenesis. 1984;5:221–3.
20. Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL et al. Levels of tobacco-specific nitro-

samines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev. 2008;17(12):3366–71.

21. Hecht SS, Yuan JM, Hatsukami DK. Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. Chem Res Toxicol. 2010;23:1001–8.

22. Hecht SS, Stepanov I, Carmella SG. Exposure and metabolic activation biomarkers of carcinogenic tobacco-specific nitrosamines. Acc Chem Res. 2016;49(1):106–14.

23. Yan W, Byrd GD, Brown BG, Borgerding MF. Development and validation of a direct LC-MS-MS method to determine the acrolein metabolite 3-HPMA in urine. J Chromatogr Sci. 2010;48(3):194–9.

24. Park SL, Carmella SG, Chen M, Patel Y, Stram DO, Haiman CA et al. Mercapturic acids derived from the toxicants acrolein and crotonaldehyde in the urine of cigarettes smokers from five ethnic groups with differing risks for lung cancer. PLoS One. 2015;10:e0124841.

25. Dougherty D, Garte S, Barchowsky A, Zmuda J, Taioli E. NQO1, MPO, CYP2E1, GSTT1 and GSTM1 polymorphisms and biological effects of benzene exposure – a literature review. Toxicol Lett. 2008;182(1–3):7–17.

26. Roethig HJ, Munjal S, Feng S, Liang Q, Sarkar M, Walk RA et al. Population estimates for biomarkers of exposure to cigarette smoke in adult US cigarette smokers. Nicotine Tob Res. 2009;11(10):1216–25.

27. Haiman CA, Patel YM, Stram DO, Carmella SG, Chen M, Wilkens L et al. Benzene uptake and glutathione S-transferase T1 status as determinants of S-phenylmercapturic acid in cigarette smokers in the Multiethnic Cohort. PLoS One. 2016;11(3):e0150641.

28. Suwan-ampai P, Navas-Acien A, Strickland PT, Agnew J. Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons in the United States, 1999 to 2002. Cancer Epidemiol Biomarkers Prev. 2009;18(3):884–93.

29. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005;57(1):79–115.

30. Benowitz N, Hall SM, Stewart S, Wilson M, Dempsey D, Jacob P III. Nicotine and carcinogen exposure with smoking of progressively reduced nicotine content cigarette. Cancer Epidemiol Biomarkers Prev. 2007;16(11):2479–85.

31. Le Marchand L, Derby KS, Murphy SE, Hecht SS, Hatsukami D, Carmella SG et al. Smokers with the CHRNA lung cancer-associated variants are exposed to higher levels of nicotine equivalents and a carcinogenic tobacco-specific nitrosamine. Cancer Res. 2008;68(22):9137–40.

32. Lowe FJ, Gregg EO, McEwan M. Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never-smokers. Clin Chem Lab Med. 2009;47(3):311–20.

33. Shepperd CJ, Eldridge AC, Mariner DC, McEwan M, Errington G, Dixon M. A study to estimate and correlate cigarette smoke exposure in smokers in Germany as determined by filter analysis and biomarkers of exposure. Regul Toxicol Pharmacol. 2009;55(1):97–109.

34. Morin A, Shepperd CJ, Eldridge AC, Poirier N, Voisine R. Estimation and correlation of cigarette smoke exposure in Canadian smokers as determined by filter analysis and biomarkers of exposure. Regul Toxicol Pharmacol. 2011;61(3 Suppl):S3–12.

35. Xia Y, Bernert JT, Jain RB, Ashley DL, Pirkle JL. Tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers in the United States: NHANES 2007–2008. Biomarkers. 2011;16(2):112–9.

36. Appleton S, Olegario RM, Lipowicz PJ. TSNA exposure from cigarette smoking: 18 years of urinary NNAL excretion data. Regul Toxicol Pharmacol. 2014;68(2):269–74.

37. Murphy SE, Park S-SL, Thompson EF, Wilkens LR, Patel Y, Stram DO et al. Nicotine N-glucuronidation relative to N-oxidation and C-oxidation and UGT2B10 genotype in five ethnic/racial groups. Carcinogenesis. 2014;35:2526–33.

38. Sakaguchi C, Kakehi A, Minami N, Kikuchi A, Futamura Y. Exposure evaluation of adult male

Japanese smokers switched to a heated cigarette in a controlled clinical setting. Regul Toxicol Pharmacol. 2014;69(3):338–47.

39. Czoli C, Hammond D. TSNA exposure: levels of NNAL among Canadian tobacco users. Nicotine Tob Res. 2015;17(7):825–30.

40. Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG et al. Randomized trial of reduced-nicotine standards for cigarettes. N Engl J Med. 2015;373(14):1340–9.

41. Theophilus EH, Coggins CR, Chen P, Schmidt E, Borgerding MF. Magnitudes of biomarker reductions in response to controlled reductions in cigarettes smoked per day: a one-week clinical confinement study. Regul Toxicol Pharmacol. 2015;71(2):225–34.

42. Wei B, Blount BC, Xia B, Wang L. Assessing exposure to tobacco-specific carcinogen NNK using its urinary metabolite NNAL measured in US population: 2011–2012. J Expo Sci Environ Epidemiol. 2016;26(3):249–56.

43. Carmella SG, Chen M, Han S, Briggs A, Jensen J, Hatsukami DK et al. Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. Chem Res Toxicol. 2009;22(4):734–41.

44. Park SL, Carmella SG, Ming X, Stram DO, Le Marchand L, Hecht SS. Variation in levels of the lung carcinogen NNAL and its glucuronides in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. Cancer Epidemiol Biomarkers Prev. 2015;24:561–9.

45. Church TR, Anderson KE, Caporaso NE, Geisser MS, Le C, Zhang Y et al. A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. Cancer Epidemiol Biomarkers Prev. 2009;18:260–6.

46. Yuan JM, Butler LM, Stepanov I, Hecht SS. Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. Cancer Res. 2014;74(2):401–11.

47. Yuan JM, Nelson HH, Carmella SG, Wang R, Kuriger-Laber J, Jin A et al. CYP2A6 genetic polymorphisms and biomarkers of tobacco smoke constituents in relation to risk of lung cancer in the Singapore Chinese Health Study. Carcinogenesis. 2017;38(4):411–8.

48. Ashley DL, O'Connor RJ, Bernert JT, Watson CH, Polzin GM, Jain RB et al. Effect of differing levels of tobacco-specific nitrosamines in cigarette smoke on the levels of biomarkers in smokers. Cancer Epidemiol Biomarkers Prev. 2010;19(6):1389–98.

49. Church TR, Anderson KE, Caporaso NE, Geisser MS, Le CT, Zhang Y et al. A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. Cancer Epidemiol Biomarkers Prev. 2009;18(1):260–6.

50. Yuan JM, Knezevich AD, Wang R, Gao YT, Hecht SS, Stepanov I. Urinary levels of the tobacco-specific carcinogen N´-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. Carcinogenesis. 2011;32(9):1366–71.

51. Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine. Bethesda (MD): Department of Health and Human Services, National Institutes of Health, National Cancer Institute; 2001.

52. Stratton K, Shetty P, Wallace R, Bondurant S, editors. Clearing the smoke: assessing the science base for tobacco harm reduction. Washington, DC: Institute of Medicine; 2001.

53. Hatsukami DK, Biener L, Leischow SJ, Zeller MR. Tobacco and nicotine product testing. Nicotine Tob Res. 2012;14(1):7–17.

54. Berman ML, Connolly G, Cummings KM, Djordjevic MV, Hatsukami DK, Henningfield JE et al. Providing a science base for the evaluation of tobacco products. Tob Regul Sci. 2015;1(1):76–93.

55. WHO Study Group on Tobacco Product Regulation. Advisory note: global nicotine reduction strategy (WHO Technical Report Series No. 989). Geneva: World Health Organization; 2015.

56. Nicotine without smoke: tobacco harm reduction. London: Royal College of Physicians; 2016.

57. Benowitz NL, Fraiman JB. Cardiovascular effects of electronic cigarettes. Nat Rev Cardiol. 2017;14(8):447–56.

58. Shahab L, Goniewicz ML, Blount BC, Brown J, McNeill A, Alwis KU et al. Nicotine, carcinogen, and toxin exposure in long-term e-cigarette and nicotine replacement therapy users: a cross-sectional study. Ann Intern Med. 2017;166(6):390–400.

59. Donny EC, Houtsmuller E, Stitzer ML. Smoking in the absence of nicotine: behavioral, subjective and physiological effects over 11 days. Addiction. 2007;102(2):324–34.

60. Benowitz NL, Dains KM, Dempsey D, Herrera B, Yu L, Jacob P III. Urine nicotine metabolite concentrations in relation to plasma cotinine during low-level nicotine exposure. Nicotine Tob Res. 2009;11(8):954–60.

61. Hatsukami DK, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen JA et al. Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. Addiction. 2010;105:343–55.

62. Benowitz NL, Dains KM, Hall SM, Stewart S, Wilson M, Dempsey D et al. Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. Cancer Epidemiol Biomarkers Prev. 2012;21(5):761–9.

63. Hatsukami DK, Hertsgaard LA, Vogel RI, Jensen JA, Murphy SE, Hecht SS et al. Reduced nicotine content cigarettes and nicotine patch. Cancer Epidemiol Biomarkers Prev. 2013;22(6):1015–24.

64. Higgins ST, Heil SH, Sigmon SC, Tidey JW, Gaalema DE, Hughes JR et al. Addiction potential of cigarettes with reduced nicotine content in populations with psychiatric disorders and other vulnerabilities to tobacco addiction. JAMA Psychiatry. 2017;74(10):1056–64.

65. Ding YS, Richter P, Hearn B, Zhang L, Bravo R, Yan X et al. Chemical characterization of mainstream smoke from SPECTRUM variable nicotine research cigarettes. Tob Regul Sci. 2017;3(1):81–94.

66. The health consequences of smoking. Nicotine addiction. A report of the Surgeon General. Rockville (MD): Department of Health and Human Services; 1988.

67. How tobacco smoke causes disease: the biology and behavioural basis for smoking-attributable disease. A report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention; 2010.

6. The science of flavour in tobacco products

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Contents

6.1 Introduction

Most conventional, traditional and new or emerging tobacco products, including cigarettes, cigars, cigarillos, smokeless tobacco products (including *snus*), waterpipe tobacco and e-cigarettes with nicotine (also known as electronic nicotine delivery systems – ENDS) and without nicotine (also known as electronic non-nicotine delivery systems ENNDS) contain flavourings. Broadly defined, flavour is the sensory experience produced when something is ingested or inhaled through the mouth *(1)*. Tobacco itself imparts a flavour (e.g. "natural tobacco" flavour), which depends on the type of tobacco and the curing process *(2)*, and many, if not most, products include added flavours. Some flavourings are derived from natural products, such as cocoa, liquorice, honey and sucrose, while other are created synthetically, such as pyrazines for chocolate flavour and sucralose for sweetness. In the context of tobacco product regulation, flavours that are present at levels that impart a strong non-tobacco smell or taste are considered "characterizing". While use of the term "characterizing" is much debated and it is not used worldwide, the European Union Tobacco Product Directive *(3)* defined a characterizing flavour as a:

> clearly noticeable smell or taste other than one of tobacco, resulting from an additive or 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.

The FDA does not directly define "characterizing flavours" but used the following language in guidance on the flavoured cigarette ban in 2009 *(4)*:

> "… a cigarette or any of its component parts (including the tobacco, filter, or paper) from containing as a constituent (including a smoke constituent) or additive, an artificial or natural flavor (other than tobacco or menthol) or an herb or spice, including strawberry, grape, orange, clove, cinnamon, pineapple, vanilla, coconut, liquorice, cocoa, chocolate, cherry, or coffee, that is a characterizing flavor of the tobacco product or tobacco smoke."

The tobacco industry has introduced flavours into tobacco products to increase the appeal of tobacco products. It is believed that flavours are added to these products to reduce their harshness, increase their appeal and improve their palatability. Further, the display of the names or graphic representations of flavours on packaging and advertising materials may enhance the attractiveness of a product.

We provide here a brief overview of the epidemiology of use of flavoured tobacco and nicotine products worldwide and perceptions of these products. We also discuss technological innovations that have been applied to flavoured products, including how they enhance appeal. We provide a general overview of the sensory processes underlying the perception of flavour, known pharmacological targets for flavour chemicals and evidence on the potential toxicity of flavours. Throughout, implications for research and regulation are highlighted.

6.2 Epidemiology of use of flavoured tobacco and nicotine products

Flavoured tobacco and nicotine products are used throughout the world. While there is limited systematic evidence about the availability and use of these products, preferences and use are often specific to countries and regions. For example, in Indonesia and other south Asian countries, traditional clove cigarettes (*kretek*), which contain clove pieces, oils and flavours, are highly popular. In India, spiced smokeless tobacco (*pan masala*, *gutka*) is used, which contains tobacco mixed with food spices and oils, flavourings, betel nut and other ingredients. Hookah smoking, which originated in India and the Middle East and is now increasingly popular among young people in Europe and north America, involves use of heavily flavoured, sweetened tobacco, known as *maassel*.

The tobacco industry has long used flavours in tobacco products as a marketing strategy *(5)*, including towards young people *(6)*. For example, in the USA in 2013, the flavoured form of non-cigarette tobacco products comprised 52.3% "cigarette-sized cigars", 81.3% "cigar wraps" ("blunt wraps", "tobacco wraps" and "wraps"), 55.1% moist snuff, 86.1% *shisha* and 81.5% dissolvable tobacco

products (e.g. Ariva, Camel Orb/Sticks) *(7)*. A longitudinal study of market trends showed that the presence of flavours accounted for 59.4% of the growth in smokeless tobacco sales between 2005 and 2011 *(8)*. More recently introduced nicotine-containing e-cigarettes are now available in many flavours *(9, 10)*.

Surveys indicate high rates of flavoured tobacco use, although there is little systematic evidence on the use of these products throughout the world. The National Adult Tobacco Survey in the USA in 2013–2014 revealed that an estimated 10.2 million e-cigarette users (68.2%), 6.1 million hookah users (82.3%), 4.1 million cigar smokers (36.2%) and 4.0 million smokeless tobacco users (50.6%) had used flavoured products in the past 30 days *(11)*. The addition of multiple flavours (menthol/mint, clove/spice/herb, fruit, alcohol, candy/chocolate/other sweet flavours) was also assessed. The most prevalent flavours used by type of tobacco product were: smokeless tobacco: menthol/mint (76.9%); hookah: fruit (74.0%); cigars, cigarillos, filtered little cigars: fruit (52.4%), candy, chocolate and other sweet flavours (22.0%) and alcohol (14.5%); e-cigarettes: fruit (44.9%), menthol/mint (43.9%) and candy, chocolate and other sweet flavours (25.7%); and pipes: fruit (56.6%), candy, chocolate and other sweet flavours (26.5%) and menthol/mint (24.8%) *(11)*. In Poland, 26% of female smokers and 10.5% of male smokers reported current use of flavoured cigarettes (menthol, vanilla or other flavour) according to an analysis of data from the Global Adult Tobacco Survey conducted in 2009–2010 *(12)*.

Flavoured tobacco products are very popular among young people *(13–15)*. In a nationally representative sample of Canadian young people who used a variety of tobacco products (cigarettes, pipes, cigars, cigarillos, *bidis*, smokeless tobacco, hookah, blunts, roll-your-own cigarettes), 52% reported using flavoured products *(16)*. Similarly, evidence from a national survey conducted in Poland showed that younger smokers were more likely to use flavoured cigarettes *(12)*. Evidence from the Population Assessment of Tobacco Health study *(15)*, a longitudinal national survey conducted in the USA, indicated that use of flavoured tobacco products was highest (80%) among children aged 12–17 years, 73% among tobacco users aged 18–24 years and lowest in users aged ≥ 65 years (29%). Most of the children (81%) and young adults (86%) and only 54% of adults aged ≥ 25 years reported that their first product had been flavoured. Furthermore, first use of a flavoured tobacco product was associated with a higher prevalence of current tobacco use among young people and adults.

Summary: future regulations on flavoured products should consider the significant differences in the availability (marketing and access) and use of flavoured tobacco by country and by demographic group. Unfortunately, current surveillance tools may be inadequate for monitoring use of these products. For example, the Global Youth Tobacco Survey *(17)*, an important global tool for monitoring tobacco use by young people, does not request information

specifically about flavoured tobacco use. In the Global Adult Tobacco Survey *(18)*, use of flavoured and unflavoured tobacco is investigated only for waterpipe tobacco, and information about flavoured cigarettes can be obtained only by analysing text responses about the brand of cigarette smoked. To address this information gap, additional questions about flavoured and unflavoured products could be added to iterations of these surveys. Surveillance tools should also assess different use of flavours. Given that menthol is often regulated separately from other flavours, it would be advisable that questions distinguish menthol or mint from other flavours.

6.3 Flavoured products: perceptions, experimentation, uptake and regulation

There is a general perception that flavoured tobacco products are less harmful than other tobacco products *(15, 19–21)*. Favourable perceptions of flavoured tobacco and nicotine products were recorded among both users and non-users of a wide range of products (e.g. cigars, e-cigarettes, hookah, *kreteks*, *bidis* and smokeless tobacco) *(22)*. Flavoured non-menthol tobacco products (hookahs, little cigars and cigarillos, e-cigarettes) were perceived to be less harmful than cigarettes *(20)*.

Perhaps not surprisingly, the presence of flavours in tobacco and nicotine products is associated with greater willingness to experiment with these products *(20, 23, 24)*, an important consideration for young people. The availability of flavours has been associated with initiation of use of a variety of tobacco products, including hookah, e-cigarettes and cigars *(25–27)*. The presence of flavours may also promote a move from experimentation to regular use; the presence of more flavours in e-cigarettes was associated with greater frequency of e-cigarette use among adolescents but not among adults *(28)*. Further, advertising for sweet and fruit flavours was shown to activate brain reward areas in young adults and to interfere with recall of health warnings *(29)*. For example, adolescents in the USA who had tried flavoured tobacco were almost three times more likely to be current cigarette smokers than those who had not tried these products *(30)*, and more than 80% of current tobacco or nicotine users reported that they had first used a flavoured product *(15)*.

In order to reduce appeal, experimentation and uptake, many countries have regulated flavoured tobacco products, as summarized by the Tobacco Legal Control Consortium *(31)*. Some countries ban cigarettes with characterizing flavours, other than menthol (for example in European Union countries), while others have extended the ban to flavours in other tobacco products, e.g. the USA, Canada (little cigars, blunt wraps) and Ethiopia (all products). Several countries have enacted legislation also banning menthol as a flavour additive in cigarettes and other tobacco products. For example, Turkey has banned the addition of

menthol at any level and of any menthol derivatives (e.g. mint); and Canada has finalized a ban on the addition of any menthol in cigarettes, blunt wraps and most cigars sold on the Canadian market. Canada has also banned the use of any promotional materials, including packaging that depicts the e-liquid containing confectionery, desserts, cannabis, soft drinks or energy drink flavours.

Summary: both the presence of specific flavours in tobacco and nicotine products and the presence of flavour descriptors on packaging and in advertising have been linked to the appeal and uptake of tobacco and nicotine products, particularly among young people. Given these associations, many countries have regulated flavoured tobacco products. In view of differences in the timing and specificities of regulations in different countries and local ordinances, there is an opportunity for surveillance research (for example, within the International Tobacco Control project or other surveillance systems) to study the impact of regulations on the uptake of tobacco by youth, tobacco sales and use patterns and the subsequent response from the tobacco industry to the regulations.

6.4 A brief history of the development of flavoured tobacco by the tobacco and e-cigarette industry

The tobacco industry has been at the forefront in integrating flavour science and technology to increase product appeal and marketability. An early example is the addition of saccharin to chewing tobacco in the 1890s by the Reynolds Tobacco Company. This high-potency artificial sweetener replaced the more expensive sugar and increased the product's shelf life and uniformity. The industry continued to improve tobacco breeding, selection, curing and manufacturing methods to control the harshness of tobacco smoke, added fillers, casings, humectants (moisteners) and designed wrapper papers and filter systems to improve appeal and the uniformity of flavour delivery.

For decades, tobacco companies have been the major consumers of cocoa and liquorice on the world market. Both are added to cigarette tobacco as casings, not to dominate the flavour experience but to increase taste fullness and reduce the harshness of smoke. In contrast, menthol in menthol cigarettes is a characterizing flavour, an added non-tobacco flavour listed on the product label, which dominates the flavour experience *(32)*. Menthol cigarettes were first marketed in the USA in the 1920s, and they remain popular. Many cigarettes that are not labelled as mentholated may also contain low levels *(33)*, which may be sufficient to enhance the appeal of the product *(34)*.

While most early products contained natural flavours, including menthol extracted from mint, fruit extracts and natural terpenes and aldehydes, the increasing demand of the tobacco industry for standardized flavourings and the limited supply from natural sources prompted suppliers to develop and optimize chemical synthetic processes to deliver bulk amounts of key flavourings. For

example, most of today's menthol supply is produced by three major chemical companies that developed procedures for stereo-selective synthesis or purification of L-menthol, the minty, cooling form of menthol *(35)*. Similarly, chemicals in non-menthol products, including vanillin (vanilla flavour), pyrazines (coffee and chocolate flavourings), aldehydes such as benzaldehyde (a berry and candy flavouring) and many other tobacco flavourings are chemically synthesized, often from petrochemical hydrocarbon precursors *(35)*. Modern flavoured tobacco products contain finely tuned mixtures of purified and recombined flavourings to create characterizing flavours, which are marketed after exhaustive testing by industry flavour panels and consumer volunteers.

Flavourings are usually added to tobacco products in the tobacco casing, sprayed on tobacco in humectants, in the filter and in the foil liner of tobacco packaging. The tobacco and flavour industries continue to introduce new flavours and flavour delivery systems to diversify products and increase their appeal. For example, the introduction of flavour capsules within the filter has resulted in a new category of cigarettes that release bursts of flavour when the filter is pressed. Cigarettes with a menthol capsule were first introduced in 2007 in Japan, then in 2008 in Europe and the USA and in 2011–2012 in Australia and Mexico *(36)*. Most capsules in products contain menthol, but they may also contain aldehydes or other flavour chemicals to create flavours like spearmint, lemon mint, apple mint and strawberry mint *(37)*. Some brands contain two flavour capsules, one containing menthol and the other a tangy, fruity flavour or more menthol. The capsules can be crushed individually, giving the user control over the dual flavour experience. The presence of flavour capsules was shown to enhance the appeal of cigarettes to young people *(37)*.

After the ban on cigarettes with characterizing flavours (except for mentholated products) in some countries, the industry quickly developed alternative flavoured products, such as small cigars, some of which share the basic design of cigarettes and have tobacco in the wrapping paper. Flavoured small cigars are highly popular among young people. Patents filed by the tobacco industry describe innovative techniques for the application and enrichment of flavourings, specifically sweeteners, to cigar wrapping papers and mouthpieces to ensure that the product is perceived as sweet. In one study, high levels of saccharin and other synthetic high-intensity sweeteners were found in many popular small cigars or cigarillos on the United States market. Saccharin levels were especially high in the mouth sections and tips of the small cigars, with a sweetness intensity exceeding that of sugar *(38)*. Small cigars of all flavour categories, regardless of whether they were labelled as sweet, contained saccharin *(39, 40)*.

Tobacco industry patents also describe the use of recombinant, sweet taste-stimulating proteins like thaumatin, which is 2000 times sweeter than table sugar, as sweeteners and of synthetic menthol derivatives as innovative cooling agents. After

the ban on menthol cigarettes in Canada, the tobacco industry began marketing cigarettes without menthol in packaging with designs almost identical to those of previously marketed menthol cigarettes, signalling the same cool freshness *(41)*. It is unclear whether these cigarettes contain cooling additives to replace menthol.

Many of the smokeless tobacco products found in Asia (e.g. *zarda*, *quiwam*, *gutkha*, *khaini*) contain flavour chemicals *(42, 43)*. Novel smokeless tobacco products have also been introduced in several countries, including *snus* (previously marketed only in Sweden) and new varieties of moist snuff. *Snus* and snuff are sold in many flavours, including menthol/mint, cherry, vanilla and strawberry, and it has been suggested that the significant rise in sales of moist snuff is related to the introduction of flavours *(8, 44)*. Newly marketed *snus* products have also been found to contain very high levels of sucralose (Splenda®), a synthetic high-intensity sweetener that tastes more like sugar than saccharin, which is still used in snuff *(45)*.

Flavoured waterpipe tobacco (*maassel*), is a sweetened, flavoured form of tobacco manufactured by fermentation of tobacco with molasses, glycerol and fruit essence. It is the preferred form of tobacco used in waterpipes, especially by adolescents and young adults *(46)*.

E-cigarettes and ENDS, which appeared on the market in 2004, in Europe in 2006 and in the USA in 2007, are available in an ever-expanding range of e-liquid flavours, and the customized flavour and nicotine combinations are attractive to users. These products electrically heat and vaporize e-liquids and are available in a variety of devices, including cigarette-like products, vape or hookah pens, advanced devices known as "mods" or personal vaporizers and more discrete pod-devices like JUUL. In 2013–2014, 466 brands of e-cigarette were available online, with over 7000 unique flavour names; 242 new flavours were added each month *(9)*. By 2016–2017, the number had doubled to over 15 000 flavours and flavour combinations *(10)*. The counterbalancing effects of flavour options on promoting a switch from combustibles to e-cigarettes or ENDS against increasing the risk for uptake among young people is a hotly debated topic.

Summary: the tobacco and now the e-cigarette industry is constantly introducing innovative additives, such as flavourings and sweeteners, to increase the appeal of tobacco products, including waterpipes, smokeless tobacco, e-cigarettes and ENDS. Regulatory surveillance to detect and follow such product manipulations is critical to curtail the appeal of tobacco products.

6.5 Sensory systems that contribute to flavour

To understand the appeal of flavours in tobacco products, it is important to understand the role of the sensory components that contribute to the experience of flavour. The senses of taste and smell (olfaction) play the largest role in flavour perception; however, flavour also includes sensations of temperature, touch and

chemaesthesis (i.e. sensations that are produced by chemical stimulation of the senses of temperature, touch and pain) *(47)*.

Taste: the sense of taste derives from taste buds located on the tongue and soft palate, which provide the sensations of sweetness, saltiness, sourness, bitterness and savoury. Because of the bitter taste of nicotine *(48)*, bitterness is a dominant quality of the flavour of tobacco. The addition of flavours to tobacco products may serve to mask the bitter taste and improve flavour and appeal.

Olfaction: the sense of smell provides information not only about odours in the environment (orthonasal olfaction) but also about odours emanating from the mouth and airways (retronasal olfaction), which contribute significantly to the flavour of tobacco products. An opening at the rear of the nasal cavity that is connected to the back of the oral cavity *(49, 50)* allows odours from products that are taken into or inhaled through the mouth to stimulate olfactory receptors during exhalation.

Temperature: temperature can contribute to the flavour of tobacco in at least two ways: directly, via sensations of warmth and heat produced by combustible or heated tobacco products, and indirectly, by modulating the rate of release of volatile flavour molecules that are sensed by retronasal olfaction.

Touch: in tobacco smoking, touch provides the oral "feel" of cigarettes, cigars, pipes and mouthpieces during smoking or vaping as well as the texture of smokeless tobacco products in the mouth. Touch also senses changes in oral mucosal surfaces such as dryness and astringency *(51)* that can be caused by inhaled or oral tobacco products.

Chemaesthesis: in addition to evoking tastes and smells, some chemicals can produce sensations of temperature, touch or pain by stimulating receptors in the mucous membranes that normally respond to either weak or strong (noxious) mechanical or thermal stimulation *(47)*. Chemaesthesis plays a key role in the flavour of tobacco products, including the harshness of nicotine, acrolein and other chemical irritants in tobacco smoke and the coolness and "burning" cold sensations of menthol.

Interactive effects of flavour: a defining characteristic of flavour is integration of its multisensory components into coherent perceptions in the mouth. A particularly important interaction is the referral of retronasal odours to the mouth *(52, 53)*, which leads to mislabelling of odours as tastes, most commonly for odours that have taste-like qualities, such as "sweet" odours like vanilla, cherry and strawberry *(54)*. This is important, because it means that characterizing flavours often emerge as a seamless combination of sensations evoked by multiple, interacting sensory systems.

Flavour constituents of tobacco and e-cigarette products can also be important for their masking or inhibitory effects, such as masking a bitter taste by a sweet taste *(55)*. Some studies have found evidence that sweeteners also suppress

pain, particularly in infants *(56, 57)*. Sweeteners are present in smokeless tobacco products, for example, at levels that exceed those in confectionery products *(45)*. Patents filed by the tobacco industry describe procedures for adding artificial sweeteners to cigar wrappings and mouthpieces to ensure that the consumer perceives the product as sweet. Similarly, the appeal of mentholated products owes much to the appeal of their cool, minty flavour, but menthol also has analgesic effects that can reduce the sensory irritation and harshness of nicotine *(34, 58)* and other constituents of tobacco smoke *(59)* and smokeless tobacco products *(58, 60, 61)*.

  Summary: the multimodal, integrative characteristics of flavour make it an important contributor to the appeal of tobacco products, ranging from essential tactile and thermal qualities in the mouth and throat to both taste and olfactory constituents in chemically derived characterizing flavours. While regulating individual well-known flavouring ingredients such as menthol and artificial sweeteners could have a straightforward effect on the appeal of tobacco products, interactions among flavour constituents designed to heighten appeal through combined sweetness or mechanisms like masking and analgesia pose more difficult problems for designing regulatory strategies. As flavour perception integrates sensory experience and is subjective, both human sensory testing and chemical analysis will be required to identify "the concentrations above which an additive will impart a characterizing flavor" *(3)* or increase the appeal of a product by masking aversive tastes or sensations. For products in which the flavour is perceptible but ambiguous, labelling may serve to boost the perception and identification of the flavour.

6.6 Flavour receptors: a new science of flavour sensing and coding

Molecular, genetic, pharmacological and behavioural approaches have revolutionized flavour research, including the discovery of flavouring receptors. Olfactory receptors, also called odorant receptors, were identified first in nerve endings in the nasal olfactory epithelium. These receptors play a dominant role in the retronasal sensing of volatile flavourings in tobacco and nicotine products. Taste receptors were identified in the taste papillae of the tongue and palate. Humans have a single sweet taste receptor made up of two protein subunits (TAS1R2 and TAS1R3) that bind sugars such as sucrose (table sugar) and, with much greater affinity, artificial high-potency sweeteners, such as saccharin and sucralose. Bitter taste receptors, the TAS2R receptors, of which humans express 38 different versions, signal the presence of potentially poisonous chemicals and are probably involved in the perception of nicotine and other tobacco alkaloids as bitter. The presence of polymorphisms of the gene encoding a human bitter receptor, TAS2R38, has been linked with menthol cigarette smoking, suggesting

perhaps that smokers who are more sensitive to bitter tastes may use menthol to mask the bitter taste of nicotine or cigarette smoke *(62, 63)*.

Receptors that mediate the chemaesthetic properties of flavours and other tobacco constituents, the transient receptor potential (TRP) ion channels, are located on nerves that transmit noxious and innocuous chemical, mechanical and thermal stimuli from the oral and nasal passages and airways. TRPA1 is the receptor for noxious aldehydes in tobacco smoke, eliciting burning and irritating sensations. TRPA1 also mediates the irritating effects of nicotine and flavouring aldehydes such as cinnamaldehyde and benzaldehyde, present in many fruity flavours. TRPM8 is the receptor for menthol and mediates its cooling and soothing effects. Experiments in mice indicated that TRPM8 is essential for the suppression of the irritating and aversive effects of tobacco smoke and nicotine by menthol *(60, 64)*.

Summary: with more knowledge about flavour receptors and their pharmacology, the flavour industry can use molecular and pharmacological approaches to develop new, highly optimized flavour receptor modulators. These include novel sweet taste enhancers, bitter blockers (to reduce bitter taste), savoury (non-glutamate) taste enhancers and novel cooling agents. Several are approved as food additives, and more are in development. The tobacco industry has experimented with synthetic cooling agents that activate the menthol receptor, TRPM8, with a less minty odour, reduced irritancy and greater stability. The identification of flavouring receptors may provide an opportunity to regulate flavourings on the basis of their receptor-mediated pharmacological and behavioural effects in humans and animal model systems. For example, instead of regulating an individual flavour, such as menthol, which can be substituted quickly by alternative cooling agents, regulators could decide instead to control all TRPM8 receptor agonists in tobacco as a receptor-specific flavouring class.

6.7 Toxicological effects of flavours

While legislation and regulations vary by country, in many, inclusion of flavours as food additives requires scientific premarket review. In the European Union, flavouring additives are regulated by the European Food Safety Authority. In the USA, the FDA has designated the generally recognized as safe (GRAS) classification for food additives, in which stakeholders submit data on safety, which is reviewed for the intended use of a flavouring. The GRAS declaration for use of a flavour in confectionery products does not, however, automatically apply to its use in tobacco products, especially products that are inhaled, including cigarettes, cigars, hookah and electronic cigarettes. For example, cinnamaldehyde, the GRAS cinnamon flavouring widely used in baked goods and confectionaries, is added at very high concentrations to some electronic cigarette fluids *(65)*. In toxicological studies, vapours from these liquids damaged lung epithelial cells and caused pulmonary

inflammation in mice *(66)*. Other sweet flavours, including characterizing banana and cherry flavours, were also found to damage cells and expose e-cigarette users to benzaldehyde, a key component of many berry flavour mixes, with known toxic effects on the respiratory system *(67, 68)*. Diacetyl, a flavour chemical commonly found in buttery flavours, also has a well-known toxic effect on respiration *(69, 70)*. Flavour chemicals can react with solvents and other components of e-liquids to form irritant compounds with unknown toxicological effects *(71)*.

Despite these concerns, some manufacturers of e-cigarette and ENDS have used GRAS labelling in their advertising, implying that the flavourings in their liquids are safe because they were previously approved for addition to food. Such health claims were strongly refuted by the Flavor and Extracts Manufacturing Association, a body of the United States flavour industry that submitted GRAS applications to the FDA, which stated that use of flavouring in e-cigarettes is not considered an intended use *(72)*.

The lack of a regulatory process for evaluating the safety of flavouring agents is also a limitation for tobacco products such as *snus*, snuff and flavoured cigars, cigarettes and hookah tobacco, which may result in ingestion or inhalation of toxic levels of flavourings situations by consumers. For example, analytical studies showed that wintergreen-flavoured snuff products can expose regular users to levels of the wintergreen flavouring, methyl salicylate, that exceed the acceptable daily intake determined by the Food and Agriculture Organization of the United Nations and WHO for food by 12 times *(73)*. Smokeless tobacco products (including *zarda*, *quiwam*, *gutka* and *khaini* varieties) have been found to have high levels of flavour chemicals such as eugenol, coumarin, camphor and diphenyl ether *(43)*. Some *snus* products contain such high levels of sweeteners that regular use might exceed the recommended daily consumption of sugar if users consume other sweetened products at the same time *(45)*.

There is little information about the fate of flavourings in combusted tobacco products (cigarettes, cigars, hookah) or heated nicotine solutions (as in e-cigarettes). The tobacco industry reported no major differences in the levels of toxic constituents in the smoke of menthol capsules or *kretek* (clove) cigarettes and in equivalent cigarettes without flavouring *(74, 75)*; however, any toxic effects of flavours may have been masked by the overwhelming contributions of the other toxic constituents of cigarettes. In other studies, increased levels of VOCs were found in the smoke of flavoured cigarettes, and industry documents provide evidence that the industry knew about the carcinogenicity of some types of flavoured cigarettes *(76, 77)*. In these studies, only the main smoke constituents (such as TSNAs and PAHs) were measured; the specific chemical products resulting from combusted flavourings were not examined. Analysis of the chemical fate of e-cigarette flavourings may provide information on the toxicity of flavours. When high-voltage settings are used in e-cigarettes,

exposure to flavourings increases, and the levels of oxidation products such as formaldehyde exceed toxic levels. As cigarettes, cigars and hookah tobacco burn at higher temperatures than vapour-producing e-cigarettes, the possibility that larger amounts of flavouring oxidation products and other toxic chemical species may be formed is a concern. Evidence obtained for e-liquid flavours suggests that flavour chemicals can have toxic effects *(65, 78, 79)*.

Summary: regulations to specify acceptable levels of flavourings depend on the type of tobacco product, the level at which the flavouring is delivered to the consumer's oral and respiratory systems and the potential for chemical changes during storage, heating and combustion. The chemical fate of flavourings, especially when heated at high temperatures, should be investigated further. This may result in the addition of flavourings and their chemical products to the lists of HPHCs curated by national regulators such as the FDA and in TobReg reports on toxicants in tobacco products. Future regulation of tobacco products might require specific reporting of the levels and toxicity of flavour chemicals.

6.8   Conclusions

Critical questions remain about the role of flavours in the appeal of and addiction to tobacco and nicotine products. Surveillance should be conducted worldwide to assess the use of flavoured tobacco products and also perceptions about the appeal and addictive potential of flavoured and unflavoured products. Flavours and sweeteners are chemicals that can be appealing independently and can also increase the use of tobacco products by enhancing the palatability of nicotine and other bitter or harsh constituents. A flavour like menthol may do this by pharmacologically attenuating the aversive effects of exposure to nicotine (e.g. cough, harshness, heat), and the presence of menthol in cigarettes is probably associated with increased rates of initiation of and progression to regular cigarette smoking, development of addiction and difficulty in quitting smoking *(80)*. Much less is known about whether and how other flavour constituents influence preferences for and use of tobacco and nicotine products.

As flavours are complex and subjective, testing of flavoured tobacco and nicotine products for their appeal will require human behavioural and toxicological evaluations, in combination with chemical analyses. Methods for determining the presence and levels of flavourings should be based on what is delivered to the consumer (e.g. in smoke or vapour) and should also be used to determine potential changes in flavourings during storage, heating and combustion. Both the appeal and toxicity of flavourings should be evaluated. While it is important to determine the optimal doses of flavours that produce these effects, it is also important to understand the influence of the concentrations of flavours. All this information should be used to further refine the definition of a "characterizing flavour".

Surveillance and testing methods will have to keep up with the constant innovations of the tobacco industry designed to enhance the appeal of flavoured tobacco products, including use of new synthetic compounds and strategic placement of flavouring molecules in tobacco products. Declaration of flavourings and their concentrations on tobacco product labels should be considered. Additionally, regulatory work will have to contend with alternative marketing strategies introduced by the tobacco industry to deal with regulations on flavours, such as manipulation of packaging to continue to convey brand features associated with flavours *(41)*.

Future regulations should account for the possibility that some flavours and flavour constituents, like sweeteners, are also positively reinforcing in themselves and, when combined with nicotine, might enhance the rewarding effects of low-dose nicotine and promote a transition to higher levels of nicotine, leading to addiction. Experimental evidence and monitoring of these complex issues will be crucial to ensure that regulations are designed to reduce the appeal and addictive potential of tobacco products. Better understanding of the science of flavours and how they enhance the appeal and addictive potential of tobacco products will be required to regulate these molecules.

6.8.1   Recommended priorities for research

- Systematically monitor the global epidemiology of flavoured conventional, traditional, new and emerging tobacco and nicotine products.

- Identify flavour chemicals, their reaction products and their concentrations in tobacco and nicotine products and in aerosols, vapours and smoke.

- Determine how depiction of flavours on product packaging and in marketing alter public perceptions and the appeal of tobacco and nicotine products, especially among young people.

- Evaluate whether the presence of specific flavours and their concentrations in tobacco and nicotine products alters their appeal and abuse potential.

- Among smokers, investigate the role of the availability of flavoured products as a motive for switching to less harmful tobacco products (relative to the degree of harm reduction).

- Determine the toxicity and health effects of various concentrations of inhaled flavour chemicals and their metabolites and adducts.

- Monitor the use of alternative chemical moieties to replace prohibited flavours, and determine their appeal, toxicity and health effects.

6.8.2 Recommended policies

- Consider obtaining systematic global evidence on the use of flavoured tobacco and e-cigarette and ENDS products.

- Consider banning the use of flavours, including menthol, in harmful combusted products.

- Consider limiting the levels, number of and/or specific flavours allowed in tobacco and nicotine products for which there is evidence of modified or reduced risk, to reduce initiation by young people and support cessation of use of combusted tobacco products.

- Consider requiring that the types and concentrations of flavour chemicals in various products be listed among the product constituents.

6.9 References

1. Small DM, Green BG. A model of flavor perception. In: Murray MM, Wallace MT, editors. The neural bases of multisensory processes. New York: CRC Press; 2012:717–38.

2. Talhout R, van de Nobelen S, Kienhuis AS. An inventory of methods suitable to assess additive-induced characterising flavours of tobacco products. Drug Alcohol Depend. 2016;161:9–14.

3. Directive 2014/40/EU of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products. Brussels: European Union; 2014 (http://ec.europa.eu/health/sites/health/files/tobacco/docs/dir_201440_en.pdf, accessed 15 May 2019).

4. Federal Food, Drug, and Cosmetic Act. Section 907. Tobacco product standard. General questions and answers on the ban on cigarettes that contain certain characterizing flavors (edition 2). Silver Spring (MD): Center for Tobacco Products, Food and Drug Administration; 2009.

5. Carpenter CM, Wayne GF, Pauly JL, Koh HK, Connolly GN. New cigarette brands with flavors that appeal to youth: tobacco marketing strategies. Health Affairs. 2005;24(6):1601–10.

6. Connolly GN. Sweet and spicy flavours: new brands for minorities and youth. Tob Control. 2004;13(3):211–2.

7. Morris DS, Fiala SC. Flavoured, non-cigarette tobacco for sale in the USA: an inventory analysis of Internet retailers. Tob Control. 2015;24(1):101–2.

8. Delnevo CD, Wackowski OA, Giovenco DP, Manderski MT, Hrywna M, Ling PM. Examining market trends in the United States smokeless tobacco use: 2005–2011. Tob Control. 2014;23(2):107–12.

9. Zhu SH, Sun JY, Bonnevie E, Cummins SE, Gamst A, Yin L et al. Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. Tob Control. 2014;23(Suppl 3):iii3–9.

10. Hsu G, Sun JY, Zhu SH. Evolution of electronic cigarette brands from 2013–2014 to 2016–2017: analysis of brand websites. J Med Internet Res. 2018;20(3):e80.

11. Bonhomme MG, Holder-Hayes E, Ambrose BK, Tworek C, Feirman SP, King BA et al. Flavoured non-cigarette tobacco product use among US adults: 2013–2014. Tob Control. 2016;25(Suppl 2):ii4–13.

12. Kaleta D, Usidame B, Szosland-Faltyn A, Makowiec-Dabrowska T. Use of flavoured cigarettes in Poland: data from the global adult tobacco survey (2009–2010). BMC Public Health. 2014;14:127.

13. Corey CG, Ambrose BK, Apelberg BJ, King BA. Flavored tobacco product use among middle

and high school students – United States, 2014. Morbid Mortal Wkly Rep. 2015;64(38):1066–70.

14. King BA, Dube SR, Tynan MA. Flavored cigar smoking among US adults: findings from the 2009–2010 National Adult Tobacco Survey. Nicotine Tob Res. 2013;15(2):608–14.

15. Villanti AC, Johnson AL, Ambrose BK, Cummings KM, Stanton CA, Rose SW et al. Flavored tobacco product use in youth and adults: findings from the first wave of the PATH study (2013–2014). Am J Prev Med. 2017;53(2):139–51.

16. Minaker LM, Ahmed R, Hammond D, Manske S. Flavored tobacco use among Canadian students in grades 9 through 12: prevalence and patterns from the 2010–2011 youth smoking survey. Prev Chronic Dis. 2014;11:E102.

17. Global Youth Tobacco Survey (GYTS). Core questionnaire with optional questions, version 1.0. Geneva: World Health organization; Atlanta (GA): Centers for Disease Control and Prevention; 2012.

18. Global Adult Tobacco Survey (GATS). Core questionnaire with optional questions, version 2.0. Atlanta (GA): Centers for Disease Control and Prevention; 2010.

19. Ashare RL, Hawk LW Jr, Cummings KM, O'Connor RJ, Fix BV, Schmidt WC. Smoking expectancies for flavored and non-flavored cigarettes among college students. Addict Behav. 2007;32(6):1252–61.

20. Kowitt SD, Meernik C, Baker HM, Osman A, Huang LL, Goldstein AO. Perceptions and experiences with flavored non-menthol tobacco products: a systematic review of qualitative studies. Int J Environ Res Public Health. 2017;14(4):338.

21. Sokol NA, Kennedy RD, Connolly GN. The role of cocoa as a cigarette additive: opportunities for product regulation. Nicotine Tob Res. 2014;16(7):984–91.

22. Feirman SP, Lock D, Cohen JE, Holtgrave DR, Li T. Flavored tobacco products in the United States: a systematic review assessing use and attitudes. Nicotine Tob Res. 2016;18(5):739–49.

23. Choi K, Fabian L, Mottey N, Corbett A, Forster J. Young adults' favorable perceptions of snus, dissolvable tobacco products, and electronic cigarettes: findings from a focus group study. Am J Public Health. 2012;102(11):2088–93.

24. Manning KC, Kelly KJ, Comello ML. Flavoured cigarettes, sensation seeking and adolescents' perceptions of cigarette brands. Tob Control. 2009;18(6):459–65.

