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WHO study group on tobacco product regulation

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



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Report on the Scientific Basis of Tobacco Product Regulation:
Seventh Report of a WHO Study Group



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

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TobReg thanks the Convention Secretariat of the WHO FCTC for facilitating drafting of the requests of the COP, which served as a basis for some of the background papers.

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
M RTP	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'-nitrosornicotine
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)

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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

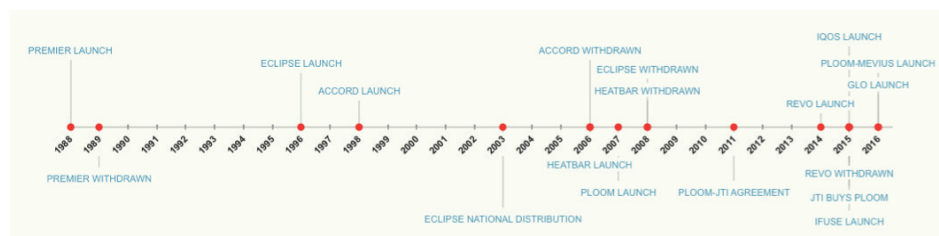
Company	Trade name®	Brief description	Current status
Philip Morris USA / Altria	Accord	THS consisting of small cigarettes placed in an external heating device. Available in tobacco and menthol flavours. Introduced in 1998 in test markets in Richmond, Virginia, USA, and Japan. Advertising focused on reduced 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. MRTD 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 °C. 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 °C). 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 °C. 2200-mAh battery. Two colours (black, red).	Available online.
	Atom	Rectangular device. Temperature range, 182, 210, 240 °C. 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 °C; 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/cigarette) HCl (28)	Eclipse (µg/cigarette) ISO (21)	EHCSS (µg/cigarette) ISO (44)	THS 2.2 (µg/cigarette) HCl (45)	THP1.0 (µg/cigarette) HCl (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[<i>a</i>]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; HCl: Health Canada intense smoking regimen; ISO: International Organization for Standardization smoking regimen; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N*'-nitrosornornicotine; 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)			
		Ciga- rettes	EHCSS	Absti- nence	Ciga- rettes	EHCSS	Absti- nence	Ciga- rettes	EHCSS-K6	EHCSS-K3	Absti- nence
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
5-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[<i>a</i>]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[<i>a</i>]pyrene	Benzo[<i>a</i>]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; 5-PMA: 5-phenylmercapturic acid. *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	Abstinence	Cigarette	EHCSS-K6 ^a	EHCSS-K3 ^a	Abstinence
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[<i>a</i>]pyrene (ng/24 h)	143.6	127.0	181.6	71.9	73.1	75.1
3-OH-Benzo[<i>a</i>]pyrene	Benzo[<i>a</i>]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	Cigarettes	Abstinence	THS	Cigarettes	Abstinence
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	<i>N</i> '-nitrosanornicotine (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[<i>a</i>]pyrene (pg/mg creatinine)	73.02	149.62	62.99	81.22	182.85	85.13
3-OH-benzo[<i>a</i>]pyrene	Benzo[<i>a</i>]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'-nitrososornicotine; 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*'-nitrosornicotine; 1-OHP: 1-hydroxypyrene; S-PMA: S-phenylmercapturic acid.

When a compensation formula

$$1 - ((\ln(\text{marker1}) - \ln(\text{marker0})) / (\ln(\text{yield1}) - \ln(\text{yield0})))$$

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, redacted² 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

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

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.

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3. Clinical pharmacology of nicotine in electronic nicotine delivery systems

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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.

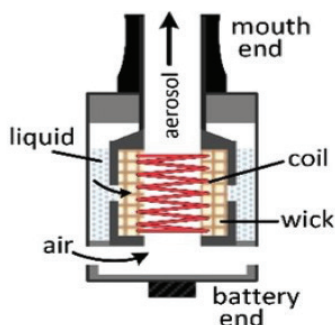
The purpose of this background paper is to review the literature at the time of writing with some additions after review between March and December 2018 on the nicotine content and nicotine delivery of ENDS and to explore factors that influence the emissions of nicotine and non-nicotine toxicants. In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