25. Hammal F, Wild TC, Nykiforuk C, Abdullahi K, Mussie D, Finegan BA. Waterpipe (hookah) smoking among youth and women in Canada is new, not traditional. Nicotine Tob Res. 2016;18(5):757–62.

26. Kong G, Morean ME, Cavallo DA, Camenga DR, Krishnan-Sarin S. Reasons for electronic cigarette experimentation and discontinuation among adolescents and young adults. Nicotine Tob Res. 2015;17(7):847–54.

27. Delnevo CD, Giovenco DP, Ambrose BK, Corey CG, Conway KP. Preference for flavoured cigar brands among youth, young adults and adults in the USA. Tob Control. 2015;24(4):389–94.

28. Morean ME, Butler ER, Bold KW, Kong G, Camenga DR, Cavallo DR et al. Preferring more e-cigarette flavors is associated with e-cigarette use frequency among adolescents but not adults. PLoS One. 2018;4:13(1):e0189015.

29. Garrison KA, O'Malley SS, Gueorguieva R, Krishnan-Sarin S. A fMRI study on the impact of advertising for flavored e-cigarettes on susceptible young adults. Drug Alcohol Depend. 2018;186:233–41.

30. Farley SM, Seoh H, Sacks R, Johns M. Teen use of flavored tobacco products in New York City. Nicotine Tob Res. 2014;16(11):1518–21.

31. How other countries regulate flavored tobacco products. Saint Paul (MN): Tobacco Control Legal Consortium; 2015 (http://www.publichealthlawcenter.org/sites/default/files/resources/tclc-fs-global-flavored-regs-2015.pdf, accessed 15 May 2019).

32. Henkler F, Luch A. European Tobacco Product Directive: How to address characterizing flavors as a matter of attractiveness? Arch Toxicol. 2015;89(8):1395–8.

33. Giovino GA, Sidney S, Gfroerer JC, O'Malley PM, Allan JA, Richter PA et al. Epidemiology of menthol cigarette use. Nicotine Tob Res. 2004;6 Suppl 1:S67–81.

34. Krishnan-Sarin S, Green BG, Kong G, Cavallo DA, Jatlow P, Gueorguieva R. Studying the interactive effects of menthol and nicotine among youth: an examination using e-cigarettes. Drug Alcohol Depend. 2017;180:193–9.

35. Noyori R. Asymmetric catalysis: science and opportunities (Nobel lecture). Angew Chem Int Ed Engl. 2002;41(12):2008–22.

36. Thrasher JF, Abad-Vivero EN, Moodie C, O'Connor RJ, Hammond D, Cummings KM et al. Cigarette brands with flavour capsules in the filter: trends in use and brand perceptions among smokers in the USA, Mexico and Australia, 2012–2014. Tob Control. 2016;25(3):275–83.

37. Abad-Vivero EN, Thrasher JF, Arillo-Santillan E, Pérez-Hernández R, Barrientos-Guitierrez I, Kollath-Cattano C et al. Recall, appeal and willingness to try cigarettes with flavour capsules: assessing the impact of a tobacco product innovation among early adolescents. Tob Control. 2016;25(e2):e113–9.

38. Erythropel HC, Kong G, deWinter TM, O'Malley SS, Jordt SE, Anastas PT et al. Presence of high intensity sweeteners in popular cigarillos of varying flavor profiles. JAMA. 2018;2:320(13):1380–3

39. Sweeney WR, Marcq P, Pierotti J, Thiem D. Sweet cigar. Google Patents; 2013.

40. Cundiff RH. Letter to Dr Paul Whiter, Wilkinson-Sword Ltd, Berkeley Heights (NJ)., 24 March 1975. Winston-Salem (NC): R.J. Reynolds Tobacco Co.; 1975 (Menthol Derivatives. RJ Reynolds Records. 1975 (https://www.industrydocumentslibrary.ucsf.edu/tobacco/docs/#id=ngdp0061, accessed January 2019).

41. Brown J, DeAtley T, Welding K, Schwartz R, Chaiton M, Kittner DL et al. Tobacco industry response to menthol cigarette bans in Alberta and Nova Scotia, Canada. Tob Control. 2017;26(e1):e71–4.

42. Stanfill SB, Croucher RE, Gupta PC, Lisko JG, Lawler TS, Kuklenyik P et al. Chemical characterization of smokeless tobacco products from South Asia: nicotine, unprotonated nicotine, tobacco-specific N´-nitrosamines, and flavor compounds. Food Chem Toxicol. 2018;118:626–34.

43. Lisko JG, Stanfill SB, Watson CH. Quantitation of ten flavor compounds in unburned tobacco products. Anal Meth. 2014;6(13):4698–704.

44. Kostygina G, Ling PM. Tobacco industry use of flavourings to promote smokeless tobacco products. Tob Control. 2016;25(Suppl 2):ii40–9.

45. Miao S, Beach ES, Sommer TJ, Zimmerman JB, Jordt SE. High-intensity sweeteners in alternative tobacco products. Nicotine Tob Res. 2016;18(11):2169–73.

46. Maziak W, Taleb ZB, Bahelah R, Islam F, Jaber R, Auf R et al. The global epidemiology of waterpipe smoking. Tob Control. 2015;24(Suppl 1):i3–12.

47. Green BG. Chemesthesis: pungency as a component of flavor. Trends Food Sci Technol. 1996;7:415–20.

48. Oliveira-Maia AJ, Phan THT, Melone PD, Mummalaneni S, Nicolelis MAL, Simon SA et al. Nicotinic acetylcholine receptors (NACHRS): novel bitter taste receptors for nicotine. Chem Senses. 2008;33(8):S113.

49. Lim J, Johnson MB. Potential mechanisms of retronasal odor referral to the mouth. Chem Senses. 2011;36(3):283–9.

50. Hummel T. Retronasal perception of odors. Chem Biodivers. 2008;5(6):853–61.

51. Green BG. Oral astringency: a tactile component of flavor. Acta Psychol. 1993;84(1):119–25.

52. Rozin P. "Taste–smell confusions" and the duality of the olfactory sense. Percept Psychophys. 1982;31:397–401.

53. Lim J, Green BG. Tactile interaction with taste localization: influence of gustatory quality and intensity. Chem Senses. 2008;33(2):137–43.

54. Small DM, Prescott J. Odor/taste integration and the perception of flavor. Exp Brain Res. 2005;166(3–5):345–57.

55. Green BG, Lim J, Osterhoff F, Blacher K, Nachtigal D. Taste mixture interactions: suppression, additivity, and the predominance of sweetness. Physiol Behav. 2010;101(5):731–7.

56. Kassab M, Foster JP, Foureur M, Fowler C. Sweet-tasting solutions for needle-related procedural pain in infants one month to one year of age. Cochrane Database Syst Rev. 2012;12:CD008411.

57. Schobel N, Kyereme J, Minovi A, Dazert S, Bartoshuk L, Hatt H. Sweet taste and chorda tympani transection alter capsaicin-induced lingual pain perception in adult human subjects. Physiol Behav. 2012;107(3):368–73.

58. Rosbrook K, Green BG. Sensory effects of menthol and nicotine in an e-cigarette. Nicotine Tob Res. 2016;18(7):1588–95.

59. Ferris Wayne G, Connolly GN. Application, function, and effects of menthol in cigarettes: a survey of tobacco industry documents. Nicotine Tob Res. 2004;6(Suppl 1):S43–54.

60. Fan L, Balakrishna S, Jabba SV, Bonner PE, Taylor SR, Picciotto MR et al. Menthol decreases oral nicotine aversion in C57BL/6 mice through a TRPM8-dependent mechanism. Tob Control. 2016;25(Suppl 2):ii50–4.

61. Liu B, Fan L, Balakrishna S, Sui A, Morris JB, Jordt SE. TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. Pain. 2013;154(10):2169–77.

62. Oncken C, Feinn R, Covault J, Duffy V, Dornela E, Kranzler HR et al. Genetic vulnerability to menthol cigarette preference in women. Nicotine Tob Res. 2015;17(12):1416–20.

63. 6Risso D, Sainz E, Gutierrez J, Kirchner T, Niaura R, Drayna D. Association of TAS2R38 haplotypes and menthol cigarette preference in an African American cohort. Nicotine Tob Res. 2017;19(4):493–4.

64. Willis DN, Liu B, Ha MA, Jordt SE, Morris JB. Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. FASEB J. 2011;25(12):4434–44.

65. Behar RZ, Davis B, Wang Y, Bahl V, Lin S, Talbot P. Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids. Toxicol In Vitro. 2014;28(2):198–208.

66. Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip D, McIntosh S et al. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PloS One. 2015;10(2):e0116732.

67. Gerloff J, Sundar IK, Freter R, Sekera ER, Friedman AE, Robinson R et al. Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography–mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts. Appl In Vitro Toxicol. 2017;3(1):28–40.

68. Kosmider L, Sobczak A, Prokopowicz A, Kurek J, Zaciera M, Knysak J et al. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. Thorax. 2016;71(4):376–7.

69. Allen JG, Flanigan SS, LeBlanc M, Vallarino J, MacNaughton P, Stewart JH et al. Flavoring chemicals in e-cigarettes: diacetyl, 2,3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes. Environ Health Perspect. 2016;124(6):733–9.

70. Klager S, Vallarino J, MacNaughton P, Christiani DC, Lu Q, Allen JG. Flavoring chemicals and aldehydes in e-cigarette emissions. Environ Sci Technol. 2017;51(18):10806–13.

71. Erythropel HC, Jabba SV, deWinter TM, Mendizabal M, Anastas PT, Jordt SE et al. Formation of flavorant–propylene glycol adducts with novel toxicological properties in chemically unstable e-cigarette liquids. Nicotine Tob Res. 2018. doi: 10.1093/ntr/nty192.

72. Safety assessment and regulatory authority to use flavors – focus on electronic nicotine delivery systems and flavored tobacco products. Washington (DC): Flavor and Extract Manufacturers Association; 2018 (https://www.femaflavor.org/safety-assessment-and-regulatory-authority-use-flavors-focus-electronic-nicotine-delivery-systems, accessed 15 May 2019).

73. Chen C, Isabelle LM, Pickworth WB, Pankow JF. Levels of mint and wintergreen flavorants: smokeless tobacco products vs. confectionery products. Food Chemical Toxicol. 2010;48(2):755–63.

74. Dolka C, Piade JJ, Belushkin M, Jaccard G. Menthol addition to cigarettes using breakable capsules in the filter. Impact on the mainstream smoke yields of the health Canada list constituents. Chem Res Toxicol. 2013;26(10):1430–43.

75. Roemer E, Dempsey R, Hirter J, Deger Evans A, Weber S, Ode A et al. Toxicological assessment of kretek cigarettes. Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol. 2014;70(Suppl 1):S66–80.

76. Gordon SM, Brinkman MC, Meng RQ, Anderson GM, Chuang JC, Kroeger RR et al. Effect of cigarette menthol content on mainstream smoke emissions. Chem Res Toxicol. 2011;24(10):1744–53.

77. Hurt RD, Ebbert JO, Achadi A, Croghan IT. Roadmap to a tobacco epidemic: transnational tobacco companies invade Indonesia. Tob Control. 2012;21(3):306–12.

78. Behar RZ, Luo W, McWhirter KJ, Pankow JF, Talbot P. Analytical and toxicological evaluation of flavor chemicals in electronic cigarette refill fluids. Sci Rep. 2018;8(1):8288.

79. Bengalli R, Ferri E, Labra M, Mantecca P. Lung toxicity of condensed aerosol from e-cig liquids: influence of the flavor and the in vitro model used. Int J Environ Res Public Health. 2017;14(10):1254.

80. Menthol cigarettes and public health: review of the scientific evidence and recommendations. Washington (DC): Tobacco Products Advisory Committee, Food and Drug Administration; 2011.

7. Sugar content of tobacco products

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Contents

7.1 Introduction

Sugar is a component of tobacco products and is often present in large amounts. Sugars are naturally present in tobacco leaves and may also be added during manufacture *(1–3)*. Unprocessed tobacco leaves contain many types of sugar, including glucose, fructose and sucrose. Drying (curing) of the leaf can affect these levels; while air-cured tobacco contains virtually no sugars, flue-cured tobacco may contain up to 25% of its weight *(4, 5)*. In addition, during manufacture, various types of sugar and sugar-containing ingredients, such as honey and fruit syrups, may be added to the casing. The amount added depends on the tobacco blend and the product type. Added sugars can serve as binders, casing ingredients,

flavours, formulation aids or humectants *(3, 4, 6)*. When sugars are burnt during combustion of tobacco products, they yield many toxic products, some of which have been hypothesized to affect the addictiveness of tobacco products *(7)*. Sugars also contribute significantly to the flavour of tobacco products *(3, 8)*. In smokeless tobacco, the sugars themselves flavour the tobacco product, whereas, in combusted tobacco products, sugars react with other components in the tobacco to produce caramel flavours and make the smoke milder to inhale *(3, 9)*. Some casing materials that contain sugars have been referred to as "ameliorants" by the tobacco industry and are claimed to "… smooth out harshness and bitterness and/or eliminate pungent aromas from tobaccos" *(10)*.

As sugar is one of the main components of tobacco products and may be harmful to consumers' health by increasing the toxicity, addictiveness and/or attractiveness of products, it may be an appropriate candidate for regulation of tobacco contents. We review the evidence on the presence and effects of sugars in different tobacco products, including smokeless and waterpipe tobacco. First, we describe the variety of sugars naturally present in tobacco leaves, those that result from curing and those that are added to processed tobacco during manufacture. We also report the percentages of products with added sugars and the amounts of sugars added. Secondly, we summarize the evidence on the effect of sugars on the levels of emissions from tobacco products. Thirdly, we review the health effects of sugars in tobacco products, as assessed by their toxicity, addictiveness and attractiveness. Finally, we highlight implications for research and regulation.

7.2 Sugars in different types of tobacco product

Tobacco is a natural plant, and its leaves contain high levels of carbohydrates such as sugars (mono- and disaccharides), starch, cellulose and pectin (polysaccharides) *(4)*. During drying (curing) of the green leaves into usable brown, dried tobacco, enzymes degrade starch into sugars. The curing conditions depend on the tobacco type. Virginia tobacco is flue-cured in humid conditions and at elevated temperatures, which allows starch to be degraded into sugar and stops enzymatic degradation of sugar, resulting in high levels (8–30%) in the cured tobacco *(11)*. Oriental tobacco is sun-cured in a process similar to flue-curing but less controlled, resulting in sugar levels of 10–20% in cured tobacco *(2)*. In contrast, Burley tobacco is air-cured at ambient temperature in a slow process that results in degradation of starch into sugars and breakdown of the sugars by enzymes, resulting in a low sugar content (≤ 0.2%) in the cured tobacco *(4, 11)*. As tobacco products contain one or a blend of tobacco varieties, the sugar levels of tobacco products differ widely, depending on the blend. In general, the sugars found most abundantly in the cured tobacco leaf are glucose, fructose and sucrose *(4, 11)*.

Besides mono- and disaccharides, tobacco also contains considerable amounts of (added or naturally present) polysaccharides, such as cellulose, pectin and starch *(9, 12)*. A conventional cigarette blend contains about 10% cellulose, 10% pectin and 2% starch *(4, 12)*. Thus, carbohydrates may comprise over 40% of tobacco and therefore have a substantial impact on the chemical composition of tobacco smoke *(3)*.

7.2.1   Types of sugars and sugar-containing additives

Besides the sugars present in natural tobacco leaves, various types of sugar and sugar-containing ingredients are added to tobacco products, primarily in the casing. The types of sugars depend on the many natural sources and processing methods used. The sugars most often added to combusted and smokeless tobacco products are sucrose and invert sugar (a mixture of fructose and glucose) *(2, 13, 14)*. Other tobacco additives that contain large amounts of sugars are fruit juices, corn and maple syrups, molasses extracts, honey and caramel *(3, 4, 6, 15)*. Other substances may also have a sweet taste and characteristics similar to those of sugar but are not classified as such. These are acesulfame K, aspartame, ethyl maltol, glycerol, propylene glycol, maltitol, maltol, saccharin, sorbitol and thaumatin.

Sugars in tobacco products identified from manufacturers' lists of ingredients in the electronic database EMTOC *(16)* are: brown sugar, caramel, (corn) syrup (solids), dextrin, dextrose, (fruit) concentrate, (fruit) juice, glucose, high-fructose sugar, honey, invert sugar, lactose, malt extract, maltodextrin, (maple) syrup, molasses, (partially) inverted sugar, sucrose (syrup), sugar cane (syrup), sugar syrup and unspecified sugar. Monosaccharides are simple sugars such as fructose, galactose and glucose, with the general formula $C_6H_{12}O_6$. Disaccharides are formed by the combination of two monosaccharide molecules with the exclusion of a molecule of water; these include sucrose, lactose and maltose, with the general formula $C_{12}H_{22}O_{11}$.

7.2.2   Amounts of sugars and sugar-containing additives

Sugars are usually added to tobacco leaves as a casing ingredient, although in some cases they are added in very small amounts to non-tobacco material like paper, filter and glue *(16)*. The amount of sugar added to conventional cigarettes represents 2–18% of the weight of the tobacco *(2, 12, 17, 18)*. The levels in waterpipe tobacco (*maassel*) are much higher, representing 50–70% of the weight of the product *(19)*. Little information is available on the amounts and types of sugars in tobacco products. In the Netherlands, manufacturers are obliged to disclose the ingredients of all tobacco products on the Dutch market annually to an electronic database (EMTOC) *(16)*. Analysis of these data shows that, in 2015, sugars were added to a substantial proportion of all types of tobacco product *(20)*.

Further analysis of the data for the purposes of this paper showed that most roll-your-own products (56%), cigarettes (77%), pipe tobacco (86%) and waterpipe products (100%) contained added sugars (Fig. 7.1A), whereas few cigars (7%) or oral tobacco products (22%) did so. The sugars used most frequently in all tobacco products are invert sugar and glucose, with sugar-containing ingredients such as caramel, honey, syrups and fruit juices (Fig. 7.1B–G). The weight percentage of sugar added was 2% in cigars, 3% in roll-your-own tobacco and in cigarettes and 12% in pipe tobacco. Remarkably, an average waterpipe tobacco product contained added sugars that represented 164% of the tobacco weight, indicating more sugar than tobacco in the final product. Although the amount of sugar added to oral tobacco was only 10% of the tobacco weight in this analysis, the final sugar levels in these smokeless tobacco products may be about 35% *(21)* because of the high levels of natural sugars present in tobacco leaf. These numbers depend on the types of tobacco used in products in different countries.

Fig. 7.1. Concentrations of sugars in tobacco products
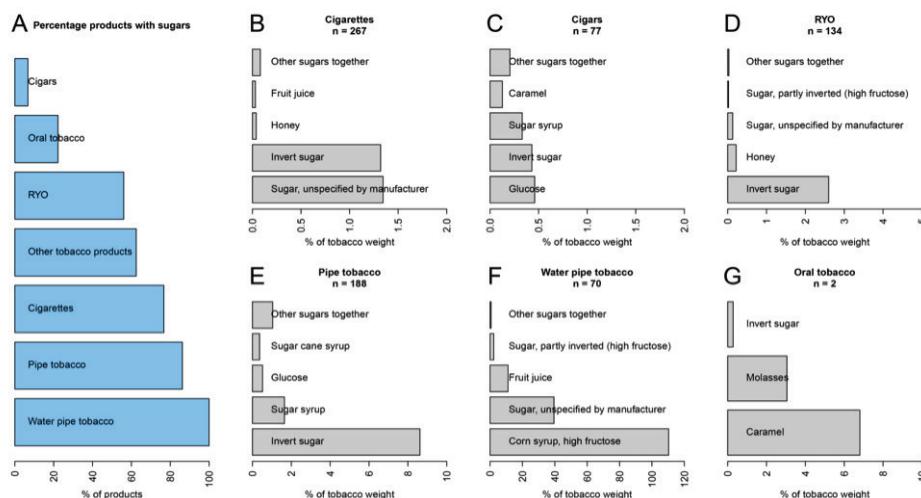
RYO, roll-your-own. A: percentage of products with sugar added to tobacco according to the total number of products containing additives, for each category of tobacco product; B–G, average amount of the sugars (percentage per mg tobacco) used most often for each category of tobacco product; n, number of products containing added sugar; "other sugars together" shows all remaining sugars in the product group. *Source*: EMTOC database *(16)*, in which manufacturers register all marketed tobacco products in the Netherlands annually and which contains information on additive composition, quantities added and function.

7.2.3   Total levels of added and endogenous sugars

Natural tobacco contains large amounts of sugar formed during growing and curing, as discussed above, besides the sugars added during manufacture. Therefore, it is more informative to measure or report the final sugar levels in

marketed tobacco products rather than that on the list of added sugars, which will provide an underestimate of the final level *(22)*. Few reports of laboratory analyses of total sugar levels in tobacco products have been published. Chemical analysis of final tobacco products for the most commonly added carbohydrates (glucose, fructose and sucrose) indicated total sugar levels of ≤ 19% of the weight of tobacco in cigarettes *(23)*, 0.1% in cigars, 0.03% in snuff and 27.7% in chewing tobacco *(24)*.

7.2.4   Regional and cultural differences in tobacco varieties, products and use

The variety of tobacco products marketed and used depends on regional and cultural preferences. In a study of 2the patterns of tobacco use among adolescents in 32 countries *(25)*, the national prevalence of current cigarette smoking ranged from 1.8% in Rwanda to 32.9% in Latvia, whereas the prevalence of current smokeless tobacco use ranged from 1.1% in Montenegro to 14.4% in Lesotho. In most European countries and the USA, the prevalence of current smoking was significantly higher than that of smokeless tobacco use, in contrast to the patterns observed in low- and middle-income countries such as in south-east Asia. Eurobarometer reported that, in Europe, smokeless tobacco is particularly popular in Sweden, whereas waterpipe use is popular in other northern European countries and northern Africa *(26)*. Experience with tobacco use may vary by culture due to differences in availability, affordability and acceptability *(25)*.

More information is available on the variety and blends of tobacco used in cigarettes, although little is known about the sugar content of specific products and brands. The use of sugars differs by brand and even within a brand because of national preferences *(2)*. The variety of tobacco also differs by region; in general, however, the cigarette market appears to be dominated by two varieties: flue-cured cigarettes and American-blend cigarettes. According to PMI, in 2008, Virginia-style (flue-cured, high in sugar and few additives) cigarettes dominated the market in Canada (99%), New Zealand (95%), Australia (92%), the United Kingdom (91%), Ireland (87%) and South Africa (76%). American-blend cigarettes (a blend of Virginia, Burley and Oriental tobacco and many additives, including sugars) are preferred in continental western European countries, with a market share of 85–100%, and in the USA, with 99% *(1)*.

7.3   Effects of sugars on levels of emissions from tobacco products

7.3.1   Smokeless tobacco products

Users of smokeless tobacco products are exposed to emissions resulting from chewing or sucking. While smokeless tobacco contains high levels of sugars (see above), the amounts extracted during use and the resulting exposure to sugars are unknown.

7.3.2 Pyrolysis products of sugars and simple mixtures

Combustion in a burning cigarette has been simulated in pyrolysis studies *(27, 28)* in order to understand precursor–smoke constituent relations in cigarette mainstream smoke *(1)*. Studies with this method indicate that only small amounts of nonvolatile sugars in tobacco (approximately 0.5%) are transferred intact into cigarette mainstream smoke, whereas most of the sugar combusts, pyrolyses or is part of other pyrosynthesis processes *(1, 3)*. Combustion processes such as caramelization result in many different chemical compounds, including aldehydes (e.g. acetaldehyde, acrolein, 2-furfural), furan derivatives, VOCs, organic acids, acrylamide and PAHs (usually at high temperatures). The pyrolysis conditions used in these studies only approximate the temperature and other conditions in a burning cigarette, however, and do not account for the interaction of other tobacco and/or smoke components with sugars during combustion. For example, sugars can react with amines in tobacco (ammonium compounds, amino acids, proteins) to form Maillard products (see also section 7.2), which can break down into chemical species such as aldehydes, ketones (e.g. diacetyl), acids, acrylamide, pyrazines and pyridines *(1, 3)*.

7.3.3 Cigarettes and other combusted tobacco products

An independent review of the effects of the addition of sugar on smoke composition in 2006 concluded that adding sugars to cigarette tobacco primarily enhances the levels of aldehydes and ketones, especially formaldehyde, acetaldehyde, acetone, acrolein, 2-furfural and other furans *(3)*. Additionally, the concentration of total acids in mainstream smoke was increased, resulting in a lower pH and less free-base nicotine. Cheah et al. *(29, 30)* also showed that the addition of sugars to Burley tobacco, which contains virtually no natural sugars, increases the concentrations of the aldehydes acetaldehyde, acrolein, crotonaldehyde, propionaldehyde and butanal in mainstream tobacco smoke. The increase is specific for aldehydes, as much smaller increases were observed in tar, nicotine and CO levels.

While many of these conclusions have been discussed in tobacco industry papers and reviews published subsequently, there has been considerable debate about whether sugars increase the levels of acetaldehyde and, to a lesser extent, of acrolein. For instance, Seeman et al. *(12)* pointed out that cellulose rather than sugar is the main precursor of acetaldehyde in smoke but that this by itself does not imply that sugars do not also contribute. In a review of cigarette tobacco additives, Klus et al. *(1)* concluded (from the results of many studies) that adding sugars to tobacco increases the concentrations of phenol, furans, organic acids, 2-furfural and formaldehyde and results in more acidic smoke. He did not report an association between sugars and acetaldehyde. Baker *(31, 32)* reported an increase in formaldehyde and found that sugars increase the levels of 2-furfural in mainstream cigarette smoke but not those of acetaldehyde or acrolein**.** Coggins

et al. *(33)* found that the addition of carbohydrates and sugar-containing natural products to tobacco resulted in only minimal changes in smoke chemistry but consistently resulted in a small increase in formaldehyde over that in the smoke of additive-free cigarettes. Roemer et al. *(2)* reported that, while most of the smoke constituents determined did not show any change in yield (per mg nicotine), increasing added sugar levels either increased (formaldehyde, acrolein, 2-butanone, isoprene, benzene, toluene, benzo[*k*]fluoranthene) or decreased (4-aminobiphenyl, *N*-nitrosodimethylamine, NNN) the levels of constituents. Hahn and Schaub *(34)* reported an increase in the concentration of formaldehyde but not that of acetaldehyde after addition of 5% sucrose as compared with a reference tobacco blend consisting of 50% Virginia tobacco (flue-cured), 20% Burley tobacco (air-cured), 20% tobacco stems and 10% Oriental tobacco (sun-cured). As Virginia tobacco contains high levels of natural sugars, relative increases in sugar and resulting aldehyde levels after addition of sugar are less evident.

O'Connor and Hurley *(35)* found that the relation between sugar and acetaldehyde levels is obscured by normalizing acetaldehyde yields to tar or total particulate matter, both of which are directly related to design features such as ventilation and mass of tobacco. They re-analysed a study by Zilkey et al. *(36)* and found that sugar accounted for over 50% of the variation in acetaldehyde levels in smoke. Re-analysis of the data of Phillpotts et al. *(37)* showed that sugars in tobacco blends accounted for an additional 11% variation in aldehyde levels, while total particulate matter accounted for 23% of the variation.

Waterpipe tobacco contains the highest levels of sugar of all tobacco products. Although this may result in higher levels of volatile aldehydes in smoke *(38, 39),* no data were available on the relation between sugar content and the composition of the smoke of waterpipe tobacco or of any other combusted tobacco products. Furthermore, correlations have been observed between sugar levels in e-cigarette liquids and aldehyde levels in their emissions *(40)*.

7.4 Effects of sugars on the toxicity of tobacco products

Sugars are GRAS additives when used in food products but not when used in tobacco *(3)*. When tobacco products are smoked, sugars are combusted to yield many toxic and carcinogenic reaction products. Furthermore, compounds are generally more toxic when inhaled than when ingested, because the respiratory system largely lacks the detoxifying metabolic pathways of the digestive system *(3)*.

7.4.1 Smokeless tobacco

Commercially available smokeless tobacco contains 0.5–2 g of sugars per chew of about 10 g *(41)*. The product is kept in the oral cavity for an average of 30 min and used repeatedly each day *(42, 43)*. Smokeless tobacco causes several types of cancer, cardiovascular disease, adverse reproductive outcomes and

adverse effects on oral health, including oral mucosal lesions, leukoplakia and periodontal disease *(44)*, but little is known about the contribution of the sugar content in smokeless tobacco to these diseases. Use of smokeless tobacco has been associated with dental caries, and a plausible cause is the high level of sugars, although other causes related to tobacco use cannot be excluded. The sugar in smokeless tobacco forms an acid that may eat away the tooth enamel, cause cavities and result in mouth sores *(42)*. Furthermore, the high levels of fermentable sugar can stimulate the growth of cariogenic bacteria *(14, 43)*.

Sugars in smokeless tobacco may also influence blood glucose. A case study showed that blood glucose levels in a patient with diabetes who stopped swallowing the juice resulting from chewing tobacco dropped by 50% (from 300–400 mg/dL to 160–200 mg/dL) *(41)*.

7.4.2   Cigarettes with and without added sugar

Many pyrolysis products of sugar have been reported to have toxic effects *(45, 46)*. These include formaldehyde (irritant, carcinogenic), acrolein (irritant) and PAHs (carcinogenic). The extent to which they contribute to the total toxicity of combusted tobacco products is unknown.

In a review of tobacco additives, Klus et al. *(1)* concluded that "sugars and the kind of sugar used as additive have – if any – only small and unimpressive effects on cigarette mainstream smoke toxicity". Mainstream cigarette smoke resulting from the combustion of cigarette tobacco with various levels of sugars was investigated in assays for cytotoxicity, mutagenicity and genotoxicity in vitro, in subchronic studies of inhalation toxicity in vivo and in studies of dermal tumorigenicity. None of the studies showed significant differences in markers of toxicity *(2, 33)*. According to the Scientific Committee on Emerging and Newly Identified Health Risks of the European Union *(47)*, however:

comparative toxicity testing strategies, …, are not considered suitable to address the properties outlined in the terms of reference with the currently available methodology. Indeed, at present, these studies lack discriminative power due to the high background toxicity of tobacco products and their results cannot be generalized to all products and brands, having a different composition with respect to tobacco type, blend and additives.

7.5   Effects of sugar on the addictiveness of tobacco products

No information was available on the role of sugar in the addictiveness of smokeless tobacco products. Sugar may, however, increase craving and reinforcement of use of combusted tobacco products, in addition to the well documented addictive effects of nicotine *(7)*. The addictive potency of sugar in combusted tobacco products may be increased by several direct and indirect pathways, including the pH of smoke, free nicotine levels and the formation of pharmacologically active compounds.

7.5.1 pH and free nicotine

The amount of nicotine that reaches the brain depends on the availability of free-base nicotine (the uncharged, volatile form), which formed at specific pH. High pH results in more free-base nicotine *(48, 49)*, which readily crosses the cell membranes of the oral cavity and lung epithelium, resulting in higher levels of nicotine that reach the brain. Tobacco products and brands with high carbohydrate or sugar contents generate more acidic smoke, resulting in lower concentrations of free-base nicotine *(50)*. To maintain a satisfying level of nicotine absorption from cigarettes with lower levels of free-base nicotine, smokers inhale more deeply and/or more frequently or increase their cigarette smoking frequency *(51, 52)*. This leads to higher exposure to carcinogenic and toxic compounds. (For other effects of changed smoke pH see section 7.7.) Other studies suggest, however, that the pH-buffering capacity of the lung epithelium diminishes the effect of changed nicotine bioavailability when the pH of tobacco or smoke is changed *(1)*.

7.5.2 Formation of pharmacologically active compounds in tobacco and smoke

Although sugars may have no addictive potential per se, combustion of sugar in a tobacco product results in several compounds in tobacco smoke that may have addictive potential. Of these, acetaldehyde is the most important *(12, 53, 54)*, as it increases the firing rate of dopaminergic neurons in the ventral tegmental area *(54)*. In experimental animals, intravenous acetaldehyde was addictive and synergistically enhanced the reinforcing effects of nicotine *(53, 56–60)*. Although acetaldehyde clearly reinforces the effects in rodents, it is not known whether these effects also occur in humans and at the concentrations found in cigarette smoke.

Acetaldehyde exerts its addictive potential by reacting with the amino acids tryptamine and tryptophan, present in tobacco or in the body, resulting in the condensation product harman (and norharman when amino acids reacts with formaldehyde) *(61, 62)*. Harman inhibits the enzyme monoamine oxidase, which degrades neurotransmitters involved in drug addiction, like dopamine, noradrenaline and serotonin *(56, 61, 63)*. Exposure to harman therefore increases the amounts of dopamine and serotonin in the nucleus accumbens and potentiates the action of nicotine *(64–66)*. Interestingly, an L-cysteine lozenge that reacts with acetaldehyde in saliva lowers the levels available for the formation of harman and showed potential for use as smoking cessation therapy in an initial double-blind, randomized, placebo-controlled trial *(67)*.

Several studies have shown that the levels of harman and norharman in smokers are related to the number of cigarettes smoked *(68–70)*, and lower activity of monoamine oxidase in tobacco smokers increased nicotine self-administration *(57)* and maintenance of behavioural sensitization to nicotine *(56, 71)* as compared with nonsmokers.

7.6   Dependence and quitting

Sugar in tobacco products can contribute to maintenance of tobacco use by enhancing dependence and making it difficult to quit. The tobacco industry performed several intercountry comparisons to investigate the influence of additives on the addictiveness of tobacco. A meta-analysis of clinical studies on smoking cessation rates did not show a significant difference in quit rates between tobacco markets dominated by Virginia flue-cured cigarettes (high in natural sugar but few added ingredients and no added sugars) and by American-blended cigarettes (many added ingredients including sugars) *(72, 73)*. An important assumption in the study, however, was that the difficulty of quitting smoking is a valid measure of tobacco addictiveness, and no correction is needed for country-specific factors such as product availability and cessation programmes *(74)*. Also, no relevant differences were found between the two markets in smoking prevalence, intensity, some markers of dependence, nicotine uptake or mortality from smoking-related lung cancer or chronic obstructive pulmonary disease *(73)*. According to Klus et al. *(1)*, no effect of sugars was to be expected, given that the total sugar level (naturally present and added) in the tobacco of American-blend and Virginia cigarettes is comparable.

Little is known about the dependence and quitting rates of smokers of specific types of tobacco products with different sugar contents *(72)*. Much of the research on the abuse potential associated with acetaldehyde is based on studies of ethanol, as acetaldehyde is also a metabolite of ethanol *(75)*, and no proper experiments have been conducted to evaluate withdrawal from acetaldehyde vapour *(76)*. Nevertheless, the synergistic action of nicotine and acetaldehyde was observed only in young and not in adult rats *(58)*, which may support the observation that, in humans, adolescents appear to be more prone to tobacco addiction than adults *(58, 65, 77)*.

7.7   Effects of sugars on the attractiveness of tobacco products

7.7.1   Perception: sensory characteristics

The sensory characteristics of tobacco products, such as taste and smell, significantly influence their attractiveness *(7)*. For instance, initial attraction may be established by the presence of flavourings like sugar and other sweeteners *(3, 9, 78)*. Manufacturers select tobacco for certain chemical criteria, such as sugar and nicotine content, and the annual variation in tobacco leaf composition is equilibrated by the use of sugars and other additives to ensure the consistent taste of a product over time *(7)*. Sugar in smokeless tobacco products is perceived by the taste receptors in the oral cavity with olfactory stimulation. In contrast, users of combusted tobacco products perceive only the pyrolysis products of sugar. Overall, manufacturers of tobacco products adjust the taste and characteristics of products to the desires of consumers and to create a "pleasant experience" *(79)*.

7.7.2   Smoking experience and behaviour: flavour, palatability, ease of inhalation, frequency of use

Sugars can improve several characteristics of tobacco products, such as masking the harshness of the smoke and improving the taste. Volatile basic components like ammonia, nicotine and alkaloids give tobacco smoke a harsh taste, which prevents smokers from inhaling *(3, 80)*. Combustion of sugar during smoking results in acids, which reduce the pH of inhaled smoke *(50)* and thus decrease its harshness and irritability *(4, 9)*, increase the palatability of the product and facilitate inhalation. More frequent, deeper inhalation, which is eased by the addition of sugar, increases exposure to nicotine and other smoke chemicals. Sugars also play an important role in tobacco flavour *(4, 6, 81)*. In smokeless tobacco, the sugar itself is consumed. During curing, storing, processing and smoking of tobacco, amino acids or ammonia react with sugars (Maillard reaction) to produce chemicals with highly diverse structures and flavouring potential. Many of these products are heterocyclic compounds, which include aromatic pyrazines, an important class responsible for the characteristic taste of certain cigarette brands. In addition, caramelization of the sugar improves the taste and smell of the tobacco smoke for both users and bystanders *(79)*. This is, unlike the Maillard reactions, a non-enzymatic browning reaction of sugar (no amines involved).

7.7.3   Initiation

The acceptance of tobacco smoke by smokers is partly proportional to the sugar level in the tobacco *(3, 8)*. The extent to which sugar in tobacco products and its level influence initiation of tobacco consumption has not been investigated. As sugar reduces pH, masking the bitter taste of cigarette smoke, new users experience less harshness. Furthermore, the sweet taste of the caramel flavours generated by the combustion of sugars is particularly attractive to adolescents *(53, 82)*. Thus, the presence of sugar in tobacco products may encourage adolescents to start smoking earlier, continue smoking for longer and increase their tobacco use *(3)*. The addition of sugars to cigarettes to stimulate or enhance the sensory attributes of cigarette smoke and encourage smoking initiation and maintenance is not only scientifically plausible but has been discussed by industry as part of their marketing strategy *(74, 83)*.

7.8   Regulation of sugars according to jurisdiction

Several international and national authorities regulate additives in tobacco products. The most important of them and the main regulations and implementations are listed below.

- ▪ **WHO FCTC**: Article 9 addresses the testing and measurement of the contents and emissions of tobacco products and their regulation, and

Article 10 addresses the regulation of tobacco product disclosures, by allowing Parties to the WHO FCTC to adopt and implement effective legislative, executive, administrative or other measures requiring manufacturers and importers of tobacco products to disclose to government authorities information about the contents and emissions of tobacco products. The Framework facilitates adoption of comprehensive tobacco control measures by States Parties, which may include reporting and disclosure, such as the requirement to submit information on sugar levels (and the emission of sugar-related compounds) in tobacco products *(84)*.

- **Health Canada**: the use of many ingredients, including sugars, in cigarettes, little cigars and blunt wraps has been prohibited since 2009 in an amendment to Bill C-32 *(85)*. Moreover, Canada requires manufacturers and importers to provide information on tobacco product ingredients, as set out in the Tobacco Reporting Regulations *(86)*.

- **The European Tobacco Product Directive:** in accordance with Directive 2014/40/EU, a ban on characterizing flavours in cigarette and roll-your-own products (but not in other tobacco products) entered into force in 2014 *(87)*. Furthermore, manufacturers in each European Union Member State are required to disclose all marketed tobacco products and their composition (e.g. amount and type of added sugars, tobacco variety or blend, curing method) annually to an electronic database (EU-CEG). The Directive states that the sugars lost during curing of leaves may be replaced during manufacture; however, the initial sugar content of the tobacco leaf and loss during processing are not defined, which makes it difficult to regulate the amounts of sugar that can be added.

- In the **United Kingdom** in 2003, a voluntary agreement with the tobacco industry set limits on the use of several sugars (sucrose, invert sugar, syrups and molasses), with a maximum of 10% weight of the product in cigarettes, roll-your-own tobacco and cigars and 15% weight in pipe tobacco *(88)*. The rationale for determining these limits was not clear. The agreement has been superseded since 2014 by the revised European Tobacco Product Directive *(89)*.

- **Brazilian Health Regulatory Agency (Anvisa):** the use of some additives in tobacco products has been restricted in a regulatory resolution with legal power (RDC 14/2012) *(90)*. The resolution prohibits the use of sweeteners, honey, molasses, (any product originating from) fruits and substances that can impart a sweet flavour, apart from sugars. An exception was made for sugar, allowing manufac-

turers to restore the quantity lost during tobacco leaf curing. In September 2013, this resolution was suspended by a lawsuit filed by the National Confederation of Industries on behalf of tobacco product manufacturers *(90)*. A working group of independent experts recommended that RDC 14/2012 be amended such that sugars would no longer be excluded from the ban on additives *(91)*. As of June 2015, the Supreme Court injunction allowing the use of additives remained in force, with a final decision pending.

- **FDA:** the Family Smoking Prevention and Tobacco Control Act in 2009 included a ban on cigarettes containing certain characterizing flavours *(92)*. Restrictions are considered in the use and sale of certain flavored e-liquids *(93)*. No specific requirements or limits are set for the sugar content of cigarettes or other tobacco products.

7.9 Conclusions

Added sugar is one of the main ingredients in many types of tobacco product. The amount may be substantial, making up 2–164% of the weight of tobacco, depending on the type of product. As tobacco leaves themselves may contain high levels of natural sugar, the total amount of sugar (natural and added) in a tobacco product may be quite high. Limited data were available on the total sugar levels in different types of tobacco products.

There is little evidence that sugar contributes to the toxicity and addictiveness of smokeless tobacco products, apart from effects on dental health and possibly diabetes; however, sugars contribute to the attractiveness of the products by improving their taste.

Burnt and unburnt sugars contribute to the appeal of all tobacco products, and the combustion of sugar in tobacco products may contribute to the formation of toxic, carcinogenic and addictive compounds.

Some jurisdictions, such as Canada and Brazil, regulate the addition of sugars to tobacco products, and limits have been set in the United Kingdom. Stricter control measures are needed to prevent the harmful effects on health of the use of tobacco products containing both naturally occurring and added sugars.

7.10 Recommendations

7.10.1 Further research

There are still large gaps in understanding the health effects of sugar in tobacco and tobacco products, as stressed by the editor of *Nicotine and Tobacco Research (94)*. In particular, more information is required on:

- the relation between sugars in waterpipe tobacco and toxicants in smoke;

- biomarkers of exposure relevant to the effects of sugars on health and the impact of changing sugar levels on those biomarkers;

- the role of sugars in the attractiveness of tobacco products to children and young adults, e.g. by monitoring market shares according to sugar content and by marketing studies;

- the amount of sugars that alters the overall sweetness of emissions and imparts a noticeable characterizing flavour;

- the effect of sugars on overall rates of initiation, addictiveness, dependence and quitting according to the type of tobacco product;

- the role of acetaldehyde, in the amounts measured in tobacco smoke, on the addictive potential of (nicotine in) tobacco through monoamine inhibitors such as harman and formation of acetaldehyde–biogenic amine condensation products in vivo; and

- the effects of sugars on the overall toxicity of tobacco products, including:

 – for smokeless tobacco products, more information on the toxic effects of the sugars themselves, e.g. whether they are associated with diabetes and dental caries;

 – for combusted tobacco products, more information on the role of sugars in inhalation toxicity; and

 – interactions of sugars with other smoke components that may increase the toxicity of smoke.

7.10.2 Policy

Several policy measures may be considered for reducing the negative effects of sugar (and its pyrolysis products) on product toxicity, addictiveness and attractiveness.

Mandate disclosure of sugar levels

Manufacturers of tobacco products should disclose the levels of sugar (and other ingredients) in all types of tobacco product. This would provide valuable information for future regulations and regulatory decisions. Simple, fast methods are available for verifying reported levels, e.g. extraction of tobacco, followed by high-performance liquid chromatography–evaporative light-scattering detection *(23)*.

Mandate lowering of sugars in final tobacco products

Defining and regulating the maximum sugar levels in tobacco products might best protect consumers, as the actual source of sugars present naturally in tobacco or added during manufacture does not define its health effects. If desired and

supported by sufficient scientific evidence, the maximum sugar level could be lowered to virtually 0, the sugar level present in Burley tobacco leaves.

Mandate lowering of sugar in all tobacco products

Canada has banned added sugar in cigarettes, little cigars and blunt wraps but not in other products, such as waterpipe and smokeless tobacco. Regulation of sugar in only some tobacco products is likely to encourage users to shift to other products. Thus, the ban on flavouring additives in cigarettes in Canada and the USA increased the market and consumption of flavoured small cigars *(86, 95)*.

Mandate disclosure and lowering of the most harmful components of tobacco smoke

To decrease the negative effects of smoking, upper limits could be set for the most harmful components resulting from the combustion of sugar in tobacco smoke *(3)*. An analytical method for aldehydes, the most harmful emission compounds resulting from the combustion of sugar, is validated by WHO TobLabNet, and TobReg has included aldehydes on its list of smoke components proposed for mandated lowering *(45)*. The COP identified these as priority toxicants for which methods should be validated. Cunningham et al., of British American Tobacco, supported the inclusion of aldehydes as a priority. In their paper on segregation of tobacco smoke toxicants for risk assessment and management purposes, acetaldehyde, acrolein and formaldehyde, with a margin of exposure < 10 000, are considered high priorities for research on reducing exposure *(96)*.

Support research on the toxicity, addictiveness and attractiveness of sugar

Regulators could support research and reporting on compounds that result from the combustion of sugar in tobacco products and their toxicity, carcinogenicity, mutagenicity, reproductive toxicity, addictiveness and attractiveness. Scientific guidelines and recommendations have been issued for assessing the impact of tobacco additives on toxicity *(97)*, addictiveness *(98)* and attractiveness *(99)*.

Change the status and definition of "sugar"

Another important means of increasing attention to and scientific research on sugar would be to include it on national and international lists of target ingredients and constituents, e.g. the European priority list *(100)*, in the next intended round of revision. The list was established in line with Article 13 of the European Union Tobacco Products Directive *(87)* and includes additives for which more scientific experimental reporting is required on cigarettes and roll-your-own tobacco, including their toxicity, addictiveness and carcinogenic, mutagenic or reproductive toxicity in unburnt and burnt forms. Sugar is not yet included, although it meets all the selection criteria (Articles 6.1 and 6.2 of the Directive).

Require disclosure, and monitor levels of sweeteners in tobacco products, in addition to sugar

Besides sugar, the (high-intensity) sweetener content of tobacco products is used efficiently to control product palatability and to increase initiation among adolescents. High-intensity sweeteners are several hundred times sweeter than sucrose *(101)* and are present in many alternative tobacco products. Regulation of sweetener content might therefore be a means of controlling the palatability of a wide range of products and reducing initiation of tobacco product use. For instance, Canada has already banned sweeteners and several flavouring additives, in addition to sugar.

7.11 Acknowledgements

7.12 References

1. Klus H, Scherer G, Muller L. Influence of additives on cigarette related health risks. Contrib Tob Res. 2012;25(3):412–93.
2. Roemer E, Schorp MK, Piadé JJ, Seeman JI, Leyden DE, Haussmann HJ. Scientific assessment of the use of sugars as cigarette tobacco ingredients: a review of published and other publicly available studies. Crit Rev Toxicol. 2012;42(3):244–78.
3. Talhout R, Opperhuizen A, van Amsterdam JG. Sugars as tobacco ingredient: effects on mainstream smoke composition. Food Chem Toxicol. 2006;44(11):1789–98.
4. Leffingwell JC. Leaf chemistry: basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis DL, Nielsen MT, editors. Tobacco: production, chemistry and technology. Oxford: Blackwell Science; 1999:265–84.
5. Cahours X, Verron T, Purkis S. Effect of sugar content on acetaldehyde yield in cigarette smoke. Beitr Tabakforsch Int. 2014;25(2):381–95.
6. Seeman JI, Laffoon SW, Kassman AJ. Evaluation of relationships between mainstream smoke acetaldehyde and "tar" and carbon monoxide yields in tobacco smoke and reducing sugars in tobacco blends of US commercial cigarettes. Inhal Toxicol. 2003;15(4):373–95.
7. Addictiveness and attractiveness of tobacco additives. Brussels: European Commission Scientific Committee on Emerging and Newly Identified Health Risks; 2010 (https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_029.pdf, accessed October 2018).
8. Bernasek PF, Furin OP, Shelar GR. Sugar/nicotine study. Industry documents library, 1992 (ATP 92-210:22) (https://www.industrydocumentslibrary.ucsf.edu/tobacco/docs/#id=fxbl0037, accessed 1 January 2019).
9. Rodgman A. Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials and humectants. Beitr Tabakforsch Int. 2002;20(4):83–103.
10. Jenkins CR et al. British American Tobacco Company limited product seminar. Tobacco documents 760076123–408, 1997 (https://www.industrydocumentslibrary.ucsf.edu/tobacco/docs/#id=rjgf0205, accessed 1 January 2019).
11. Fisher P. Tobacco blending. In: Davis DL, Nielsen MT, editors. Tobacco: production, chemistry and technology. Oxford: Blackwell Science; 1999:346–52.
12. Seeman JI, Dixon M, Haussmann HJ. Acetaldehyde in mainstream tobacco smoke: formation and

occurrence in smoke and bioavailability in the smoker. Chem Res Toxicol. 2002;15(11):1331–50.

13. Hsu SC, Pollack RL, Hsu AF, Going RE. Sugars present in tobacco extracts. J Am Dent Assoc. 1980;101(6):915–8.

14. Going RE, Hsu SC, Pollack RL, Haugh LD. Sugar and fluoride content of various forms of tobacco. J Am Dent Assoc. 1980;100(1):27–33.

15. Rustemeier K, Stabbert R, Haussmann HJ, Roemer E, Carmines EL. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: chemical composition of mainstream smoke. Food Chem Toxicol. 2002;40(1):93–104.

16. Electronic Model Tobacco Control (EMTOC). Amsterdam: Rijks Instituut voor Volksgezondheiden Milieu and Ministerium für Gesundheit und Frauen; 2015.

17. Newson JR. Evaluation of competitive cigarettes. Liggett & Myers; 1975 (https://industrydocuments.library.ucsf.edu/tobacco/docs/#id=zslf0014, accessed 10 February 2016).

18. Gordon DL. PM's global strategy: Marlboro product technology. A summary developed with input from Batco, Batcf, Souza Cruz & B&W. Winston Salem (NC): Brown & Williamson; 1992.

19. Soussy S, El-Hellani A, Baalbaki R, Salman R, Shihadeh A, Saliba NA. Detection of 5-hydroxymethylfurfural and furfural in the aerosol of electronic cigarettes. Tob Control. 2016;25(Suppl 2):ii88–93.

20. van Nierop LE, Pennings JLA, Schenk E, Kienhuis AS, Talhout R. Analysis of manufacturer's information on tobacco product additive use. Tob Regul Sci. (accepted).

21. Lawler TS, Stanfill SB, Zhang L, Ashley DL, Watson CH. Chemical characterization of domestic oral tobacco products: total nicotine, pH, unprotonated nicotine and tobacco-specific N-nitrosamines. Food Chem Toxicol. 2013;57:380–6.

22. van Nierop LE, Talhout R. Sugar as tobacco additive tastes "bitter". J Addict Res Ther. 2016;7(4):2.

23. Jansen EHJM, Cremers J, Borst S, Talhout R. Simple determination of sugars in cigarettes. Anal Bioanal Tech. 2014;5(6):219.

24. Clarke MB, Bezabeh DZ, Howard CT. Determination of carbohydrates in tobacco products by liquid chromatography–mass spectrometry/mass spectrometry: a comparison with ion chromatography and application to product discrimination. J Agric Food Chem. 2006;54(6):1975–81.

25. Agaku IT, Ayo-Yusuf OA, Vardavas CI, Connolly G. Predictors and patterns of cigarette and smokeless tobacco use among adolescents in 32 countries, 2007–2011. J Adolesc Health. 2014;54(1):47–53.

26. Attitudes of Europeans towards tobacco and electronic cigarettes (Special Eurobarometer 458). Brussels: European Commission; 2017.

27. Busch C, Streibel T, Liu C, McAdam KG, Zimmermann R. Pyrolysis and combustion of tobacco in a cigarette smoking simulator under air and nitrogen atmosphere. Anal Bioanal Chem. 2012;403(2):419–30.

28. Baker RR, Bishop LJ. The pyrolysis of tobacco ingredients. J Anal Appl Pyrolysis. 2004;71(1):223–311.

29. Cheah NP. Volatile aldehydes in tobacco smoke: source, fate and risk. Maastricht: Maastricht University; 2016:2016846.

30. Cheah NP, Borst S, Hendrickx L, Cremers H, Jansen E, Opperhuizen A et al., Effect of adding sugar to Burley tobacco on the emission of aldehydes in mainstream tobacco smoke. Tob Regul Sci. 2018;4(2):61–72.

31. Baker RR. Sugars, carbonyls and smoke. Food Chem Toxicol. 2007;45(9):1783–6.

32. Baker RR. The generation of formaldehyde in cigarettes – overview and recent experiments. Food Chem Toxicol. 2006;44(11):1799–822.

33. Coggins CR, Wagner KA, Weley MS, Oldham MJ. A comprehensive evaluation of the toxicology of cigarette ingredients: carbohydrates and natural products. Inhal Toxicol. 2011;23(Suppl 1):13–40.

34. Hahn J, Schaub J. Influence of tobacco additives on the chemical composition of mainstream

smoke. Beitr Tabakforsch Int. 2014;24(3):101–16.

35. O'Connor RJ, Hurley PJ. Existing technologies to reduce specific toxicant emissions in cigarette smoke. Tob Control. 2008;17(Suppl 1):i39–48.

36. Zilkey B, Court WA, Binns MR, Walker EK, Dirks VA, Basrur PK. Chemical studies on Canadian tobacco and tobacco smoke. 1. Tobacco, tobacco sheet and cigarette smoke. Chemical analyses on various treatments of Bright and Burley. Tob Int. 1982;184:83–9.

37. Phillpotts DF, Spincer D, Westcott DT. The effect of the natural sugar content of tobacco upon the acetaldehyde concentration found in cigarette smoke. London: British American Tobacco; 1974 (Bates Number: 505242960).

38. Shihadeh A, Salman R, Jaroudi E, Saliba N, Sepetdjian E, Blank MD et al. Does switching to a tobacco-free waterpipe product reduce toxicant intake? A crossover study comparing CO, NO, PAH, volatile aldehydes, tar and nicotine yields. Food Chem Toxicol. 2012;50(5):1494–8.

39. Al Rashidia M, Shihadeh A, Saliba NA. Volatile aldehydes in the mainstream smoke of the narghile waterpipe. Food Chem Toxicol. 2008;46(11):3546–9.

40. Fagan P, Pokhrel P, Herzog TA, Moolchan ET, Cassel KD, Franke AA et al. Sugar and aldehyde content in flavored electronic cigarette liquids. Nicotine Tob Res. 2018;20(8):985–92.

41. Pyles ST, Van Voris LP, Lotspeich FJ, McCarty SA. Sugar in chewing tobacco. N Engl J Med. 1981;304(6):365.

42. Vellappally, S, Fiala Z, Smejkalová J, Jacob V, Shriharsha P. Influence of tobacco use in dental caries development. Centr Eur J Public Health. 2007;15(3):116–21.

43. Tomar SL, Winn DM. Chewing tobacco use and dental caries among US men. J Am Dent Assoc. 1999;130(11):1601–10.

44. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: fifth report of a WHO study group (WHO Technical Report Series, No. 989). Geneva: World Health Organization; 2015.

45. Burns DM, Dybing E, Gray N, Hecht S, Anderson C, Sanner T et al. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. Tob Control. 2008;17(2):132–41.

46. Talhout, R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8(2):613–28.

47. Opinion on additives used in tobacco products (opinion 2). Tobacco additives II. Brussels: European Commission Scientific Committee on Emerging and Newly Identified Health Risks; 2016.

48. Willems EW, Rambali B, Vleeming W, Opperhuizen A, van Amsterdam JG. Significance of ammonium compounds on nicotine exposure to cigarette smokers. Food Chem Toxicol. 2006; 44(5):678–88.

49. Elson LA, Betts TE. Sugar content of the tobacco and pH of the smoke in relation to lung cancer risks of cigarette smoking. J Natl Cancer Inst. 1972;48(6):1885–90.

50. Wayne GF, Carpenter CM. Tobacco industry manipulation of nicotine dosing. Handb Exp Pharmacol. 2009;192:457–85.

51. Hammond, D, Fong GT, Cummings KM, O'Connor RJ, Giovino GA, McNeill A. Cigarette yields and human exposure: a comparison of alternative testing regimens. Cancer Epidemiol Biomarkers Prev. 2006;15(8):1495–501.

52. Jarvis MJ, Boreham R, Primatesta P, Feyerabend C, Bryant A. Nicotine yield from machine-smoked cigarettes and nicotine intakes in smokers: evidence from a representative population survey. J Natl Cancer Inst. 2001;93(2):134–8.

53. Bates C, Jarvis M, Connolly G. Tobacco additives: cigarette engineering and nicotine addiction. A survey of the additive technology used by cigarette manufacturers to enhance the appeal and addictive nature of their product. London: Action on Smoking and Health, Imperial Cancer Research Fund; 1999.

54. Paschke T, Scherer G, Heller W. Effects of ingredients on cigarette smoke composition and biological activity: a literature overview. Beitr Tabakforsch Int. 2002;20(3):107–47.

55. Foddai M, Dosia G, Spiga S, Diana M. Acetaldehyde increases dopaminergic neuronal activity in the VTA. Neuropsychopharmacology. 2004;29(3):530–6.

56. Villegier AS, Blanc G, Glowinski J, Tassin JP. Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. Pharmacol Biochem Behav. 2003;76(2):267–74.

57. Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M et al. Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. J Neurosci. 2005;25(38):8593–600.

58. Belluzzi JD, Wang R, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology. 2005;30(4):705–12.

59. Gray N. Reflections on the saga of tar content: why did we measure the wrong thing? Tob Control. 2000;9(1):90–4.

60. Henningfield JE, Benowitz NL, Connolly GN, Davis RM, Gray N, Myers ML et al., Reducing tobacco addiction through tobacco product regulation. Tob Control. 2004;13(2):132–5.

61. Herraiz T, Chaparro C. Human monoamine oxidase is inhibited by tobacco smoke: beta-carboline alkaloids act as potent and reversible inhibitors. Biochem Biophys Res Commun. 2005;326(2): 378–86.

62. Pfau W, Skog K. Exposure to beta-carbolines norharman and harman. J Chromatogr B. 2004;802(1):115–26.

63. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci. 1992;13(5):177–84.

64. Fowler JS, Logan J, Wang GJ, Volkow ND. Monoamine oxidase and cigarette smoking. Neurotoxicology. 2003;24(1):75–82.

65. Talhout R, Opperhuizen A, van Amsterdam JG. Role of acetaldehyde in tobacco smoke addiction. Eur Neuropsychopharmacol. 2007;17(10):627–36.

66. Berlin I, Anthenelli RM. Monoamine oxidases and tobacco smoking. Int J Neuropsychopharmacol. 2001;4(1):33–42.

67. Syrjanen K, Salminen J, Aresvuo U, Hendolin P, Paloheimo L, Eklund C et al. Elimination of cigarette smoke-derived acetaldehyde in saliva by slow-release L-cysteine lozenge is a potential new method to assist smoking cessation. A randomised, double-blind, placebo-controlled intervention. Anticancer Res. 2016;36(5):2297–306.

68. Spijkerman R, van den Eijnden R, van de Mheen D, Bongers I, Fekkes D. The impact of smoking and drinking on plasma levels of norharman. Eur Neuropsychopharmacol. 2002;12(1):61–71.

69. Rommelspacher H, Meier-Henco M, Smolka M, Kloft C. The levels of norharman are high enough after smoking to affect monoamine oxidase B in platelets. Eur J Pharmacol. 2002;441(1–2):115–25.

70. Breyer-Pfaff U, Wiatr G, Stevens I, Gaertner HJ, Mundle G, Mann K. Elevated norharman plasma levels in alcoholic patients and controls resulting from tobacco smoking. Life Sci. 1996;58(17):1425–32.

71. Rose JE, Mukhin AG, Lokitz SJ, Turkington TG, Herskovic J, Behm FM et al. Kinetics of brain nicotine accumulation in dependent and nondependent smokers assessed with PET and cigarettes containing ^{11}C-nicotine. Proc Natl Acad Sci U S A. 2010;107(11):5190–5.

72. Sanders E, Weitkunat R, Utan A, Dempsey R. Does the use of ingredients added to tobacco increase cigarette addictiveness? A detailed analysis. Inhal Toxicol. 2012;24(4):227–45.

73. Lee PN, Forey BA, Fry JS, Hamling JS, Hamling JF, Sanders EB et al. Does use of flue-cured rather than blended cigarettes affect international variation in mortality from lung cancer and COPD? Inhal Toxicol. 2009;21(5):404–30.

74. Ferreira CG, Silveira D, Hatsukami DK, Paumgartten FJ, Fong GT, Glória MB et al. The effect of to-

bacco additives on smoking initiation and maintenance. Cad Saude Publica. 2015;31(2):223–5.

75. Correa M, Salamone JD, Segovia KN, Pardo M, Longoni R, Spina L et al. Piecing together the puzzle of acetaldehyde as a neuroactive agent. Neurosci Biobehav Rev. 2012;36(1):404–30.

76. Hoffman AC, Evans SE. Abuse potential of non-nicotine tobacco smoke components: acetaldehyde, nornicotine, cotinine, and anabasine. Nicotine Tob Res. 2013;15(3):622–32.

77. Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB et al. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. J Neurosci. 2003;23(11):4712–6.

78. Leffingwell JC. Nitrogen components of leaf and their relationship to smoking quality and aroma. Recent Adv Tob Sci. 1976;2:1–31.

79. Opinion on additives used in tobacco products (opinion 1). Brussels: European Commission Scientific Committee on Emerging and Newly Identified Health Risks; 2016.

80. Hoffmann D, Hoffmann I. The changing cigarette, 1950–1995. J Toxicol Environ Health. 1997;50(4):307–64.

81. Weeks WW. Relationship between leaf chemistry and organoleptic properties of tobacco smoke. In: Davis DL, Nielsen MT, editors. Tobacco: production, chemistry and technology. Oxford: Blackwell Science; 1999:304–12.

82. Fowles J. Chemical factors influencing the addictiveness and attractiveness of cigarettes in New Zealand. Wellington: Institute of Environmental Science and Research; 2001 (http://www.moh.govt.nz/notebook/nbbooks.nsf/0/D0B68B2D9CB811ABCC257B81000D9959/$file/chemicalfactorsaddictivenesscigarettes.pdf, accessed 15 May 2019).

83. Truth Tobacco Industry Documents. San Francisco (CA): University of California at San Francisco Library and Center for Knowledge Management (https://www.industrydocumentslibrary.ucsf.edu/tobacco/, accessed 15 May 2019).

84. WHO Framework Convention on Tobacco Control. 2013 Guidelines for implementation. Article 5.3, Article 8, Articles 9 and 10, Article 11, Article 12, Article 13, Article 14. Geneva: World Health Organization; 2013 (http://apps.who.int/iris/bitstream/10665/80510/1/9789241505185_eng.pdf, accessed 1 October 2018).

85. An act to amend the Tobacco Act, Bill C-32. Ottawa: Government of Canada; 2009.

86. Tobacco Reporting Regulations (SOR/2000-273). Tobacco and Vaping Products Act, Tobacco Act. Ottawa: Government of Canada; 2006.

87. Tobacco Product Directive (2014/40/EU) of the European Parliament and of the Council. Brussels: European Commission; 2014 (http://ec.europa.eu/health/tobacco/docs/dir_201440_en.pdf, accessed 15 May 2019).

88. Permitted additives to tobacco products in the United Kingdom. London: Department of Health; 2003.

89. Consultation on implementation of the revised Tobacco Products Directive (2014/40/EU), section 5.11. London: Department of Health; 2015 (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/440991/TPD_Consultation_Doc.pdf, accessed 16 January 2019).

90. Richter AP. Best practices in implementation of Article 9 of the WHO FCTC Case study: Brazil and Canada (National Best Practices Series, Paper No. 5). Geneva: World Health Organization; 2015.

91. Report of the Working Group on Tobacco Additives. Brasilia: Agência Nacional de Vigilância Sanitária; 2014 (http://portal.anvisa.gov.br/documents/106510/106594/Report+Working+Group+Tobacco+Additives/b99ad2e7-23d9-4e88-81cd-d82c28512199, accessed 15 May 2019).

92. Family Smoking Prevention and Tobacco Control Act. Washington (DC): Food and Drug Administration; 2009 (http://www.fda.gov/tobaccoproducts/labeling/rulesregulationsguidance/ucm246129.htm#ingredients, accessed 1 January 2019).

93. Gottlieb S. FDA statement from FDA Commissioner Scott Gottlieb, MD, on proposed new steps to protect youth by preventing access to flavored tobacco products and banning menthol in cigarettes. Washington (DC): Food and Agriculture Administration; 2018 (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/UCM625884.htm, accessed 10 January 2019).

94. Munafo M. Understanding the role of additives in tobacco products. Nicotine Tob Res. 2016;18(7):1545.

95. Corey CG, Ambrose BK, Apelberg BJ, King BA. Flavored tobacco product use among middle and high school students – United States, 2014. Morb Mortal Wkly Rep. 2015;64(38):1066–70.

96. Cunningham FH Fiebelkorn S, Johnson M, Meredith C. A novel application of the Margin of Exposure approach: segregation of tobacco smoke toxicants. Food Chem Toxicol. 2011;49(11):2921–33.

97. Kienhuis AS, Staal YC, Soeteman-Hernández LG, van de Nobelen S, Talhout R. A test strategy for the assessment of additive attributed toxicity of tobacco products. Food Chem Toxicol. 2016;94:93–102.

98. van de Nobelen S, Kienhuis AS, Talhout R. An inventory of methods for the assessment of additive increased addictiveness of tobacco products. Nicotine Tob Res, 2016;18(7):1546–55.

99. Talhout R, van de Nobelen S, Kienhuis AS. An inventory of methods suitable to assess additive-induced characterising flavours of tobacco products. Drug Alcohol Depend. 2016;161:9–14.

100. European Commission. Priority list of additives contained in cigarettes and roll-your-own. Off J Eur Union. 2016:C/2016/2923.

101. Miao S, Beach ES, Sommer TJ, Zimmerman JB, Jordt SE. High-intensity sweeteners in alternative tobacco products. Nicotine Tob Res. 2016;18(11):2169–73

8. Updated priority list of toxicants in combusted tobacco products

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Contents

8.1 Introduction

At the fifth meeting of the COP of the WHO FCTC, WHO was requested to compile, make available to Parties and update a non-exhaustive list of toxic contents and emissions of tobacco products and provide advice about how such information could best be used by Parties. TobReg at its meeting in Rio de Janeiro, Brazil, on 4–6 December 2013 provided an updated list of priority toxicants for reporting and regulation.

In this paper, we re-evaluate the list, as it has been four years since the updated list was issued, and new knowledge on the subject matter has become available. In particular, this report includes:

- background information on the current TobReg priority list of toxicants, including the criteria used to select specific contents and emissions (8.2);

- an overview of new data related to the priority list, including new publications on the toxicity of specific constituents, new or modified analytical methods and new data on variations in levels of toxicant in combustible tobacco brands (8.3–8.5);

- a discussion of the criteria for future re-evaluation of toxicants on the list (8.6);

- a discussion of criteria for selecting new toxicants for the list (8.7); and

- research needs and regulatory recommendations for testing selected contents and emissions in combusted tobacco products (8.8).

Literature was searched primarily in the PubMed database and SciFinder, which retrieves citations from the Medline and CAplus databases. Relevant articles cited in publications obtained in the database search were also included. In addition, the websites of the United States Centers for Disease Control and Prevention, the United States Environmental Protection Agency, the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) and other relevant websites that provide information on the toxicology of the constituents of interest and on methods were used.

8.2 Background of preparation of the priority list

Regulation of tobacco products requires establishment of a metric or set of metrics by which tobacco products can be assessed. The measurements that have been made most commonly for cigarettes have been machine-measured tar, nicotine and CO yields per cigarette, based on the ISO regimen and the United States Federal Trade Commission (FTC) method. It is well established, however, that such measures do not provide valid estimates of human exposure or toxicity and thereby cause harm by misleading smokers and most regulators *(1, 2)*. New product assessment approaches for setting regulatory measures were therefore considered necessary.

The new approach proposed by TobReg requires quantification of the levels of known harmful toxicants that accompany a specified amount of nicotine, the principal addictive substance in smoke sought by smokers, as measured in a standardized machine testing regimen. Normalization of specific toxicants to the nicotine yield allows measurement of the toxicity of smoke generated in a

standardized regimen rather than the quantity of smoke generated. Standardized measures of toxicant yields will allow regulators to reduce the levels of identified priority toxicants in tobacco smoke, consistent with other regulatory approaches that mandate reductions of known toxicants in products used by humans. Selection of high-priority smoke toxicants for product assessment is a critical step in this strategy.

The current non-exhaustive priority list of toxic contents and emissions of combustible tobacco products was drawn up by TobReg from among more than 7000 chemicals found in cigarette smoke, after an evaluation of lists of harmful and toxic chemicals associated with cancer, cardiovascular and pulmonary diseases published by several regulatory bodies. Consideration was also given to constituents in both the particulate and the gas phases of smoke and in different chemical classes. As the list represents only a small fraction of the total complex mixture of chemicals present in tobacco smoke, the overall toxicity of the emissions of tobacco products is not necessarily characterized by the toxicity of these chemicals. Regulation of a very large number of toxicants would, however, lead to significant distortions in the existing market and increase the complexity of regulatory oversight. Limiting the number of toxicants to a carefully selected priority list recognizes the practical reality of a regulatory structure.

8.2.1 Criteria for selection of toxicants for the priority list

The following criteria were used to select priority tobacco contents and emissions of cigarette smoke for testing, reporting and future regulation.

The presence of specific chemicals in cigarette smoke at levels that are toxic for smokers, as determined by well established scientific toxicity indices

Evidence of toxicity was the most important criterion. The toxicologically important constituents considered were those on the list reported by law to Health Canada for the year 2004 *(3)* and measured by Counts et al. *(4)* by comparing several international brands manufactured by Philip Morris. The list was selected by Health Canada to represent those constituents of tobacco smoke that contribute most to its toxicity.

Quantitative data for characterizing the hazards of the reviewed toxicants were generated by calculating "toxicant animal carcinogenicity indices" and non-cancer response indices with a modification of a simplified system presented by Fowles and Dybing *(5)*. For these calculations, published toxicant yields (obtained with the modified intense smoking regimen) were normalized per milligram of nicotine and multiplied by cancer and non-cancer potency factors. "Cancer potency factors" were defined as T_{25} per milligram $(1/T_{25})$, where T_{25} is the long-term daily dose that will produce tumours at a specific tissue site above the background rate in 25% of animals *(6)*. For non-cancer potency factors,

the long-term reference exposure levels published by the California Office of Environmental Health Hazard Assessment (USA) in February 2005 (http://www.oehha.ca.gov/air/chronic_rels/AllChrels.html) were used. Toxicant yields measured in a consistent manner with the modified intense smoking regimen are available from three sources: Counts et al. *(4)*, a set of Canadian brands *(3)* and a set of Australian brands *(7)*.

Variations in concentrations among cigarette brands that are substantially greater than the variation in repeated measurements of the toxicant in a single brand

Mandated reductions are likely to have the greatest impact on lowering the mean levels per milligram of nicotine for those toxicants with the broadest variation in levels around the midpoint. The variation in levels of different toxicants can be expressed as the coefficient of variation, which is the standard deviation of the measurement across brands divided by the mean value for all the brands. As the reproducibility of testing can vary substantially for different toxicants and with different testing methods, repeated measurements are used to estimate the mean value for a brand, and the variation of the repeated measurements defines the CI around that mean value. A second approach is direct determination of the range of toxicants in brands by determining the maximum and minimum values for each toxicant in the brand data set, expressed as the ratio of the median value for that toxicant. This does not include adjustment for variation in replicate measurements of the toxicant. Another useful approach to assessing variations in toxicant levels is to compare the mean levels of toxicants in different data sets. This analysis can identify levels of toxicants that differ within the same brand sold in different markets or by different manufacturers, which indicates that it is clearly possible to manufacture cigarettes that yield lower levels of that particular toxicant.

The availability of technology to reduce the concentration of a given toxicant in smoke, should an upper limit be mandated

The ability of the tobacco industry to modify their products to comply with lower toxicant levels is another factor to be considered in selecting toxicants for tobacco product regulation. For instance, the levels of the carcinogenic TSNAs NNK and NNN in smoke can be reduced by changing agricultural practices, curing and tobacco blending *(8)*. The levels of volatile toxicants, such as acetaldehyde, acrolein and formaldehyde, can be reduced by reducing the concentration of sugars added to tobacco or using charcoal filters or other filter modifications *(9)*. Reductions in benzo[*a*]pyrene yields can be achieved by treating, extracting or modifying the tobacco blend *(10)*.

8.2.2 **Key decisions of the Conference of the Parties on priority contents and emissions of combustible tobacco**

Table 8.1 summarizes the key COP decisions and TobReg's progress in regulating the contents and emissions of combustible tobacco products under WHO FCTC Articles 9 and 10. The initial list of nine priority emissions identified by TobReg at the third COP and for which validation of testing methods in cigarette smoke was recommended are listed in Table 8.2. These toxicants were selected among 43 toxicologically relevant compounds for which the hazard indices were calculated and other criteria were reviewed as described above. Nicotine was not included in the list of emissions but was recommended for testing in tobacco filler (contents). Following the COP mandate, analytical methods for these selected priority emissions were developed and validated by the WHO Tobacco Laboratory Network (TobLabNet).

Table 8.1. History of COP decisions and TobReg progress relevant to the testing and reporting of emissions

COP session	COP decisions	TobReg progress and reports on contents and emissions
COP 1 (Geneva, 2006)	*COP/1/INF.DOC./3*: set up a working group to prepare guidelines pursuant to Articles 9 and 10 of the WHO FCTC. First phase to comprise testing and measuring tobacco product contents and emissions.	
COP 2 (Bangkok, 2007)	*COP/2/DIV/9*: continue the work of TobReg, including on product characteristics, such as design features, to the extent that they affect the objectives of the WHO FCTC.	*COP/2/8*: TobReg presented a progress report that included the proposal that the provisional list of cigarette emissions consist of 44 "Hoffmann analytes".
COP 3 (Durban, 2008)	*COP/3/DIV/3*: decisions relevant to the priority list included: • validate, within 5 years, analytical chemical methods for testing and measuring cigarette contents and emissions identified as priorities in the progress report of the working group, with two smoking regimens (ISO and modified intense method with blocked ventilation holes). • when appropriate, design and validate methods for testing and measuring product characteristics identified in the progress report of the working group.	*COP/3/6*: the new progress report presented by TobReg identified: • three contents for which methods for testing and measuring should be validated as a priority (nicotine, ammonia, humectants); • nine emissions for which methods for testing and measuring should be validated as a priority (NNK, NNN, acetaldehyde, acrolein, benzene, benzo[a]pyrene, 1,3-butadiene, CO, formaldehyde); • the smoking regimens for validation of the test methods: (i) ISO 3308:2000 and (ii) a modified intense method with blocked ventilation holes; and • a provisional list of product characteristics for testing and disclosure.
COP 4 (Punta del Este, 2010)	*COP/4/DIV/6*: continue validation of analytical chemical methods for testing and measuring cigarette contents and emissions.	*COP/4/INF.DOC./2*: three analytical methods have been validated: • CO in emissions; • NNN and NNK in emissions; and • nicotine content of tobacco.
COP 5 (Seoul, 2012)	COP/5/DIV/5: continue validation of the analytical chemical methods for testing and measuring cigarette contents and emissions; compile a non-exhaustive list of toxic contents and emissions of tobacco products.	*COP/5/INF.DOC./1*: work in progress: • validation of methods for humectants and ammonia in cigarette tobacco filler; • validation of the method for benzo[a]pyrene in mainstream cigarette smoke.

COP 6 (Moscow, 2014)	*COP6(12)*: finalize validation of the analytical chemical methods for testing and measuring cigarette contents and emissions.	*COP/6/14*: TobReg proposed: • a non-exhaustive list of 39 toxicants in tobacco products for monitoring and eventual regulation; • a shorter list of nine toxicants for mandated lowering; • development of the following standardized methods by TobLabNet: cadmium and lead content; nicotine in the smoke of waterpipes (shisha); and nicotine, NNN, NNK and benzo[a] pyrene in smokeless tobacco products.
COP 7 (Delhi, 2016)	COP7(14): finalize validation of the analytical chemical methods for aldehydes and VOCs in cigarette emissions; assess the availability of validated analytical methods for the extended list of toxicants in contents and emissions of tobacco products, as reported in FCTC/COP/6/14.	*COP/7/INF.DOC./1*: method validation is completed for all mandated contents and emissions, except for aldehydes and VOCs.
COP 8 (Geneva, 2018)	COP8(21): encourage Parties to acknowledge and implement TobLabNet methods.	*COP/8/8:* Method validation is completed for aldehydes and VOCs. All mandated contents and emissions are now validated. TobReg identified the following opportunities for extending the list of toxicants in contents and emissions: • extend SOPs to include the remaining aldehydes and VOCs; • prepare a SOP for the metal content of cigarette tobacco filler; • devise methods for the analysis of waterpipe tobacco and charcoal.

CO: carbon monoxide; COP: Conference of the Parties; ISO: International Organization for Standardization; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N*´-nitrosonornicotine; SOP: standard operating procedure; VOC: volatile organic compound.

Table 8.2. Emissions of combusted tobacco products considered and evaluated for inclusion in the lists of priorities for testing, reporting and regulation

	Carcinogenicity or toxicity data		Inclusion on priority lists		
			COP/3/6	COP/6/14	
Toxicants evaluated by TobReg	TACI	TNCRI	**Toxicants selected for testing and measuring**	**Expanded priority list for monitoring and regulation**	**Proposed for mandatory reduction**
Alkaloids					
Nicotine[a]	–	–	X	x	–
Aldehydes					
Acetaldehyde	6.1	67.1	X	x	x
Acrolein	–	1099	X	x	x
Formaldehyde	–	19.8	X	x	x
Crotonaldehyde[a]	–	–	–	x	–
Propionaldehyde	–	–	–	x	–
Butyraldehyde	–	–	–	x	–
Aromatic amines					
3-Aminobiphenyl	–	–	–	x	–
4-Aminobiphenyl[a]	–	–	–	x	–
1-Aminonaphthalene	0.00036	–	–	x	–

2-Aminonaphthalene	0.00068	–	–	x	–
Hydrocarbons					
Benzene	2.6	0.64	X	x	x
1,3-Butadiene	9.9	2.4	X	x	x
Isoprene	3.7	–	–	x	–
Styrene[b]	–	0.01	–	–	–
Toluene	–	0.22	–	x	–
PAHs					
Benzo[*a*]pyrene[a]	0.0086	–	X	x	x
TSNAs[a]					
NNK	3.4	–	X	x	x
NNN	0.29	–	X	x	x
NAB	–	–	–	x	–
NAT	–	–	–	x	–
Phenols					
Catechol	0.58	–	–	x	–
m- and *p*-Cresol	–	0.01	–	x	–
o-Cresol	–	0.01	–	x	–
Phenol	–	0.07	–	x	–
Hydroquinone	1.2	–	–	x	–
Resorcinol	–	–	–	x	–
Other organic compounds					
Acetone	–	–		x	
Acrylonitrile	1.4	2.1		x	
Quinoline	–	–		x	
Pyridine	–	–		x	
Metals and metalloids					
Arsenic[c]	–	0.16			
Cadmium	1.7	2.6		x	
Chromium[b]	–	–			
Lead	0.00	–		x	
Mercury	–	0.02		x	
Nickel[b]	–	–			
Selenium[b]	–	–			
Other constituents					
Ammonia	–	0.07		x	
CO[a]	–	1.3	X	x	x
Hydrogen cyanide	–	17.2		x	
Nitrogen oxides	–	3.1		x	

CO: carbon monoxide; NAB: *N'*-nitrosoanabasine; NAT: *N'*-nitrosoanatabine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N'*-nitrosonornicotine; PAH: polycyclic aromatic hydrocarbon; TACI: toxicant animal carcinogenicity index; TNCRI: toxicant non-cancer response index; TSNA: tobacco-specific N-nitrosamine.

[a]Justification for inclusion on the priority lists (initial and/or expanded) of some toxicants: nicotine was not on the initial priority list of emissions but was recommended for testing in tobacco (contents) and was subsequently listed with other toxicants in the extended list. Benzo[*a*]pyrene was included despite its low TACI because it is a proxy for the family of PAHs found in smoke and because there is a wealth of evidence for the carcinogenicity of many of these PAHs. The toxicant 4-aminobiphenyl was added because it is a human carcinogen, although experimental data did not allow proper calculation of T_{25}. NNK and NNN were included as they had already been identified in the first report on mandating reductions in toxicant yields *(12)*. Crotonaldehyde was included because of its reactive α,β-unsaturated aldehyde structure, although a tolerable level value was lacking. CO was also included even though it has a relatively low toxicant non-cancer response index, as it is thought to be mechanistically related to cardiovascular disease.

[b] Originally considered *(11)* but not included in the final recommended list because it occurs at low levels and is not considered to contribute appreciably to hazard indices.

[c] Added after the expanded list was reported in document COP/6/14.

In response to the subsequent COP request to compile a non-exhaustive list of toxic contents and emissions of tobacco products, TobReg at its meeting in Rio de Janeiro, Brazil, on 4–6 December 2013 re-evaluated the priority toxicant list by examining the lists of harmful and toxic chemicals published by several regulatory bodies, including Health Canada, the National Institute for Public Health and the Environment in the Netherlands (RIVM) and the FDA, and reviewing the list of toxicants assessed in an earlier WHO technical report on the scientific basis of tobacco product regulation *(11)*. TobReg subsequently drew up a non-exhaustive list by adding toxicants that satisfied the criteria for sufficient evidence of carcinogenicity or with known respiratory or cardiac toxicity, variations in levels in different brands in different countries that were readily measurable and possibilities for lowering yields in a product (Table 8.2). The list, composed of 37 such toxicants plus nicotine, was presented to the COP for discussion at its sixth session. Only a few toxicants on the originally assessed list of 43 constituents were not included in the updated list. For instance, chromium, nickel and selenium occur either at low levels or could not be quantified in the three analysed data sets; therefore, although these elements are quite toxic, they were not considered to contribute appreciably to hazard indices and were not included.

In the same report (COP/6/14), TobReg proposed that the nine compounds originally selected for method validation (emissions) should be considered for mandatory reduction. TobReg concluded that these compounds are the most hazardous toxicants in cigarette smoke that could be reduced in emissions; they represent different chemical families of toxicants and different phases of smoke, are toxic to the pulmonary and cardiovascular systems and are carcinogenic. The nine toxicants are: acetaldehyde, acrolein, benzene, benzo[*a*] pyrene, 1,3-butadiene, CO, formaldehyde, NNK and NNN (Table 8.2). The remaining toxicants on the priority list of 39 should be measured and reported.

8.3 Overview of new scientific knowledge on toxicity

8.3.1 Aldehydes (acetaldehyde, acrolein, formaldehyde, crotonaldehyde, propionaldehyde, butyraldehyde)

Aldehydes are toxic to the respiratory system *(13)*. Acetaldehyde and formaldehyde are also toxic to the cardiovascular system and are respiratory tumorigens *(14, 15)*. Acrolein is an intense irritant, is toxic to lung cilia and has been proposed as a lung carcinogen *(16, 17)*. Crotonaldehyde is a potent irritant and a weak hepatocarcinogen and forms DNA adducts in the human lung *(18)*.

New studies on mechanisms of toxicity and carcinogenicity

The genotoxicity of acetaldehyde, acrolein and formaldehyde in human lung cells was demonstrated in the γH2AX assay, which detects early cellular response

to DNA double-strand breaks and is used as a biomarker of DNA damage in human epidemiology *(19)*. All three aldehydes were genotoxic in a dose- or time-dependent manner, acrolein having the strongest potential to induce DNA damage, followed by formaldehyde and acetaldehyde. A computational fluid dynamics modelling study of simulated human oral breathing, representative aldehyde yields from cigarette smoke and lifetime average daily doses indicated that the order of concern for human exposure is acrolein > formaldehyde > acetaldehyde *(20)*. An analysis of the mode of action of the toxicity of acrolein in the lower respiratory system, reflecting the exposure of smokers who inhale tobacco smoke, suggested that the mechanisms of acrolein toxicity include oxidative stress, chronic inflammation, necrotic cell death, necrosis-induced inflammation, tissue remodelling and destruction and subsequent loss of lung elasticity and enlarged lung airspaces *(21)*. These processes are consistent with the inflammation and necrosis in the middle and lower regions of the respiratory tract that occur in chronic obstructive pulmonary disease. The findings of another study suggested that acrolein contributes to the dysfunctional innate immune responses observed in the lung during cigarette smoking *(22)*. In addition, acrolein may contribute to bladder carcinogenesis in smokers. Lee et al. *(23)* analysed acrolein–deoxyguanosine adducts in normal human urothelial mucosa and in bladder tumour tissues and measured their mutagenicity in human urothelial cells. The adduct levels in both types of tissue were higher than the levels of those due to the known bladder carcinogen 4-aminobiphenyl, and they induced mutation signatures and spectra that appeared to be much more mutagenic than those due to 4-aminobiphenyl. The levels of acrolein–deoxyguanosine were two times higher in bladder tumour tissues than in normal cells.

A study of transcription responses to aldehyde exposure in human lung carcinoma lines showed a different ranking from that in the studies described above *(24)*. Formaldehyde gave the strongest response, with differential expression of 66 genes (mostly involved in apoptosis and DNA damage) by more than 1.5 times. Acetaldehyde dysregulated 57 genes, while acrolein caused upregulation of only one gene involved in oxidative stress.

While formation of DNA adducts is a critical factor in the mutagenicity and carcinogenesis of aldehydes, protein modifications may also play a role. For instance, in addition to N^2-ethylidene-2′-deoxyguanosine, its major DNA adduct, acetaldehyde also formed hybrid protein adducts with malondialdehyde, a product of lipid peroxidation induced by toxic constituents and reactive species in cigarette smoke. Formation of hybrid malondialdehyde–acetaldehyde protein adducts in the lung has been shown to initiate several pathological conditions, including inflammation and inhibition of wound healing *(25)*. Antibodies (immunoglobulins) involved in the immune response to these adducts (i.e. IgM, IgG and IgA) are predictive of progression of atherosclerotic disease and

of cardiovascular events such as acute myocardial infarction or coronary artery bypass grafting *(26)*.

It is also important to consider the effect of gene polymorphisms on the response of humans to toxicants. For instance, risk assessments of acetaldehyde have so far been based on thresholds determined in animal toxicology studies, which do not account for the genetic-epidemiological and biochemical evidence that *ALDH2*-deficient humans are highly vulnerable to the carcinogenic effects of this chemical. In a study on exposure to acetaldehyde through alcohol consumption, *ALDH2* inactivity was associated with odds ratios of up to 7 for head-and-neck and oesophageal cancers *(27)*. A study of a Japanese cohort of patients with myocardial infarction and stable angina and matching controls suggested that the inactive *ALDH2* genotype may increase the risk for myocardial infarction in smokers *(28)*.

Additional potential mechanisms of the toxicity and carcinogenicity of aldehydes have been investigated. Acrolein has been shown to affect the metabolism and fate of aromatic amines by interacting with enzymes responsible for their acetylation *(29)*. A study of potential interactions between acrolein and the antiretroviral drug zidovudine, which is used widely in limited-resource countries but is associated with hepatotoxicity, strongly suggest that exposure to acrolein through smoking and/or alcohol consumption can contribute to the major mechanisms by which zidovudine induces hepatotoxicity *(30)*. Another study suggested that exposure to acetaldehyde and formaldehyde may induce carcinogenesis in carriers of the *BRCA2* mutation *(31)*. Mutations in *BRCA2*, which is a tumour suppressor gene, increase the risk for breast cancer and are also associated with susceptibility to ovarian, pancreatic and other cancers. Both aldehydes selectively deplete *BRCA2* by proteasomal degradation, which may trigger spontaneous mutagenesis during DNA replication *(31)*.

New studies on health effects other than respiratory toxicity and carcinogenicity

Addictiveness: there is accumulating evidence that acetaldehyde also contributes to the addictiveness of tobacco *(32)*. Studies of self-administration in laboratory animals add to understanding of the behavioural correlates of acetaldehyde administration and possible interactions with the neurotransmitters for motivation, reward and stress-related responses, such as dopamine and endocannabinoids *(33)*. Another possible mechanism is via the formation of harman, a condensation product of acetaldehyde and amines in saliva. Harman is a monoamine oxidase inhibitor and can help to maintain behavioural sensitization to nicotine. In support of this hypothesis, it was shown that smokers given a lozenge containing L-cysteine (an amino acid that reacts with acetaldehyde) had higher rates of smoking cessation than those treated with placebo *(34)*. It should be noted, however, that harman is also present in tobacco and cigarette smoke.

Cardiovascular effects: the cardiovascular effects of acrolein have been reviewed *(35)*. In vitro and in vivo, cardiovascular tissues appear to be particularly sensitive to the toxic effects of acrolein, which can generate oxidative stress in the heart, form protein adducts with myocyte and vascular endothelial cell proteins and cause vasospasm. Therefore, chronic exposure to acrolein could contribute to cardiomyopathy and cardiac failure in humans. This conclusion is supported by the results of a study of mice exposed chronically to acrolein, which suggested that even relatively low exposure to acrolein, such as that from second-hand smoke or e-cigarettes, could increase cardiovascular risk by reducing endothelium repair, suppressing immune cells or both *(36)*. Exposure to acrolein was also assessed in 211 participants in the Louisville Healthy Heart Study who were at moderate-to-high risk for cardiovascular disease *(37)*. Exposure to acrolein was associated with platelet activation, suppression of circulating angiogenic cell levels and increased cardiovascular disease risk.

Glucose and lipid metabolism: in rats, exposure to acrolein caused metabolic impairment by inducing hyperglycaemia and glucose intolerance, accompanied by a significant increase in the level of corticosterone and modest but insignificant increases in the level of adrenaline *(38)*. The association between urinary levels of acrolein metabolites and diabetes and biomarkers of insulin resistance was investigated in 2027 adults who participated in the 2005–2006 National Health and Nutrition Examination Survey in the USA *(39)*. A positive association was found between the biomarkers analysed, suggesting a potential role of acrolein in the etiology of type-2 diabetes and insulin resistance in humans.