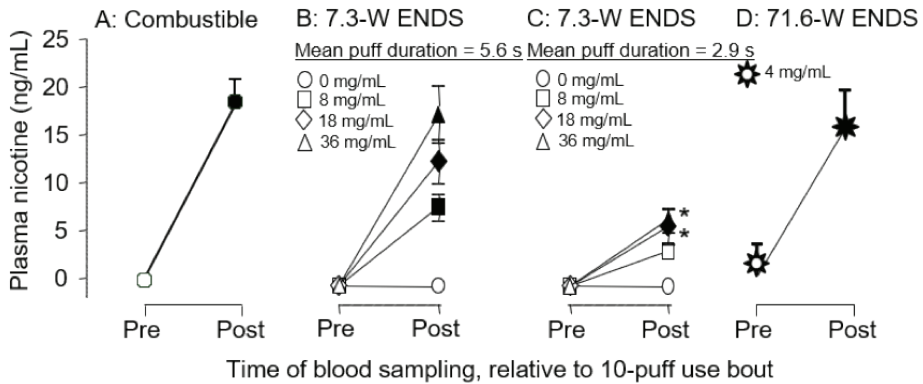


Source: Dr Alan Shihadeh, American University of Beirut, Lebanon.

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-naïve 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
Goniewicz (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	Samples			Nicotine concentration in samples containing > 0.1 mg/mL
	Analysed	Nicotine > 0.1 mg/mL	Nicotine > 10 mg/mL	
Beauval (13)	2	0	0	–
Cheah (10)	2	0	0	–
Davis (16)	10	0	0	–
Goniewisz (21)	28	3	0	0.8–0.9
Kim (22)	20	0	0	–
Lisko (25)	5	0	0	–
Omaie (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).

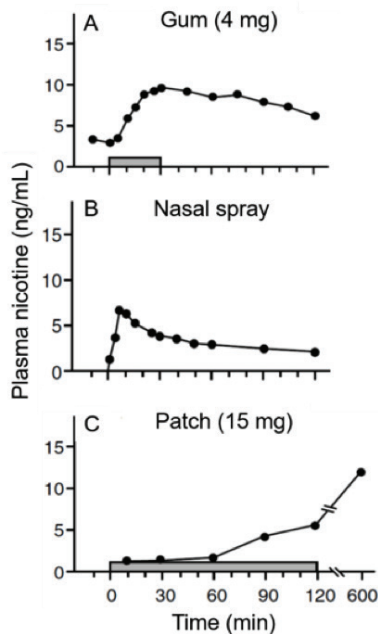
Puff duration is also a factor in ENDS nicotine delivery: Panel C (8) shows the same device and e-liquid nicotine concentration as in Panel B, but the study participants took shorter puffs (2.9 s on average). When the puff duration is shorter and all other device and e-liquid characteristics are constant, 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

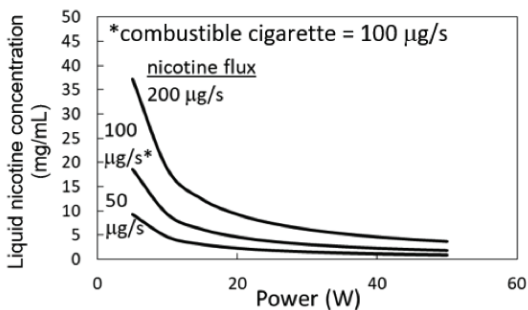
The “yield” of nicotine from ENDS is the amount (in mg) of nicotine in the aerosol produced by an ENDS under a specific puffing regimen. Knowing the yield of nicotine from ENDS has been considered important for understanding the pharmacokinetics of nicotine in ENDS users. One review of the literature (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 $\mu\text{g/s}$) 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 $100 \mu\text{g/s}$, 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	ElDib (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.	Sample		Product		Measures				Results			
	Country and baseline	Age range (years)	No. at baseline	Follow-up	Retention rate at follow-up	EN&NNDS type	Nicotine in e-liquid	EN&NNDS use		Nicotine dependence	Criterion for smoking abstinence	
								Frequency	Quantity	Duration	Reasons	
118	USA. Nationally representative sample of smokers in the general population	≥ 18	5255	1 year	63%	NM	NM	Ever used vs Never used	NM	NM	M	Association between EN&NNDS use and quitting smoking
119	USA Smokers Quit-line callers	≥ 18	2758	7 months	35%	NM	NM	Ever use vs use for ≥ 1 month vs use for < 1 month	NM	NM	M NU	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.
120	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	M	Never ENDS users, 31.3% Use < 1 month, 16.6% Use > 1 month, 21.7% (P < .001). Not adjusted for use of NRT. 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.