Other effects: acrolein may affect intestinal epithelial integrity. After oral exposure of mice to acrolein, damage was found to the intestinal epithelial barrier, resulting in increased permeability and subsequent bacterial translocation *(40)*. Song et al. *(41)* provided additional evidence that acrolein is a risk factor for otitis media. The developmental and reproductive toxicity of aldehydes has also been studied. Amiri & Turner-Henson *(42)* reported the results of a cross-sectional study of a convenience sample of 140 healthy pregnant women, in which the relation between exposure to formaldehyde and fetal growth during the second trimester was examined. A linear regression model showed that the dichotomized level of formaldehyde exposure (< 0.03 and > 0.03 parts per million) was a significant predictor of biparietal diameter percentile after control for maternal race (P <.006). In a study in an animal model, exposure to 5 mg/kg acrolein in utero resulted in significantly decreased testosterone synthesis in male offspring *(43)*.

New epidemiological evidence

A study of more than 2200 smokers in the Multiethnic Cohort study in the USA showed that urinary biomarkers of acrolein and crotonaldehyde exposure were significantly different among five ethnic groups *(44)*. Native Hawaiians had the highest and Latinos the lowest geometric mean levels of urinary biomarkers of both aldehydes after adjustment for confounders. These results are consistent with the findings of an epidemiological study in this cohort, in which native Hawaiians had a higher risk for lung cancer and Latinos a lower risk as compared with Whites, for the same number of cigarettes smoked *(45)*. These results suggest that acrolein and crotonaldehyde may be involved in the etiology of lung cancer in smokers.

8.3.2 Aromatic amines (3-aminobiphenyl, 4-aminobiphenyl, 1-aminonaphthalene, 2-aminonaphthalene)

Exposure to aromatic amines is associated with bladder cancer *(46)*, and 2-aminonaphthalene and 4-aminobiphenyl are known human bladder carcinogens *(15)*. In one study, the mean level of 4-aminobiphenyl was 4.8 times higher in smokers (> 20 cigarettes/day) than nonsmokers, reaffirming that tobacco smoke is a major source of exposure to this carcinogen *(47)*.

Aromatic amines may present a risk for non-urological cancer. A cohort of 224 male workers at a single factory who were followed from 1953 to 2011 had high risks for both lung cancer and bladder cancer associated with exposure to 2-aminonaphthalene *(48)*. A systematic review and meta-analysis of studies on the risk for lung cancer among workers exposed to 2-aminonaphthalene showed a significantly increased lung cancer risk, the effect estimates being similar in studies with and without concomitant occupational exposure to other lung toxicants and carcinogens *(49)*. Exposure to benzidine and 2-aminonaphthalene was monitored in both studies.

8.3.3 Hydrocarbons (benzene, 1,3-butadiene, isoprene, toluene)

Benzene and butadiene cause cancers of the haematolymphatic organs and are classified as known human carcinogens *(15)*. Isoprene causes tumours at various sites in laboratory animals *(50)*. 1,3-Butadiene and toluene are respiratory toxicants, and toluene is also toxic to the central nervous system and is a reproductive toxicant. These compounds are present in high amounts in cigarette smoke and probably play a role in lung cancer in smokers *(14, 15, 51)*.

The tobacco smoke-related health effects of 1,3-butadiene and the possible impacts of risk reduction strategies were evaluated from the ratio (margin of exposure) between the most sensitive toxicity end-point and appropriate estimates of exposure to 1,3-butadiene in mainstream and second-hand tobacco

smoke *(52)*. The authors concluded that the risks for cancer (leukaemia) and non-cancer (ovarian atrophy) could be significantly reduced by lowering the levels of 1,3-butadiene in smoke. They proposed that analysis of the margin of exposure is a practical means for assessing the impact of risk reduction strategies on human health. In a review of non-cancer health effects of benzene, it was concluded that exposure to benzene can have numerous outcomes in the reproductive, immune, nervous, endocrine, cardiovascular and respiratory systems *(53)*.

Cardiovascular effects

A potential association between exposure to benzene and an increased risk for cardiovascular disease has been investigated in mice and humans *(54)*. The effects of benzene in mice were assessed by direct inhalation, while the effects in humans were assessed in 210 people with mild-to-high risks for cardiovascular disease risk by measuring urinary biomarkers. Mice had significantly reduced levels of circulating angiogenic cells and higher plasma levels of low-density lipoprotein than control mice that breathed filtered air. In humans, smokers and people with dyslipidaemia had higher exposure to benzene, which was negatively correlated with populations of circulating angiogenic cells and associated with the risk for cardiovascular disease assessed on the Framingham risk score.

New epidemiological evidence

The results of a study with a sample of adult participants in the Gulf Long-term Follow-up Study in the USA during 2012 and 2013 suggested that ambient exposure to benzene and toluene is associated with haematological effects, including decreased haemoglobin and mean corpuscular haemoglobin concentration and increased red cell distribution width *(55)*. The evidence was particularly strong for benzene.

In the Multiethnic Cohort study, benzene uptake was compared in smokers in five different ethnic groups by analysing urinary *S*-phenylmercapturic acid, a specific biomarker of exposure to benzene *(56)*. African Americans had significantly higher and Japanese Americans significantly lower levels of *S*-phenylmercapturic acid than Whites. While benzene is not generally considered to cause lung cancer, these differences are consistent with those for lung cancer risk in this cohort.

8.3.4 Polycyclic aromatic hydrocarbons (benzo[a]pyrene)

PAHs are formed during incomplete combustion of organic matter and always occur as mixtures. Many PAHs are potent carcinogens or toxicants in laboratory animals *(57)*, and many are present in cigarette smoke, including the prototypic PAH benzo[*a*]pyrene, classified as a human carcinogen by a working group convened by the International Agency for Research on Cancer (IARC) *(57, 58)*.

PAHs are widely accepted to be major contributors to lung cancer in smokers *(57, 59–61)*. A study of benzo[*a*]pyrene and the TSNA NNK in A/J mice showed dose-dependent tumorigenesis at lower doses than previously reported *(62)*.

Zaccaria and McClure *(63)* analysed published studies on benzo[*a*] pyrene and other PAHs and found a correlation between the derived relative potency factors for immune suppression for nine PAHs and their potency factors for cancer, confirming previous observations of an association between the carcinogenicity of PAHs and immunosuppression.

Environmental exposure to benzo[*a*]pyrene is correlated with impaired learning and memory in adults and poor neurodevelopment in children. A comprehensive literature review was conducted to determine the potential mechanism of neurotoxicity by benzo[*a*]pyrene *(64)*. The results suggest that neurotoxic effects are observed at lower exposure than those associated with cancer. It was proposed that benzo[*a*]pyrene binding to the aryl hydrocarbon receptor results in loss of neuronal activity and decreased long-term potentiation, compromising learning and memory.

8.3.5 Tobacco-specific N-nitrosamines

TSNAs are important constituents of tobacco products, and two, NNK and NNN, are probably responsible for cancers of the lung, pancreas, oral cavity and oesophagus in tobacco users *(65, 66)*. Both have been classified as human carcinogens by working groups at IARC *(66, 67)*. These nitrosamines are formed from tobacco alkaloids during tobacco processing. The amounts that are formed depend on tobacco type, nitrate content and tobacco processing techniques, resulting in wide variation in the amounts in various cigarette brands *(65, 68–70)*. In smokers, increases in smoke TSNA yields due to changes in cigarette design, including filter ventilation, were accompanied by an increase in the incidence of lung adenocarcinoma, the type of lung cancer that is induced by NNK in laboratory animals *(71, 72)*.

New evidence in laboratory animals

The carcinogenicity of NNK and its metabolite NNAL was studied in male F-344 rats treated for 70 weeks. Both compounds induced a high incidence of lung tumours, and metastases were observed from primary pulmonary carcinomas to the pancreas. The results clearly demonstrate the potent pulmonary carcinogenicity and DNA damaging activity of NNK and NNAL in rats *(73)*. In another study by the same group *(74)*, NNN induced 96 oral cavity tumours and 153 oesophageal tumours in 20 male F-344 rats treated chronically with this carcinogen in their drinking water. This study showed for the first time the carcinogenic potency of NNN in the oesophagus and identified NNN as a strong oral cavity carcinogen present in tobacco.

New evidence in humans

Consistent with the data on carcinogenicity in animals, a positive association was found between prospectively measured exposure to NNN and NNK and risks for oesophageal and lung cancer, respectively, in smokers in the Shanghai Cohort Study. Additional analyses of the same cohort indicated that exposure to NNK was not associated with oesophageal cancer, and exposure to NNN was not associated with the risk of smokers for lung cancer *(75)*. Together, these results reaffirm the organ specificity of NNN and NNK towards the oesophagus and the lung, respectively, in smokers, consistent with the findings in F-344 rats. The uptake of NNK was also measured in 2252 smokers in the Multiethnic Cohort study *(76)*. After adjustment for age at urine collection, sex, creatinine and total nicotine equivalents, a marker of total nicotine uptake, the highest exposure to NNK was found for African Americans and the lowest for Japanese Americans. These findings are consistent with the findings on lung cancer risk of smokers in these groups.

Mechanistic studies and studies in laboratory animals indicate that DNA adduct formation is a critical step in NNN- and NNK-induced carcinogenesis. Higher levels of these adducts were found in the oral cells of smokers with head-and-neck squamous cell carcinoma than in cancer-free smokers *(77)*.

8.3.6   Alkaloids (nicotine)

Nicotine is the major known addictive agent in tobacco and cigarette smoke *(78)* and is a key driver of tobacco use. Nicotine content can greatly influence the extent and pattern of product use and can also define the category of users to whom a product will appeal. Further, it affects exposure to other toxicants and carcinogens in the product.

New evidence on the effects of nicotine reduction in cigarette smoke

Reduction of the nicotine content of cigarettes to a minimal or non-addictive levels has been recommended for consideration by TobReg and proposed by the FDA as an approach for reducing or eliminating the use of combusted tobacco products *(79, 80)*. The feasibility of this approach is confirmed by the results of a number of studies. In a double-blind, parallel, randomized clinical trial conducted between June 2013 and July 2014 at 10 sites in the USA, 840 participants were randomly assigned to smoke either their usual brand of cigarettes or one of six types of investigational cigarettes with a nicotine content ranging from 0.4 mg/g to 15.8 mg/g of tobacco (comparable to the nicotine content of commercial brands) for 6 weeks *(81)*. At the end of the study, participants assigned to cigarettes containing 2.4, 1.3 or 0.4 mg of nicotine per gram of tobacco had smoked a smaller average number of cigarettes per day than those assigned to their usual

brand or to cigarettes containing 15.8 mg/g (*P* <.001). Cigarettes with a lower nicotine content than control cigarettes reduced exposure to and dependence on nicotine and also reduced craving during abstinence from smoking, without significantly increasing the expired CO level or total puff volume, suggesting minimal compensation.

In a follow-up randomized, parallel-arm 8-week study by Hatsukami et al. *(82)*, smokers who were unwilling to quit were randomly assigned to normal or VLNC cigarettes, and use of alternative nicotine products, smoking behaviour and biomarkers of tobacco exposure were assessed. The offer of and instructions for use of reduced-nicotine cigarettes led to reduced smoking rates, reduced biomarkers of exposure to smoke toxicants and greater use of alternative tobacco or nicotine products than continued use of cigarettes with normal nicotine.

Health effects other than addiction

Nicotine can contribute to acute cardiovascular events and accelerated atherogenesis in tobacco users, probably due to stimulation of the sympathetic nervous system, decreasing coronary blood flow, impairment of endothelial function and other pharmacological effects *(83)*. A systematic review of studies in humans and animals on the health effects of exposure to nicotine during pregnancy and adolescence indicated that nicotine contributes critically to adverse effects, including reduced pulmonary function, auditory processing defects and impaired infant cardiorespiratory function, and it may contribute to cognitive and behavioural deficits in later life *(84)*. The study also found that exposure to nicotine during adolescence is associated with deficits in working memory, attention and auditory processing, as well as increased impulsivity and anxiety, and studies in animals suggest that nicotine increases the liability for addiction to other drugs.

8.3.7 Phenols (catechol, m-, p- and o-cresols, phenol, hydroquinone, resorcinol)

Catechol is a co-carcinogen *(85)* that is present in high amounts in cigarette smoke *(66)*. The United States Environmental Protection Agency has classified *m*-, *o*- and *p*-cresols as possible human carcinogens on the basis of genetic toxicity and increased incidences of skin and nasal tumours in rodents. Cresols are also respiratory toxicants. Hydroquinone is mutagenic in vitro and in vivo, including in a study in which it had significant genotoxicity in vitro in the γH2AX assay *(19)*. It has been shown reproducibly to induce benign neoplasms in the kidneys of male F-344 rats dosed orally, but the data on humans are inadequate. Phenol is a respiratory toxicant, elicits cardiovascular effects and is a tumour promoter. Resorcinol was reported to have a range of toxic effects in various studies and is considered to be a respiratory toxicant.

8.3.8   **Other organic compounds (acetone, acrylonitrile, pyridine, quinolone)**

Acetone is a respiratory toxicant and can irritate the respiratory tract. Acrylonitrile is a respiratory toxicant and is classified by the IARC as possibly carcinogenic to humans (Group 2B) *(14)*. It readily forms adducts with proteins, and the levels of such adducts are higher in smokers than in nonsmokers *(14, 86)*. Acrylonitrile is also mutagenic in some assays *(14)*. Few studies have been conducted on the toxicity of pyridine; some suggest effects on the respiratory tract, the central nervous system and the liver. Quinoline is an irritant after acute exposure and showed liver toxicity and carcinogenicity in animals. No significant new toxicological findings on these organic compounds have been identified since 2013.

8.3.9   **Metals and metalloids (arsenic, cadmium, lead, mercury)**

Arsenic and cadmium are human carcinogens *(87)*. These metals are lung carcinogens and could also play a role in bladder (arsenic) and kidney (cadmium) cancers. Arsenic also has cardiovascular and reproductive effects, and cadmium is a neurological and respiratory toxicant. Lead is a neurological, reproductive and cardiovascular toxicant and a probable human carcinogen *(88)*. Mercury is classified by IARC in Group 2B and is also a reproductive toxicant *(89)*. These elements are present in varying amounts in cigarette smoke *(66)* and smokeless tobacco *(90)*; the levels are probably affected by their concentrations in the soils in which the tobacco is grown. Pinto et al. *(91)* detected significantly higher levels of arsenic, lead and cadmium in the lung tissue of smokers than nonsmokers. The exposure of young children to second-hand tobacco smoke can result in blood lead levels that are associated with decreased IQ and cognition *(92)*.

Carcinogenicity

The association between long-term exposure to cadmium, measured in urine, and mortality from cancer was investigated in 3792 American Indians in Arizona, Oklahoma and North and South Dakota (USA) who participated in the Strong Heart Study during 1989–1991. Exposure to cadmium was associated with mortality from all cancers and with that from cancers of the lung and pancreas *(93)*. In a review of the literature, Feki-Tounsi & Hamza-Chaffai *(94)* concluded from the available in vitro and epidemiological studies that exposure to cadmium is associated with an increased risk of bladder cancer and may be involved in urothelial toxicity and carcinogenesis.

Neurotoxicity

Cadmium and lead are neurotoxicant components of tobacco smoke and could contribute to depression associated with smoking. The association between blood cadmium and lead levels and current depressive symptoms was investigated in a

cross-sectional study of adult participants in the National Health and Nutrition Examination Survey 2011–2012 in the USA (N = 3905). Blood cadmium was associated with higher odds for depressive symptoms in male participants aged 20–47 years, and blood lead, cigarette smoking and obesity were associated with depressive symptoms in female participants in this age range *(95)*. The finding of effects on spatial and nonspatial working memory, anxiety-related behaviour and motor activities in female adolescent mice exposed to cadmium and/or nicotine supports these conclusions *(96, 97)*. Nicotine and cadmium increased the metabolism, food intake and weight of treated mice as compared with controls. Nicotine administration increased motor function, while cadmium decreased motor activity. Both compounds induced a reduction in the memory index. Combined treatment with nicotine and cadmium induced decreases in weight and motor activity, increased anxiety and a significant decrease in nonspatial working memory.

Cardiovascular disease

It has been hypothesized that cadmium contributes to the cardiovascular risk associated with smoking by injuring vascular endothelial cells *(98)*. In a systematic review of epidemiological studies of the association between exposure to cadmium and cardiovascular disease, the pooled relative risks (95% CIs) for cardiovascular disease, coronary heart disease, stroke and peripheral arterial disease were: 1.36 (1.11, 1.66), 1.30 (1.12, 1.52), 1.18 (0.86, 1.59) and 1.49 (1.15, 1.92), respectively *(99)*. With the experimental evidence, the review supports an association between exposure to cadmium and cardiovascular disease, especially coronary heart disease.

Other health effects

Mice exposed to sodium arsenite for 90 days showed increased micronucleated polychromatic erythrocytes and an increase in genotoxic and germ-cell toxic effects in liver, kidney and intestinal tissues as compared with a control group. Combined treatment with smokeless tobacco extract induced a significant increase in sperm head abnormality as compared with either material alone *(100)*.

The effect of exposure to lead in cigarette smoke on fetal growth was studied by measuring blood lead concentrations in 150 healthy pregnant women *(101)*. The birth weight of the infants of mothers who smoked was significantly lower than that of infants born to non-smoking mothers ($P <.001$) and was negatively correlated with lead levels in plasma ($r = -0.38$; $P <.001$) and in whole blood ($r = -0.27$; $P <.001$).

Exposure to mercury has been hypothesized to lead to metabolic syndrome and diabetes mellitus. Reviews of the literature indicated that, while epidemiological data suggest a possible association between total mercury

concentrations in biological matrices and the incidence of these health outcomes, the relation is not consistent *(102, 103)*. A more comprehensive review of the health effects associated with exposure to mercury suggests that chronic exposure, even to low concentrations of mercury, can cause cardiovascular, reproductive and developmental toxicity, neurotoxicity, nephrotoxicity, immunotoxicity and carcinogenicity *(104)*.

8.3.10 Other constituents (ammonia, carbon monoxide, hydrogen cyanide, nitrogen oxides)

Ammonia is a respiratory irritant and toxicant, which increases the prevalence of respiratory symptoms, asthma and impaired pulmonary function in various industrial and agricultural settings *(105–108)*. Limited studies suggest that people with asthma are more sensitive to the respiratory effects of ammonia *(108, 109)*. CO is a well established cardiovascular toxicant, which competes with oxygen for binding to haemoglobin. In smokers, it is considered to reduce oxygen delivery, cause endothelial dysfunction and promote the progression of atherosclerosis and other cardiovascular diseases *(110–112)*.

Hydrogen cyanide is a well-known toxic agent, its primary targets being the cardiovascular, respiratory and central nervous systems. It acts by inhibiting cytochrome oxidase in the respiratory chain. Cigarette smoke can reduce detoxification of hydrogen cyanide, leading to chronic exposure of smokers to this toxin and consequent amblyopia, retrobulbar neuritis, sterility and a potential contribution to impaired wound healing *(112, 113)*.

Nitrogen oxides are respiratory and cardiovascular toxicants. Nitric oxide, the primary form in fresh cigarette smoke, induces vasodilation and causes DNA strand breaks and lipid peroxidation, possibly contributing to carcinogenesis *(114)*. It may also contribute to nicotine addiction by increasing nicotine absorption, reducing symptoms of stress and increasing post-synaptic dopamine levels *(115)*. Nitrogen dioxide is a pulmonary irritant.

8.4 Availability of analytical methods

8.4.1 Standardized WHO TobLabNet methods for the analysis of priority toxicants

To ensure implementation of Articles 9 and 10 of the WHO FCTC, laboratory capacity must be available that meets the highest standards of excellence, transparency, reliability and credibility *(116)*. Standardized, reliable, accurate analytical methods are required by laboratories to conduct the scientifically rigorous testing required for tobacco products globally *(117)*. Consensus on a set of methods may partly depend on successful transfer of such methods to other laboratories. Established, validated WHO TobLabNet standard operating procedures (SOPs) as of October 2018 are summarized in Table 8.3.

Table 8.3. Standardized WHO TobLabNet standard operating procedures for the analysis of priority toxicants

SOP	Title	Priority toxicants	Year of publication
SOP 01	Intense smoking of cigarettes	Necessary for mainstream cigarette smoke generation	2012
SOP 02	Validation of analytical methods of tobacco product contents and emissions	Describes method validation	2017
SOP 03	Determination of tobacco-specific nitrosamines in mainstream cigarette smoke under ISO and intense smoking conditions	Mainstream NNK, NNN (emissions)	2014
SOP 04	Determination of nicotine in cigarette tobacco filler	Nicotine (content)	2014
SOP 05	Determination of benzo[*a*]pyrene in mainstream cigarette smoke	Mainstream benzo[*a*]pyrene (emissions)	2015
SOP 06	Determination of humectants in cigarette tobacco filler	Propylene glycol, glycerol, triethylene glycol (content)	2016
SOP 07	Determination of ammonia in cigarette tobacco filler	Ammonia (content)	2016
SOP 08	Determination of aldehydes in mainstream cigarette smoke under ISO and intense smoking conditions	Acetaldehyde, acrolein, formaldehyde (emissions)	2018
SOP 09	Determination of volatile organics in mainstream cigarette smoke under ISO and intense smoking conditions	1,3-Butadiene, benzene (emissions)	2018
SOP 10	Determination of nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions	Mainstream nicotine and CO (emissions)	2016

CO: carbon monoxide; ISO: International Organization for Standardization; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N′*-nitrosonornicotine; SOP: standard operating procedure.

8.4.2 Overview of methods for the remaining priority toxicants

The priority toxicants for which standardized WHO TobLabNet SOPs are not available are described below, with published methods for their quantification in tobacco products and mainstream smoke.

Aldehydes (butyraldehyde, crotonaldehyde, propionaldehyde).

Ding et al. *(118)* reported a significant improvement in terms of ease of use, efficiency and environmental friendliness to previous methods for the analysis of acetaldehyde, acrolein, acetone, crotonaldehyde, formaldehyde, propionaldehyde and methyl ethyl ketone. The method involves use of a double filter (Cambridge pads, one pre-treated with dinitrophenylhydrazine and one dry) to trap and derivatize carbonyls in mainstream smoke simultaneously. The hydrazones are then quantified by ultra-high-pressure liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) with a 4-min run time. The accuracy of the method, determined from spiking at low, medium and high levels, was 83–106%, and its precision, determined from 30 replicate measurements of reference cigarette smoke (3R4F), was < 20% relative standard deviation for all target analytes. The limits of detection of the WHO priority toxicants were 2.7 µg for acetaldehyde, 0.1 µg for acrolein, 0.2 µg for crotonaldehyde, 2.4 µg for

formaldehyde and 0.6 μg for propionaldehyde. This method is ideal for regulatory analyses, as it can be used with linear smoking machines, which greatly increases sample throughput.

Researchers at the China National Tobacco Corporation established a simple method for rapid determination of acrolein, acetone, propionaldehyde, crotonaldehyde, butanone and butyraldehyde in mainstream smoke *(119)*. Although the analysis time is short (4 min), data mining of the full and daughter scans must be conducted to overcome difficulties in separating and quantifying isomers of acetone–propionaldehyde and butanone–butyraldehyde with this atmospheric pressure chemical ionization MS/MS technique. The results obtained with a reference cigarette (3R4F, 35-mL puff volume, eight puffs) are consistent with those reported in the literature. The limits of detection of the WHO priority toxicants were 0.007 μg/L for acrolein, 0.021 μg/L for acetone, 0.008 μg/L for propionaldehyde, 0.004 μg/L for crotonaldehyde, 0.012 μg/L for butanone and 0.006 μg/L for butyraldehyde. Modifications were made to the chemical ionization source in order to introduce the gas sample directly into the ionization region.

In another reported method, mainstream smoke from cigarettes was collected in sulfuric acid (20%) and ascorbic acid (25 mmol/L) impingers *(120)*, and a dispersive liquid–liquid microextraction method was used to simultaneously extract the solution and convert benzaldehyde, butyraldehyde and furfural into their hydrazone derivatives, which were then quantified by high-performance LC. The matrix spike recovery was 88.0–109%, and the relative standard deviation for inter- and intra-day assays were < 8.50%. The limits of detection of the WHO priority toxicants were 14.2 μg/L for benzaldehyde, 21.3 μg/L for butyraldehyde and 7.92 for furfural. This method is quicker, simpler and less expensive than previously published liquid phase microextraction methods for the analytes tested.

Aromatic amines (1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl)

In another method, the gas (0.6 M hydrochloric acid impinger) and particulate (glass-fibre filter) phases of mainstream smoke were collected, and the resulting acid solution was used to extract the filter in an ultrasonic bath *(121)*. The extract was cleaned by passage through two solid-phase extraction cartridges of different polarity and separated on a phenyl-hexyl column. Nine aromatic amines were quantified by LC–MS/MS. The method was validated by spiking the target analytes into the extract from a reference cigarette (3R4F) at three levels (low, medium, high). Recovery of the WHO priority toxicants was 84.8–97.8%, and intra- and inter-day precision was < 9% and < 14%, respectively. The limits of detection for the WHO priority toxicants were 0.08 ng/cigarette for 1-aminonaphthalene, 0.09 ng/cigarette for 2-aminonaphthalene, 0.05 ng/cigarette for 3-aminobiphenyl and

0.03 ng/cigarette for 4-aminobiphenyl. This method is similar to that reported by Xie et al. *(122)*, but gave better separation and baseline resolution of the target aromatic amines.

Deng et al. *(123)* used a novel dispersive liquid–liquid microextraction clean-up method and faster ultraperformance convergence chromatography MS/MS to quantify nine aromatic amines in mainstream smoke. This approach resulted in an instrument run time of 5 min, a substantial improvement over the 30 min required for the high-performance LC method of Zhang et al. *(121)*. In this method, supercritical CO_2 is used as the primary mobile phase to ensure higher flow rates with a lower pressure drop across the column and faster run times. The method was validated with a reference cigarette (3R4F). The recovery from spiked (low, medium, high) mainstream smoke extracts of the 3R4F was 69.4–120% for the WHO priority toxicants. The limits of detection of the WHO priority toxicants were 0.29 ng/cigarette for 1-aminonaphthalene, 0.21 ng/cigarette for 2-aminonaphthalene, 0.02 ng/cigarette for 3-aminobiphenyl and 0.03 ng/cigarette for 4-aminobiphenyl.

Hydrocarbons (isoprene, toluene)

Sampson et al. *(124)* reported an automated, high-throughput method for accurate quantification of a broad range of hazardous VOCs, including acrylonitrile, benzene, butyraldehyde, 1,3-butadiene and toluene, in mainstream smoke. The gas phase was collected into a polyvinyl fluoride gas sampling bag, and isotopically labelled analogues were added as internal standards to account for any losses due to handling and ageing. The particle phase was collected onto a glass-fibre filter and spiked with internal standards. After heating, the bags and filter headspace were sampled by automated solid-phase microextraction and quantified by gas chromatography (GC) with mass selective detection. The method was validated with reference cigarettes (1R5F and 3R4F) smoked under ISO and HCI puffing regimens. The results were comparable to those in other reports, except for toluene, which was found at a level ~30% lower than in previous reports. The inter- and intra-run precision was ≤a20%. There was a high correlation (0.97) between toluene and *m*- and *p*-xylene levels in mainstream smoke. This method was later used by the same group to quantify the target analytes in 50 US commercial brands *(125)*. The limits of detection of the WHO priority toxicants were not reported in either article; however, the lowest calibration standards used were 0.14 parts per billion by volume for all WHO priority toxicants except benzene, which was 0.1 parts per billion by volume.

Phenols (catechol, m-, p-, o-cresols, phenol, hydroquinone, resorcinol)

A simple, precise method for quantifying WHO priority phenols in mainstream smoke was reported by Saha et al. *(126)*. The method involves single-drop

microextraction and LC–MS/MS to reduce organic solvent consumption, shorten sample preparation time and eliminate the derivatization steps required in previously published GC–MS methods. The limits of detection of the WHO priority toxicants were 0.30 ng/mL for catechol, 0.05 ng/mL for *o*- and *p*-cresol, 0.15 ng/mL for phenol, 0.30 ng/mL for hydroquinone and 0.20 ng/mL for resorcinol. The method reported by Wu et al. at Labstat International *(127)* with LC–MS/MS improved on the Health Canada methods (T-114, T-211) by use of a shorter analytical column with smaller particle size to resolve the three cresol positional isomers fully.

Other organic compounds (acetone, acrylonitrile, pyridine, quinoline)

A multi-analyte method that includes acrylonitrile is discussed above in the section on hydrocarbons, and a multi-analyte method that includes acetone is discussed in the section on aldehydes. Qualitative two-dimensional chromatography time-of-flight MS methods for the analysis of organic compounds in the vapour *(128)* and particle phases *(129)* of mainstream tobacco smoke were reported by tobacco industry researchers. These methods are not suitable for testing emissions but could be useful for regulatory purposes to differentiate mainstream tobacco smoke emissions resulting from different product designs or to identify adulteration.

Metals and metalloids (arsenic, cadmium, lead, mercury)

Traditional sample collection methods for quantifying cadmium in mainstream smoke were critically evaluated *(130)*. A platinum trap was used to determine breakthrough in two different sample collection methods: electrostatic precipitation and Cambridge filter pad. The detection limit of cadmium that had passed through the primary traps to the platinum traps was 0.30 ng/cigarette. Cadmium breakthrough from the Cambridge filter was significant (4–23% of the sample) but was negligible with electrostatic precipitation (< 1%). This technique was used by Fresquez et al. *(131)* in a high-throughput method for the analysis of mercury in the gas phase of cigarette and little cigar smoke emitted by ISO and HCI puffing regimens. The method is much quicker, simpler and more environmentally friendly than previously reported methods, because it eliminates the need for impingers, strong oxidizing agents (e.g. permanganate) and strong acids (e.g. sulfuric acid). The limit of detection was 0.27 ng cadmium/g in tobacco and 0.097 ng mercury/cigarette. The same team used high-throughput methods to establish the amounts of all WHO priority toxicant metals in the tobacco filler (content) of 50 brands of cigarettes on the United States market *(132)*. Microwave digestion was used for sample preparation, except for mercury, which was introduced directly into the analyser. The analytical instrumentation included a direct combustion analyser for mercury and an inductively coupled plasma MS for all other elements except arsenic and selenium, which were run

separately with a different inductively coupled plasma MS method. The accuracy of the method was determined with standard tobacco reference materials, and the results were within the target ranges and the results for WHO priority toxicant metals, except for lead, which was slightly lower (4%) than the lower range of the target value for the Oriental tobacco reference material. The limits of detection of the WHO priority toxicants in tobacco were 0.082 µg/g (magnetic sector) and 0.25 µg/g (quadrupole) for arsenic, 0.23 µg/g for cadmium, 0.16 µg/g for lead and 0.00063 µg/g for mercury.

Other constituents (hydrogen cyanide, nitric oxides)

Hydrogen cyanide was quantified in mainstream smoke by collecting smoke samples on a sodium hydroxide-treated Cambridge filter pad and quantified by ion chromatography with pulsed amperometric detection *(133)*. In comparison with the traditional continuous flow analyser method, the filter-based method offers convenience, greater accuracy and a wider linear quantification range. The method performance was evaluated by spiking hydrogen cyanide onto filters containing particulate from commercial cigarettes made of blended and flue-cured tobaccos. The mean recovery was 97%, and the intra- and inter-day relative standard deviation was < 6%. The limit of detection of hydrogen cyanide was 3 µg/L for a 25-µL injection loop. Mahernia et al. *(134)* used polarography to measure hydrogen cyanide concentrations in 50 large cigars and cigarettes purchased in the Islamic Republic of Iran. Mainstream smoke was drawn from each tobacco product with a pump and passed through a glass tube containing sodium hydroxide (0.1 M). The concentration of the cyanide was determined by fortifying the solution with known quantities of cyanide and using the standard addition method. The hydrogen cyanide level in the tobacco products was 17.6–1550 µg per rod. Limits of detection for hydrogen cyanide were not provided. No recent methods for the analysis of nitric oxides were found.

8.5   Update on variations in toxicants among brands

The variations in toxicants reported in mainstream tobacco smoke generated by standardized machine smoking methods, the HCI puffing regimen for cigarettes and little cigars and the Beirut puffing regimen for waterpipe tobacco are summarized in Table 8.4. Variations in toxicants in the tobacco filler (content) of cigarettes, little cigars, waterpipe tobacco and waterpipe charcoal are summarized in Table 8.5. More research is needed on emissions of WHO priority toxicants from the increasingly popular, newer tobacco products (in comparison with cigarettes) in order to determine the 10th, 25th, 75th and 90th percentiles, so that global comparisons can be made as a first step towards establishing limits for the WHO priority toxicants.

Table 8.4. Variations in WHO priority toxicants, expressed as mass per rod or session (waterpipe), in mainstream smoke from combustible products

Toxicant	Cigarettes[a]	Little cigars[a]	Waterpipes
Aldehydes			
Acetaldehyde (µg)	1198–1947 *(118)* (USA)	NR	120–2520 *(135)*
Acetone (µg)	385–724 *(118)* (USA)	NR	20.2–118 *(135)*
Acrolein (µg)	107 –169 *(118)* (USA)	105–185 *(136)* (USA)	10.1–892 *(135)*
Crotonaldehyde (µg)	25–72 *(118)* (USA)	NR	NR
Formaldehyde (µg)	55–108 *(118)* (USA)	NR	36–630 *(135)*
Propionaldehyde (µg)	116–232 *(118)* (USA)	NR	5.71–403 *(135)*
Aromatic amines			
1-Aminonaphthalene (ng)	4.31–33.37 *(121)* (China)	NR	6.20 *(135)*
2-Aminonaphthalene (ng)	0.89–4.60 *(121)* (China)	NR	2.84 *(135)*
3-Aminobiphenyl (ng)	1.46–4.68 *(121)* (China)	NR	< 3.30 *(137)* (Germany)
4-Aminobiphenyl (ng)	0.38–5.95 *(121)* (China)	NR	
Hydrocarbons			
Benzene	58.7–128.5 *(125)* (USA)	NR	271 *(135)*
1,3-Butadiene	67.8–118.3 *(125)* (USA)	NR	Present *(138)*[a]
Isoprene	NR	NR	4.00 *(135)*
Toluene	81–178 *(125)* (USA)	NR	9.92 *(135)*
Polycyclic aromatic hydrocarbons			
Benzo[*a*]pyrene (ng)	NR	17–32 *(139)* (USA)	LOD–307 *(135)*
Pyrene[b] (ng)	NR	NR	30–12 950 *(135)*
Tobacco-specific *N*-nitrosamines			
NAB (ng)	NR	NR	8.45 *(135)*
NAT (ng)	NR	NR	103 (135)
NNK (ng)	NR	438–995 *(139)* (USA)	LOD–46.4 *(135)*
NNN (ng)	NR	434–1550 *(139)* (USA)	34.3 *(135)*
Alkaloids			
Nicotine, mg	NR	1.85–6.15 *(140)* (USA)	> 0.01–9.29 *(135)*
Phenols			
Catechol (µg)	49.6–118 *(127)* (Canada)	NR	166–316 *(135)*
m-Cresol (µg)	1.93–6.92 *(127)* (Canada)	NR	NR
p-Cresol (µg)	5.28–17.7 *(127)* (Canada)	NR	NR
m+*p*-Cresol (µg)	7.24–24.6 *(127)* (Canada)	NR	2.37–4.66 *(135)*
o-Cresol (µg)	2.21–7.93 *(127)* (Canada)	NR	2.93–4.41 *(135)*
Phenol (µg)	8.34–32.7 *(127)* (Canada)	NR	3.21–58.0 *(135)*
Hydroquinone (µg)	60.1–140 *(127)* (Canada)	NR	21.7–110.7 *(135)*
Resorcinol (µg)	1.25–2.46 *(127)* (Canada)	NR	1.69–1.87 *(135)*
Other organic compounds			
Acetone	NR	NR	20.2–118 *(135)*
Acrylonitrile (µg)	19.7–37.7 *(125)* (USA)	NR	Present *(138)*[a]
Pyridine	NR	NR	4.76 *(135)*
Quinoline	NR	NR	BLQ
Metals and metalloids			
Arsenic (ng)	NR	NR	165 *(135)*
Cadmium (ng)	NR	NR	< 100 *(141)* (UK)

Chromium[c] (ng)	0.60–1.03 *(142)* (USA)	NR	250–1340 *(135)*
Lead (ng)	NR	NR	200–6870 *(135)*
Mercury (ng)	NR	5.2–9.6 *(131)* (USA)	< 100 *(141)* (UK)
Other priority toxicants			
Carbon monoxide, mg	NR	NR	5.7 - 367 *(135)*
Hydrogen cyanide	NR	NR	NR
Nitric oxide, mg	140.9–266.8 *(143)* (Russian Federation)	NR	0.325–0.440 *(135)*

BLQ: below limit of quantification; LOD: below the limit of detection; NAB: *N´*-nitrosoanabasine; NAT: *N´*-nitrosoanatabine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N´*-nitrosonornicotine; NR: not reported; UK: United Kingdom.
[a] Health Canada intense puffing regimen: 55 mL puff volume, 2-s puff duration every 30 s, 100% vent blocking).
[b] Present, presence in mainstream smoke extrapolated from urinary biomarker.
[c] Currently not a TobReg priority toxicant.

Table 8.5. Variations in WHO priority toxicant content in tobacco filler or charcoal (waterpipe) of combustible tobacco products, expressed as mass of chemical per mass of tobacco (g)

Toxicant	Cigarettes	Little cigars	Waterpipe tobacco	Waterpipe charcoal
Nicotine (mg)	16.2–26.3 *(144)* (USA) 10.5–17.8 *(145)* (Pakistan)	10.3–19.1 *(144)* (USA)	0.48–2.28 *(146)* (USA) 1.8–41.3 *(147)* (Jordan)	NA
Benzo[a]pyrene (ng)	NR	NR	NR	1–26 *(148, 149)* (Lebanon, USA)
Pyrene (ng)	NR	NR	NR	6–170 *(148, 149)* (Lebanon, USA)
Arsenic (µg)	0.22–0.36 *(132)* (USA)	NA	0.062 (0.023)[b] *(150)* (Egypt)	0.018 (0.013) *(150)* (Egypt)
Cadmium (µg)	1.0–1.7 *(132)* (USA)	NA	0.34 (0.007) *(150)* (Egypt)	0.005 (0.017)[b] *(150)* (Egypt)
Chromium[a] (µg)	1.3–3.1 *(132)* (USA)	NR	0.15–0.37 *(151)* (USA, Middle East)	0.161–8.32 *(152)* (Worldwide)
Lead (µg)	0.60–1.16 *(132)* (USA)	NR	0.15 (0.008)[b] *(150)* (Egypt)	0.97 (0.01)[b] *(150)* (Egypt)
Mercury (µg)	0.013–0.020 *(132)* (USA)	0.017–0.024 *(132)* (USA)	NR	NR

NR: not reported. [a] Currently not a TobReg priority toxicant. [b] Mean (relative standard deviation).

8.6 Criteria for future re-evaluation of toxicants on the list

The criteria for selecting priority toxicants and the overall recommendations of TobReg indicate four areas that should guide periodic re-evaluation of the priority list.

Toxicological profile of specific contents and emissions: the priority list of toxicants was identified to help WHO FCTC Parties and Member States to fulfil the requirements of Articles 9 and 10 of the WHO FCTC. Monitoring of the priority list is viewed as an initial step towards regulating the contents and emissions of combustible tobacco products. Up-to-date scientific knowledge on the toxicity of the selected emissions is critical to ensure the continuous relevance of the priority list and to inform future regulatory measures.

Validated methods: the contents and emissions of priority toxicants must be measured according to WHO TobLabNet SOPs to ensure accurate, reproducible results that can serve as a basis for regulatory measures. Development of standardized methods by TobLabNet is guided by robust, well-characterized, published protocols, which are reviewed, tested and further validated by TobLabNet. Advances in analytical chemistry and toxicological data are reviewed periodically by TobLabNet to identify priorities for testing methods.

Brand variations: according to the partial guidelines on Articles 9 and 10 to the WHO FCTC, the tobacco industry is ultimately responsible for generating and reporting data on priority contents and emissions for each brand. Such data can be generated according to the WHO TobLabNet SOPs, as noted in the decision of the eighth COP of the WHO FCTC in October 2018 (FCTC/COP8(21)). Emissions of many priority chemicals have not been measured systematically in all commercially available products. Thus, periodic review of new reports on the chemical composition of combustible tobacco products establishes a frame of reference for understanding variations in emissions among product types and the lowest practicably achievable levels of priority toxicants.

Correlations among constituents within a brand: a positive correlation among several toxicants would suggest that the levels of one could serve as a proxy for several other toxicants in a regulatory strategy. A negative correlation would suggest that mandatory lowering of one toxicant could result in increases in the levels of negatively correlated toxicants. While caution should be exercised in relying on these assumptions, close monitoring of the relations among constituents could provide insight for future selection of toxicants for mandatory reduction.

8.7 Criteria for selection of new toxicants

Addition of new constituents to the non-exhaustive priority list should be considered in the future. Several criteria for the selection of new toxicants are listed below.

Substantial evidence of risk to human health: for instance, Talhout et al. *(153)* listed 98 hazardous smoke constituents on the basis of the risk for human inhalation. In addition, the FDA identified 93 HPHCs for potential reporting and regulation *(154)*. The HPHC list comprises chemicals and chemical compounds in tobacco or tobacco smoke that are taken into the body (inhaled, ingested or absorbed) and cause or have the potential to cause direct or indirect harm to users and non-users of tobacco products. The FDA selected constituents that had been identified as known, probable or possible human carcinogens by either the IARC, the United States Environmental Protection Agency or the United States National Toxicology Program, as well as those identified by the United States National Institute for Occupational Safety and Health as potential occupational

carcinogens. Constituents identified by the United States Environmental Protection Agency or the Agency for Toxic Substances and Disease Registry as having adverse respiratory or cardiac effects, constituents identified by the California Environmental Protection Agency as reproductive or developmental toxicants and constituents reported in the literature as contributing to abuse liability were also included. Forty toxic and carcinogenic constituents are listed on both the FDA HPHC list and by Talhout et al. *(153)* but are not currently on the TobReg list (Table 8.6). These constituents could be prioritized for future consideration by TobReg, for instance by applying hazard indices and generating data on variations in their levels among brands.

Table 8.6. Constituents to be considered for future inclusion on the TobReg priority toxicant list

Constituent	Health effect *(154)*	Risk associated with inhalation (mg/m³) *(153)*
Acetamide	CA	5.0×10^{-4} (cancer)
Acrylamide	CA	8.0×10^{-3} (cancer)
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole	CA	1.4×10^{-6} (cancer)
2-Amino-3-methyl)-9*H*-pyrido[2,3-*b*]indole	CA	2.9×10^{-5} (cancer)
2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole	CA	7.1×10^{-6} (cancer)
2-Amino-3-methylimidazo[4,5-*f*]quinoline	CA	2.5×10^{-5} (cancer)
2-Amino-9*H*-pyrido[2,3-*b*]indole	CA	8.8×10^{-5} (cancer)
2-Aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole	CA	2.5×10^{-5} (cancer)
Benz[*a*]anthracene	CA, CT	9.1×10^{-5} (cancer)
Beryllium	CA	4.2×10^{-6} (cancer)
Chromium	CA, RT, RTD	8.3×10^{-7} (cancer)
Chrysene	CA, CT	9.1×10^{-4} (cancer)
Cobalt	CA, CT	5.0×10^{-4} (respiratory)
Dibenz[*a,h*]anthracene	CA	8.3×10^{-6} (cancer)
Dibenzo[*a,e*]pyrene	CA	9.1×10^{-6} (cancer)
Dibenzo[*a,h*]pyrene	CA	9.1×10^{-7} (cancer)
Dibenzo[*a,i*]pyrene	CA	9.1×10^{-7} (cancer)
Dibenzo[*a,l*]pyrene	CA	9.1×10^{-7} (cancer)
Ethyl benzene	CA	7.7×10^{-1} (liver and kidney)
Ethyl carbamate	CA, RDT	3.5×10^{-5} (cancer)
Ethylene oxide	CA, RT, RDT	1.1×10^{-4} (cancer)
Hydrazine	CA, RT	2.0×10^{-6} (cancer)
Indeno[*1,2,3-cd*]pyrene	CA	9.1×10^{-5} (cancer)
Methyl ethyl ketone	RT	5.0 (developmental)
1-Methyl-3-amino-5*H*-pyrido[4,3-*b*]indole	CA	1.1×10^{-5} (cancer)
5-Methylchrysene	CA	9.1×10^{-6} (cancer)
Naphthalene	CA, RT	3.0×10^{-3} (nasal)
Nickel	CA, RT	9.0×10^{-5} (lung fibrosis)
N-Nitrosodiethanolamine	CA	1.3×10^{-5} (cancer)
N-Nitrosodiethylamine	CA	2.3×10^{-7} (cancer)
N-Nitrosodimethylamine	CA	7.1×10^{-7} (cancer)
N-Nitrosomethylethylamine	CA	1.6×10^{-6} (cancer)
N-Nitrosopiperidine	CA	3.7×10^{-6} (cancer)

N-Nitrosopyrrolidine	CA	1.6×10^{-5} (cancer)
Polonium-210	CA	925.9 (cancer)
Propylene oxide	CA, RT	2.7×10^{-3} (cancer)
Selenium	RT	8.0×10^{-4} (respiratory)
Styrene	CA	9.2×10^{-2} (neurotoxicity)
Vinyl acetate	CA, RT	2.0×10^{-1} (nasal)
Vinyl chloride	CA	1.1×10^{-3} (cancer)

CA: carcinogen; CT: cardiovascular toxicant; RDT: reproductive or developmental toxicant; RT: respiratory toxicant.

Constituents in the same chemical class as current priority toxicants: the rationale for including such constituents is that several compounds can be analysed simultaneously with the same analytical technique. Examples include metals and other trace elements, as well as PAHs. The elements beryllium, chromium, cobalt, nickel, polonium-210 and selenium, listed in Table 8.6, can be analysed by a multi-element method with cadmium and other metals already on the TobReg priority list. At least 23 different PAHs could be analysed with the same analytical technique *(155)*. Eight PAHs listed in Table 8.4 – benz[*a*]anthracene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*] pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[*1,2,3-cd*]pyrene and naphthalene – could be analysed with benzo[*a*]pyrene. It has been demonstrated that the levels of these PAHs correlate negatively with those of nitrate and TSNAs; however, the relations depend on tobacco type *(156)*. In addition, in many studies of human exposure, urinary levels of 1-hydroxypyrene or phenanthrene tetraols, which are biomarkers of exposure to the non-carcinogenic PAHs pyrene and phenanthrene, respectively, are analysed to assess exposure to PAHs *(157, 158)*. As these two compounds are present in tobacco smoke at much higher levels than benzo[*a*]pyrene and other carcinogenic PAHs, simultaneous analysis of pyrene and phenanthrene with benzo[*a*]pyrene could provide information for future monitoring of changes in human exposure due to reductions in the levels of these constituents in smoke.

      Chemicals or chemical compounds that are precursors to toxic emissions in tobacco smoke: for instance, nitrite and nitrate in tobacco leaf are precursors to the carcinogenic nitrosamines NNK and NNN in tobacco and smoke and to nitrogen oxides in smoke. Well established data from both academic and industry researchers show that nitrate and nitrite in tobacco significantly affect the composition of tobacco smoke *(159, 160)*. In addition, nitrate levels in tobacco affect the levels of ammonia in cigarette smoke, which in turn influences smoke pH and the bioavailability of nicotine. Therefore, nitrite and nitrate levels in tobacco filler of cigarettes are important predictors of the toxicological properties of tobacco smoke. The current TobReg priority list includes nitric oxides in the gas phase of tobacco smoke; however, analysis of such constituents of tobacco smoke may be complicated, with inconsistent results

among laboratories. Therefore, analysis of nitrites and nitrates in tobacco filler would be a robust, informative alternative.

Constituents that contribute to the palatability and/or addictiveness of tobacco products: the impact of additives on the attractiveness and abuse liability of tobacco products has been reviewed *(161)*. For instance, sugars in tobacco filler of combustible products are a group of additives that could be considered for future monitoring and regulation. Sugars in tobacco contribute to the formation of acetaldehyde in tobacco smoke *(162, 163)*, potentially contributing to the addictive potential of tobacco smoke, either directly or through the formation of harman *(32–34)*.

8.8 Research needs and regulatory recommendations

8.8.1 Research needs, data gaps and future work

This review of the toxicity, analytical methods and reports on the levels of the priority toxicants in combustible tobacco products indicates that the following areas require research.

Despite toxicological evidence of the importance of the 39 priority toxicants and the availability of analytical methods, **brand- and product-specific information on the levels of emissions of toxicants in diverse combustible tobacco products is lacking**. Information on variations in the levels of toxicants in different brands and types of combustible tobacco products (e.g. cigarettes, cigarillos, waterpipes) could justify regulation of specific emissions. In view of the widespread global use of combustible products manufactured locally (e.g. *bidis*) or made by consumers (e.g. roll-you-own cigarettes), better characterization of these products is essential.

Evaluation of standardized methods for the analysis of certain constituents in tobacco filler (contents) is a more robust alternative to measurements in cigarette smoke in some cases. For instance, nitrate and nitrite levels in tobacco strongly influence the levels of nitric oxides in tobacco smoke. Analysis of these constituents in tobacco filler will require less time and resources and could provide more consistent data from laboratories than analysis of nitric oxide emissions. Furthermore, the validated methods can be used directly for the analysis of smokeless tobacco products. Carcinogenic TSNAs are formed in tobacco during processing, and the contribution of pyrolysis to their levels in smoke, if any, is minimal. Therefore, the levels in tobacco are strong predictors of the levels in cigarette smoke *(68, 86, 164, 165)*.

While TobReg previously recommended that upper limits for emissions of toxicants in tobacco products should be set on the basis of established toxicological principles *(166)*, **the extent to which toxicant levels must be reduced, as a complex mixture or singly, in order to minimize the harm caused by tobacco products remains unknown.** Laboratory in vitro and in vivo studies are required

to better understand the effect of the complex chemistry of tobacco products on the carcinogenic and toxic potency and the toxicity thresholds of individual constituents. Studies of human exposure and molecular epidemiology, including studies of prospective cohorts, would indicate the optimal reductions in tobacco toxicant levels necessary to achieve the maximum public health benefit.

Research is needed to **better understand which ingredients or constituents contribute to the addictiveness and palatability of tobacco products,** either independently or by increasing the bioavailability of nicotine. This information will have an impact on future regulatory approaches. For instance, it has been suggested that measures to reduce the attractiveness and palatability of tobacco products might have a greater impact on public health than reducing toxic emissions *(167)*.

Current work to prioritize toxic emissions and the development of methods for product testing should be **extended to human exposure and health outcomes.** The smoker–cigarette interaction is more complex than any single machine-based regimen *(1)*, and it is not clear whether reductions in per-milligram nicotine emissions will lead to corresponding reductions in human exposure. Laboratory capacity should be built for analysing biomarkers of the priority toxicants in human biological samples. Analyses of spent cigarette filters might be considered as a less expensive measure of human toxicant intake *(69, 168–170)*. In addition, research should be conducted to identify suitable biomarkers of potential harm that could be used to evaluate the long-term health impact of future product standards.

8.8.2 Regulatory recommendations and support to Parties

TobLabNet has completed validation of methods for measuring selected emissions in cigarette tobacco filler and in mainstream cigarette smoke, and the SOPs for these methods are available on the WHO Tobacco Product Regulation website. It is recommended that Parties consider requiring cigarette manufacturers to conduct emission testing in accordance with the TobLabNet SOPs and to report the results to national authorities. This recommendation is part of a decision by the eighth COP (FCTC/COP8(21)), which encourages Parties to acknowledge and implement the WHO TobLabNet methods as appropriate and emphasizes the need for further support of TobLabNet's capacity-building activities by WHO.

Assessment of studies on the toxicity of the extended list of priority toxicants has demonstrated their continued role in the toxic and addictive potential of combustible tobacco products. The methods validated by TobLabNet can be used for other toxicants on the extended priority list, as reviewed in this report. Therefore, it is recommended that Parties consider requesting, as applicable and appropriate, manufacturers to report on the emissions of the additional priority toxicants with methods based on the TobLabNet SOPs.

SOPs for the analysis of priority contents and emissions for which methods validated by TobLabNet are not yet available should be developed next, as recommended by TobReg *(166)*: cadmium and lead in tobacco; nicotine in waterpipe smoke; and nicotine, TSNAs and benzo[*a*]pyrene in smokeless tobacco.

Of the constituents not currently on the priority list, it is recommended that Parties consider requiring that manufacturers report on the levels of nitrate and nitrite in tobacco filler (content), as a potential proxy for nitric oxide emissions. An easy, cost–effective method for the analysis of nitric oxide in tobacco smoke is not yet available. Nitrate and nitrite in aqueous tobacco extracts can be analysed by adapting the TobLabNet method for ammonia in tobacco filler (SOP 07). In addition, given their importance to human biomonitoring research, pyrene and phenanthrene (and potentially other carcinogenic PAHs listed in Table 8.6) could be monitored simultaneously with benzo[*a*]pyrene by the TobLabNet method.

It is recommended that reference tobacco product materials be made available to TobLabNet laboratories participating in WHO method validation to aid in determining the success of method transfer, in terms of the accuracy, repeatability and reproducibility of each method. Suitable sample matrix-matched certified and standard reference materials can be analysed at the same time as test materials as a form of quality control for the evaluation of data generated in interlaboratory testing. Commercially available certified and standard reference materials are listed in Table 8.7.

Table 8.7. Certified and standard reference materials suitable for quality control of samples

Name	Description	Issuer	Certified values
SRM 3222	Cigarette tobacco filler	NIST	Nicotine, NNN, NNK, VOCs
1R6F	Reference cigarette	KTRDC	Mainstream smoke emissions, puff count for ISO and HCl puffing, filler content, physical properties
RT5	High-TSNA ground tobacco	KTRDC	Nicotine, nornicotine, anabasine, anatabine, NNN, NAT, NAB, NNK, moisture
RT4	Burley tobacco		
RTDAC	Dark, air-cured, ground tobacco		
RT3	Turkish Oriental ground tobacco		
RT2	Flue-cured ground tobacco		
RTDFC	Dark fire-cured ground tobacco		
1R6F (RT1)	Ground filler		
1R5F (RT7)	Ground filler		
STRP 1S1	Loose leaf chewing tobacco	NCSU	Content values are not certified, but may be reported in the scientific literature
STRP 2S1	Loose leaf chewing tobacco		
STRP 1S2	Dry snuff		
STRP 1S3	Moist snuff		
STRP 2S3	Moist snuff		

CRP1	Snus
CRP2	Moist snuff
CRP3	Dry snuff
CRP4	Loose leaf chewing tobacco

HCI: Health Canada intense; ISO: International Organization for Standardization; KTRDC: University of Kentucky Center for Tobacco Reference Products; NAB: N'-nitrosoanabasine; NAT: N'-nitrosoanatabine; NCSU: North Carolina State University Smokeless Tobacco Reference Products; NIST: National Institute of Standards and Technology; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: N'-nitrosonornicotine; VOCs: volatile organic compounds.

It is recommended that a research agenda be prepared on which constituents contribute to the addictiveness and palatability of tobacco products, with identification or development of methods for their quantification in tobacco and smoke and establishment of ranges of emissions in various cigarette brands.

8.9 References

1. Djordjevic MV, Stellman SD, Zang E. Doses of nicotine and lung carcinogens delivered to cigarette smokers. J Natl Cancer Inst. 2000;92:106–11.
2. Jarvis MJ, Boreham R, Primatesta P, Feyerabend C, Bryant A. Nicotine yield from machine-smoked cigarettes and nicotine intakes in smokers: evidence from a representative population survey. J Natl Cancer Inst. 2001;93:134–8.
3. Health Canada data for 2004 using the intense smoking protocol. Ottawa: Health Canada Tobacco – Reports and Publications; 2004.
4. Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. Regul Toxicol Pharmacol. 2005;41:185–227.
5. Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tob Control. 2003;12:424–30.
6. Dybing E, Sanner T, Roelfzema H, Kroese D, Tennant RW. T25: a simplified carcinogenic potency index. Description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. Pharmacol Toxicol. 1997;80:272–9.
7. Cigarette emissions data, 2001. Canberra: Australian Department of Health and Aging; 2001 (http://www.health.gov.au/internet/main/publishing.nsf/content/tobacco-emis, accessed 15 May 2019).
8. Stepanov I, Hatsukami D. Call to establish constituent standards for smokeless tobacco products. Tob Regul Sci. 2016;2:9–30.
9. Talhout R, Richter PA, Stepanov I, Watson CV, Watson CH. Cigarette design features: effects on emission levels, user perception, and behavior. Tob Regul Sci. 2018;4:592–604.
10. Rodgman A. Studies of polycyclic aromatic hydrocarbons in cigarette mainstream smoke: identification, tobacco precursors, control of levels: a review. Beitr Tabakforsch Int. 2001;19:361–79.
11. WHO Study Group on Tobacco Product Regulation. The scientific basis of tobacco product regulation. Second report of a WHO study group (WHO Technical Report Series, No. 951). Geneva: World Health Organization; 2008:45–125.
12. WHO Study Group on Tobacco Product Regulation. Setting maximal limits for toxic constituents in cigarette smoke. Geneva: World Health Organization; 2001 (http://www.who.int/tobacco/global_interaction/tobreg/tsr/en/index.html, accessed 15 May 2019).
13. O'Brien PJ, Siraki AG, Shangari N. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Crit Rev Toxicol. 2005;35:609–62.
14. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide (IARC Mono-

graphs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 71). Lyon: International Agency for Research on Cancer; 1999.

15. A review of human carcinogens: chemical agents and related occupations (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100F), Lyon: International Agency for Research on Cancer; 2012.

16. Dry cleaning, some chlorinated solvents and other industrial chemicals (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 63). Lyon: International Agency for Research on Cancer; 1995:337–72.

17. Feng Z, Hu W, Hu Y, Tang MS. Acrolein is a major cigarette-related lung cancer agent. Preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci USA. 2006;103:15404–9.

18. Zhang S, Villalta PW, Wang M, Hecht SS. Analysis of crotonaldehyde- and acetaldehyde-derived 1,N^2-propanodeoxyguanosine adducts in DNA from human tissues using liquid chromatography-electrospray ionization–tandem mass spectrometry. Chem Res Toxicol. 2006;19:1386–92.

19. Zhang S, Chen H, Wang A, Liu Y, Hou H, Hu Q. Assessment of genotoxicity of four volatile pollutants from cigarette smoke based on the in vitro gH2AX assay using high content screening. Environ Toxicol Pharmacol. 2017;55:30–6.

20. Corley RA, Kabilan S, Kuprat AP, Carson JP, Jacob RE, Minard KR et al. Comparative risks of aldehyde constituents in cigarette smoke using transient computational fluid dynamics/physiologically based pharmacokinetic models of the rat and human respiratory tracts. Toxicol Sci. 2015;146:65–88.

21. Yeager RP, Kushman M, Chemerynski S, Weil R, Fu X, White M et al. Proposed mode of action for acrolein respiratory toxicity associated with inhaled tobacco smoke. Toxicol Sci. 2016;151:347–64.

22. Takamiya R, Uchida K, Shibata T, Maeno T, Kato M, Yamaguchi Y et al. Disruption of the structural and functional features of surfactant protein A by acrolein in cigarette smoke. Sci Rep. 2017;7:8304.

23. Lee HW, Wang HT, Weng MW, Hu Y, Chen WS, Chou D et al. Acrolein- and 4-aminobiphenyl-DNA adducts in human bladder mucosa and tumor tissue and their mutagenicity in human urothelial cells. Oncotarget. 2014;5:3526–40.

24. Cheah NP, Pennings JL, Vermeulen JP, van Schooten FJ, Opperhuizen A. In vitro effects of aldehydes present in tobacco smoke on gene expression in human lung alveolar epithelial cells. Toxicol In Vitro. 2013;27:1072–81.

25. Sapkota M, Wyatt TA. Alcohol, aldehydes, adducts and airways. Biomolecules. 2015;5:2987–3008.

26. Antoniak DT, Duryee MJ, Mikuls TR, Thiele GM, Anderson DR. Aldehyde-modified proteins as mediators of early inflammation in atherosclerotic disease. Free Radical Biol Med. 2015;89:409–18.

27. Lachenmeier DW, Salaspuro M. ALDH2-deficiency as genetic epidemiologic and biochemical model for the carcinogenicity of acetaldehyde. Regul Toxicol Pharmacol. 2017;86:128–36.

28. Morita K, Miyazaki H, Saruwatari J, Oniki K, Kumagae N, Tanaka T et al. Combined effects of current-smoking and the aldehyde dehydrogenase 2*2 allele on the risk of myocardial infarction in Japanese patients. Toxicol Lett. 2015;232:221–5.

29. Bui LC, Manaa A, Xu X, Duval R, Busi F, Dupret JM et al. Acrolein, an a,ß-unsaturated aldehyde, irreversibly inhibits the acetylation of aromatic amine xenobiotics by human arylamine N-acetyltransferase 1. Drug Metab Dispos. 2013;41:1300–5.

30. Chare SS, Donde H, Chen WY, Barker DF, Gobejishvilli L, McClain CJ et al. Acrolein enhances epigenetic modifications, FasL expression and hepatocyte toxicity induced by anti-HIV drug zidovudine. Toxicol In Vitro. 2016;35:76.

31. Tan SLW, Chadha S, Liu Y, Gabasova E, Perera D, Ahmed K et al. A class of environmental and endogenous toxins induces BRCA2 haploinsufficiency and genome instability. Cell. 2017;169:1105–18.

32. Hoffman AC, Evans SE. Abuse potential of non-nicotine tobacco smoke components: acetalde-hyde, nornicotine, cotinine, and anabasine. Nicotine Tob Res. 2013;15:622–32.

33. Brancato A, Lavanco G, Cavallaro A, Plescia F, Cannizzaro C. Acetaldehyde, motivation and stress: behavioral evidence of an addictive ménage à trois. Front Behav Neurosci. 2017;11:23.

34. Syrjänen K, Salminen J, Aresvuo U, Hendolin P, Paloheimo L, Eklund C et al. Elimination of ciga-rette smoke-derived acetaldehyde in saliva by slow-release L-cysteine lozenge is a potential new method to assist smoking cessation. A randomised, double-blind, placebo-controlled intervention. Anticancer Res. 2016;36:2297–306.

35. Henning RJ, Johnson GT, Coyle JP, Harbison RD. Acrolein can cause cardiovascular disease: a review. Cardiovasc Toxicol. 2017;17:227–36.

36. Conklin DJ, Malovichko MV, Zeller I, Das TP, Krivokhizhina TV, Lynch BH et al. Biomarkers of chronic acrolein inhalation exposure in mice: implications for tobacco product-induced toxic-ity. Toxicol Sci. 2017;158:263–74.

37. DeJarnett N, Conklin DJ, Riggs DW, Myers JA, O'Toole TE, Hamzeh I et al. Acrolein exposure is associated with increased cardiovascular disease risk. J Am Heart Assoc. 2014;3:e000934.

38. Snow SJ, McGee MA, Henriquez A, Richards JE, Schladweiler MC, Ledbetter AD et al. Respira-tory effects and systemic stress response following acute acrolein inhalation in rats. Toxicol Sci. 2017;158:454–64.

39. Feroe AG, Attanasio R, Scinicariello F. Acrolein metabolites, diabetes and insulin resistance. Environ Res. 2016;148:1–6.

40. Chen WY, Wang M, Zhang J, Barve SS, McClain CJ, Joshi-Barve S. Acrolein disrupts tight junc-tion proteins and causes endoplasmic reticulum stress-mediated epithelial cell death leading to intestinal barrier dysfunction and permeability. Am J Pathol. 2017;187(12):2686–97.

41. Song JJ, Lee JD, Lee BD, Chae SW, Park MK. Effect of acrolein, a hazardous air pollutant in smoke, on human middle ear epithelial cells. Int J Pediatr Otorhinolaryngol. 2013;77:1659–64.

42. Amiri A, Turner-Henson A. The roles of formaldehyde exposure and oxidative stress in fetal growth in the second trimester. J Obstet Gynecol Neonatal Nurs. 2017;46:51–62.

43. Yang Y, Zhang Z, Zhang H, Hong K, Tang W, Zhao L et al. Effects of maternal acrolein expo-sure during pregnancy on testicular testosterone production in fetal rats. Mol Med Rep. 2017;16:491–8.

44. Park SL, Carmella SG, Chen M, Patel Y, Stram DO, Haiman CA et al. Mercapturic acids derived from the toxicants acrolein and crotonaldehyde in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. PLoS One. 2015;10:e0124841.

45. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE et al. Ethnic and racial differences in the smoking-related risk of lung cancer. N Engl J Med. 2006;354:333–42.

46. Some aromatic amines, organic dyes, and related exposures (IARC Monographs on the Evalu-ation of Carcinogenic Risks to Humans, Vol. 99). Lyon: International Agency for Research on Cancer; 2010.

47. Cai T, Bellamri M, Ming X, Koh WP, Yu MC, Turesky RJ. Quantification of hemoglobin and white blood cell DNA adducts of the tobacco carcinogens 2-amino-9H-pyrido[2,3-b]indole and 4-aminobiphenyl formed in humans by nanoflow liquid chromatography/ion trap multistage mass spectrometry. Chem Res Toxicol. 2017;30:1333–43.

48. Tomioka K, Obayashi K, Saeki K, Okamoto N, Kurumatani N. Increased risk of lung cancer as-sociated with occupational exposure to benzidine and/or beta-naphthylamine. Int Arch Occup Environ Health. 2015;88:455–65.

49. Tomioka K, Saeki K, Obayashi K, Kurumatani N. Risk of lung cancer in workers exposed to benzidine and/or beta-naphthylamine: a systematic review and meta-analysis. J Epidemiol. 2016;26:447–58.

50. Some industrial chemicals (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 60). Lyon: International Agency for Research on Cancer; 1994:215–32.

51. Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Chow WH et al. Mortality among benzene-exposed workers in China. Environ Health Perspect. 1996;104(Suppl 6):1349–52.

52. Soeteman-Hernandez LG, Bos PM, Talhout R. Tobacco smoke-related health effects induced by 1,3-butadiene and strategies for risk reduction. Toxicol Sci. 2013;136:566–80.

53. Bahadar H, Mostafalou S, Abdollahi M. Current understandings and perspectives on non-cancer health effects of benzene: a global concern. Toxicol Appl Pharmacol. 2014;276:83–94.

54. Abplanalp W, DeJarnett N, Riggs DW, Conklin DJ, McCracken JP, Srivastava S et al. Benzene exposure is associated with cardiovascular disease risk. PLoS One. 2017;12:e0183602.

55. Doherty BT, Kwok RK, Curry MD, Ekenga C, Chambers D, Sandler DP et al. Associations between blood BTEXS concentrations and hematologic parameters among adult residents of the US Gulf States. Environ Res. 2017;156:579–87.

56. Haiman CA, Patel YM, Stram DO, Carmella SG, Chen M, Wilkens LR et al. Benzene uptake and glutathione S-transferase T1 status as determinants of S-phenylmercapturic acid in cigarette smokers in the Multiethnic Cohort. PLoS One. 2016;11(3):0150641.

57. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 92). Lyon: International Agency for Research on Cancer; 2010.

58. Ding YS, Ashley DL, Watson CH. Determination of 10 carcinogenic polycyclic aromatic hydro-carbons in mainstream cigarette smoke. J Agric Food Chem. 2007;55:5966–73.

59. Hecht SS. Tobacco carcinogens, their biomarkers, and tobacco-induced cancer. Nature Rev Cancer. 2003;3:733–44.

60. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene. 2002;21:7435–51.

61. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999;91:1194–210.

62. Onami S, Okubo C, Iwanaga A, Suzuki H, Iida H, Motohashi Y et al. Dosimetry for lung tumo-rigenesis induced by urethane, 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and benzo[a]pyrene (B[a]P) in A/JJmsSlc mice. J Toxicol Pathol. 2017;30:209–16.

63. Zaccaria KJ, McClure PR. Using immunotoxicity information to improve cancer risk assessment for polycyclic aromatic hydrocarbon mixtures. Int J Toxicol. 2013;32:236–50.

64. Chepelev NL, Moffat ID, Bowers WJ, Yauk CI. Neurotoxicity may be an overlooked consequence of benzo[a]pyrene exposure that is relevant to human health risk assessment. Mutat Res Rev Mutat Res. 2015;764:64–89.

65. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol. 1998;11:559–603.

66. Tobacco smoke and involuntary smoking (IARC Monographs on the Evaluation of Carcino-genic Risks to Humans, Vol. 83). Lyon: International Agency for Research on Cancer; 2004.

67. Smokeless tobacco and tobacco-specific nitrosamines (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 89). Lyon: International Agency for Research on Cancer; 2007.

68. Wu W, Zhang L, Jain RB, Ashley DL, Watson CH. Determination of carcinogenic tobacco-spe-cific nitrosamines in mainstream smoke from US-brand and non-US-brand cigarettes from 14 countries. Nicotine Tob Res. 2005;7:443–51.

69. Ashley DL, O'Connor RJ, Bernert JT, Watson CH, Polzin GM, Jain RB et al. Effect of differing levels of tobacco-specific nitrosamines in cigarette smoke on the levels of biomarkers in smokers. Cancer Epidemiol Biomarkers Prev. 2010;19:1389–98.

70. Stepanov I, Knezevich A, Zhang L, Watson CH, Hatsukami DK, Hecht SS. Carcinogenic to-bacco-specific N-nitrosamines in US cigarettes: three decades of remarkable neglect by the tobacco industry. Tob Control. 2012;21:44–8.

71. Hoffmann D, Djordjevic MV, Hoffmann I. The changing cigarette. Prev Med. 1997;26:427–34.

72. Burns DM, Anderson CM, Gray N. Do changes in cigarette design influence the rise in adeno-

carcinoma of the lung? Cancer Causes Control. 2011;22:13–22.

73. Balbo S, Johnson CS, Kovi RC, James-Yi SA, O'Sullivan MG, Wang M et al. Carcinogenicity and DNA adduct formation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in F-344 rats. Carcinogenesis. 2014;35:2798–806.

74. Balbo S, James-Yi S, Johnson CS, O'Sullivan MG, Stepanov I, Wang M et al. (S)-N´-Nitrosonornicotine, a constituent of smokeless tobacco, is a powerful oral cavity carcinogen in rats. Carcinogenesis. 2013;34:2178–83.

75. Stepanov I, Sebero E, Wang R, Gao YT, Hecht SS, Yuan JM. Tobacco-specific N-nitrosamine exposures and cancer risk in the Shanghai Cohort Study: remarkable coherence with rat tumor sites. Int J Cancer. 2014;134:2278–83.

76. Park SL, Carmella SG, Ming X, Stram DO, LeMarchand L, Hecht SS. Variation in levels of the lung carcinogen NNAL and its glucuronides in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. Cancer Epidemiol Biomarkers. 2015;24:561–9.

77. Khariwala SS, Ma B, Ruszczak C, Carmella SG, Lindgren B, Hatsukami DK et al. High level of tobacco carcinogen-derived DNA damage in oral cells is an independent predictor of oral/head and neck cancer risk in smokers. Cancer Prev Res. 2017;10:507–13.

78. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005;57:79–115.

79. WHO Study Group on Tobacco Product Regulation. Global nicotine reduction strategy. Advisory note. Geneva: World Health Organization; 2015.

80. Food and Drug Administration. Tobacco product standard for nicotine level of combusted cigarette. Fed Regist. 2018;83.

81. Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG et al. Randomized clinical trial of reduced-nicotine standards for cigarettes. N Engl J Med. 2015;373:1340–9.

82. Hatsukami DK, Luo X, Dick L, Kangkum M, Allen SS, Murphy SE et al. Reduced nicotine content cigarettes and use of alternative nicotine products: exploratory trial. Addiction. 2017;112:156–67.

83. Benowitz NL, Burbank AD. Cardiovascular toxicity of nicotine: implications for electronic cigarette use. Trends Cardiovasc Med. 2016;26:515–23.

84. England LJ, Aagaard K, Bloch M, Conway K, Cosgrove K, Grana R et al. Developmental toxicity of nicotine: a transdisciplinary synthesis and implications for emerging tobacco products. Neurosci Biobehav Rev. 2017;72:176–89.

85. Van Duuren BL, Goldschmidt BM. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. J. Natl Cancer Inst. 1976;56:1237–42.

86. Fennell TR, MacNeela JP, Morris RW, Watson M, Thompson CL, Bell DA. Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-null and M1-null genotypes. Cancer Epidemiol Biomarkers Prev. 2000;9:705–12.

87. A review of human carcinogens: arsenic, metals, fibres, and dusts (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100C). Lyon: International Agency for Research on Cancer; 2012.

88. Inorganic and organic lead compounds (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 87). Lyon: International Agency for Research on Cancer; 2006.

89. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 58). Lyon: International Agency for Research on Cancer; 1993.

90. Pappas RS, Stanfill SB, Watson CH, Ashley DL. Analysis of toxic metals in commercial moist snuff and Alaskan iqmik. J Anal Toxicol. 2008;32:281–91.

91. Pinto E, Cruz M, Ramos P, Santos A, Almeida A. Metals transfer from tobacco to cigarette smoke: evidences in smokers' lung tissue. J Hazard Mater. 2017;5:31–5.

92. Richter PA, Bishop EE, Wang J, Kaufmann R. Trends in tobacco smoke exposure and blood

lead levels among youths and adults in the United States: the National Health and Nutrition Examination Survey, 1999–2008. Prev Chronic Dis. 2013;10:E213.

93. Garcia-Esquinas E, Pollan M, Tellez-Plaza M, Francesconi KA, Goessler W, Guallar E et al. Cadmium exposure and cancer mortality in a prospective cohort: the Strong Heart Study. Environ Health Perspect. 2014;122:363–70.

94. Feki-Tounsi M, Hamza-Chaffai A. Cadmium as a possible cause of bladder cancer: a review of accumulated evidence. Environ Sci Pollut Res Int. 2014;21:10561–73.

95. Buser MC, Scinicariello F. Cadmium, lead, and depressive symptoms: analysis of National Health and Nutrition Examination Survey 2011–2012. J Clin Psychiatry. 2017;78:e515–21.

96. Adeniyi PA, Olatunji BP, Ishola AO, Ajonijebu DC, Ogundele OM. Cadmium increases the sensitivity of adolescent female mice to nicotine-related behavioral deficits. Behav Neurol. 2014;2014:36098.

97. Ajonijebu C, Adekeye AO, Olatunji BP, Ishola AO, Ogundele OM. Nicotine–cadmium interaction alters exploratory motor function and increased anxiety in adult male mice. Neurodegener Dis. 2014;359436. doi: 10.1155/2014/359436.

98. Hecht EM, Landy DC, Ahn S, Hlaing WM, Hennekens CH. Hypothesis: cadmium explains, in part, why smoking increases the risk of cardiovascular disease. J Cardiovasc Pharmacol Ther. 2013;18:550–4.

99. Tellez-Plaza M, Jones MR, Dominguez-Lucas A, Guallar E, Navas-Acien A. Cadmium exposure and clinical cardiovascular disease: a systematic review. Curr Atheroscler Rep. 2013;15:356.

100. Das S, Upadhaya P, Giri S. Arsenic and smokeless tobacco induce genotoxicity, sperm abnormality as well as oxidative stress in mice in vivo. Genes Environ. 2016;38:4.

101. Chelchowska M, Ambroszkiewicz J, Jablonka-Salach K, Gajewska J, Maciejewski TM, Bulska E et al. Tobacco smoke exposure during pregnancy increases maternal blood lead levels affecting neonate birth weight. Biol Trace Elem Res. 2013;155:169–75.

102. Tinkov AA, Ajsuvakova OP, Skalnaya MG, Popova EV, Sinitskii AI, Nemereshina ON et al. Mercury and metabolic syndrome: a review of experimental and clinical observations. Biometals. 2015;28:231–54.

103. Roy C, Tremblay PY, Ayotte P. Is mercury exposure causing diabetes, metabolic syndrome and insulin resistance? A systematic review of the literature. Environ Res. 2017;156:747–60.

104. Genchi G, Sinicropi MS, Carocci A, Lauria G, Catalano A. Mercury exposure and heart diseases. Int J Environ Res Public Health, 2017;14:pii: E74.

105. Rahman MH, Bratveit M, Moen BE. Exposure to ammonia and acute respiratory effects in a urea fertilizer factory. Int J Occup Environ Health. 2007;13:153–9.

106. Casas L, Zock JP, Torrent M, Garcia-Esteban R, Garcia-Lavedan E, Hyvarinen A et al. Use of household cleaning products, exhaled nitric oxide and lung function in children. Eur Respir J. 2013;42:1415–8.

107. Arif AA, Delclos GL. Association between cleaning-related chemicals and work-related asthma and asthma symptoms among healthcare professionals. Occup Environ Med. 2012;69:35–40.

108. Loftus C, Yost M, Sampson P, Torres E, Arias G, Breckwich Vasquez V et al. Ambient ammonia exposures in an agricultural community and pediatric asthma morbidity. Epidemiology. 2015;26:794–801.

109. Petrova M, Diamond J, Schuster B, Dalton P. Evaluation of trigeminal sensitivity to ammonia in asthmatics and healthy human volunteers. Inhal Toxicol. 2008;20:1085–92.

110. The health consequences of smoking: a report of the Surgeon General. Rockville (MD): Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health; 2004.

111. Ludvig J, Miner B, Eisenberg MJ. Smoking cessation in patients with coronary artery disease. Am Heart J. 2005;149:565–72.

112. Scherer G. Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monox-

ide and hydrogen cyanide in tobacco smoke. Exp Toxicol Pathol. 2006;58:101–24.

113. Silverstein P. Smoking and wound healing. Am J Med. 1992;93:22S–24S.

114. Weinberger B, Laskin DL, Heck DE, Laskin JD. The toxicology of inhaled nitric oxide. Toxicol Sci. 2001;59:5–16.

115. Vleeming W, Rambali B, Opperhuizen A. The role of nitric oxide in cigarette smoking and nicotine addiction. Nicotine Tob Res. 2002;4:341–8.

116. WHO Study Group on Tobacco Product Regulation. Guiding principles for the development of tobacco product research and testing capacity and proposed protocols for the initiation of tobacco product testing: Recommendation 6. Geneva: World Health Organization; 2004.

117. WHO Tobacco Laboratory Network standard operating procedure for validation of analytical methods of tobacco product contents and emissions. WHO TobLabNet Official Method SOP02. Geneva: World Health Organization; 2017.

118. Ding YS, Yan X, Wong J, Chan M, Watson CH. In situ derivatization and quantification of seven carbonyls in cigarette mainstream smoke. Chem Res Toxicol. 2016;29:125–31.

119. Zhao W, Zhang Q, Lu B, Sun S, Zhang S, Zhang J. Rapid determination of six low molecular carbonyl compounds in tobacco smoke by the APCI-MS/MS coupled to data mining. J Anal Meth Chem. 2017:8260860. doi: 10.1155/2017/8260860.

120. Ahad BT, Abdollahi A. Dispersive liquid–liquid microextraction for the high performance liquid chromatographic determination of aldehydes in cigarette smoke and injectable formulations. J Hazard Mater. 2013;254–255:390–6.

121. Zhang J, Bai R, Zhou Z, Liu X, Zhou J. Simultaneous analysis of nine aromatic amines in mainstream cigarette smoke using online solid-phase extraction combined with liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2017;409:2993–3005.

122. Xie F, Yu J, Wang S, Zhao G, Xia G, Zhang X et al. Rapid and simultaneous analysis of ten aromatic amines in mainstream cigarette smoke by liquid chromatography/electrospray ionization tandem mass spectrometry under ISO and "Health Canada intensive" machine smoking regimens. Talanta. 2013;115:435–41.

123. Deng H, Yang F, Li Z, Bian Z, Fan Z, Wang Y et al. Rapid determination of 9 aromatic amines in mainstream cigarette smoke by modified dispersive liquid liquid microextraction and ultraperformance convergence chromatography tandem mass spectrometry. J Chromatogr A. 2017;1507:37–44.

124. Sampson MM, Chambers DM, Pazo DY, Moliere F, Blount BC, Watson CH. Simultaneous analysis of 22 volatile organic compounds in cigarette smoke using gas sampling bags for high-throughput solid-phase microextraction. Anal Chem. 2014;86:7088–95.

125. Pazo DY, Moliere F, Sampson MM, Reese CM, Agnew-Heard KA, Walters MJ et al. Mainstream smoke levels of volatile organic compounds in 50 US domestic cigarette brands smoked with the ISO and Canadian intense protocols. Nicotine Tob Res. 2016;18:1886–94.

126. Saha S, Mistri R, Ray BC. A rapid and selective method for simultaneous determination of six toxic phenolic compounds in mainstream cigarette smoke using single-drop microextraction followed by liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2013;405:9265–72.

127. Wu J, Rickert WS, Masters A. An improved high performance liquid chromatography-fluorescence detection method for the analysis of major phenolic compounds in cigarette smoke and smokeless tobacco products. J Chromatogr A. 2012;1264:40–7.

128. Savareear B, Brokl M, Wright C, Focant JF. Thermal desorption comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry for vapour phase mainstream tobacco smoke analysis. J Chromatogr A. 2017;1525:126–37.

129. Brokl M, Bishop L, Wright CG, Liu C, McAdam K, Focant JF. Analysis of mainstream tobacco smoke particulate phase using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. J Separation Sci. 2013;36:1037–44.

130. Pappas RS, Fresquez MR, Watson CH. Cigarette smoke cadmium breakthrough from traditional filters: implications for exposure. J Anal Toxicol. 2014;39:45–51.

131. Fresquez MR, Gonzalez-Jimenez N, Gray N, Watson CH, Pappas RS. High-throughput determination of mercury in tobacco and mainstream smoke from little cigars. J Anal Toxicol. 2015;39:545–50.

132. Fresquez MR, Pappas RS, Watson CH. Establishment of toxic metal reference range in tobacco from US cigarettes. J Anal Toxicol. 2013;37:298–304.

133. Zhang ZW, Xu YB, Wang CH, Chen KB, Tong HW, Liu SM. Direct determination of hydrogen cyanide in cigarette mainstream smoke by ion chromatography with pulsed amperometric detection. J Chromatogr A. 2011;1218:1016–9.

134. Mahernia S, Amanlou A, Kiaee G, Amanlou M. Determination of hydrogen cyanide concentration in mainstream smoke of tobacco products by polarography. J Environ Health Sci Eng. 2015;13:57.

135. Shihadeh A, Schubert J, Klaiany J, El Sabban M, Luch A, Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. Tob Control. 2015;24:i22–30.

136. Cecil TL, Brewer TM, Young M, Holman MR. Acrolein yields in mainstream smoke from commercial cigarette and little cigar tobacco products. Nicotine Tob Res. 2017;19:865–70.

137. Schubert J, Kappenstein O, Luch A, Schulz TG. Analysis of primary aromatic amines in the mainstream waterpipe smoke using liquid chromatography–electrospray ionization tandem mass spectrometry. J Chromatogr A. 2011;1218:5628–37.

138. Jacob P III, Abu Raddaha AH, Dempsey D, Havel C, Peng M, Yu I et al. Comparison of nicotine and carcinogen exposure with water pipe and cigarette smoking. Cancer Epidemiol Biomarkers Prev. 2013;22:765–72.

139. Hamad SH, Johnson NM, Tefft ME, Brinkman MC, Gordon SM, Clark PI et al. Little cigars vs 3R4F cigarette: physical properties and HPHC yields. Tob Regul Sci. 2017;3:459–78.

140. Goel R, Trushin N, Reilly SM, Bitzer Z, Muscat J, Foulds J et al. A survey of nicotine yields in small cigar smoke: influence of cigar design and smoking regimens. Nicotine Tob Res. 2018;20(10):1250–7.

141. Apsley A, Galea KS, Sanchez-Jimenez A, Semple S, Wareing H, van Tongeren M. Assessment of polycyclic aromatic hydrocarbons, carbon monoxide, nicotine, metal contents and particle size distribution of mainstream shisha smoke. J Environ Health Res. 2011;11:93–103.

142. Fresquez MR, Gonzalez-Jimenez N, Gray N, Valentin-Blasini L, Watson CH, Pappas RS. Electrothermal vaporization-QQQ-ICP-MS for determination of chromium in mainstream cigarette smoke particulate. J Anal Toxicol. 2017;41:307–12.

143. Ashley M, Dixon M, Prasad K. Relationship between cigarette format and mouth-level exposure to tar and nicotine in smokers of Russian king-size cigarettes. Regul Toxicol Pharmacol. 2014;70:430–7.

144. Lawler TS, Stanfill SB, de Castro BR, Lisko JG, Duncan BW, Richter P et al. Surveillance of nicotine and pH in cigarette and cigar filler. Tob Regul Sci. 2017;3:101–16.

145. Mahmood T, Zaman M. Comparative assessment of total nicotine content of the cigarette brands available in Peshawar, Pakistan. Pak J Chest Med. 2015;16.

146. Kulak JA, Goniewicz ML, Giovino GA, Travers MJ. Nicotine and pH in waterpipe tobacco. Tob Regul Sci. 2017;3:102–7.

147. Hadidi KA, Mohammed FI. Nicotine content in tobacco used in hubble-bubble smoking. Saudi Med J. 2004;25:912–7.

148. Sepetdjian E, Saliba N, Shihadeh A. Carcinogenic PAH in waterpipe charcoal products. Food Chem Toxicol. 2010;48:3242–5.

149. Nguyen T, Hlangothi D, Matrinez RA, Jacob D, Anthony K, Nance H et al. Charcoal burning as a source of polyaromatic hydrocarbons in waterpipe smoking. J Environ Sci Health B. 2013;48:1097–102.

150. Schubert J, Muller FD, Schmidt R, Luch A, Schultz TG. Waterpipe smoke: source of toxic and carcinogenic VOCs, phenols and heavy metals? Arch Toxicol. 2015;89:2129–39.

151. Saadawi R, Figueroa JAL, Hanley T, Caruso J. The hookah series part 1: total metal analysis in hookah tobacco (narghile, shisha) – an initial study. Anal Meth. 2014;4:3604–11.

152. Saadawi R, Hachmoeller O, Winfough M, Hanley T, Caruso JA, Figueroa JAL. The hookah series part 2: elemental analysis and arsenic speciation in hookah charcoals. J Anal Atom Spectrom. 2014;29:2146–58.

153. Talhout R, Schulz T, Florek E, Van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8:613–28.

154. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed Regist. 2012;77, No. 64:20034–7.

155. Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami DK, Hecht SS. Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry. Chem Res Toxicol. 2010;23:66–73.

156. Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL et al. Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev. 2008;17:3366–71.

157. Hecht SS, Chen M, Yoder A, Jensen J, Hatsukami D, Le C et al. Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. Cancer Epidemiol Biomarkers Prev. 2005;14:2969–74.

158. Hochalter JB, Zhong Y, Han S, Carmella SG, Hecht SS. Quantitation of a minor enantiomer of phenanthrene tetraol in human urine: correlations with levels of overall phenanthrene tetraol, benzo[a]pyrene tetraol, and 1-hydroxypyrene. Chem Res Toxicol. 2011;24:262–8.

159. Hoffmann D, Adams JD, Brunnemann KD, Hecht SS. Assessment of tobacco-specific N-nitrosamines in tobacco products. Cancer Res. 1979;39:2505–9.

160. DeRoton C, Wiernik A, Wahlberg I, Vidal B. Factors influencing the formation of tobacco-specific nitrosamines in French air-cured tobaccos in trials and at the farm level. Beitr Tabakforsch Int. 2005;21:305–20.

161. van de Nobelen S, Kienhuis AS, Talhout R. An inventory of methods for the assessment of additive increased addictiveness of tobacco products. Nicotine Tob Res. 2016;18:1546–55.

162. Talhout R, Opperhuizen A, van Amsterdam JG. Sugars as tobacco ingredient: effects on mainstream smoke composition. Food Chem Toxicol. 2006;44:1789–98.

163. O'Connor RJ, Hurley PJ. Existing technologies to reduce specific toxicant emissions in cigarette smoke. Tob Control. 2008;18:139–48.

164. Fischer S, Spiegelhalder B, Eisenbarth J, Preussmann R. Investigations on the origin of tobacco-specific nitrosamines in mainstream smoke of cigarettes. Carcinogenesis. 1990;11:723–30.

165. Djordjevic MV, Sigountos CW, Brunnemann KD, Hoffmann D. Formation of 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid in vitro and in mainstream cigarette smoke. J Agric Food Chem. 1991;39:209–13.

166. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation. Fifth report of a WHO study group (WHO Technical Report Series, No. 989). Geneva: World Health Organization; 2015.

167. Gray N, Borland R. Research required for the effective implementation of the Framework Convention on Tobacco Control, Articles 9 and 10. Nicotine Tob Res. 2013;15:777–88.

168. Pauly JL, O'Connor RJ, Paszkiewicz GM, Cummings KM, Djordjevic MV, Shields PG. Cigarette filter-based assays as proxies for toxicant exposure and smoking behavior – a literature review. Cancer Epidemiol Biomarkers Prev. 2009;18:3321–33.

9. Approaches to measuring and reducing toxicant concentrations in smokeless tobacco products

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Contents

9.1 Introduction

This section presents possible means of measuring and decreasing the levels of certain harmful agents in smokeless tobacco products, in conformity with the decision at the seventh session of the Conference of the Parties to the WHO FCTC in 2016 (COP7) *(1)*. Global estimates suggest that 367 million people in all six WHO regions use smokeless tobacco *(2)*, comprising almost 90% of adult tobacco consumption in south-east Asia (mainly Bangladesh and India), where its use exceeds that of combustible tobacco products *(3)*. This section builds on several reports on smokeless tobacco that provide more detail on product chemistry *(4, 5)*.