<p>121</p> <p>2011–12</p> <p>127</p>	<p>18–65</p> <p>two United States metropolitan areas</p>	<p>1374</p> <p>2-years</p> <p>51%</p>	<p>NM</p> <p>NM</p> <p>M</p>	<p>Ever used an e-cigarette or not; if so, on how many of the past 30 days</p> <p>Currently used e-cigarettes every day, or not at all. If not at all, ever used e-cigarettes “fairly regularly”</p> <p>E-cigarette use:</p> <p>Intensive (daily ≥ 1 month); intermittent (\geq once but not daily for ≥ 1 month); no use or at most once or twice.</p>	<p>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 SR 1 month at follow-up</p> <p>NM</p> <p>NM</p> <p>M</p> <p>up</p> <p>significant.</p>	<p>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.</p> <p>SR 1 month at follow-up</p> <p>NM</p> <p>NM</p> <p>M</p> <p>up</p>
<p>122</p> <p>2012</p> <p>128</p>	<p>30–52</p> <p>free NRT, no access)</p>	<p>3</p> <p>3-408 months</p> <p>62%</p>	<p>NM</p> <p>NM</p> <p>NM</p>	<p>Ever used during follow-up vs never used</p>	<p>SR 1 month at follow-up</p> <p>NM</p> <p>NM</p> <p>M</p> <p>up</p>	<p>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.</p> <p>SR 1 month at follow-up</p> <p>NM</p> <p>NM</p> <p>M</p> <p>up</p>

123	2012–2013	USA In-treatment cancer patients who were current smokers and had received cessation treatment	NR	781	1 year	53%	NM	NM	NM	NM	M	SR 1 day at follow-up	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.
													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.
124	2012–2013	Great Britain. Current smokers	≥ 18	1759	1 year	44%	NM	NM	NM	NM ^b	NM ^b	SR "1" stopped smoking completely in the past year"	
111	2013–2015	Italy. Convenience sample of daily smokers for ≥ 6 months, EN&NDS users for ≥ 6 months	75	932	2 years	69%	NM	NM	NM	NM	NM	SR 30 days at follow-up with CO-tested subsample	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	NM	NM	M	M	SR 7-day point prevalence of abstinence	Any EN&NDS use in the 3 months before follow-up 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.

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	NM	NM	M	EN&NDS 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. 7 days at 6-month follow-up Validated salivary cotinine Baseline dual users were not more likely than exclusive tobacco smokers to have quit smoking at follow-up (12.5% versus 9.5%, <i>P</i> = .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.
105	2014–2015	France. Exclusive smokers and dual users	15–85	2057	6 months	695	NM	NM	NM	NM	NM	Use of e-cigarettes: regular use in past 30 days. No use: used e-cigarettes sometimes, rarely or never SR 7 days at 6-month follow-up Number of days used EN&NDS in past 30 days Classified as 0, none 1–5 6–20 30, daily Quit rate of daily EN&NDS 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.
107	2014	USA. Smokers seeking treatment enrolled in a quitline programme offering counselling and NRT	≥ 18	5672	NR	NR	NR	NR	NR	NM	NM	Daily EN&NDS users were eight times more likely to quit than non-users when using non-cartridge, refillable tanks. Experimenters were half as likely as non-users to quit. Self-reported 30-day complete abstinence at follow-up 1 month before follow-up Time to first cigarette up
108	2013–2015	USA. Current cigarette smokers with no current use of EN&NDS	≥ 25	5124	1 year	83%	NM	NM	NM	NM	NM	Re-reported use of cartridges, refills and tanks Number of days used EN&NDS in past 30 days Time to first cigarette up Self-reported 30-day complete abstinence at follow-up 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.

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	680	6 months	75% for controls 77% for intervention	NM	NM	Any use of EN&NDS 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&NDS non-users than among users in the 7 days before follow-up, but the difference was statistically nonsignificant.
125	2014–2016	USA. Smokers of ≥55 cigarettes/day for ≥ 1 year, ENDS-naive, not seeking treatment to quit	18	21	3 months	Control= 73%; arm 1=76%; arm 2=71%. EMA compliance 58%	NM	NM	Arm 1= 3-week provision of 16 mg/mL nicotine Arm 2= 24 mg/mL nicotine Control= no provision of ENDS. No ENDS provided but not precluded from buying them	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.	2015-2016	ENDS-naive	≥ 55	per arm	70	Arm 1 = ENDS plus support Placebo = ENDS 3.3-4.2 V plus support Control = no ENDS	Arm 2 = ENDS plus support Placebo = ENDS 3.3-4.2 V plus support Control = no ENDS	Quitting rate higher among ENDS users than in control group; however, there was no difference between ENDS and ENDS users. Authors indicate that ENDS users had an e-liquid that delivered very little nicotine in the aerosol (0.1 mg/puff).
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AOR: adjusted odds ratio; CI: confidence interval; CO: carbon monoxide; ENDS: electronic nicotine and non-nicotine delivery systems; MI: measured; NMI: not measured; NRT: nicotine replacement therapy; NU: not used in analysis; SR: self-reported; U: used in analysis. ^aThe 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. ^b Instead, they measured desire to quit. ^c 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	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
Berry (108)	^a Adjusted for age, sex, race, region, income, frequency, intensity of cigarette use, nicotine dependence and previous quit attempts.			

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.

Hitchman (124)

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

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

Biener (121)

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.

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).

Manzoli (111)

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.

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.

Rigotti (109)

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.

Zawartallo (110)

AOR: adjusted odds ratio; CI: confidence interval; ENDS: electronic nicotine delivery systems; ENNDS: 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 ENDS, 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 ENDS 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 ENDS. 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 ENDS in England has been associated with the increasing success of quit attempts (139).

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

3.7 Potential health impact of ENDS

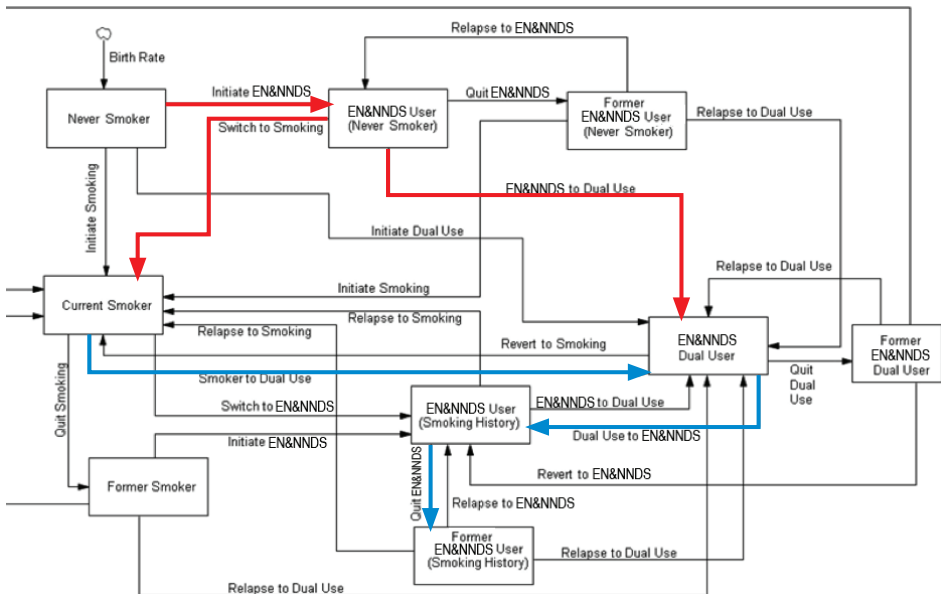
As some ENDS may help some smokers to quit, what is their potential health benefit for the population? The overall impact of using ENDS on population

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&NDS, 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 EN&NNDS 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 use: 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.