Smokeless tobacco products are complex and contain both inorganic and organic chemicals that contribute to their addictive, toxic or carcinogenic effects *(6)*. Of the harmful chemicals in smokeless tobacco, TSNAs are the most abundant and include potent compounds, such as NNN and NNK, which are known human carcinogens; NNN is known to cause oral cancer *(6)*. During cultivation and production, the levels of these agents or their precursors can be increased by soil uptake, biosynthesis of alkaloids and microbial activity, including the formation of mycotoxins, nitrite and TSNAs *(4, 7)*. In certain product types, fire-curing can

increase the levels of harmful agents, such as VOCs and PAHs, while the inclusion of certain additives (areca nut, tonka bean, *khat*, diphenyl ether, caffeine, pH-boosting agents) and chemicals, can increase addiction and carcinogenicity *(4, 5)*. Microorganisms play an important role throughout production, resulting in chemical changes during the production of tobacco products. Harmful and carcinogenic agents are generated throughout production *(8, 9, 10)*.

The wide variety of smokeless tobacco products ranges from simple to elaborate hand-made and industrial preparations consisting of tobacco mixed with a wide spectrum of non-tobacco plant materials and chemical additives. Manufactured and cottage-industry products include tobacco leaves, loose flaked tobacco, finely minced tobacco, pulverized tobacco, pressed cakes, tars, gel-like pastes, tobacco-containing toothpaste and pressed pellets. Smokeless tobacco products can be generally subdivided into four main categories on the basis of key ingredients (a similar scheme was presented previously) *(4, 6, 11)*:

- category 1, which contains tobacco with few or no alkaline modifiers;
- category 2, which contains tobacco and substantial amounts of alkaline agents;
- category 3, which contains tobacco, one or more alkaline agents and areca nut; and
- category 4, which contains tobacco mixed with other chemical or plant ingredients with
- additional bioactivity, such as stimulants.

Because of the constituents it contains, each product category harms health. The tobacco contained in these products contributes to their toxicity, carcinogenicity and addictiveness. Approximately 70 *Nicotiana* species are known; however, only a few are used to make smokeless tobacco products. In general, *Nicotiana* species have different levels of alkaloids *(12, 13)*; the most commonly used species, *N. tabacum* (cultivated tobacco), has a moderate amount of nicotine, whereas *N. rustica* has extremely high levels *(14)*. Some smokeless tobacco products contain *N. glauca* (tree tobacco), which has high concentrations of the tobacco alkaloid anabasine; accidental poisoning and fatalities have been associated with use of this species in a few cases *(15, 16)*. The presence of different species of tobacco can result in exposure to different proportions of tobacco alkaloids, which can contribute to addictiveness, toxicity and carcinogenicity, as tobacco alkaloids are necessary precursors of TSNAs *(10, 12, 14, 17, 18)*. The presence of nicotine, common to all smokeless tobacco products, promotes continued use and can result in repeated, often daily, exposure to carcinogens and toxicants *(4)*.

Although tobacco contains thousands of chemicals *(19)*, many of the carcinogenic agents in smokeless tobacco products are not present or are present

at very low concentrations in newly transplanted tobacco *(17)* but form and accumulate between cultivation and the finished product. Certain chemical constituents of tobacco are synthesized from metals and nitrate, which are absorbed by tobacco during growth *(18)*. According to the soil characteristics and the environment in which tobacco is grown, certain microorganisms may occur naturally inside the plant (endophytes) or on its surface (epiphytes) *(20, 21)*. During cultivation, harvesting and processing, other microorganisms may be deposited on tobacco from air, water, soil or manure (if used) or introduced by human handling or as additives. The microbial communities present during production and in the final product can affect the product constituents. Smokeless tobacco production also includes steps such as fire-curing of green leaves, which can introduce additional chemical agents, such as VOCs, phenolic compounds and PAHs *(22–24)*. During processes such as fermentation, ageing and storage, microorganisms are viable and metabolically active in tobacco *(8–10, 25)*, and their presence can result in the generation of reactive agents such as nitrite and other harmful by-products (aflatoxins, endotoxins, TSNAs, other nitrosamines, ethyl carbamate) *(9, 26, 27)* (Table 9.1). Studies published by tobacco industry scientists have shown that toxicant levels can be lowered by changes in growing practices, manufacturing processes and continuous monitoring *(19, 28)*.

Table 9.1. Potential sources of carcinogens, toxicants and biologically active compounds in smokeless tobacco products, originating mainly from soil, microbial action, fire-curing and additives

Agent class	IARC-classified carcinogens (groups 1, 2A, 2B), toxicants or biologically active compounds	Potential source or cause
Metals and metalloids	Group 1: arsenic, beryllium, cadmium, nickel compounds, polonium-210 Group 2A: inorganic lead compounds Group 2B: cobalt sensitization: aluminium, chromium, cobalt, nickel Dermal irritants: barium, mercury Copper in areca nut may contribute to oral submucosal fibrosis	Absorption from the soil or by deposition of soil particles on tobacco leaf surfaces; potentially present in other ingredients (betel leaf, slaked lime) used with tobacco
Nitrosation agents	Group 2B: nitrite	Generated by microorganisms
Mycotoxins	Group 1: aflatoxins (mixtures of) Group 2B: aflatoxin M1, ochratoxin A, sterigmatocystin	Formed by fungi (*Aspergillus*)
Nitrosamines Tobacco-specific *N´*-nitrosoamines	Group 1: *N´*-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	Formed by microbial-generated nitrite followed by nitrosation during curing, fermentation and ageing (nitrite reacts with alkaloids)
Volatile *N´*-nitrosoamines	Group 2A: *N*-nitrosodimethylamine Group 2B: *N*-nitrosopyrrolidine, *N*-nitrosopiperidine, *N*-nitrosomorpholine, *N*-nitrosodiethanolamine	Formed by microbial-generated nitrite followed by nitrosation during curing, fermentation and ageing (nitrite reacts with certain secondary and tertiary amines)
Nitrosoacids	Group 2B: *N*-nitrososarcosine	
Carbamates	Group 2A: ethyl carbamate	Formed during fermentation (reaction of urea and ethanol)

Polycyclic aromatic hydrocarbons	Group 1: benzo[*a*]pyrene Group 2A: dibenz[*a,h*]anthracene 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 Group 2B: acetaldehyde	Deposited on tobacco during fire-curing
Non-tobacco plant materials	Group 1: areca nut Liver toxicant: tonka bean Stimulant: *khat,* caffeine	Additives

Source: reference *29*.

Smokeless tobacco users are exposed to inorganic, organic and biological agents and their interactions. The areas of concern depend on product types and the region of the world because of different soil constituents (metals, nitrate, microbial communities), tobacco species grown, agricultural practices, product processing steps and permissible inclusion of certain additives.

9.2   Product composition

Tobacco products are highly complex mixtures that contain nicotine, Group 1 carcinogens (as evaluated by IARC working groups) *(6)*) and toxic metals, such as arsenic, cadmium and lead *(30)*. These products also contain nitrate, which can be metabolized by microbes to nitrite; nitrite initiates the formation of TSNAs and other nitrosamines *(9, 31)*. Certain NO_x gases present during fire-curing can also cause nitrosation (*32*). In particular, nitrosation of nornicotine yields *N´*-nitrosonornicotine, a known human carcinogen (IARC Group 1) which is known to cause oral cancer *(6)*. The concentrations of these agents in various smokeless tobacco products have been surveyed extensively *(4–6, 33)*.

The concentrations of total and free nicotine vary widely among product types. Detectable concentrations of total nicotine in smokeless tobacco products range from < 0.2 mg/g to 95 mg/g *(11, 14)*. High nicotine concentrations are found in products such as dry snuff and Sudanese *toombak (14, 34)*. The high concentrations in certain products, such as *toombak*, *gul*, tobacco leaf and *zarda*, are attributed to use of the *N. rustica* variety of tobacco, a nicotine-enriched tobacco species *(11, 14)*. For a given product, the percentage of nicotine that is unprotonated is calculated from the pH of the product and the appropriate pK_a of nicotine (8.02) with the Henderson–Hasselbach equation *(35)*. The pH of smokeless tobacco products has been reported to range from pH 4.6 to pH 11.8; in this range, < 0.1–99.9% of the nicotine present would be free nicotine *(3)*. The highest pH values were observed for *nass (36)* and also *iqmik* which is used in Alaska *(23)*. As nicotine is converted to free nicotine, it is more readily released from tobacco during use and passes across biological membranes *(37–39)*.

Limiting both total nicotine concentrations and the permissible pH could maintain lower free nicotine concentrations in smokeless tobacco products.

Metals and metalloids are naturally present in soil *(40)*, and a number of these and other inorganic compounds are absorbed into tobacco plant tissue *(41)* and are also present in smokeless tobacco products *(4, 42–45)*. In a study by Golia et al. *(41)*, certain metals, including cadmium, lead, zinc and copper, were in higher concentrations on tobacco leaf surfaces near the ground (primers) than on leaves higher up the tobacco stalk. This was the case for Oriental, flue and Burley tobaccos. It is possible that soils containing metals may be splashed on to tobacco leaves during rain or may be deposited on tobacco leaves that are spread out on the ground.

The levels of metals in smokeless tobacco products vary among countries. The amounts in products may be influenced by the soil content, pH *(46)* and industrial contamination *(43, 47–48)*. The concentration of metals varies by product type and country of origin. High concentrations have been reported in products from Ghana, India and Pakistan, with higher levels of lead, nickel and chromium in products from Pakistan than in those from Ghana and India. Products from India had high iron and copper levels, while those from Ghana had higher levels of copper, iron and aluminium than those from the other countries *(43)*. Swedish *snus* has a very low metal content *(49)* due to deliberate selection of tobacco *(28)* or other processes, such as washing tobacco leaves *(28, 50)*.

The concentrations of total TSNAs range from < 0.5 to 12 630 µg/g, with the highest concentration in Sudanese *toombak* products. The highest values are consistently seen in products that undergo fermentation (e.g. *toombak, khaini*, dry snuff, moist snuff) *(11, 14, 27, 51–53)*. Fermentation is used in tobacco processing to enhance taste but is characterized by microbial proliferation and active metabolism to nitrite, which reacts with natural tobacco alkaloids to form TSNAs *(8, 9)*. The lowest TSNA concentrations are found in products such as *snus* and dissolvable oral tobacco that do not contain fermented tobacco. ("Dissolvables" are products that essentially include tobacco and other ingredients compressed into chewable tablets, wafer-like strips or elongated sticks.) *Snus* products that are not fermented but are pasteurized eliminate microorganisms and result in low TSNA concentrations such as that in moist snuff. Some products in India that are labelled "*snus*" have high TSNA levels *(53)*.

A wide variety of microbes, including bacteria (*Staphylococcus, Corynebacterium, Lactobacillus, Enterobacter, Clostridium, Bacillus, Serratia* and *Escherichia*) and fungi (*Aspergillus, Fusarium, Cladosporium, Candida, Alternaria and Acremonium*), can generate nitrite. Some of these organisms are potentially harmful or pathogenic *(26, 54, 55)*. Microbial production of nitrite is a major determinant of the concentrations of TSNAs and other nitrosamines in tobacco *(9, 36, 56)*. A number of species, including *Staphylococcus, Corynebacterium,*

Lactobacillus and genera in the *Enterobacteriaceae* family, present in smokeless tobacco products contain a respiratory nitrate reductase and associated transporters that export nitrite (encoded in the *nar* gene operon) *(57)*. Dry snuff also contains a periplasmic nitrate reductase (*nap* gene), mainly in species in the *Enterobacteriaceae* family, which may also result in the release of nitrite *(58)*. In some species, *nar* genes are activated when oxygen levels decrease *(59)*.

As tobacco is cured, tobacco cells rupture and release nitrate *(31)*. Nitrate-reducing microbes can convert nitrate to nitrite under low-oxygen conditions. Certain bacteria can convert nitroalkanes (if present) to nitrite in the presence of oxygen *(60)*. Nitrite that is not further metabolized is generally expelled due to its toxicity. Fermentation, ageing, storage and tightly sealed packaging, with low oxygen, provide conditions in which respiration by nitrate-reducing bacteria involves generating and exporting nitrite *(8–10)*. Regardless of how nitrite is formed, once formed, it can combine with alkaloids and other secondary and tertiary amines to form nitrosamines *(31)*. Certain secondary or tertiary amines can also react with nitrite to form volatile nitrosamines, such as *N*-nitrosodimethylamine, and nitrosoacids *(31)*.

9.3 Agricultural practices and manufacturing processes that result in the formation and accumulation of harmful compounds

Many of the harmful agents in tobacco products are at lower levels or almost entirely absent from freshly transplanted tobacco *(61)* but begin accumulating in the early stages of cultivation and curing. Growing tobacco involves a number of agronomic decisions – type or species of tobacco to be grown, harvest timing and procedures, types of fertilizers and agrichemicals and application rates – and environmental factors such as soil composition, climate and rainfall, which collectively determine the chemistry of a product *(4)*.

The constituents of a finished smokeless tobacco product, including nicotine (in its various ionic forms), toxicants and carcinogens, result from the presence of inorganic, organic and biological agents and their interactions *(4, 19, 31, 40, 42, 43)*. Naturally occurring organic constituents of tobacco used in smokeless tobacco products include lignins, fatty acids, sugars, alkaloids, terpenoids, polyphenols, cembranoids and carotenoid pigments. The breakdown products of some of these constituents contribute to volatile flavour chemicals and the colouration of cured tobacco *(18, 19, 61)*.

Methods of tobacco growing, harvesting and curing vary geographically, which may affect product chemistry *(19)*. During the growing season, tobacco can absorb metals *(41, 43)*, as noted above. Addition of nitrate fertilizer boosts plant mass and also increases the concentrations of nitrate, nicotine and other alkaloids in leaves – all precursors of TSNAs *(18, 31, 61, 62, 63)*. Agricultural

plant protection chemicals applied to tobacco may also persist on the tobacco at harvest *(64)*. Exposure to the soil and atmosphere during cultivation and harvesting can result in the introduction of microbes and also fungi that generate mycotoxins. At harvesting, tobacco may be laid on the ground (*18*) or piled in a field for extended periods (≤ 45 days) (*65*). Contact with the soil offers an opportunity for the introduction of microorganisms and other organisms (insects) into the tobacco.

After harvest, tobacco leaves are cured by sun, air, flue or fire, the four primary means for traditional products (except products such as dissolvables). Sun-curing involves drying tobacco in the sun, whereas air-curing involves hanging tobacco in a well-ventilated barn. Flue-curing is done by exposing the leaves to elevated temperatures fuelled by wood, coal, oil or liquid petroleum gas in a tightly constructed building equipped with ventilators and flues *(18)*. During fire-curing, leaves are dried in smoke from the burning of wood or sawdust, when smoke-derived chemicals such as PAHs and VOCs can accumulate on the tobacco leaves (*22, 23, 24*). The levels of PAHs in fire-cured tobacco exceed those in air-cured tobacco (*23*) (see Fig. 9.1).

Fig. 9.1. Effects of type of curing on levels of polycyclic aromatic hydrocarbons in tobacco used to make smokeless products
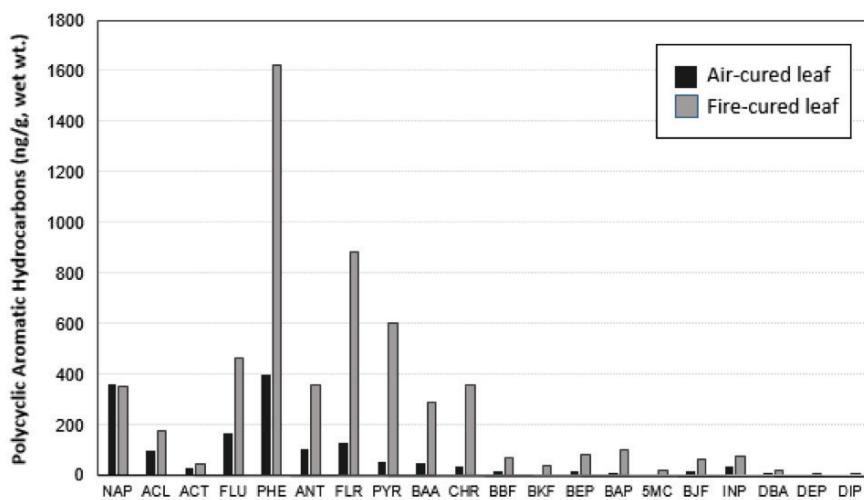
5MC: 5-methylchrysene; ACL: acenaphthylene; ACT: acenaphthene; ANT: anthracene; BAA: benz[*a*]anthracene; BAP: benzo[*a*]pyrene; BBF: benzo[*b*]fluoranthrene; BEP: benzo[*e*]pyrene; BJF: Benzo[*j*]fluoranthene; BKF: benzo[*k*]fluoranthrene; CHR: chrysene; DBA: Dibenz[*ah*]anthracene; DEP: Dibenzo[*ae*]pyrene; DIP: Dibenzo[*ai*]pyrene; FLR: fluoranthene; FLU: fluorene; INP: Indeno[1,2,3-*cd*]pyrene; NAP: naphthalene; PHE: phenanthrene; PYR: pyrene. BAA, BAP, BBF and BKF are on the FDA list of HPHCs *(60)*. *Source*: reference *23*.

After curing, tobacco may undergo further ageing, fermentation or long-term storage, and these production stages may increase microbial proliferation in anaerobic conditions. During these phases, dramatic chemical changes occur that include rapid catalysis of sugars, increases in pH and temperature and increases in the concentrations of nitrite and TSNAs *(8)*. Microbes may be deliberately added during fermentation *(9)*. Also, viable microorganisms are present in purchased products (*25, 55*). Aflatoxins, carcinogens produced by certain *Aspergillus* mould species, may accumulate during cultivation or subsequent production steps and have been found in certain smokeless tobacco products *(7, 66)*. Microbial action is clearly a driver of the evolving product chemistry.

9.4 Product additives

During manufacture, almost all products are augmented with some level of additives, including flavouring compounds that are not necessarily toxic but may add to the attractiveness of products, thus promoting initiation or fostering use. Flavourings used in these products are drawn from extracts, oleoresins, spice powders, individual compounds (e.g. menthol, vanillin) and more than 60 essential oils *(67)*. Some substances used as additives that have known adverse health effects include *khat* (an addictive plant), tonka bean (a liver toxin) and areca nut (an addictive psychoactive substance); areca nut is known to cause cancer and oral malformations, such as oral submucosal fibrosis *(68, 69)*. In some cases, areca nut and other ingredients are added at substantial levels to tobacco-containing products (*zarda, rapé*) or hand-made preparations (e.g. betel quid, *tombol*, *dohra*, *moawa* and *mainpuri*) *(3, 7, 70, 71)*.

9.5 Harmful agents in smokeless tobacco products and methods to reduce their effects

Damaging agents in smokeless tobacco products can be reduced in several ways, including by changes to smokeless tobacco production *(19)*. Some of these measures may help decrease TSNA levels *(9, 28, 72)*. Means of altering the levels of harmful agents may include the following.

- **Fertilizers**: decreasing the use of nitrate-containing fertilizers or using other fertilizers (e.g. urea or other non-nitrate fertilizers late in the growing season) could limit the formation of nitrosamines by decreasing the accumulation of nitrate at harvest (*18, 61*).

- **Surface disinfection of harvested tobacco:** washing harvested leaf material with a dilute bleach solution (hyprochlorite:water solution) can disinfect leaf surfaces. This procedure removes not only microorganisms but also soil *(50,56)*. Protocols effective in disinfecting food

products, such as leafy greens, may be useful for eliminating surface contamination. Disinfection of tobacco leaves have been shown reduce the levels of surface microbes *(28, 50, 56)*.

- Practices used effectively in the food industry can **reduce contamination of tobacco by microorganisms and soil**. In Sweden, *snus* must meet food regulatory standards, which has led to a product with reproducibly lower toxicant levels *(28)*.

- **Electron beam technology:** high-energy electron beam irradiation is a non-thermal, chemical-free technology, in which compact linear accelerators generate highly energetic (10 MeV) electrons, that has been used to pasteurize foods and sterilize medical devices. The technology is often called "cold pasteurization", because it irradiates products without generating excess heat, which might cause undesirable product changes *(73)*. Although the appropriate dose of irradiation may depend on the product, electron beam technology could eliminate viable organisms present in tobacco that generate mycotoxins or nitrite and remove viable and potentially harmful organisms in smokeless tobacco. Because electron beam technology does not generate heat, it may be possible to use it to sterilize tobacco, ingredients, packaging material and the final product. Irradiation of tobacco early in production, especially before fermentation and ageing, may eliminate some microorganisms that generate nitrite resulting in lower TSNA as compared to non-irradiated tobacco.

- **Changes related to fire-curing and air-curing:** the levels of PAHs and volatile aldehydes are higher in fire-cured tobacco *(22, 23, 30, 74)*; the process of fire-curing should be omitted, if possible. During air-curing, microbial treatment with *Bacillus amyloliquefaciens* DA9, an organism that efficiently accumulates nitrite, may decrease nitrite and TSNA concentrations *(75)*. During curing, conditions of high humidity (70%) and temperatures ranging from 10°C to 32°C that may be found in poorly ventilated curing facilities are conducive to mould growth and the potential formation of mycotoxins, such as aflatoxins and ochratoxins *(11, 76)*. Efforts should be made to prevent these conditions and monitor to ensure mould growth is prevented.

- **Pasteurization:** the Swedish Match Company uses pasteurization in the preparation of Swedish *snus*, a smokeless tobacco product with a low TSNA concentration *(28, 77)*. Ground, blended leaf tobacco is mixed with water and sodium chloride in closed process blenders, then heat-treated with hot water and steam injection to achieve temperatures up to 80–100 °C for several hours. The mixture is cooled,

and other ingredients, such as flavours and humectants, are added before the product is packaged. This process changes taste, reduces microbial activity and yields a product with a shelf life of 14–30 weeks when refrigerated, according to a manufacturer (*28, 76). Snus* products continue to have low TSNA levels *(28)*, which have been reduced and maintained over several decades. In 2017, the average level of NNN plus NNK in the products of the Swedish Match Company was 0.47 µg/g product, dry weight *(28, 77)*.

- **Modified fermentation:** fermentation is the main process in the production of tobacco products that increases levels of nitrite and TSNAs *(8)*, and it should be modified *(9)* or avoided entirely. During fermentation, microbial populations can rapidly proliferate or decrease and can increase pH, oxalate, nitrite and TSNA levels *(8, 9)*. The steps in the formation of TSNAs are shown in Fig. 9.2. Cleaning of fermentation equipment before use and addition of non-nitrite-producing microbes at that stage can reduce TSNA levels *(9)*. Use of oxygen-rich endothermic fermentation *(78)* may also decrease the anaerobic respiratory nitrate reduction that occurs during tobacco fermentation *(8, 9)*. Addition of suitable fermentation organisms that do not produce nitrite (*9*) may be required after irradiation. A product free of microbes (as is the case for *snus*) would generally be seen as beneficial (*8, 9, 28*) as compared with products harbouring unknown microorganisms, including those that generate nitrite or are otherwise harmful to the user.

- **Microwave technology:** this technology has been used safely over the past 50 years for cooking, drying, pasteurizing, sterilizing, bacterial destruction and enzyme deactivation in food products, but has only recently been used in continuous in-line processing in the United States. The principle of microwave technology is simple: when a substance is subjected to microwave radiation, water molecules in the material absorb the energy and internal heat is generated volumetrically by their molecular vibration. Continuous microwave heating equipment and technology was first used for aseptic processing of food products in a North Carolina processing facility in 2008. Microwave technology has been used in producing pharmaceutical and nutraceutical products. In India, commercial ready-to-eat meals were processed in-pack using proprietary microwave technology. Microwave energy likely increases temperature inside of the microbial cell, denaturing critical biomolecules and resulting in reduced cell efficacy and often death (*79–83*). Although not used for tobacco presently, microwave technology that has been used successfully with food

products may be assessed for its applicability in reducing or eliminating microorganisms in tobacco or tobacco products.

- **Nitrite scavengers:** although the most efficient approach to minimizing nitrite in smokeless tobacco products is complete elimination of nitrite-generating organisms, the use of agents that act as nitrite scavengers has been investigated *(28)*. Certain polyphenols, vitamin C, tocopherol, green tea extract, a green tea component (epigallocatechin gallate) and morpholine could be investigated as potential nitrite scavengers to neutralize nitrite generated during smokeless tobacco processing *(28, 84, 85)*.

- **Product refrigeration:** continuing growth of microbial populations and potential formation of nitrosamine compounds in products can be controlled by refrigeration. One manufacturer encourages refrigeration of its products, including at points of sale *(28)*.

Fig. 9.2. Processes that can increase the concentrations of nitrosamines in smokeless tobacco products
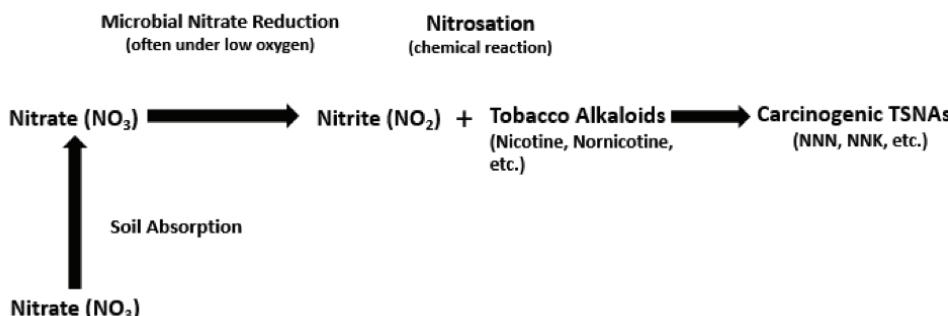
NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N*´-nitrosonornicotine.
Source: references *9, 10, 18, 31, 61*

Smokeless tobacco production can result in the presence of nitrosamines and microbes, including bacteria and fungi. Tobacco contains nitrate, tobacco alkaloids and certain secondary and tertiary amines, which play a role in the formation of nitrosamines. The most abundant carcinogens in tobacco products are TSNAs, but they also include other nitrosamines such as *N*-nitrosamino acids and volatile *N*-nitrosamines. Nitrosamines can be formed at various stages in smokeless tobacco production. One TSNA, NNN, is a human carcinogen known to cause oral cancer *(6, 54)*.

9.6　Detection of microorganisms

Culture counts in standard culture media: microbial contamination can be assessed on culture media plates, but this method is time-consuming, as it involves pouring media plates, streaking and interpreting growth; it may be difficult to find the appropriate media for testing specific organisms in tobacco *(87)*. Many different microorganisms grow in tobacco, and they can be cultured on various media, including tryptic soy agar *(55)*, sheep blood agar, mannitol salt agar and MacConkey agar plates *(25)*. According to the "great plate anomaly" *(87)*, certain taxa may be overrepresented, such that less abundant yet important taxa remain unidentified, and no single medium will capture the microbial diversity of tobacco products. The expertise, volume of supplies and requirement for sterile facilities to set up, inoculate, incubate and correctly interpret media plates may be too costly in certain localities, and less expensive, less complicated methods may have practical advantages.

Culture counts on disposable microbiological films: an alternative to culture media is a small two-layer film. The upper film is lifted open so that the inoculum can be spread on the lower film surface, which is then incubated and read for the extent of microbial growth. Although other products may exist, media for testing a wide variety of bacteria are made by 3M Corp.

9.6.1　Rapid detection of live microorganisms by cell viability

Plate reader format: it would be helpful to know the extent of microbial contamination on tobacco at various points during cultivation and production and in final tobacco products. An assay of microbial viability based on luciferase (e.g. BacTiter-Glo) indicates the number of viable microorganisms by quantification of ATP, a molecule present in all living cells (*88*). A single reagent causes cell lysis and generation of a luminescent signal, which is proportional to the ATP concentration and is directly proportional to the number of viable cells. This assay detects a variety of bacteria and fungi. Data can be recorded 5 min after addition and mixing of the reagent. The assay can detect as few as 10 bacterial cells on a luminometer or a CCD camera. The application can be used with a 96-well plate reader (*88*).

Handheld luminometer: ATP measurement has been miniaturized in handheld readers made by at least six manufacturers. Handheld luminometers to test for ATP allow determination of the extent of microbial contamination in 5–20 seconds. Several manufacturers make ATP luminometers, and their capability has been compared. One manufacturer makes devices to enumerate specific bacterial groups, total viable count, *Enterobacteriaceae*, coliform, *E. coli* and *Listeria* spp. These devices are used in various industries (e.g. health care, pharmaceuticals, water treatment, dairy, meat, produce). A portable unit could be used to test tobacco products, preparations or related ingredients on site within minutes (*90, 91*).

Detection of organisms by qPCR: organisms could also be detected by quantitative polymerase chain reaction (qPCR), a technique in which probes against specific gene regions bind, replicate and can be quantified. These and other organisms can be detected with the use of properly designed molecular probes. Molecular probes can be designed for qPCR that are specific for nitrate-reducing organisms and genes (e.g. *narG*, *napA*, etc.) and used quantification by qPCR *(89)*.

Detection of nitrate-reducing organisms: in addition to enumeration of microorganisms to assess the extent of contamination, actual nitrate-reducing organisms in tobacco products should be identified. One means may involve growing microbes on nitrate agar with an added nitrite indicator. Another approach is use of Greise reagent (produced from sulfanilamide with NO_2, followed by addition of naphthylethylenediamine) *(92)*.

9.7 Overview of analytical methods used to measure toxicants in smokeless tobacco

Analytical methods for measuring tobacco constituents (Table 9.2) depend mainly on GC and LC linked to various types of mass spectrometers (single and triple quad; MS, MS/MS) and other detectors, such as inductively coupled plasma and flame ionization detectors. This is not an exhaustive list, but it shows some recent papers describing approaches utilized by various laboratories for analysing tobacco products.

Table 9.2. Methods for measuring tobacco constituents

Analyte	Measurement method
Nicotine *(35, 93)*	GC-MS, GC/FID
Minor alkaloids *(13)*	GC-MS/MS
Flavours and non-tobacco plant constituents, including coumarin and camphor *(94)*	GC-MS
PAHs *(22, 23, 24)*	GC-MS
VOCs *(22)*	GC-MS
Toxic metals *(42)*	ICP-MS
TSNA *(11, 52, 53)*	LC–MS/MS
Aflatoxins *(7)*	UHPLC-MS/MS
Areca-nut-related compounds *(95)*	LC/MS /MS
pH *(34, 35)*	Ion probe
Nitrate and nitrite *(96)*	Ion chromatography
Fungi *(76)*	Culture media specific to fungi
Bacteria *(25)*	Culture media specific to bacteria
Identification of metals and alkaline agents *(97)*	X-ray fluorescence (XRF)
Identification of tobacco species (*Nicotiana tabacum, N. rustica, N. glauca*), non-tobacco plant materials (e.g. areca nut, tonka bean) and alkaline agents (magnesium carbonate, slake lime) *(11, 52, 71)*	Fourier transform/infrared spectroscopy (FT/IR)

GC: gas chromatography; LC: liquid chromatography; MS: mass spectrometry; PAHs: polycyclic aromatic hydrocarbons; TSNAs: tobacco-specific nitrosamines; VOCs: volatile organic compounds.

Other techniques that have been used to measure these analytes could be substituted, with appropriate validation, if necessary. For regulatory agencies with limited funding or space, compact or portable instrumentation is available, including GC-MS systems that could allow the detection and quantification of nicotine, minor alkaloids, arecoline (areca nut), flavours and non-tobacco plant constituents (coumarin, diphenyl ether, camphor). Some GC-MS systems are miniaturized, mobile and cost less than conventional bench systems.

9.8 Regulatory approaches and responses

Various regulatory and other responses have been used. While the European Union regulates smokeless tobacco under directive 2001/37/EC *(98)*, Sweden is exempted from the prohibition on marketing of certain types of tobacco for oral use. In the United States, smokeless tobacco has been regulated by the FDA since the passage of the Family Smoking Prevention and Tobacco Control Act in 2009 *(99)*. Elsewhere, there is limited regulation of smokeless tobacco use, such as prohibiting products or regulating the contents. Despite attempts by the states of Andhra Pradesh, Bihar, Goa, Maharashtra and Tamil Nadu in India to prohibit sales of *gutkha* and *pan masala* as food products *(3)*, manufacturers have circumvented the ban *(100)*, in the case of *gutkha* by dividing the product into attached packages with tobacco flakes in one and the other constituents in the other. However, Goa has reportedly sustained a ban on smokeless tobacco products through the Goa Public Health (Amendment) Act, 2005 *(101)*.

An example of a targeted approach to reducing toxicants in smokeless tobacco is the rule proposed by the FDA in January 2017 for NNN in finished smokeless tobacco products *(72)*, which was based on the carcinogenicity of NNN, a major contributor to elevated oral cancer risks associated with smokeless tobacco use. Elements of the standard include:

- required expiration date and, if applicable, storage conditions (e.g. re-frigeration at point of sale) for finished smokeless tobacco products;
- a mean level of NNN in any batch of finished smokeless tobacco products of ≤ 1 μg/g of tobacco (dry weight) at any time up to the expiration date;
- testing to assess the stability of NNN levels in finished smokeless tobacco products and to establish and verify the product's expiration date and storage conditions; and
- batch-testing to determine whether the products conform to the proposed NNN level.

The FDA also proposed various evidence-based, broadly stated considerations with regard to this regulation *(72)*, which might be helpful for other regulatory bodies pursuing toxicant reduction in smokeless tobacco products.

- Regulation of smokeless tobacco toxicants may affect consumer perceptions of the harm of smokeless tobacco use.
- Users of smokeless tobacco products might interpret a reduction in one or more toxicants as resulting in less harm, which could reduce their motivation to quit. FDA, however, does not expect the proposed product standard to appreciably discourage cessation of smokeless tobacco products in such a way as to offset the beneficial public health impact from reduced cancer risk.
- Health messages must continue to educate consumers with evidence-based advice about all products.

Another approach is the GothiaTek standard instituted by Swedish Match, a *snus* manufacturer, in the late 1990s. It set maximum allowable concentrations of nitrite, TSNAs (NNN and NNK), *N*-nitrosodimethylamine, benzo[*a*]pyrene, aflatoxins, agrochemicals and various metals, and these have been met *(28, 102)*. *Snus* is made with air-cured tobacco that is pasteurized, fire-curing and fermentation are omitted, and products are refrigerated at points of sale and have very low TSNA concentrations *(22)*.

In contrast to the FDA product standard for NNN, GothiaTek limits are set on an "as is" (wet weight) basis *(22)*. Other components include:

- standards for raw materials, including guidance levels for agricultural residues
- quality control and quality assurance measures during manufacture
- heat treatment of tobacco
- reduced water content
- flavourings consistent with the Swedish Food Act for additives and flavourings.

The GothiaTek standard does not regulate product pH or nicotine content *(102)*, but, for certain harmful and carcinogenic agents, it serves as a reference for what is possible if tobacco product makers or regulators wish to decrease toxicant levels. The levels of most GothiaTek toxicants decreased or remained stable between 2009 and 2017. The agents included are related to soil content, curing, microbial activity, and agrochemical use and are shown in Table 9.3.

Table 9.3. Systematic decreases in toxicant levels regulated under GothiaTek in 2009, 2015 and 2017

Potential source and chemical component	2009 level[a]	2015 level[b]	2017 level[b]	Limit (2009)	Limit (2015, 2017)
Soil metal content					
Arsenic (µg/g)	0.1	< 0.06	0.06	0.5	0.25
Cadmium (µg/g)	0.6	0.28	0.27	1	0.5
Chromium (µg/g)	0.8	0.46	0.46	3	1.5
Lead (µg/g)	0.3	0.15	0.15	2	1
Mercury (µg/g)	NA	< 0.02	< 0.02	NA	0.02
Nickel (µg/g)	1.3	0.87	0.82	4.5	2.25
Curing[c]					
Acetaldehyde[d] (µg/g)	NA	6.5	6.3	NA	25
Crotonaldehyde[d] (µg/g)	NA	< 0.10	< 0.10	NA	0.75
Formaldehyde[d] (µg/g)	NA	2.3	2.3	NA	7.5
Benzo[*a*]pyrene[e] (ng/g)	1.1	< 0.6	< 0.6	20	1.25
Microorganisms (fungi)					
Aflatoxin (ng/g)	NA	< 2.1	< 2.1	NA	2.5
Ochratoxin A (ng/g)	NA	2.3	2	NA	10
Microorganisms (bacteria)					
Nitrite (µg/g)	2.0	1.7	1.5	7	3.5
NNN+NNK (µg/g)	1.6	0.39	0.47	10	0.95
NDMA (ng/g)	0.7	< 0.6	< 0.6	10	2.5
Agrochemicals (µg/g)	NA	[b]	[b]	NA	[a]

NA: not available; NDMA: *N*-nitrosodimethylamine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N´*-nitrosonornicotine. These analytes are derived primarily from soil uptake (toxic metals), curing and the presence and activity of fungi and bacteria. Units are converted from per kilogram to per gram.
[a] In accordance with the Swedish Match Agrochemical Management Programme *(28)*.
[b] Below Swedish Match internal limits *(102)*.
[c] *Snus* is not fire-cured; these analytes are often formed during fire-curing in the manufacture of other products (dry and moist snuff).
[d] These aldehydes are volatile organic compounds.
[e] This compound is a polycyclic aromatic hydrocarbon.

9.9   Policy recommendations and summary

In this section, we have described research to identify the major characteristics of concern and approaches to measuring and decreasing the levels of certain harmful agents in smokeless tobacco products. We concur with the recommendations of TobReg in 2015 *(29)* for the establishment of regulatory limits for carcinogens in smokeless tobacco products, which are still valid and are reiterated below.

- **Reduce toxicity**: decrease or eliminate use of *N. rustica*; limit bacterial contamination, which can promote nitrosation and carcinogen formation; require that tobacco be flue- or sun-cured rather than fire- or air-cured to avoid bacterial growth; kill bacteria by pasteurization; improve storage conditions, such as by refrigerating products before sale; affix the date of manufacture; eliminate ingredients such as areca nut and tonka bean that are known to be carcinogenic.

- **Impose product standards**: set an upper limit of 2 μg/g (dry weight) for NNN plus NNK and an upper limit of 5 ng/g (dry weight) for benzo[*a*]pyrene; and monitor the levels of arsenic, cadmium and lead in tobacco.

- **Reduce appeal and addictiveness**: take steps to reduce the appeal of and addiction to tobacco products, including by prohibiting addition of sweeteners and flavourings (including herbs, spices and flowers) and setting limits on free nicotine and pH.

- **Apply uniform standards for transnational products**: hold exported smokeless tobacco products to the same (or higher) standards as in the country of manufacture.

WHO TobReg also recommended that communications about smokeless tobacco products be regulated to prevent unsubstantiated claims about exposure or disease reduction *(103)*.

We have summarized below some concerns associated with the carcinogenic, toxic and addictive agents in smokeless tobacco products and propose ways of lowering the concentrations of these agents. Decreasing the levels of nicotine, free nicotine, arsenic, cadmium, lead, benzo[*a*]pyrene and NNN plus NNK will not make smokeless tobacco products safe; however, it is prudent to decrease the levels of known addictive, toxic or carcinogenic agents.

The concerns associated with smokeless tobacco products include:

- use of high-nicotine tobacco, including *N. rustica*;

- inclusion of alkaline agents that substantially raise the pH, increase free nicotine concentrations and contribute to higher blood nicotine levels, cardiovascular risk and addiction;

- presence of toxic metals in tobacco due to soil uptake or deposited from contaminated soil or the environment;

- use of fire-curing, which can introduce chemicals from smoke, such as PAHs (including benzo[*a*]pyrene), phenols and volatile aldehydes;

- presence of microbial contamination (bacteria and fungi) on tobacco leaves, especially those organisms that promote the formation of aflatoxins, ochratoxin A, nitrite or nitrosamines (particularly TSNAs), and the conditions most conducive to their formation;

- presence of microbes (bacteria and fungi) that can cause infectious disease, become resistant to antibiotics, alter oral biofilms or displace the healthy microflora of the gastrointestinal tract;

- soil fertilization practices that result in elevated levels of nitrate, leading to increased levels of nitrite and nitrosamines in tobacco at harvest;

- presence of harmful agricultural chemical residues on tobacco at harvest;

- presence of viable microorganisms (including pathogens) in purchased products that can be transferred to users and may become established in the oral cavity or the gastrointestinal tract;

- use of fermentation, ageing, product storage or other processes that provide anaerobic conditions that can contribute to rapid formation of nitrite and TSNA;

- presence of high residual levels of nitrate in finished tobacco products;

- presence of areca nut (IARC Group 1 human carcinogen) and other additives of recognized toxicity or carcinogenicity;

- presence of flavouring additives that have toxic effects; and

- presence of flavouring additives that enhance initiation of tobacco use by adding appeal, impeding cessation or masking the recognition or severity of disease symptoms.

Attention should also be paid to the contribution of non-tobacco ingredients and plant materials in smokeless tobacco products. Steps that might decrease the level of TSNAs, the most abundant carcinogens in smokeless tobacco products, are shown in Fig. 9.3.

Fig. 9.3. Countermeasures that might contribute to reducing concentrations of TSNAs and other harmful agents in tobacco products
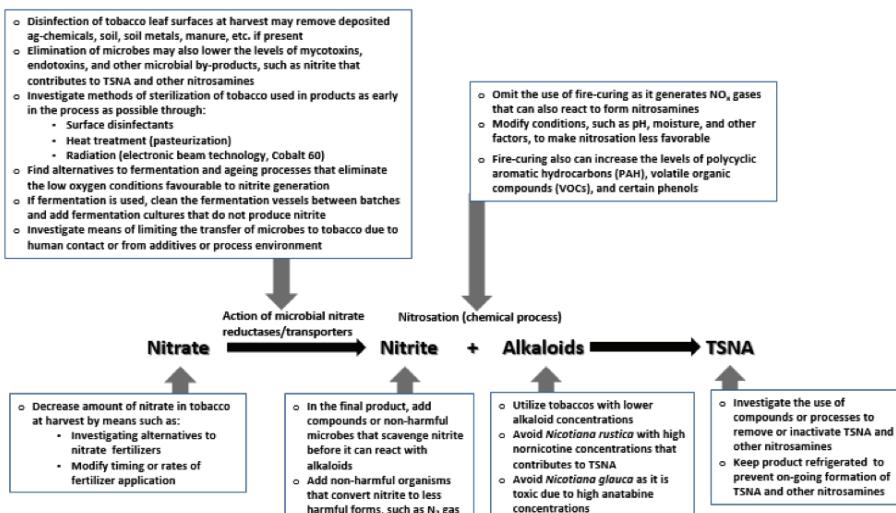
N_2: nitrogen; NO_x: nitrogen oxide; TSNA: tobacco-specific N'-nitrosamines.
To address the concerns listed above, in addition to the steps recommended by TobReg and specific product standards, product manufacturers could take a number of steps to decrease the concentrations of harmful agents in tobacco products.

- Because nornicotine is the precursor of NNN, genetically screen tobacco cultivars for lower nornicotine content as a means of reducing the formation of NNN.
- Screen soils for metal contamination and nitrate levels.
- Investigate agronomic means for minimizing the concentrations of nitrate and agricultural chemicals in or on plants at harvest.
- Use gloves when harvesting or handling tobacco to prevent the transfer of organisms on the skin (e.g. *Staphylococcus*) to the tobacco and also to protect workers from "green tobacco sickness".
- Wash harvested tobacco with dilute bleach solution (1:250 hyprochlorite:water) to remove soil, soil metals, manure, agricultural chemicals, insects and microorganisms.
- Ensure that curing facilities are clean so that other organisms (especially nitrate-reducing organisms) are not introduced.
- Identify harmless chemical or biological agents that could be added to prevent microbial growth.
- Screen cured tobacco for elevated nitrite or the presence of nitrite-generating organisms (e.g. *Staphylococcus*, *Corynebacterium*) before further processing.
- Pasteurize or heat-treat products.
- Clean fermentation vats before use.
- Add non-nitrate-reducing fermentation organisms before fermentation.
- Investigate means for lowering nitrate levels during fermentation (selective filtration, denitrification, cycle liquid from fermentation through a cell that reacts nitrite with chemicals scavengers or denitrifying organisms).
- Add nitrite scavengers (vitamin C, tocopherol, green tea extract, Kunlun tea extract, cysteine) before fermentation.
- Assay final products for microbial growth.
- Keep products in refrigerated storage before sale.

These observations provide additional support for the feasibility of monitoring and controlling the levels of some carcinogenic and other harmful agents in smokeless tobacco products.

9.9.1 Disclaimer

The findings and conclusions in this chapter are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the United States Department of Health and Human Services.

9.10 References

1. Decision FCTC/COP7(14). Further development of the partial guidelines for implementation of Articles 9 and 10 of the WHO FCTC (Regulation of the contents of tobacco products and Regulation of tobacco product disclosures). In: Report of the seventh session of the Conference of the Parties to the WHO Framework Convention on Tobacco Control. Geneva: World Health Organization; 2016) (http://www.who.int/fctc/cop/cop7/FINAL_COP7_REPORT_EN.pdf, accessed 15 May 2019).

2. WHO global report on trends in prevalence of tobacco smoking 2000–2025, 2nd edition. Geneva: World Health Organization; 2018.

3. Eriksen M, Mackay J, Schluger N, Gomeshtapeh FI, Drope J, editors. Tobacco atlas. Fifth edition. Atlanta (GA): American Cancer Society and World Lung Foundation; 2015 (https://tobaccoatlas.org/, accessed 16 July 2019).