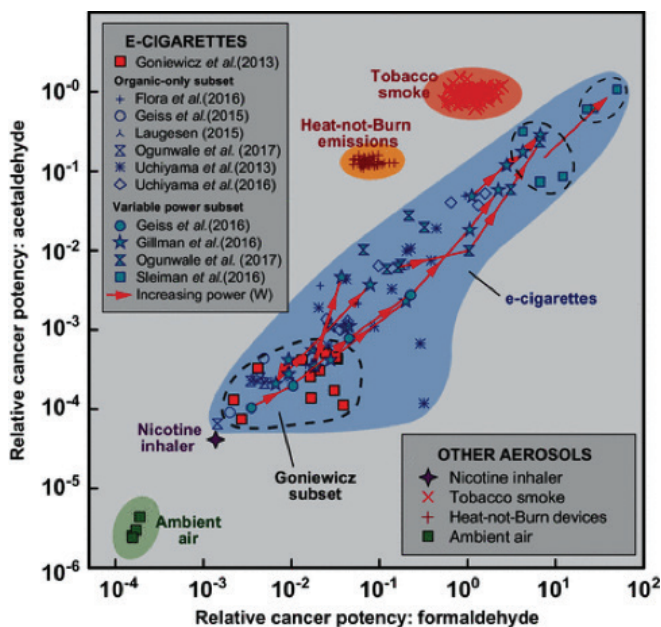
3.7.2 Harm from ENDS and electronic non-nicotine delivery systems

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
Technology	<p>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:</p> <ul style="list-style-type: none"> • 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. 	<p>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.</p> <p>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.</p> <p>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.</p>	<p>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.</p> <p>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).</p> <p>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.</p>
Effective nicotine delivery	<p>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:</p>	<p>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.</p>	Toxicant profile information
Relation between toxicant profile of ENDS emissions and nicotine flux	<p>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.</p>	<p>device construction: including general design, metals used, wicking materials.</p>	<p>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.</p>

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.

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.

<p>Effectiveness of ENDS as a smoking cessation aid</p> <p>ENDS can be effective for cessation for some smokers under some circumstances. It may have the opposite effect for other smokers under different circumstances.</p>	<p>Better understanding is needed of the circumstances in which ENDS use can promote or be detrimental to smoking cessation, including:</p> <ul style="list-style-type: none"> features of ENDS that facilitate cessation: <ul style="list-style-type: none"> 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. <p>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.</p>
<p>The conditions that appear to affect the potential of ENDS as a smoking cessation aid include:</p> <ul style="list-style-type: none"> 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. 	

<p>The overall population health effects of using ENDS depends primarily on:</p> <ul style="list-style-type: none"> 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. <p>Potential health impact of ENDS</p>	<p>The gaps in research on the effectiveness of ENDS as smoking cessation aid are described above.</p> <p>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 non-smokers.</p> <p>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.</p>
	<p>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).</p>

3.9 References

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

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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 ≤ 2.4 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

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 ≤ 2.4 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 ≤ 0.4 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

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

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.

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 0.4 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 subjective characteristics similar to those of regular cigarettes but with reduced potential for dependence has now been reasonably established. 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.

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

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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-aminonaphthalene, 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 ($\mu\text{g}/\text{mg}$ 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
	International 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[<i>a</i>]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*'-nitrosornicotine. ^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.

Hausmann (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. Hausmann 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 HCl 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*'-nitrosornicotine. 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.

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6. The science of flavour in tobacco products

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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

chamaesthesia (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.

Chamaesthesia: 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). Chamaesthesia 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 TSNA 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. Flavourings 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.

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7. Sugar content of tobacco products

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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 ($\leq 0.2\%$) 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.

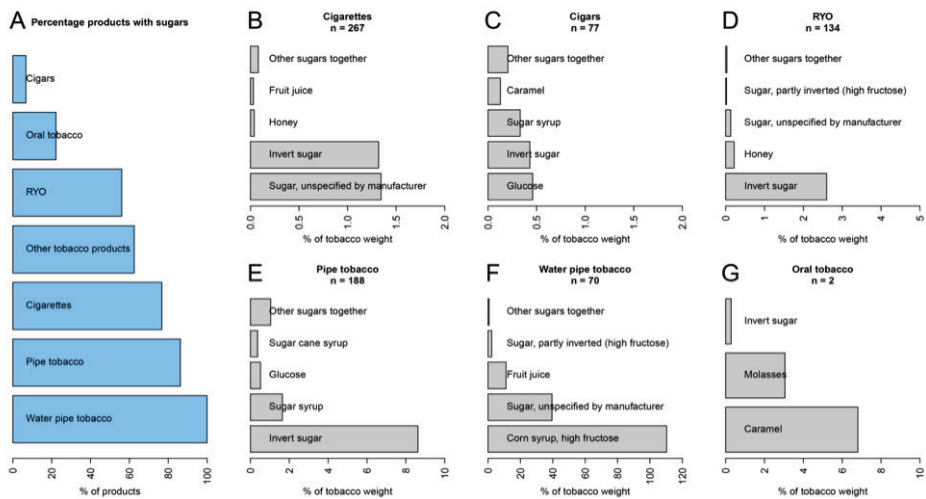
Sugars in tobacco products identified from manufacturers' lists of ingredients in the electronic database EMTOC (16) are: brown sugar, caramel, (corn) syrup (solids), dextrin, dextrose, (fruit) concentrate, (fruit) juice, glucose, high-fructose sugar, honey, invert sugar, lactose, malt extract, maltodextrin, (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 $\leq 19\%$ 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 the 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 Phillipotts 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.

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7.12 References

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8. Updated priority list of toxicants in combusted tobacco products

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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)	<i>COP/1/INF.DOC./3</i> : 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)	<i>COP/2/DIV/9</i> : 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.	<i>COP/2/8</i> : 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)	<i>COP/3/DIV/3</i> : decisions relevant to the priority list included: <ul style="list-style-type: none"> • 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. 	<i>COP/3/6</i> : the new progress report presented by TobReg identified: <ul style="list-style-type: none"> • 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)	<i>COP/4/DIV/6</i> : continue validation of analytical chemical methods for testing and measuring cigarette contents and emissions.	<i>COP/4/INF.DOC./2</i> : three analytical methods have been validated: <ul style="list-style-type: none"> • CO in emissions; • NNN and NNK in emissions; and • nicotine content of tobacco.
COP 5 (Seoul, 2012)	<i>COP/5/DIV/5</i> : 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.	<i>COP/5/INF.DOC./1</i> : work in progress: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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'-nitrosornicotine; 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

Toxicants evaluated by TobReg	Carcinogenicity or toxicity data		Inclusion on priority lists		
	TACI	TNCRI	COP/3/6	COP/6/14	
			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 ^b	–	–	–	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[<i>a</i>]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	–
<i>m</i> - and <i>p</i> -Cresol	–	0.01	–	x	–
<i>o</i> -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'*-nitrososonornicotine; PAH: polycyclic aromatic hydrocarbon; TACI: toxicant animal carcinogenicity index; TNCRI: toxicant non-cancer response index; TSNA: tobacco-specific N-nitrosamine.

^aJustification 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 T₂₅. 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.

^bOriginally 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.