4. Smokeless tobacco and public health: a global perspective. Chapter 3. Global view of smokeless tobacco products: constituents and toxicity (NIH/CDC Monograph). Bethesda (MD): Department of Health and Human Services; 2014:75–114 (NIH Pub. 14-7983) (https://cancercontrol.cancer.gov/brp/tcrb/global-perspective/SmokelessTobaccoAndPublicHealth.pdf, accessed 16 July 2019).

5. Stanfill SB. Chapter 7: Toxic contents and emissions in smokeless tobacco products. In: WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation. Sixth report of a WHO study group (WHO Technical Report Series, No. 1001). Geneva: World Health Organization; 2017:23–39. (http://www.who.int/tobacco/publications / prod_regulation/trs1001/en/, accessed 16 July 2019).

6. Smokeless tobacco and some tobacco-specific N-nitrosamines. Lyon: International Agency for Research on Cancer; 2007 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 89) (http://monographs.iarc.fr/ENG/Monographs/vol89/index.php, accessed 20 September 2018).

7. Zitomer N, Rybak ME, Li Z, Walters MJ, Holman MR. Determination of aflatoxin B1 in smokeless tobacco products by use of UHPLC-MS/MS. J Agric Food Chem. 2015;63(41):9131–8.

8. Di Giacomo M, Paolino M, Silvestro D, Vigliotta G, Imperi F, Visca P et al. Microbial community structure and dynamics of dark fire-cured tobacco fermentation. Appl Environ Microbiol. 2007;73:825–37.

9. Fisher MT, Bennett CB, Hayes A, Kargalioglu Y, Knox BL, Xu D et al. Sources of and technical approaches for the abatement of tobacco specific nitrosamine formation in moist smokeless tobacco products. Food Chem Toxicol. 2012;50:942–8.

10. Andersen RA, Burton HR, Fleming PD, Hamilton-Kemp TR. Effect of storage conditions on nitrosated, acylated, and oxidized pyridine alkaloid derivatives in smokeless tobacco products. Cancer Res. 1989;49:5895–900.

11. Stanfill SB, Connolly GN, Zhang L, Jia TL, Henningfield J, Richter P et al. Surveillance of international oral tobacco products: total nicotine, unionized nicotine and tobacco-specific nitrosamines. Tob Control. 2011;20:e2.

12. Sisson VA, Severson RF. Alkaloid composition of Nicotiana species. Beitr Tabakforsch Int. 1990;14:327–39.

13. Lisko JG, Stanfill SB, Duncan BW, Watson CH. Application of GC-MS/MS for the analysis of to-

bacco alkaloids in cigarette filler and various tobacco species. Anal Chem. 2013;85:3380−4.

14. Idris AM, Nair J, Ohshima H, Friesen M, Brouet I, Faustman EM et al. Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (toombak). Carcinogenesis. 1991;12:1115−8.

15. Steenkamp PA, van Heerden FR, van Wyk B. Accidental fatal poisoning by Nicotiana glauca: identification of anabasine by high performance liquid chromatography/photodiode array/ mass spectrometry. Forensic Sci Int. 2002;128:208–17.

16. Furer V, Hersch M, Silvetzki N, Breuer GS, Zevin S. Nicotiana glauca (tree tobacco) intoxication – two cases in one family. J Med Toxicol. 2011;7(1):47–51.

17. Bhide SV, Nair J, Maru GB, Nair UJ, Kameshwar Rao BV, Chakraborty MK et al. Tobacco-specific N-nitrosamines [TSNA] in green mature and processed tobacco leaves from India. Beitr Tabak- forsch Int. 1987;14:29–32.

18. Burton HR, Dye NK, Bush LP. Distribution of tobacco constituents in tobacco leaf tissue. 1. To- bacco-specific nitrosamines, nitrate, nitrite, and alkaloids. J Agric Food Chem. 1992;40(6):1050–5.

19. Rodgman A, Perfetti TA, editors. The chemical components of tobacco and tobacco smoke, 2nd edition. Boca Raton (FL): CRC Press; 2013.

20. Chen Z, Xia Z, Lei L, Xu S, Wang M, Cun L et al. Characteristic analysis of endophytic bacteria population in tobacco. Acta Tabacaria Sinica. 2014;20:102–7.

21. List of tobacco pests and diseases. In: Ephytia [website]. Paris: French National Institute for Agricultural Research; 2019 (http://ephytia.inra.fr/en/C/10749/Tobacco-List-of-tobacco-pests- and-diseases, accessed 16 July 2019).

22. Stepanov I, Jensen J, Hatsukami D, Hecht SS. New and traditional smokeless tobacco: compa- rison of toxicant and carcinogen levels. Nicotine Tob. Res. 2008; 10:1773–82.

23. Hearn BA, Ding YS, England L, Kim S, Vaughan C, Stanfill SB et al. Chemical analysis of Alaskan iq'mik smokeless tobacco. Nicotine Tob Res. 2013;15(7):1283–8.

24. Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami D, Hecht SS. Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography–mass spectrometry. Chem Res Toxicol. 2010;23:66–73.

25. Han J, Sanad YM, Deck J, Sutherland JB, Li Z, Walters MJ et al. Bacterial populations associated with smokeless tobacco products. Appl Environ Microbiol. 2016;82:6273–83.

26. Larsson L, Szponar B, Ridha B, Pehrson C, Dutkiewicz J, Krysińska-Traczyk E et al. Identification of bacterial and fungal components in tobacco and tobacco smoke. Tob Induced Dis. 2008;4:4.

27. Pauly JL, Paszkiewicz G. Cigarette smoke, bacteria, mold, microbial toxins, and chronic lung inflammation. J Oncol. 2011;819129.

28. Rutqvist LE, Curvall M, Hassler T, Ringberger T, Wahlberg I. Swedish snus and the GothiaTek standard. Harm Reduct J. 2011;8:11.

29. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: sixth report of a WHO study group (WHO Technical Report Series No. 1001). Geneva: World Health Organization; 2018 (https://www.who.int/tobacco/publications/ prod_regulation/trs1001/en/, accessed 16 July 2019).

30. Fact sheet, smokeless tobacco health effects. Atlanta (GA): Centers for Disease Control and Prevention; 2018 (https://www.cdc.gov/tobacco/data_statistics/fact_sheets/smokeless/, accessed 16 July 2019).

31. Spiegelhalder B, Fischer S. Formation of tobacco-specific nitrosamines. Crit Rev Toxicol. 1991;21:241.

32. Wang J, Yang H, Shi H, Zhou J, Bai R, Zhang M et al. Nitrate and nitrite promote formation of tobacco-specific nitrosamines via nitrogen oxides intermediates during postcured storage under warm temperature. J Chemistry. 2017;6135215 (https://doi.org/10.1155/2017/6135215, accessed 20 July 2019).

33. Bhisey RA, Stanfill SB. Chapter 13. Chemistry and toxicology of smokeless tobacco. In: Smokeless tobacco and public health in India. New Delhi: Ministry of Health and Family Welfare; 2017 (https://www.researchgate.net/, accessed 23 September 2018).

34. Lawler TS, Stanfill SB, Zhang L, Ashley DL, Watson CH. Chemical characterization of domestic oral tobacco products: total nicotine, pH, unprotonated nicotine and tobacco-specific N-nitrosamines. Food Chem Toxicol. 2013;57:380–6.

35. Notice regarding requirement for annual submission of the quantity of nicotine contained in smokeless tobacco products manufactured, imported, or packaged in the United States. Washington (DC): Centers for Disease Control and Prevention; Department of Health and Human Services; 1999 (https://www.gpo.gov/fdsys/pkg/FR-1999-03-23/pdf/99-7022.pdf, accessed 16 July 2019).

36. Brunnemann KD, Genoble L, Hoffmann D. N-Nitrosamines in chewing tobacco: an international comparison. J Agric Food Chem. 1985;33:1178–81.

37. Tomar SL, Henningfield JE. Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. Tob Control. 1997;6(3):219–25.

38. Fant RV, Henningfield JE, Nelson RA, Pickworth WB. Pharmacokinetics and pharmacodynamics of moist snuff in humans. Tob Control. 1999;8:387–92.

39. Alpert HR, Koh H, Connolly GN. Free nicotine content and strategic marketing of moist snuff tobacco products in the United States: 2000–2006. Tob Control. 2008;17:332–8.

40. Alloway BJ, editor. Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Dordrecht: Springer Netherlands; 2013.

41. Golia EE, Dimirkou A, Mitsios IK. Accumulation of metals on tobacco leaves (primings) grown in an agricultural area in relation to soil. Bull Environ Contam Toxicol. 2007;79:158–62.

42. Pappas RS, Stanfill SB, Watson C, Ashley DL. Analysis of toxic metals in commercial moist snuff and Alaskan iqmik. J Anal Toxicol. 2008;32(4):281–91.

43. Pappas RS. Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization. Metallomics. 2011; 3(11):1181–98.

44. Zakiullah SM, Muhammad N, Khan SA, Gul F, Khuda F, Khan H. Assessment of potential toxicity of a smokeless tobacco product (naswar) available on the Pakistani market. Tob Control. 2012;(4):396–401.

45. Gupta P, Sreevidya S. Laboratory testing of smokeless tobacco products. Final report to the India Office of the WHO (allotment No: SE IND TOB 001.RB.02). New Delhi; 2004.

46. Zaprjanova P, Ivanov K, Angelova V, Dospatliev L. Relation between soil characteristics and heavy metal content in Virginia tobacco. In: World Congress of Soil Science. Brisbane, Australia. 2010:205–8 (https://www.iuss.org/19th%20WCSS/Symposium/pdf/2027.pdf, accessed 16 July 2019).

47. Mulchi CL, Adamu CA, Bell PF, Chaney RL. Residual heavy metal concentrations in sludge amended coastal plain soils – II. Predicting metal concentrations in tobacco from soil test information. Commun Soil Sci Plant Anal.1992;23(9–10):1053–69.

48. Adamu CA, Bell PF, Mulchi CL, Chaney RL. Residual metal levels in soils and leaf accumulations in tobacco a decade following farmland application of municipal sludge. Environ Pollut. 1989;56:113–26.

49. New limit values for GothiaTek. In: Swedish Match [website]. Stockholm: Swedish Match; 2016 (https://www.swedishmatch.com/Media/Pressreleases-and-news/News/New-limit-values-for-GOTHIATEK/, accessed 16 July 2019).

50. Wahlberg I, Wiernik A, Christakopoulos A, Johansson L. Tobacco-specific nitrosamines: a multidisciplinary research area. Agro Food Industry Hi Tech. 1999;10(4);23–8.

51. Richter P, Hodge K, Stanfill S, Zhang L, Watson C. Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines. Nicotine Tob Res. 2008;

10(11):1645–52.

52. Stanfill SB, Croucher R, Gupta P, Lisko J, Lawler TS, Kuklenyik P. Chemical characterization of smokeless tobacco products from South Asia: nicotine, unprotonated nicotine, tobacco-specific N-nitrosamines and flavor compounds. Food Chem Toxicol. 2018;118:626–34.

53. Stepanov I, Gupta PC, Dhumal G, Yershova K, Toscano W, Hatsukami D et al. High levels of

54. tobacco-specific N-nitrosamines and nicotine in Chaini Khaini, a product marketed as snus.

55. Tob Control. 2015;24,e271–4.

56. Balbo S, James-Yi S, Johnson CS, O'Sullivan MG, Stepanov I, Wang M et al. (S)-N′-nitrosonornicotine, a constituent of smokeless tobacco, is a powerful oral cavity carcinogen in rats. Carcinogenesis. 2013;34:2178.

57. Smyth EM, Kulkarni P, Claye E, Stanfill SB, Tyx RE, Maddox C et al. Smokeless tobacco products harbor diverse bacterial microbiota that differ across products and brands. Appl Microbiol Biotechnol. 2017;101:5391.

58. Wiernik A, Christakopoulos A, Johansson L, Wahlberg I. Effect of air-curing on the chemical composition of tobacco. Recent Adv Tob Sci. 1995;21:39–80.

59. González PJ, Correia C, Moura I, Brondino CD, Moura JJG. Bacterial nitrate reductases: molecular and biological aspects of nitrate reduction. J Inorg Biochem. 2006;100:1015–23.

60. Tyx RE, Stanfill SB, Keong LM, Rivera AJ, Satten GA, Watson CH. Characterization of bacterial communities in selected smokeless tobacco products using 16 S rDNA analysis. PLoS One 2016;11:e0146939.

61. Nishimura T, Vertes AA, Shinoda Y, Inui M, Yukawa H. Anaerobic growth of Corynebacterium glutamicum using nitrate as a terminal electron acceptor. Appl Microbiol Biotechnol. 2007;75:889–97.

62. Gadda G, Francis K. Nitronate monooxygenase, a model for anionic flavin semiquinone intermediates in oxidative catalysis. Arch Biochem Biophysics. 2010;493:53–61.

63. Burton HR, Dye NK, Bush LP. Distribution of tobacco constituents in tobacco leaf tissue. 1. Tobacco-specific nitrosamines, nitrate, nitrite, and alkaloids. J Agric Food Chem. 1992;40(6):1050–5.

64. Burton HR, Dye NK, Bush LP. Relationship between tobacco-specific nitrosamines and nitrite from different air-cured tobacco varieties. J Agric Food Chem. 1994;42:2007–11.

65. Bush LP. Alklaloid biosynthesis. In: Davis DL, Nielsen MT, editors. Tobacco: production, chemistry and technology. Oxford: Blackwell; 1999;285–91.

66. Ghosh RK, Khan Z, Rao CVN, Banerjee K, Reddy DD, Murthy TGK et al. Assessment of organochlorine pesticide residues in Indian flue-cured tobacco with gas chromatography-single quadrupole mass spectrometer. Environ Monit Assess. 2014;186:5069–75.

67. Idris AM, Ibrahim SO, Vasstrand EN, Johannessen AC, Lillehaug JR, Magnusson B et al. The Swedish snus and the Sudanese toombak: are they different? Oral Oncol. 1998;34(6):558–66.

68. Harmful and potentially harmful constituents (HPHCs) [website].Washington (DC): Food and Drug Administration; 2018 (https://www.fda.gov/TobaccoProducts/Labeling/ProductsIngredientsComponents/ucm20035927.htm, accessed 17 January 2019).

69. Smokeless tobacco ingredient list as of April 4, 1994. Report to the Subcommittee on Health and the Environment, Committee on Energy and Commerce. Washington (DC): House of Representatives; 1994 (http://legacy.library.ucsf.edu/tid/pac33f00/pdf, accessed 16 July 2019).

70. Garg A, Chaturvedi P, Gupta PC. A review of the systemic adverse effects of areca nut or betel nut. Indian J Med Paediatr Oncol. 2014;35(1):3–9.

71. Prabhu RV, Prabhu V, Chatra L, Shenai P, Suvarna N, Dandekeri S. Areca nut and its role in oral submucous fibrosis. J Clin Exp Dent. 2014;6(5):e569–75.

72. Bao P, Huang H, Hu ZY, Haggblom MM, Zhu YG. Impact of temperature, CO2 fixation and nitrate reduction on selenium reduction, by a paddy soil clostridium strain. J Appl Microbiol. 2013;114:703–12.

73. Stanfill SB, Oliveira-Silva AL, Lisko J, Lawler TS, Kuklenyik P, Tyx R et al. Comprehensive chemical characterization of rapé tobacco products: flavor constituents, nicotine, tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons. Food Chem Toxicol. 2015;82:50–8.

74. Tobacco product standard for N-nitrosonornicotine level in finished smokeless tobacco products. Washington (DC): Department of Health and Human Services, Food and Drug Administration; 2017 (https://www.fda.gov/downloads/aboutfda/reportsmanualsforms/reports/economicanalyses/ucm537872.pdf, accessed 16 July 2019).

75. Pillai SD, Shayanfar S. Electron beam technology and other irradiation technology applications in the food industry. Top Curr Chem. 2017;375:6. doi: 10.1007/s41061-016-0093-4.

76. Idris AM, Ibrahim SO, Vasstrand EN, Johannessen AC, Lillehaug JR, Magnusson B et al. The Swedish snus and the Sudanese toombak: are they different? Oral Oncol. 1998;34(6):558–66.

77. Wei W, Deng X, Cai D, Ji Z, Wang C, Li J et al. Decreased tobacco-specific nitrosamines by microbial treatment with *Bacillus amyloliquefaciens* DA9 during the air-curing process of Burley tobacco. Agric Food Chem. 2014;62:12701–6.

78. Saleem S, Naz A, Safique M, Jabeen N, Ahsan SW. Fungal contamination in smokeless tobacco products traditionally consumed in Pakistan. J Pak Med Assoc. 2018;68(10):1471–77.

79. How long is the shelf life of snus. Stockholm: Swedish Match; 2018 (https://www.swedish-match.ch/en/what-is-snus/qa/shelf-life-of-snus/, accessed 16 July 2019).

80. Brenik W, Rudhard H. Method of endothermic fermentation of tobacco. US Patent 04343318. Washington (DC): Patent Office; 1982 (https://pubchem.ncbi.nlm.nih.gov/patent/US4343318#section=Patent-Submission-Date, accessed 16 July 2019).

81. Chandrasekaran S, Ramanathan S, Basak T. Microwave food processing—a review. Food Res Intl. 2013;52:243–61 (https://doi.org/10.1016/j.foodres.2013.02.033, accessed 16 July 2019).

82. Industrial Microwave Systems LLC [website]. New Orleans (LA): Industrial Microwave Systems LLC; 2019 (http://www.industrialmicrowave.com, accessed 16 July 2019).

83. Brinley TA, Dock CN, Truong VD, Coronel P, Kumar P, Simunovic J et al. Feasibility of utilizing bioindicators for testing microbial inactivation in sweet potato purees processed with a continuous-flow microwave system. J Food Sci. 2007;72(5):235–42.

84. David JRD, Graves RH, Szemplenski T. Handbook of aseptic processing and packaging, 2nd edition. Boca Raton (FL): CRC Press; 2013.

85. Parrott DL. Microwave technology sterilizes sweet potato puree. Food Technol. 2010:66–70 (https://ucanr.edu/datastoreFiles/608-672.pdf, accessed 20 July 2019).

86. Lee S, Kim S, Jeong S, Park J. Effect of far-infrared irradiation on catechins and nitrite scavenging activity of green tea. J Agric Food Chem. 2006;54(2):399–403.

87. Rundlöf T, Olsson E, Wiernik A, Back S, Aune M, Johansson L et al. Potential nitrite scavengers as inhibitors of the formation of N-nitrosamines in solution and tobacco matrix systems. J Agric Food Chem. 2000;48(9):4381–8.

88. Cockrell WT, Roberts JS, Kane BE, Fulghum S. Microbiology of oral smokeless tobacco products. Tob Int. 1989;191:55–7.

89. Harwani D. The great plate count anomaly and the unculturable bacteria. Int J Sci Res. 2012;2(9):350–1.

90. Technical bulletin: BacTiter-Glo™ microbial cell viability assay (document TB337). Madison (WI): Promega Corporation; 2019 (https://www.promega.com/, accessed 20 July 2019).

91. Law AD, Fisher C, Jack A, Moe LA. Tobacco, microbes, and carcinogens: correlation between tobacco cure conditions, tobacco-specific nitrosamine content, and cured leaf microbial community. Microb Ecol. 2016;72:1–10.

92. Omidbakhsh N, Ahmadpour F, Kenny N. How reliable are ATP bioluminescence meters in assessing decontamination of environmental surfaces in healthcare settings? PLoS One. 2014;9(6):e99951.

93. Performance evaluation of various ATP detecting units (Food Science Center report RPN 13922). Chicago (IL): Silliker Inc; 2010.

94. Sigma-Aldrich [website] (https://www.sigmaaldrich.com, accessed 16 July 2019).

95. Stanfill SB, Jia LT, Watson CH, Ashley DL. Rapid and chemically-selective quantification of nicotine in smokeless tobacco products using gas chromatography/mass spectrometry, J Chrom Sci. 2009;47(10):902–9.

96. Lisko JG, Stanfill SB, Watson CH. Quantitation of ten flavor compounds in unburned tobacco products. Anal Methods. 2014;6(13):4698–704. doi: 10.1039/C4AY00271G.

97. Jain V, Garg A, Parascandola M, Chaturvedi P, Khariwala SS, Stepanov I. Analysis of alkaloids in areca nut-containing products by liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 2017;65, 1977–1983 (https://doi.org/10.1021/acs.jafc.6b05140, accessed 20 July 2019).

98. Stepanov I, Hecht SS, Ramakrishnan S, Gupta PC. Tobacco-specific nitrosamines in smokeless tobacco products marketed in India. Carcinogenesis. 2015;116:16–9 (https://doi.org/10.1002/ijc.20966, accessed 16 July 2019).

99. Dalipi R, Margui E, Borgese L, Depero E. Multi-element analysis of vegetal foodstuff by means of low power total reflection X-ray fluorescence (TXRF) spectrometry. Food Chem. 2017; 218:348–55.

100. Directive 2001/37/EC of the European Parliament and of the Council (L194/26–L194/34). Brussels: European Commission; 2001 (https://ec.europa.eu/health/sites/health/files/tobacco/docs/dir200137ec_tobaccoproducts_en.pdf, accessed 22 July 2019).

101. Family Smoking Prevention and Tobacco Control Act 2009. Washington (DC): United States Congress (Public Law 111-31 111th Congress) (https://www.gpo.gov/fdsys/pkg/PLAW-111publ31/pdf/PLAW-111publ31.pdf, accessed 16 July 2019).

102. Vidhubala E, Pisinger C, Basumallik B, Prabhakar DS. The ban on smokeless tobacco products is systematically violated in Chennai, India. Indian J Cancer. 2016;53:325–30.

103. Arora M, Madhu R. Banning smokeless tobacco in India: policy analysis. Indian J. Cancer. 2012;49(4):336–41.

104. GothiaTek limits for undesired components. Stockholm: Swedish Match; 2017 (https://www.swedishmatch.com/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed 16 July 2019).

105. Gray N, Hecht S. Smokeless tobacco – proposals for regulation. Lancet. 2010;375:1589–91

10. Waterpipe tobacco smoking: prevalence, health effects and interventions to reduce use

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Contents

10.1 Introduction

At the seventh session of the COP to the WHO FCTC, document FCTC/COP/7/10 presented policy options and best practice for the control of waterpipe tobacco product use in relation to the WHO FCTC *(1)*. This section builds on the evidence presented in document FCTC/COP/7/10 in preparation for the eighth session of the COP. The purpose is to provide a comprehensive analysis of waterpipe tobacco control and to outline challenges and recommendations to improve the prevention and control of waterpipe tobacco use. The objectives are to analyse studies on waterpipe tobacco in order to:

- summarize regional and global patterns of waterpipe tobacco use, including changes in prevalence;
- evaluate the acute and chronic health effects of waterpipe tobacco use;
- describe cultural practices in relation to initiation and maintenance of use;
- explain the influence of flavourings on initiation, maintenance of use and increasing use;
- explore dependence liability with low-nicotine products;
- evaluate the evidence base for culturally relevant, waterpipe-specific interventions to prevent uptake and promote cessation;
- outline effective policies based on conceptual frameworks; and
- make recommendations for research and regulation.

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This section is limited to waterpipe tobacco and its accessories. Electronic nicotine devices with synonyms of "waterpipe" (e.g. shisha or hookah pens, electronic shisha or hookah) are not addressed. We focus on the most recent literature to minimize overlap with document FCTC/COP/7/10 and the WHO advisory note on waterpipe tobacco smoking *(2)*.

10.2 Prevalence, health effects and effective interventions to reduce use

The literature on waterpipe tobacco is relatively limited but is growing exponentially. In 2016, the number of citations in five clinical databases (Medline, Embase, Web of Science, PsychInfo, Global Health) that contained synonyms of "waterpipe" in the title or abstract was more than 10 times the number in 2006 (285 versus 25) (Fig. 10.1). Academia is continuously providing strengthened evidence for the prevention and control of waterpipe tobacco use.

Fig. 10.1. Numbers of citations in five clinical databases with synonyms of "waterpipe" in the titles or abstracts, 1990–2017
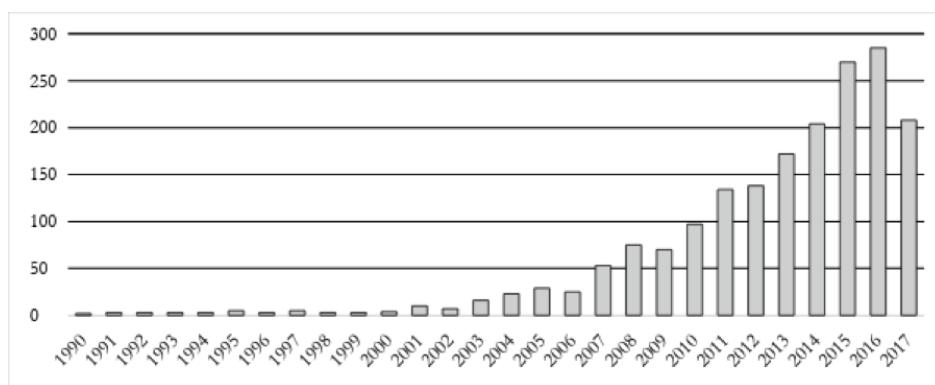
Source: databases – Embase, Medline, Global Health, PsychInfo, Web of Science; duplicate entries removed; 1 January–27 August only, in 2017.

10.2.1 Regional and global patterns of waterpipe tobacco use

Document FCTC/COP/7/10 noted that estimates of prevalence were available for some countries in all WHO regions *(1)*. Generally, studies reported high prevalence among children aged 13–15 years and university students, although these population groups were surveyed in only a few countries, and many of the studies were not nationally representative.

To the best of our knowledge, 131 studies have reported jurisdictionally representative estimates of the prevalence of waterpipe tobacco use in 84 countries *(3)*. About one third of the estimates derive from three international surveys: the Global Adult Tobacco Survey, the Global Youth Tobacco Survey and the Special

Eurobarometer Survey. Fig. 10.2 shows combined data from the latest wave of the Global Adult Tobacco Survey and Special Eurobarometer Survey (wave 87.1, 2017) *(4, 5)*, both among adults, and also the results of other studies in which similar methods were used *(6–9)*. The figure reveals two important findings: an increasing prevalence in the WHO European Region and an absence of studies with comparable survey tools in the WHO Eastern Mediterranean Region, where waterpipe tobacco use is the highest globally.

Fig. 10.2. Regular or occasional waterpipe tobacco use among adults
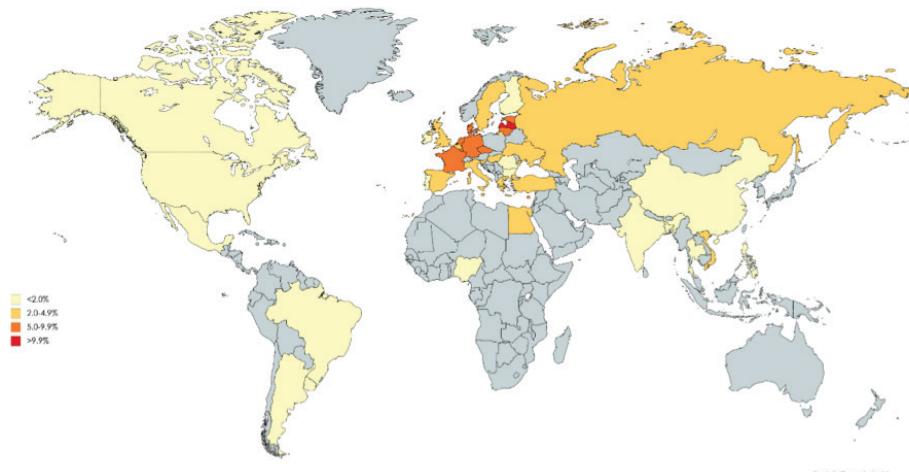
Source: references *4–9*.

The lack of data on the prevalence among adults in the WHO Eastern Mediterranean Region is in contrast to its involvement in the Global Youth Tobacco Survey. Fig. 10.3 shows data from the latest waves of the Global Youth Tobacco Survey *(5)* and other methodologically similar national school-based surveys *(10–13)*. The figure shows an estimated prevalence of 5.0–14.9% in many countries in all WHO regions, which is a concern and warrants further investigation and intervention. The highest estimated prevalence is in the Eastern Mediterranean Region (Lebanon, 37.0%; West Bank, 35.2%) and in Cyprus (33.2%) (Fig. 10.4).

Fig. 10.3. Use of waterpipe tobacco in the past 30 days by children aged 13–15 years
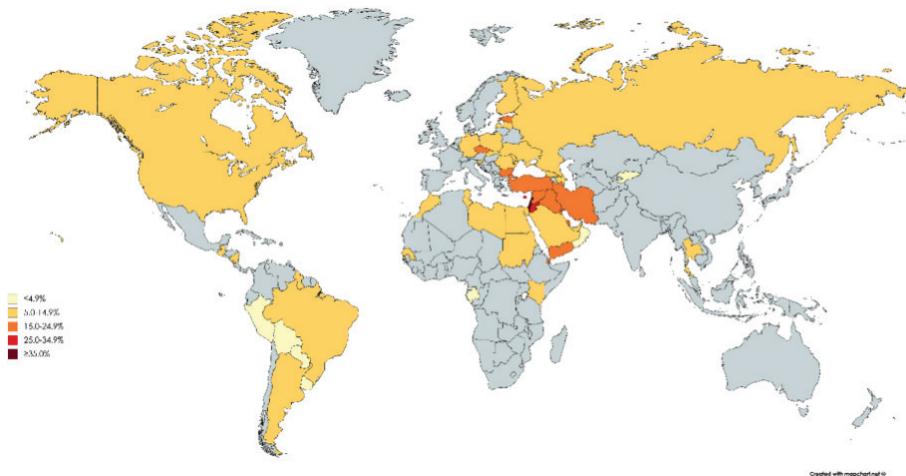
Fig. 10.4. Use of waterpipe tobacco in the past 30 days by young people, Middle East
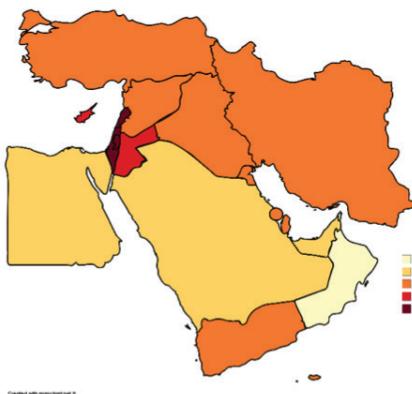
Source: references *5–10*.

Few studies have reported trends in waterpipe use. In 28 European Union Member States that were surveyed in 2009 and again in 2017, the mean prevalence of regular waterpipe tobacco use among adults increased by 11.2% *(4)*.

Waterpipe tobacco use appears to be increasingly popular among children aged 13–15 years, especially in the WHO Eastern Mediterranean Region (Fig. 10.4). In the USA, one national survey reported no or very little change in the prevalence of use in the past 30 days among young adults aged 18–24 years, which remained at 2–3% between 2011 and 2016 *(14)*. In contrast, a number of

other surveys conducted between 2008 and 2015 among young people in the USA, including the National Youth Tobacco Survey, showed absolute increases in waterpipe use in the past 30 days of 0.3–1.0% each year *(15–22)*. Similar increases were seen in repeated national school surveys in Canada and Lebanon *(23–26)*, while in Jordan much larger absolute increases were seen, of about 2.9% per year between 2008 and 2011 *(27)*. In the study in Jordan, increased use over time was associated with higher maternal education, frequent physical activity, ever cigarette use and waterpipe use by peers *(28)*. In Turkey, the Global Adult Tobacco Survey indicated a decrease in waterpipe use, from 2.3% in 2008 to 0.8% in 2010 *(29)*.

While waterpipe tobacco use is best characterized as intermittent, its interactions with other tobacco products are a potential cause for concern. In a longitudinal study of schoolchildren in Jordan, the risk of cigarette initiation was significantly higher among waterpipe tobacco smokers than those who had never smoked *(30)*. A similar conclusion was made in a study of adolescents in the USA, which was of the same methodological design but additionally controlled for baseline propensity to smoke *(31)*. In a study of university students in North Carolina (USA), those whose first tobacco product was waterpipe tobacco were more likely to be dual or multiple tobacco users at the time of the survey *(32)*.

In contrast to cigarette use, there is no clear pattern of increased waterpipe use with lower socioeconomic status. Nevertheless, inequalities by socioeconomic status may develop as both the public perception of waterpipe tobacco and the regulatory environment shift.

10.2.2 Acute and chronic health effects

Waterpipe users are exposed to toxicants from both the tobacco and the charcoal used to heat it. Toxicant yield studies have consistently found substantial levels of tar, nicotine, CO and cancer-causing chemicals in waterpipe tobacco smoke *(33)*. A population-level modelling study of exposure to cigarettes and waterpipe toxicants among 13–15-year-olds in the WHO Eastern Mediterranean Region *(34)* included estimates of the population intake of tobacco toxicants while factoring in frequencies of use and waterpipe sharing. It showed that waterpipe tobacco users were exposed to about 70% of all tobacco-derived CO and benzene, while cigarette users were exposed to only 30% *(34)*. The high levels of CO and benzene probably come from the charcoal used to heat the tobacco.

In the acute phase, the absorbed components of waterpipe tobacco and charcoal combustion cause adverse cardiovascular and respiratory changes. Increased blood pressure and heart rate *(35, 36)* are expected, given the considerable but variable nicotine content of waterpipe tobacco *(37)*. Reduced lung function is also commonly reported *(38)*. Particularly with waterpipe tobacco, CO levels rise sharply secondary to charcoal combustion, and reported

cases of CO poisoning among waterpipe tobacco users are widespread. It should be noted that non-tobacco, "herbal" waterpipe products are also heated with charcoal, resulting in a toxicological profile similar to that tobacco waterpipe products except for the absence of nicotine *(39, 40)*.

Growing evidence shows the risk of second-hand waterpipe tobacco smoke, particularly in waterpipe cafes. One pre–post study of 15 people passively exposed to waterpipe tobacco smoke showed an average increase in carboxyhaemoglobin from 0.8% (± 0.2) to 1.2% (± 0.8) after they had sat next to four to five active smokers for 30 min *(41)*. Another showed that the level of acrolein, a harmful respiratory toxicant, increased in nonsmokers in waterpipe cafes or at waterpipe smoking events in homes *(42)*. The effects of second-hand waterpipe tobacco smoke on other cardiorespiratory and toxicant parameters are less certain *(41, 43, 44)*. In an analysis of air quality, non-smoking rooms in waterpipe cafes had > 10 times the concentration of fine particulate matter as smoke-free rooms in other venues *(45)*. Assessments of poor air quality inside waterpipe cafes have been replicated in studies in the United Kingdom *(46)*.

Emerging evidence suggests that waterpipe tobacco has harmful long-term effects on health, similar to those of other forms of smoked tobacco. Table 10.1 summarizes the findings of several published systematic reviews *(36, 47–50)* and a further nine studies published after the reviews *(51–59)*, thus providing the latest evidence of long-term harm. The table shows that waterpipe tobacco use is associated with respiratory and cardiovascular disease, five types of cancer, adverse complications in pregnancy (low birth weight and intrauterine growth restriction) and a number of other diseases. Second-hand smoke from waterpipe tobacco use has been shown to induce wheezing in children *(60, 61)*. A further two cohort studies showed that waterpipe tobacco use was associated with increased overall mortality *(56, 62)*; one of these also showed an increase in mortality from cancer *(56)*. It is nevertheless difficult to draw clear conclusions from these studies because of the common practice of dual use of waterpipes and cigarettes. Many systematic reviews on this topic therefore rate the risk of bias as high. Consequently, the quality of the epidemiological studies on health and waterpipe tobacco use remains low, and further high-quality research, especially with enough statistical power to determine the relation between waterpipe tobacco smoking and health outcomes among never-cigarette-users, should be conducted to help to confirm these associations.

Table 10.1. Mortality and diseases associated with waterpipe tobacco use

Outcome	No. of studies	Odds ratio (95% CI)
Mortality	--	--
Overall	2	1.25 (1.03, 1.51)[a]
Cardiovascular disease	1	0.94 (0.43, 2.07)
Cancer	1	2.82 (1.30, 6.11)[a]
Cardiovascular disease	--	--
Coronary artery disease	3	1.47 (1.06, 2.04)[a]
Hypertension	1	1.95 (1.54, 2.48)[a]
Respiratory disease	--	--
Chronic obstructive pulmonary disease	5	2.33 (1.96, 2.77)[a]
Bronchitis	2	3.85 (1.92, 7.72)[a]
Asthma in children (active exposure)	2	1.32 (1.20, 1.46)[a]
Wheeze in children (passive exposure)	2	1.61 (1.25, 2.07)[a]
Cancer		
Bladder	6	1.28 (1.10, 1.48)[a]
Colorectal	3	1.20 (0.79, 1.82)
Gastric	4	2.35 (1.47, 3.76)[a]
Head and neck	7	3.00 (2.39, 3.76)[a]
Liver	1	1.13 (0.62, 2.78)
Lung	6	3.96 (2.96, 5.30)[a]
Oesophageal	5	2.61 (2.12, 3.26)[a]
Pancreatic	1	1.60 (0.91, 2.82)
Prostate	1	7.00 (0.88, 55.66)
Pregnancy-related diseases		
Low birthweight	4	1.54 (1.16, 2.04)[a]
Intrauterine growth restriction	1	3.50 (1.10, 12.60)[a]
Other diseases		
Diabetes	1	0.52 (0.27, 1.00)
Gastroesophageal reflux disease	2	1.31 (1.13, 1.53)[a]
Hepatitis C	3	0.98 (0.76, 1.25)
Infertility	1	2.50 (1.00, 6.30)[a]
Mental health	2	1.33 (1.25, 1.42)[a]
Multiple sclerosis	1	1.77 (1.36, 2.31)[a]
Periodontal disease	3	3.65 (2.22, 6.01)[a]

[a] Statistically significant positive association between waterpipe tobacco use and this disease in a random effects meta-analysis.

10.2.3 Cultural practices and initiation and maintenance of use

Cultural practices of waterpipe smoking are broadly embedded within a strong network of social acceptance, at the levels of family, peers and communities. Users and non-users alike have a positive attitude towards waterpipe tobacco use, which stems from the social environment in which it is consumed, the attraction towards the array of flavours and elaborately designed apparatuses and the portrayal of waterpipes in marketing media *(63–65)*.

One of several recommendations for research in the second WHO advisory note on waterpipe tobacco use *(2)* was on cultural practices and how they affect initiation and maintenance of use. For policy, the MPOWER package *(66)* is intended to assist countries in implementing effective interventions to reduce the demand for tobacco products. The six components of MPOWER are: Monitor tobacco use and prevention policies, Protect people from tobacco smoke, Offer help to quit tobacco use, Warn about the dangers of tobacco, Enforce bans on tobacco advertising, promotion and sponsorship, and Raise taxes on tobacco. Several Parties to the WHO FCTC, such as India, the Islamic Republic of Iran, Kenya, Malaysia, Pakistan, the United Arab Emirates and the United Republic of Tanzania, have gone beyond MPOWER and enforced (although, in some cases, then reversed), a total prohibition on public consumption of waterpipe tobacco, at either state or national levels. This policy may be based on social norms, as waterpipe tobacco use is embedded within the cultures of these Parties. The extent to which prohibitions on waterpipe tobacco use have been enforced and their impact on smoking prevalence remain uncertain, and this should be a key area of evaluation for these Parties. Evaluation within the International Tobacco Control Policy Evaluation Project is recommended *(67)*.

In Europe and north America, acculturation may determine waterpipe tobacco use. A study in the USA showed that immigrants from Arab countries who felt less acculturated into north American identity were more likely to use waterpipes *(68)*. Furthermore, qualitative research in Canada showed that immigrants from Arab countries saw themselves as expressing their cultural heritage by smoking waterpipes, and, in the United Kingdom, non-Arab users saw themselves as experiencing an alternative culture *(69)*.

Individual patterns of waterpipe use vary widely; the extent of within- and between-culture variation is, however, unclear. For example, in a study of waterpipe tobacco smoking behaviour in Lebanon, users on average drew an inhalation every 17 s *(70)*, whereas in a methodologically identical study in neighbouring Jordan, the frequency was 8 s and resulted in far greater exposure to toxicants. Gender differences in waterpipe smoking patterns were also noted in these studies, males tending to take larger puffs and spending more time smoking than females *(71)*.

Cultural differences are also apparent commercially. In commercial waterpipe premises, where about half of all waterpipe tobacco is consumed *(72, 73)*, café cultures range from a quiet coffee shop-like atmosphere to a busy, loud bar-like environment *(74)*. The café culture is likely to be associated with the patterns of use; for example, covert observation of commercial waterpipe premises in the USA indicated that some café owners insist that all clients entering the premises smoke a waterpipe *(74)*. In boisterous cafes that attract many customers, they are likely to associate waterpipe tobacco with socializing, whereas those that frequent quieter cafes may be more likely to indulge in solo, nicotine-driven use.

10.2.4   **Influence of flavourings**

Most of the waterpipe tobacco that is consumed is flavoured *(75, 76)*. Flavours are aggressively marketed by the waterpipe tobacco industry; in a review of 52 marketing material items from a waterpipe trade exhibition, flavours were among the most commonly elicited themes *(77)*. Alarmingly, the most common theme was that waterpipe tobacco was safe or safer than cigarettes *(77)*. Marketing practices of the waterpipe tobacco industry have indicated that tobacco and flavouring components may be sold separately to either evade flavouring bans or reduce the tobacco weight in order to lessen the excise tax *(77)*.

Waterpipe tobacco flavourings play a dominant role in perceptions of their safety and their attraction, in particular to young people. A review conducted in April 2016 of 10 qualitative studies on the role of flavours in waterpipe tobacco found that flavours were appealing or tasty *(78)*. In four studies (in Canada, the United Kingdom and the USA), young adults reported using waterpipes specifically for their flavour and because they did not wish to use other tobacco products *(78)*. There was also a perception that flavoured waterpipe tobacco was less harmful than cigarettes. In the same review, adults in Canada and Lebanon explained that young people used waterpipe tobacco because of their flavours, and another explained that flavours were responsible for initiation of waterpipe tobacco use by young adults *(78)*. In a large survey of adolescents in the USA, nearly 80% of users of waterpipes in the past 30 days reported that the flavours were a reason for use *(79)*.

These qualitative studies are supported by other research. In an experiment in the USA, 367 adult waterpipe smokers were asked to choose from menus with hypothetical combinations of different session types *(80)*. Flavoured waterpipe tobacco products were preferred significantly more than non-flavoured ones, and flavour more strongly influenced the decision to smoke waterpipes than price or nicotine content *(80)*. The association was stronger for females and for non-cigarette smokers. In another experiment in the USA, 36 adult waterpipe smokers completed two waterpipe sessions, one with their preferred flavour of waterpipe tobacco and another with a non-preferred flavour, in a randomized cross-over design *(81)*. Those who smoked their preferred flavour reported a better subjective smoking experience on a visual analogue scale, such as more interest in continued use, greater pleasure derived from smoking, increased liking and enjoyment and willingness to continue use *(81)*. The fact that the study was conducted on the premise that waterpipe tobacco users have preferred flavours is an important finding in itself. Together, these studies suggest that flavour restrictions or flavour bans might be effective in discouraging waterpipe tobacco use.

10.2.5 Dependence liability of low-nicotine products

The second WHO advisory note on waterpipe tobacco smoking clearly demarcated the role of nicotine dependence in waterpipe tobacco use *(2)*. Regular waterpipe users absorb enough nicotine to reach a dependence threshold and exhibit typical symptoms of dependence, such as craving, withdrawal symptoms and difficulty in quitting. Regular waterpipe use is, however, relatively uncommon outside the Eastern Mediterranean Region. In one study, it was estimated that users would have to smoke a waterpipe at least three times per week to become dependent *(82)*, whereas in the USA only 11.7% of young adults who had smoked a waterpipe in the past 30 days smoked at this level of frequency or more *(83)*.

There are two issues with regard to the dependence liability of low-nicotine waterpipe tobacco products. The first is that there is almost no regulation of waterpipe tobacco manufacture, which results in widely different nicotine levels among products. In an experiment in which 110 waterpipe smokers engaged in a 45-min session of smoking, brands labelled "0.05% nicotine" resulted in higher mean peak plasma levels of nicotine than brands labelled "0.5% nicotine" *(37)*. Given the lack of attention to policy on waterpipe tobacco, discussions about manufacture should prioritize flavour bans, as flavours have been shown to play a larger role in waterpipe purchase than low nicotine *(80)*.

The second issue is that waterpipe tobacco already contains less nicotine than cigarettes, and, while dependence on waterpipe tobacco is well documented, it remains relatively uncommon at a population level. When standardized to the nicotine yield per minute of smoking, waterpipe tobacco contains 2–13 ng less nicotine than cigarettes for every minute smoked (Table 10.2). Because the nicotine level is already low and dependence relatively uncommon on a population level, the justification for low-nicotine waterpipe tobacco is unclear.