^cAdded 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 γ H2AX 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 γ H2AX 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[<i>a</i>]pyrene in mainstream cigarette smoke	Mainstream benzo[<i>a</i>]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*'-nitrosornicotine; 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 CO₂ 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 ≤20%. 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[<i>a</i>]pyrene (ng)	NR	17–32 (139) (USA)	LOD–307 (135)
Pyrene ^b (ng)	NR	NR	30–12 950 (135)
Tobacco-specific			
<i>N</i>-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)
<i>m</i> -Cresol (µg)	1.93–6.92 (127) (Canada)	NR	NR
<i>p</i> -Cresol (µg)	5.28–17.7 (127) (Canada)	NR	NR
<i>m+p</i> -Cresol (µg)	7.24–24.6 (127) (Canada)	NR	2.37–4.66 (135)
<i>o</i> -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)		0.48–2.28 (146) (USA)	
	10.5–17.8 (145) (Pakistan)	10.3–19.1 (144) (USA)	1.8–41.3 (147) (Jordan)	NA
Benzo[<i>a</i>]pyrene (ng)	NR	NR	NR	1–26 (148, 149) (Lebanon, USA)
Pyrene (ng)	NR	NR	NR	6–170 (148, 149) (Lebanon, USA)
	0.22–0.36 (132) (USA)	NA	0.062 (0.023) ^b (150) (Egypt)	0.018 (0.013) (150) (Egypt)
Arsenic (µg)	1.0–1.7 (132) (USA)	NA	0.34 (0.007) (150) (Egypt)	0.005 (0.017) ^b (150) (Egypt)
Cadmium (µg)	1.3–3.1 (132) (USA)	NR	0.15–0.37 (151) (USA, Middle East)	0.161–8.32 (152) (Worldwide)
Chromium ^a (µg)	0.60–1.16 (132) (USA)	NR	0.15 (0.008) ^b (150) (Egypt)	0.97 (0.01) ^b (150) (Egypt)
Lead (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/m ³) (153)
Acetamide	CA	5.0×10^{-4} (cancer)
Acrylamide	CA	8.0×10^{-3} (cancer)
3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	CA	1.4×10^{-6} (cancer)
2-Amino-3-methyl-9H-pyrido[2,3-b]indole	CA	2.9×10^{-5} (cancer)
2-Amino-6-methyldipyrido[1,2- <i>a</i> :3',2'- <i>d</i>]imidazole	CA	7.1×10^{-6} (cancer)
2-Amino-3-methylimidazo[4,5- <i>f</i>]quinoline	CA	2.5×10^{-5} (cancer)
2-Amino-9H-pyrido[2,3- <i>b</i>]indole	CA	8.8×10^{-5} (cancer)
2-Aminodipyrido[1,2- <i>a</i> :3',2'- <i>d</i>]imidazole	CA	2.5×10^{-5} (cancer)
Benz[<i>a</i>]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[<i>a,h</i>]anthracene	CA	8.3×10^{-6} (cancer)
Dibenzo[<i>a,e</i>]pyrene	CA	9.1×10^{-6} (cancer)
Dibenzo[<i>a,h</i>]pyrene	CA	9.1×10^{-7} (cancer)
Dibenzo[<i>a,i</i>]pyrene	CA	9.1×10^{-7} (cancer)
Dibenzo[<i>a,l</i>]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- <i>cd</i>]pyrene	CA	9.1×10^{-5} (cancer)
Methyl ethyl ketone	RT	5.0 (developmental)
1-Methyl-3-amino-5H-pyrido[4,3- <i>b</i>]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)
<i>N</i> -Nitrosodiethanolamine	CA	1.3×10^{-5} (cancer)
<i>N</i> -Nitrosodiethylamine	CA	2.3×10^{-7} (cancer)
<i>N</i> -Nitrosodimethylamine	CA	7.1×10^{-7} (cancer)
<i>N</i> -Nitrosomethylethylamine	CA	1.6×10^{-6} (cancer)
<i>N</i> -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 TSNA; 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, TSNA 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; NNN: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N*'-nitrosornornicotine; 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.

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9. Approaches to measuring and reducing toxicant concentrations in smokeless tobacco products

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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 (<i>Aspergillus</i>)
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[<i>a</i>]pyrene Group 2A: dibenz[<i>a,h</i>]anthracene Group 2B: benz[<i>a</i>]anthracene, benzo[<i>b</i>]fluoranthene, benzo[<i>j</i>]fluoranthene, benzo[<i>k</i>]fluoranthene, dibenzo[<i>a,i</i>]pyrene, dibenzo[<i>a,l</i>]pyrene, indeno[1,2,3- <i>cd</i>]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: <i>khat</i> , 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'*-nitrosornicotine, 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 TSNA 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 TSNA (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 TSNA 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).

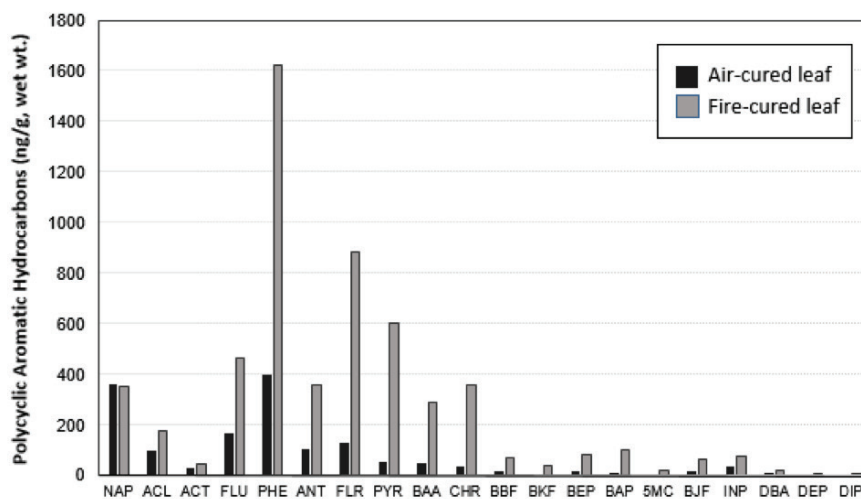
The constituents of a finished smokeless tobacco product, including nicotine (in its various ionic forms), toxicants and carcinogens, result from the presence of inorganic, organic and biological agents and their interactions (4, 19, 31, 40, 42, 43). Naturally occurring organic constituents of tobacco 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*]fluoranthrene; 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 TSNA (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 (hypochlorite: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,