Table 10.2. Nicotine yield per minute in waterpipe tobacco and cigarettes

Reference	Nicotine yield (ng/min)	No. of times less concentrated than in cigarettes[a]
34	0.11	1.9–5.6
70	0.08	2.7–8.0
84	0.05	4.4–13.2

[a] Based on data from Hoffman & Hoffman *(85)*, who estimated a nicotine yield of 1–3 ng/cigarette and an average time to consume a cigarette of 5 min.

10.2.6 Interventions

A review of interventions to reduce waterpipe tobacco use found only four individual-level and five group-level interventions *(86)*. A further search for this section found no further interventional studies, indicating lack of research in this area. Of five randomized controlled trials, only two showed statistically

significant higher quit rates in the intervention group *(87, 88)*; in one trial, a cigarette-specific intervention was tested in waterpipe tobacco users *(87)*. The details of these interventions are shown in Table 10.3. Non-randomized studies had mixed results for cessation and behavioural and knowledge outcomes and were generally of much lower methodological quality.

The behavioural interventions used in these randomized studies varied widely but were broadly based on the same principles as cigarette behavioural interventions. In the study by Asfar et al. *(89)*, the intervention consisted of three 45-min, individual and in-person counselling sessions by a trained physician and five 10-min phone calls before and after the proposed quit date. Dogar et al. *(87)* provided two structured behavioural sessions, the first lasting 30 min and the second 10 min, based on the WHO "5 As" approach, and used behavioural change techniques considered to be effective for cigarette smoking cessation. Lipkus et al. *(90)* showed participants 20 slides giving factual information about waterpipe tobacco use, including its effects on health. Mohlman et al. *(88)* delivered health promotion in villages over 12 months, including deglamourizing tobacco use in primary schools and describing its health hazards in primary schools, mosques and homes; education was also given in handling peer pressure to smoke in preparatory and secondary schools. Nakkash et al. *(91)* delivered 10 sessions to students, four of which were on knowledge and six on skill-building (e.g. media literacy, decision-making and refusal skills).

Table 10.3. Randomized interventions for waterpipe tobacco cessation

Reference	Study design	Period of study	Sample size, sex and mean age	Country	Intervention	Control	Outcome	Biochemical verification	Effect size, AOR (95% CI)
89	Two-arm, parallel trial	2007–08	N=50, 94% male, 30 years	Syrian Arab Republic	Behavioural	Usual care	Prolonged abstinence	Yes	1.46 (0.69, 3.09)
89	Two-arm, parallel trial	2007–08	N=50, 94% male, 30 years	Syrian Arab Republic	Behavioural	Usual care	Seven-day point prevalence of abstinence	Yes	1.34 (0.57, 3.35)
89	Two-arm, parallel trial	2007–08	N=50, 94% male, 30 years	Syrian Arab Republic	Behavioural	Usual care	Continuous abstinence	Yes	1.07 (0.27, 4.42)
87	Three-arm cluster trial	2011–12	N=1955, 79% male, 52 years	Pakistan	Behavioural	Usual care	Continuous abstinence	Yes	2.20 (1.30, 3.80)
87	Three-arm cluster trial	2011–12	N=1955, 79% male, 52 years	Pakistan	Behavioural and bupropion	Usual care	Continuous abstinence	Yes	2.50 (1.30, 4.70)
90	Two-arm trial	2009–10	N=91, 76% male, 20 years	USA	Behavioural (health education)	Behavioural (non-health education)	No waterpipe use in past 30 days	No	1.46 (0.81, 2.62)
88	Two-arm cluster trial	2004–06	N=7657, 45% male, 36 years	Egypt	Behavioural	No intervention	No current waterpipe use	No	3.25 (1.39, 8.89)[a]
91	Two-arm cluster trial	2011–12	N=1857, 6th–8th grade students	Lebanon and Qatar	Behavioural	No intervention	No waterpipe use in past 30 days	No	1.50 (0.89, 2.53)

AOR: adjusted odds ratio; CI: confidence interval. [a] Analysis restricted to males.

10.3 Future research

Four areas of research on waterpipe tobacco should be addressed, according to the findings in this section.

1. **Surveillance of waterpipe tobacco use should continue:** nonetheless, researchers should consider using improved, standardized tools to measure prevalence, so that estimates can be compared between countries. Simple estimates of prevalence among adults in the Eastern Mediterranean Region and young people in central Europe and in the South-East Asia Region are a priority. For researchers, a toolbox of survey items is available that covers use patterns, dependence, exposure and policy *(92)*. An often overlooked but important detail is the type of waterpipe tobacco consumed. This report summarizes studies that mainly focused on *mo'assel* tobacco, a flavoured tobacco type commonly marketed in commercial venues. Other waterpipe tobacco types, often consumed in the WHO Eastern Mediterranean and South-East Asia regions, are associated with distinct patterns of use and sociodemographic correlates *(93)*.

2. **High-quality epidemiological studies on the long-term health effects of waterpipe tobacco should be conducted:** in particular, not much is known about the long-term health effects of intermittent or infrequent use or the cumulative harm of dual use of waterpipe tobacco and cigarettes *(34)*. Furthermore, understanding of the harm due to the potential of waterpipe tobacco to act as a gateway to other tobacco use will strongly inform the policy debate and action. Modelling studies could complement traditional epidemiological approaches in filling this research gap. While evidence for the long-term health effects of waterpipe tobacco use is being collected, better characterization of the product, by charcoal and tobacco type, will be an important step to better understanding its potential harm.

3. **As Parties to the WHO FCTC continue to develop and implement policies on waterpipe tobacco, the policies should be formally evaluated:** it is important to evaluate the impact of policies on the use of and attitudes towards waterpipe tobacco and any unintended consequences and to monitor industry compliance. Sharing of experience will guide efforts to reduce the health effects of waterpipe tobacco use. Key considerations are whether enforcement in waterpipe cafes will be offset by increased use at home and whether policies to reduce waterpipe tobacco use will increase use of other tobacco products.

4. **Evidence for the effectiveness of individual and group interventions is needed to support waterpipe tobacco cessation:** while research is

lacking on effective individual and group interventions, this should not delay implementation and evaluation of population-level interventions that have been shown to work. The main gaps in individual and group interventions include the effectiveness of pharmacological cessation aids *(94)* and the extent to which interventions for cigarette users can be transposed directly for waterpipe tobacco users. Researchers who are considering conducting individual or group interventions should consult an inventory of behaviour change techniques for waterpipe tobacco use *(95)*. Population-level approaches described in the MPOWER framework should be implemented immediately.

10.4 Policy recommendations

An extensive list of policy recommendations aligned with the WHO FCTC is given in documents FCTC/COP/7/10 *(1)* and FCTC/COP7(4) *(96)*. We support these recommendations as important measures for the prevention and control of waterpipe tobacco use. Below, we provide an abridged list of policy options that build on the success of the MPOWER framework and could be considered the most pertinent. This is prudent, given that countries that have not implemented MPOWER fully have a higher prevalence of waterpipe tobacco use *(97)*. While MPOWER is applicable to all tobacco products, we call for renewed implementation explicitly incorporating the particularities of waterpipe tobacco smoking, such as the predominant use of flavours, lengthy, stationary tobacco use and regular use at commercial venues. We call this revision MPOWER-W.

In implementing the policies listed below, it should be remembered that waterpipe tobacco substitutes (e.g. "herbal" substances and steam stones) still require charcoal as the heating source *(76)* and should be classified as tobacco products because of the known chemical composition of the smoke they produce, the fact that they are marketed with waterpipe tobacco products and the fact that products claimed to be tobacco-free may nevertheless contain tobacco *(39, 98)*. Similar calls have been made for products that are used as substitutes for or mimic smokeless tobacco *(99)*.

1. **Monitor tobacco use and prevention policies (Article 20 of the WHO FCTC).**

   Surveillance of waterpipe tobacco use has improved substantially during the past decade, but many countries still do not have mechanisms to estimate the prevalence of waterpipe tobacco use, exposure to second-hand smoke and waterpipe tobacco industry activities. This section shows that comparable prevalence estimates for adults are not available for the Eastern Mediterranean Region, and prevalence esti-

mates for young people are not available for central Europe and the South-East Asia Region. High-quality surveillance is essential for the prevention and control of waterpipe tobacco use. Prevalence should be measured with standardized tools *(92)*, perhaps supplemented by routine administrative data or directories of waterpipe cafes *(100)*.

2. **Protect people from exposure to second-hand tobacco smoke (Article 8 of the WHO FCTC).**

Commercial establishments that allow customers to smoke waterpipe tobacco on their premises should be included in comprehensive smoke-free laws. There is evidence of the harm of second-hand smoke from waterpipe smoking *(41, 43, 44)*. Studies of cigarette smoking in commercial premises have shown that comprehensive smoke-free laws reduce the harm of second-hand smoke, help smokers to quit and reduce smoking among young people *(101)*. Waterpipe tobacco sessions can be lengthy (e.g. up to several hours), and movement of smokers outdoors increases their public visibility and may be noisy and create a nuisance for nearby residents *(98)*. Turkey and several countries in the Eastern Mediterranean Region have instituted zoning laws to prohibit waterpipe cafes within a certain distance of residential areas and educational establishments *(102)*. Zoning laws should be co-implemented with smoke-free laws to maximize protection of the public.

3. **Offer help to quit tobacco use (Article 14 of the WHO FCTC).**

Regular waterpipe tobacco smokers are nicotine dependent. Therefore, Parties to the WHO FCTC should integrate waterpipe tobacco cessation into traditional smoking cessation services. Although the literature on effective individual interventions is sparse, they show promising results and should be scaled up and evaluated for exchange among countries. It is also important to address the strong social components of waterpipe tobacco use.

4. **Warn about the dangers of tobacco (Articles 11 and 12 of the WHO FCTC).**

Health warnings on waterpipe tobacco packs should, as for cigarettes, cover at least 50% of the principal display areas and include graphic imagery, which is more effective in reaching people with low literacy. Health warnings should also be applied to waterpipe accessories, such as the device and charcoal. Evidence strongly suggests that waterpipe tobacco use is harmful to health *(36, 47–50)*, but the industry complies poorly with WHO FCTC recommendations for health warnings *(103, 104)*. As waterpipe devices are elaborate, well decorated and a

component of the positive affect of waterpipe tobacco smoking, the device should be regulated to a standardized size and pattern to reduce its attractiveness.

5. **Enforce bans on tobacco advertising, promotion and sponsorship (Article 13 of the WHO FCTC).**

Complete bans on waterpipe tobacco advertising, promotion and sponsorship, which are commonly seen in and around waterpipe cafes and on social media, should be enforced. In contrast to the products of transnational tobacco companies, most waterpipe tobacco is advertised and promoted by retailers or at waterpipe cafes *(77, 105, 106)*. Student offers, discounts and the display of waterpipe devices in shop windows are common approaches to encouraging waterpipe tobacco use.

6. **Raise taxes on tobacco (Article 6 of the WHO FCTC).**

Taxes on waterpipe tobacco should be raised, at least to be in line with the tax rate on cigarettes. Tobacco taxation is one of the most effective policies in tobacco control, but waterpipe tobacco is taxed at a much lower rate than cigarettes. A national survey in Lebanon indicated that a 10% increase in waterpipe tobacco taxes would result in a 14% decrease in consumption in homes *(107)*. While the impact of such a tax on use in waterpipe cafes is unknown, policy-makers should be mindful that, when waterpipe tobacco is used by a group, the effect of taxation is weakened, as the cost per user is lower. Therefore, larger increases in taxes on waterpipe tobacco will be required to achieve a similar effect to cigarette taxation.

10.4.1 Policies relevant to waterpipe tobacco use

The six policies listed above are the backbone of tobacco control policy globally as part of the comprehensive policy recommendations in the WHO FCTC; however, two further recommendations do not fall within the MPOWER framework. Introduction of MPOWER-W will guide policies to address waterpipe-specific priorities.

First, as most waterpipe tobacco is flavoured, a ban on flavours is likely to have a profound effect on the waterpipe tobacco industry *(108)*. It would reduce the attractiveness of waterpipe tobacco, promote cessation and prevent uptake, especially by young people. Policy-makers should be wary, however, that a flavour ban could be circumvented by separate sale of flavours in bottles, to be added by the user to an unflavoured tobacco mix. Such bottles are likely to be specific to waterpipe tobacco use, and any shift to this approach by the industry could be

prevented by tightly regulated, enforced policy. Flavours can also be added to charcoal and water rather than to the tobacco, and parts of the apparatus can be replaced with fruit-based accessories (such as cored fruit in place of the head, where the tobacco is held). Manipulations of tobacco flavouring in retail settings should be prohibited by policy-makers to ensure that any flavour ban is upheld throughout the supply chain.

Secondly, as many of these policies target customers of waterpipe cafes, licensing might be the most effective method for reducing the burden of enforcement on local governments and ensuring that waterpipe retailers understand their legislative responsibilities *(98)*. In addition to the above policies, a licensing framework could incorporate wider protection not directly related to tobacco, such as health and safety requirements, quality control measures to promote good sanitation (e.g. disposable hoses and mouthpieces and device cleaning procedures) and age restrictions on entry. By including tobacco control policies within this licensing framework, enforcement can be cost-effective. Furthermore, depending on the country, the terms of waterpipe tobacco licenses may be under the jurisdiction of local governments, which would facilitate implementation of local policies such as flavour bans and the categorization of non-tobacco ("herbal") products as tobacco products, without the need for changes to national licensing frameworks.

10.5   Conclusions

Research on waterpipe tobacco use is increasing exponentially, providing evidence that use of this harmful tobacco product is prevalent in many countries on all continents, particularly among children aged 13–15 years. While waterpipe tobacco users may become dependent on nicotine, most are not dependent and may therefore be more susceptible to population-level policies than individual or group interventions. The prevalence of waterpipe tobacco smoking continues to rise in many countries, mainly because of lack of regulation of the industry. Public health concern about waterpipe tobacco centres on the fact that it is predominantly a flavoured product and that flavour encourages its use and may be more important than price in the decision to smoke it. A ban on flavoured waterpipe tobacco could reduce the appeal of these products, thereby reducing demand and ultimately improving public health. The ban should, however, be complementary to tobacco control policies such as higher taxation, comprehensive smoke-free laws and public education to remove misperceptions of harm. Renewed implementation of MPOWER with incorporation of the particularities of waterpipe tobacco smoking could facilitate prevention and control of waterpipe tobacco use. In the meantime, the research priorities include continued, standardized surveillance, better-quality epidemiological studies of harm, evaluation of policies and the design of prevention and cessation interventions.

10.6   References

1.   Control and prevention of waterpipe tobacco products (FCTC/COP/7/10). Geneva: World Health Organization; 2016 (http://bit.ly/2xGlxSx, accessed 28 August 2017).

2.   Advisory note. Waterpipe tobacco smoking: health effects, research needs and recommended actions for regulators. 2nd edition. Geneva: World Health Organization; 2015 (http://bit.ly/2i-7nAwr, accessed 10 September 2017).

3.   Jawad M, Charide R, Waziry R, Darzi A, Ballout RA, Akl EA. The prevalence and trends of waterpipe tobacco smoking: A systematic review. PLoS One. 2018;13(2):e0192191.

4.   Filippidis FT, Jawad M, Vardavas CI. Trends and correlates of waterpipe use in the European Union: analysis of selected Eurobarometer surveys (2009–2017). Nicotine Tob Res. 2017. doi: 10.1093/ntr/ntx255.

5.   Yang Y, Jawad M, Filippidis FT, Millett C. The prevalence, correlates and trends of waterpipe tobacco smoking: a secondary analysis of the Global Youth Tobacco Survey (under review).

6.   Agaku IT, King BA, Husten CG, Bunnell R, Ambrose BK, Hu SS et al. Tobacco product use among adults – United States, 2012–2013. Morb Mortal Wkly Rep. 2014;63(25):542–7.

7.   Ward KD, Ahn S, Mzayek F, Al Ali R, Rastam S, Asfar T et al. The relationship between waterpipe smoking and body weight: population-based findings from Syria. Nicotine Tob Res. 2015;17(1):34–40.

8.   Aden, B, Karrar S, Shafey O, Al Hosni F. Cigarette, water-pipe, and *medwakh* smoking prevalence among applicants to Abu Dhabi's pre-marital screening program, 2011. Int J Prev Med. 2013;4(11):1190–5.

9.   Baron-Epel O, Shalata W, Hovell MF. Waterpipe tobacco smoking in three Israeli adult populations. Israel Med Assoc J. 2015;17(5):282–7.

10.   Szklo AS, Autran Sampaio MM, Masson Fernandes E, de Almeida LM. Smoking of non-cigarette tobacco products by students in three Brazilian cities: Should we be worried? Cad Saúde Pública. 2011;27(11):2271–5.

11.   Minaker LM, Shuh A, Burkhalter RJ, Manske SR. Hookah use prevalence, predictors, and perceptions among Canadian youth: findings from the 2012/2013 Youth Smoking Survey. Cancer Causes Control. 2015;26(6):831–8.

12.   Kuntz B, Lampert T. Waterpipe (shisha) smoking among adolescents in Germany: results of the KiGGS study: first follow-up (KiGGS wave 1) [in German]. Bundesgesundheitsblatt Gesundheitsforsch Gesundheitsschutz. 2015;58(4–5):467–73.

13.   Singh T, Arrazola RA, Corey CG, Husten CG, Neff LJ, Homa DM et al. Tobacco use among middle and high school students – United States, 2011–2015. Morbid Mortal Wkly Rep. 2016;65(14):361–7.

14.   Cohn AM, Johnson AL, Rath JM, Villanti AC. Patterns of the co-use of alcohol, marijuana, and emerging tobacco products in a national sample of young adults. Am J Addict. 2016;25(8):634–40.

15.   Arrazola RA, Neff LJ, Kennedy SM, Holder-Hayes E, Jones CD. Tobacco use among middle and high school students – United States, 2013. Morb Mortal Wkly Rep. 2014;63(45):1021–6.

16.   Arrazola RA, Singh T, Corey CG, Husten CG, Neff LJ, Apelberg BJ et al. Tobacco use among middle and high school students – United States, 2011–2014. Morb Mortal Wkly Rep. 2015;64(14):381–5.

17.   Singh T, Arrazola RA, Corey CG, Husten CG, Neff LJ, Homa DM et al. Tobacco use among middle and high school students – United States, 2011–2015. Morb Mortal Wkly Rep. 2016;65(14):361–7.

18.   Centers for Disease Control and Prevention. Tobacco product use among middle and high school students –United States, 2011 and 2012. Morb Mortal Wkly Rep. 2013;62(45):893–7.

19.   Bover Manderski MT, Hrywna M, Delnevo CD. Hookah use among New Jersey youth: associations and changes over time. Am J Health Behav. 2012;36(5):693–9.

20. Haider MR, Salloum RG, Islam F, Ortiz KS, Kates FR, Maziak W. Factors associated with smoking frequency among current waterpipe smokers in the United States: findings from the National College Health Assessment II. Drug Alcohol Depend. 2015;153:359–63.

21. Primack BA, Fertman CI, Rice KR, Adachi-Mejia AM, Fine MJ. Waterpipe and cigarette smoking among college athletes in the United States. J Adolesc Health. 2010;46(1):45–51.

22. Sidani JE, Shensa A, Primack BA. Substance and hookah use and living arrangement among fraternity and sorority members at US colleges and universities. J Community Health. 2013;38(2):238–45.

23. Chan WC, Leatherdale ST, Burkhalter R, Ahmed R. Bidi and hookah use among Canadian youth: an examination of data from the 2006 Canadian Youth Smoking Survey. J Adolesc Health. 2011;49(1):102–4.

24. Minaker LM, Shuh A, Burkhalter RJ, Manske SR. Hookah use prevalence, predictors, and perceptions among Canadian youth: findings from the 2012/2013 Youth Smoking Survey. Cancer Causes Control. 2015;26(6):831–8.

25. Jawad M, Lee JT, Millett C. Waterpipe tobacco smoking prevalence and correlates in 25 eastern Mediterranean and eastern European countries: cross-sectional analysis of the Global Youth Tobacco Survey. Nicotine Tob Res. 2016;18(4):395–402.

26. Saade G, Warren CW, Jones NR, Asma S, Mokdad A. Linking Global Youth Tobacco Survey (GYTS) data to the WHO Framework Convention on Tobacco Control (FCTC): the case for Lebanon. Prev Med. 2008;47(Suppl 1):S15–9.

27. McKelvey KL, Attonito J, Madhivanan P, Jaber R, Yi Q, Mzayek F et al. Time trends of cigarette and waterpipe smoking among a cohort of school children in Irbid, Jordan, 2008–11. Eur J Public Health. 2013;23(5):862–7.

28. Jaber R, Madhivanan P, Khader Y, Mzayek F, Ward KD, Maziak W. Predictors of waterpipe smoking progression among youth in Irbid, Jordan: a longitudinal study (2008–2011). Drug Alcohol Depend. 2015.153:265–70.

29. Erdol C, Ergüder T, Morton J, Palipudi K, Gupta P, Asma S. Waterpipe tobacco smoking in Turkey: policy implications and trends from the Global Adult Tobacco Survey (GATS). Int J Environ Res Public Health. 2015;12(12):15559–66.

30. Jaber R, Madhivanan P, Veledar E, Khader Y, Mzayek F, Maziak W. Waterpipe a gateway to cigarette smoking initiation among adolescents in Irbid, Jordan: a longitudinal study. Int J Tuberc Lung Dis. 2015;19(4):481–7.

31. Soneji S, Sargent JD, Tanski SE, Primack BA. Associations between initial water pipe tobacco smoking and snus use and subsequent cigarette smoking: results from a longitudinal study of us adolescents and young adults. JAMA Pediatrics. 2015;169(2):129–36.

32. Sutfin EL, Sparks A, Pockey JR, Suerken CK, Reboussin BA, Wagoner KG et al. First tobacco product tried: associations with smoking status and demographics among college students. Addict Behav 2015;51:152–7.

33. Shihadeh A, Schubert J, Klaiany J, El Sabban M, Luch A, Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. Tob Control. 2015;24:i22–30.

34. Jawad M, Eissenberg T, Salman R, Alzoubi KH, Khabour OF, Karaoghlianian N et al. Real-time in situ puff topography and toxicant exposure among singleton waterpipe tobacco users in Jordan. Tob Control. 2018 [Epub ahead of print]

35. Haddad L, Kelly DL, Weglicki LS, Barnett TE, Ferrell AV, Ghadban R. A systematic review of effects of waterpipe smoking on cardiovascular and respiratory health outcomes. Tob Use Insights. 2016;9:13–28.

36. El-Zaatari ZM, Chami HA, Zaatari GS. Health effects associated with waterpipe smoking. Tob Control. 2015;24:i31–43.

37. Vansickel AR, Shihadeh A, Eissenberg T. Waterpipe tobacco products: nicotine labelling versus nicotine delivery. Tob Control. 2012;21(3):377–9.

38. Raad D, Gaddam S, Schunemann HJ, Irani J, Abou Jaoude P, Honeine R et al. Effects of water-pipe smoking on lung function: a systematic review and meta-analysis. Chest. 2011;139(4):764–74.

39. Hammal F, Chappell A, Wild TC, Kindzierski W, Shihadeh A, Vanderhoek A et al. "Herbal" but potentially hazardous: an analysis of the constituents and smoke emissions of tobacco-free waterpipe products and the air quality in the cafes where they are served. Tob Control. 2015;24(3):290–7.

40. Shihadeh A, Salman R, Jaroudi E, Saliba N, Sepetdjian E, Blank MD, Cobb CO et al. Does switching to a tobacco-free waterpipe product reduce toxicant intake? A crossover study comparing CO, NO, PAH, volatile aldehydes, "tar" and nicotine yields. Food Chem Toxicol. 2012;50(5):1494–8.

41. Bentur L, Hellou E, Goldbart A, Pillar G, Monovich E, Salameh M et al. Laboratory and clinical acute effects of active and passive indoor group water-pipe (narghile) smoking. Chest. 2014;145(4):803–9.

42. Kassem NOF, Kassem NO, Liles S, Zarth AT, Jackson SR, Daffa RM et al. Acrolein exposure in hookah smokers and non-smokers exposed to hookah tobacco secondhand smoke: implications for regulating hookah tobacco products. Nicotine Tob Res. 2018;20(4):492–501.

43. Azar RR, Frangieh AH, Mroué J, Bassila L, Kasty M, Hage G et al. Acute effects of waterpipe smoking on blood pressure and heart rate: a real-life trial. Inhal Toxicol. 2016;28(8):339–42.

44. Kassem NOF, Kassem NO, Liles S, Jackson SR, Chatfield DA, Jacob P 3rd et al. Urinary NNAL in hookah smokers and non-smokers after attending a hookah social event in a hookah lounge or a private home. Regul Toxicol Pharmacol. 2017;89:74–82.

45. Cobb CO, Vansickel AR, Blank MD, Jentink K, Travers MJ, Eissenberg T. Indoor air quality in Virginia waterpipe cafes. Tob Control. 2013;22(5):338–43.

46. Gurung G, Bradley J, Delgado-Saborit JM. Effects of shisha smoking on carbon monoxide and $PM_{2.5}$ concentrations in the indoor and outdoor microenvironment of shisha premises. Sci Total Environ. 2016;548–9:340–6.

47. Akl EA, Gaddam S, Gunukula SK, Honeine R, Jaoude PA, Irani J. The effects of waterpipe tobacco smoking on health outcomes: a systematic review. Int J Epidemiol. 2010;39(3):834–57.

48. Waziry R, Jawad M, Ballout RA, Al Akel M, Akl EA. The effects of waterpipe tobacco smoking on health outcomes: an updated systematic review and meta-analysis. Int J Epidemiol. 2017;46(1):32–43.

49. Awan KH, Siddiqi K, Patil S, Hussain QA. Assessing the effect of waterpipe smoking on cancer outcome – a systematic review of current evidence. Asian Pac J Cancer Prev. 2017;18(2):495–502.

50. Mamtani, R, Cheema S, Sheikh J, Al Mulla A, Lowenfels A, Maisonneuve P. Cancer risk in water-pipe smokers: a meta-analysis. Int J Public Health. 2017;62(1):73–83.

51. Nasrallah MP, Nakhoul NF, Nasreddine L, Mouneimne Y, Abiad MG, Ismaeel H et al. Prevalence of diabetes in greater Beirut area; worsening over time. Endocr Pract. 2017;23(9):1091–1100.

52. Lotfi MH, Keyghobadi N, Javahernia N, Bazm S, Tafti AD, Tezerjani HD. The role of adverse life style factors in the cause of colorectal carcinoma in the residents of Yazd, Iran. Zahedan J Res Med Sci. 2016;18(9):e8171.

53. Larsen K, Faulkner GEJ, Boak A, Hamilton HA, Mann RE, Irving HM, To T. Looking beyond cigarettes: Are Ontario adolescents with asthma less likely to smoke e-cigarettes, marijuana, waterpipes or tobacco cigarettes? Respir Med. 2016;120:10–15.

54. Lai HT, Koriyama C, Tokudome S, Tran HH, Tran LT, Nandakumar A et al. Waterpipe tobacco smoking and gastric cancer risk among Vietnamese men. PLoS One. 2016;11(11):e0165587.

55. Fedele DA, Barnett TE, Dekevich D, Gibson-Young LM, Martinasek M, Jagger MA. Prevalence of and beliefs about electronic cigarettes and hookah among high school students with asthma. Ann Epidemiol. 2016;26(12):865–9.

56. Etemadi A, Khademi H, Kamangar F, Freedman ND, Abnet CC, Brennan P et al. Hazards of ciga-rettes, smokeless tobacco and waterpipe in a Middle Eastern population: a cohort study of 50 000 individuals from Iran. Tob Control. 2017;26(6):674–82.

57. Khodamoradi Z, Gandomkar A, Poustchi H, Salehi A, Imanieh MH, Etemadi A et al. Prevalence and correlates of gastroesophageal reflux disease in southern Iran: Pars Cohort Study. Middle East J Dig Dis. 2017;9(3):129–38.

58. Bandiera FC, Loukas A, Wilkinson AV, Perry CL. Associations between tobacco and nicotine product use and depressive symptoms among college students in Texas. Addict Behav. 2016;63:19–22.

59. Abdollahpour I, Nedjat S, Sahraian MA, Mansournia MA, Otahal P, van der Mei I. Waterpipe smoking associated with multiple sclerosis: a population-based incident case–control study. Mult Scler. 2017;23(10):1328–35.

60. Tamim H, Akkary G, El-Zein A, El-Roueiheb Z, El-Chemaly S. Exposure of pre-school children to passive cigarette and narghile smoke in Beirut. Eur J Public Health. 2006;16(5):509–12.

61. Mohammad Y, Shaaban R, Hassan M, Yassine F, Mohammad S, Tessier JF et al. Respiratory ef-fects in children from passive smoking of cigarettes and narghile: ISAAC phase three in Syria. Int J Tuberc Lung Dis. 2014;18(11):1279–84.

62. Wu F, Chen Y, Parvez F, Segers S, Argos M, Islam T et al. A prospective study of tobacco smoking and mortality in Bangladesh. PLoS One. 2013;8(3):e58516.

63. Akl EA, Ward KD, Bteddini D, Khaliel R, Alexander AC, Lotfi T et al. The allure of the waterpipe: a narrative review of factors affecting the epidemic rise in waterpipe smoking among young persons globally. Tob Control. 2015;24(Suppl 1):i13–21.

64. Akl EA, Jawad M, Lam WY, Co CN, Obeid R, Irani J. Motives, beliefs and attitudes towards wa-terpipe tobacco smoking: a systematic review. Harm Reduction J. 2013;10(1):12.

65. Griffiths MA, Ford EW. Hookah smoking: behaviors and beliefs among young consumers in the United States. Soc Work Public Health. 2014;29(1):17–26.

66. MPOWER. Advancing the WHO Framework Convention on Tobacco Control (WHO FCTC). Ge-neva: World Health Organization; 2018 (http://www.who.int/cancer/prevention/tobacco_im-plementation/mpower/en/, accessed 1 November 2018).

67. International Tobacco Control Policy Evaluation Project. Waterloo, Ontario; 2017 (https://bit.ly/1pe5ELk, accessed 3 August 2018).

68. El Hajj DG, Cook PF, Magilvy K, Galbraith ME, Gilbert L, Corwin M. Tobacco use among Arab immigrants living in Colorado: prevalence and cultural predictors. J Transcult Nurs. 2017;28(2):179–86.

69. Roskin J, Aveyard P. Canadian and English students' beliefs about waterpipe smoking: a quali-tative study. BMC Public Health. 2009;9:10.

70. Katurji M, Daher N, Sheheitli H, Saleh R, Shihadeh A. Direct measurement of toxicants inhaled by water pipe users in the natural environment using a real-time in situ sampling technique. Inhal Toxicol. 2010;22(13):1101–9.

71. Alzoubi KH, Khabour OF, Azab M, Shqair DM, Shihadeh A, Primack B et al. CO exposure and puff topography are associated with Lebanese waterpipe dependence scale score. Nicotine Tob Res. 2013;15(10):1782–6.

72. Bejjani N, I Bcheraoui C, Adib SM. The social context of tobacco products use among adoles-cents in Lebanon (MedSPAD-Lebanon). J Epidemiol Global Health. 2012;2(1):15–22.

73. Alzyoud S, Weglicki LS, Kheirallah KA, Haddad L, Alhawamdeh KA. Waterpipe smoking among middle and high school Jordanian students: patterns and predictors. Int J Environ Res Public Health. 2013;10(12):7068–82.

74. Carroll MV, Chang J, Sidani JE, Barnett TE, Soule E, Balbach E et al. Reigniting tobacco ritual: waterpipe tobacco smoking establishment culture in the United States. Nicotine Tob Res.

2014;16(12):1549–58.

75. Joudrey PJ, Jasie KA, Pykalo L, Singer ST, Woodin MB, Sherman S. The operation, products and promotion of waterpipe businesses in New York City, Abu Dhabi and Dubai. East Mediterr Health J. 2016;22(4):237–43.

76. Amin TT, Amr MAM, Zaza BO, Suleman W. Harm perception, attitudes and predictors of water-pipe (shisha) smoking among secondary school adolescents in Al-Hassa, Saudi Arabia. Asian Pac J Cancer Prev. 2010;11(2):293–301.

77. Jawad M, Nakkash RT, Hawkins B, Akl EA. Waterpipe industry products and marketing strate-gies: analysis of an industry trade exhibition. Tob Control. 2015;24(e4):e275–9.

78. Kowitt SD, Meernik C, Baker HM, Osman A, Huang LL, Goldstein AO. Perceptions and experi-ences with flavored non-menthol tobacco products: a systematic review of qualitative studies. Int J Environ Res Public Health. 2017;14(4):338.

79. Ambrose BK, Day HA, Rostron B, Conway KP, Borek N, Hyland A et al. Flavored tobacco product use among US youth aged 12–17 years, 2013–2014. JAMA. 2015;314(17):1871–3.

80. Salloum RG, Maziak W, Hammond D, Nakkash R, Islam F, Cheng X et al. Eliciting preferences for waterpipe tobacco smoking using a discrete choice experiment: implications for product regulation. BMJ Open. 2015;5(9):e009497.

81. Leavens EL, Driskill LM, Molina N, Eissenberg T, Shihadeh A, Brett EI, Floyd E et al. Comparison of a preferred versus non-preferred waterpipe tobacco flavour: subjective experience, smoking behaviour and toxicant exposure. Tob Control. 2018;27(3):319–24.

82. Salameh P, Waked M, Aoun Z. Waterpipe smoking: construction and validation of the Lebanon Waterpipe Dependence Scale (LWDS-11). Nicotine Tob Res. 2008.10(1):149–58.

83. Haider MR, Salloum RG, Islam F, Ortiz KS, Kates FR, Maziak W. Factors associated with smoking frequency among current waterpipe smokers in the United States: findings from the National College Health Assessment II. Drug Alcohol Depend. 2015;153:359–63.

84. Shihadeh A. Investigation of mainstream smoke aerosol of the nargileh water pipe. Food Chem Toxicol. 2003;41(1):143–52.

85. Hoffmann D, Hoffmann I. Tobacco smoke components. Beitr Tabakforsch Int. 1998;18(1):49–52.

86. Jawad M, Jawad S, Waziry RK, Ballout RA, Akl EA. Interventions for waterpipe tobacco smoking prevention and cessation: a systematic review. Sci Rep. 2016;6:25872.

87. Dogar O, Jawad M, Shah SK, Newell JN, Kanaan M, Khan MA et al. Effect of cessation interven-tions on hookah smoking: post-hoc analysis of a cluster-randomized controlled trial. Nicotine Tob Res. 2014;16(6):682–8.

88. Mohlman MK, Boulos DN, El Setouhy M, Radwan G, Makambi K, Jillson I et al. A randomized, controlled community-wide intervention to reduce environmental tobacco smoke exposure. Nicotine Tob Res. 2013;15(8):1372–81.

89. Asfar T, Al Ali R, Rastam S, Maziak W, Ward KD. Behavioral cessation treatment of waterpipe smoking: the first pilot randomized controlled trial. Addict Behav. 2014;39(6):1066–74.

90. Lipkus IM, Eissenberg T, Schwartz-Bloom RD, Prokhorov AV, Levy J. Affecting percep-tions of harm and addiction among college waterpipe tobacco smokers. Nicotine Tob Res. 2011;13(7):599–610.

91. Nakkash RT, Al Mulla A, Torossian L, Karhily R, Shuayb L, Mahfoud ZR et al. Challenges to obtain-ing parental permission for child participation in a school-based waterpipe tobacco smoking prevention intervention in Qatar. BMC Med Ethics. 2014;15:70.

92. Maziak W, Ben Taleb Z, Jawad M, Afifi R, Nakkash R, Akl EA et al. Consensus statement on as-sessment of waterpipe smoking in epidemiological studies. Tob Control. 2017;26(3):338–43.

93. Jawad M, Lee JT, Millett C. The relationship between waterpipe and cigarette smoking in low and middle income countries: cross-sectional analysis of the global adult tobacco survey. PLoS One. 2014;9(3):e93097.

94. Maziak W, Jawad M, Jawad S, Ward KD, Eissenberg T, Asfar T. Interventions for waterpipe smoking cessation. Cochrane Database Syst Rev. 2015;7:CD005549.

95. O'Neill N, Dogar O, Jawad M, Kellar I, Kanaan M, Siddiqi K. Which behaviour change techniques may help waterpipe smokers to quit? An expert consensus using a modified delphi technique. Nicotine Tob Res. 2018;20(2):154–60.

96. Decision FCTC/COP7(4). Control and prevention of waterpipe tobacco products. Geneva: World Health Organization; 2016 (https://bit.ly/2vbWd9s, accessed 3 August 2018).

97. Heydari, G, EbnAhmady A, Lando HA, Chamyani F, Masjedi M, Shadmehr MB. Third study on WHO MPOWER tobacco control scores in Eastern Mediterranean countries 2011–2015. East Mediterr Health J. 2017;23(9):598–603.

98. Jawad M. Legislation enforcement of the waterpipe tobacco industry: a qualitative analysis of the London experience. Nicotine Tob Res. 2014;16(7):1000–8.

99. Mukherjea A, Modayil MV, Tong EK. Paan (pan) and paan (pan) masala should be considered tobacco products. Tob Control. 2015;24(e4):e280–4.

100. Cawkwell PB, Lee L, Weitzman M, Sherman SE. Tracking hookah bars in New York: utilizing Yelp as a powerful public health tool. JMIR Public Health Surveill. 2015;1(2):e19.

101. Protect people from exposure to second-hand tobacco smoke. Geneva: World Health Organization, Tobacco Free Initiative; 2018 (https://bit.ly/2vbkUCU, accessed 3 August 2018).

102. Jawad M, El Kadi L, Mugharbil S, Nakkash R. Waterpipe tobacco smoking legislation and policy enactment: a global analysis. Tob Control. 2015;24:i60–5.

103. Jawad M, Darzi A, Lotfi T, Nakkash R, Hawkins B, Akl EA. Waterpipe product packaging and labelling at the 3rd international Hookah Fair; does it comply with Article 11 of the Framework Convention on Tobacco Control? J Public Health Policy. 2017;19:19.

104. Jawad M, Choaie E, Brose L, Dogar O, Grant A, Jenkinson E et al., Waterpipe tobacco use in the United Kingdom: a cross-sectional study among university students and stop smoking practitioners. PLoS One. 2016;11 (1):e0146799.

105. Primack BA, Walsh M, Bryce C, Eissenberg T. US hookah tobacco smoking establishments advertised on the internet. Am J Prev Med. 2012;42(2):150–6.

106. Griffiths MA, Harmon TR, Gilly MC. Hubble bubble trouble: the need for education about and regulation of hookah smoking. J Public Policy Market. 2011;30(1):119–32.

107. Jawad M, Lee JT, Glantz S, Millett C. Price elasticity of demand of non-cigarette tobacco products: a systematic review and meta-analysis. Tob Control. 2018; doi: 10.1136/tobaccocontrol-2017-054056.

108. Jawad M, Millett C. Impact of EU flavoured tobacco ban on waterpipe smoking. BMJ. 2014;348:2698.

11.   Overall recommendations

The WHO Study Group on Tobacco Product Regulation publishes reports to provide a scientific basis for tobacco product regulation. In line with Articles 9 and 10 of the WHO FCTC, the reports identify evidence-based approaches to the regulation of tobacco products.

At its ninth meeting, the Study Group discussed: the prevalence and health effects of waterpipe tobacco smoking and interventions to reduce use; approaches to reducing toxicant concentrations in tobacco products, including cigarettes and smokeless tobacco; the state of the science on a global nicotine reduction strategy; the clinical pharmacology of nicotine in ENDS; the sugar content of tobacco products; a regulatory strategy for reducing exposure to toxicants in cigarette smoke; an updated list of priority toxicants in tobacco products for regulatory purposes; heated tobacco products; and the science of flavours in tobacco products. The aim of the discussions was to update knowledge in these areas in order to inform policy at global level and to advance tobacco product regulation.

The report provides guidance through the Executive Board to Member States. It focuses primarily on requests of the COP to the WHO FCTC to WHO through the Convention Secretariat at its seventh session, in 2016, as articulated in decisions FCTC/COP7(4), FCTC/COP7(9) and FCTC/COP7(14). These decisions informed the content of the background papers in the above areas, for which Member States have requested technical assistance as a basis for national policies. The 10-member Study Group invited subject matter experts, who drafted background papers, contributed to discussions and provided the most up-to-date empirical data on the topics considered. Sections 2–10 of the report provide scientific information and policy recommendations to guide Member States in navigating difficult issues in tobacco product regulation. Further, the report provides guidance to Member States on the most effective evidence-based means for bridging regulatory gaps in tobacco control and for developing coordinated regulatory frameworks for tobacco products to guide international policy. Additionally, it identifies areas for further work and future research, focusing on the regulatory needs of Member States; it takes into consideration regional differences, thus providing a strategy for continued, targeted technical support to Member States.

11.1 Main recommendations

The main recommendations of this report to policy-makers and all other interested parties include the following.

- Monitor and collect reliable, independent data on heated tobacco products and alternative products in order to understand behaviour and potential risks to users and bystanders and to verify claims of reduced exposure and risk.

- Consider and examine the design features that determine nicotine flux in ENDS and the extent to which these products could promote or impede cessation of smoking, and invest in research on appropriate policies and regulations on ENDS.

- Consider a regulatory strategy for reducing exposure to toxicants in combusted tobacco product smoke that includes a nicotine level in tobacco that does not exceed 0.4 mg/g of tobacco (0.04 mg nicotine per combusted product in mainstream smoke under HCI smoking conditions). This should be accompanied by a reliable system for monitoring regulated constituents in tobacco and smoke, comprehensive tobacco control and concerted national and international efforts to prevent black markets.

- Consider a nicotine reduction policy coordinated with policies that allow adequate access to nicotine replacement therapies and other products, if and as approved by relevant authorities and with appropriate safeguards. This should be supported by population surveillance, monitoring and testing of products, enforcement of product standards, and a strong focus on protecting children and young people.

- Consider banning or restricting the use of flavours in nicotine delivery systems and tobacco products in order to reduce initiation by young people, and consider banning or restricting flavours in combusted tobacco products to promote cessation.

- Require manufacturers to disclose relevant information on sugar content, and consider lowering the level of sugars in tobacco products to reduce their effects on product toxicity, addictiveness and attractiveness.

- Require manufacturers, as applicable and appropriate, to report priority toxicants analysed with methods based on the SOPs of the WHO TobLabNet.

- Lower the levels of addictive, toxic and carcinogenic agents in tobacco products, including cigarettes and smokeless tobacco, recognizing that decreasing the levels of these agents will not make these products safe.

- Consider applying WHO FCTC provisions to waterpipe tobacco for the prevention and control of waterpipe tobacco use.

Continuing research is required to monitor product development and use, promotional strategies and other activities of the tobacco and related industry to build intelligence to protect public health. Specific recommendations on each of the topics considered in this report can be found in sections 2.9, 3.8, 4.5–4.6, 5.8, 6.8, 7.10, 8.8, 9.9 and 10.3–10.5.

11.2 Significance for public health policies

The Study Group's report provides helpful guidance for understanding the content, emissions and design features of selected products, such as smokeless tobacco, waterpipes, heated tobacco products and ENDS and describes the public health impact of these products and features. In recent years, unconventional nicotine and tobacco products have permeated several markets, for which there is no precedent, and these present unique regulatory challenges to Member States. Further, there is better understanding of the science, adverse effects, characteristics, contents and emissions of conventional products owing to advances in knowledge; therefore, the report provides updated information for Member States on novel and emerging tobacco products and nicotine delivery systems to support them in formulating effective strategies for regulating tobacco and nicotine products.

Because of the unique composition of the Study Group, with regulatory, technical and scientific experts, it can navigate and distil complex data and research and synthesize them into recommendations for policy development at country, regional and global levels. The recommendations promote international coordination of regulatory efforts and the adoption of best practices in tobacco product regulation, strengthen capacity for tobacco product regulation in all WHO regions and provide a ready, science-based resource for Member States.

11.3 Implications for the Organization's programmes

The report fulfils the mandate of the WHO Study Group on Tobacco Product Regulation to provide the Director-General with scientifically sound, evidence-based recommendations for Member States about tobacco product regulation. Tobacco product regulation is a highly technical area of tobacco control, in which Member States face complex regulatory challenges. The outcomes of the Study Group's deliberations and its main recommendations will improve Member States' understanding of tobacco and nicotine products. The report's contribution to the body of knowledge on tobacco product regulation will play a pivotal role in informing the work of the tobacco programme in the WHO Department for Prevention of Noncommunicable Diseases, especially in providing technical support to Member States. It will also contribute to further development of partial guidelines for implementation of Articles 9 and 10 of the WHO FCTC.

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 ninth meeting, where the group reviewed background papers specially commissioned for the meeting and considered the following topics:

1. Heated tobacco products (section 2);

2. Clinical pharmacology of nicotine in electronic nicotine delivery systems (section 3);

3. A global nicotine reduction strategy: state of the science (section 4);

4. A regulatory strategy for reducing exposure to toxicants in cigarette smoke (section 5);

5. The science of flavour in tobacco products (section 6);

6. Sugar content of tobacco products (section 7);

7. Updated priority list of toxicants in combusted tobacco products (section 8);

8. Approaches to measuring and reducing toxicant concentrations in smokeless tobacco products (section 9);

9. Waterpipe tobacco smoking: prevalence, health effects and interventions to reduce use (section 10).

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.