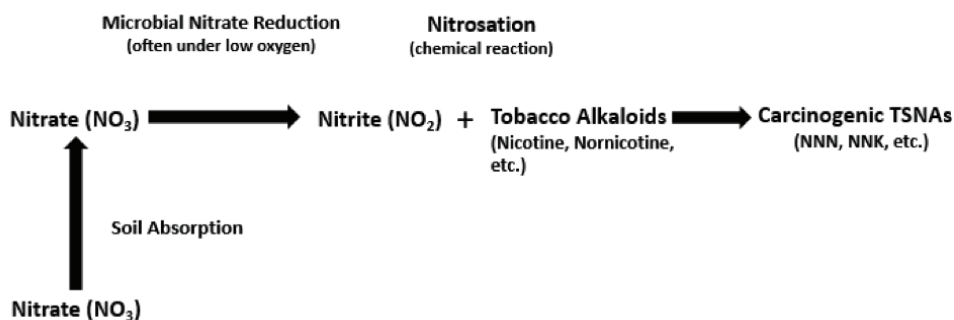
and other ingredients, such as flavours and humectants, are added before the product is packaged. This process changes taste, reduces microbial activity and yields a product with a shelf life of 14–30 weeks when refrigerated, according to a manufacturer (28, 76). *Snus* products continue to have low 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 TSNA levels (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 TSNA 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*'-nitrosornnicotine.
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 TSNA, 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 (<i>Nicotiana tabacum</i> , <i>N. rustica</i> , <i>N. glauca</i>), 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; TSNA: 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. refrigeration at point of sale) for finished smokeless tobacco products;
- a mean level of NNN in any batch of finished smokeless tobacco products of $\leq 1 \mu\text{g/g}$ 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, TSNA (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[<i>a</i>]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*'-nitrosanornicotine. 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.

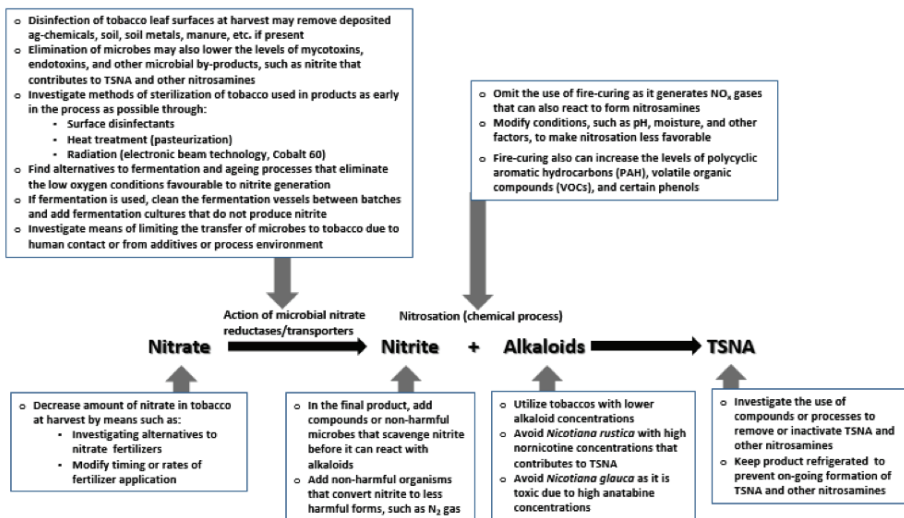
The concerns associated with smokeless tobacco products include:

- 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



N₂: 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 hypochlorite: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.

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10. Waterpipe tobacco smoking: prevalence, health effects and interventions to reduce use

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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:

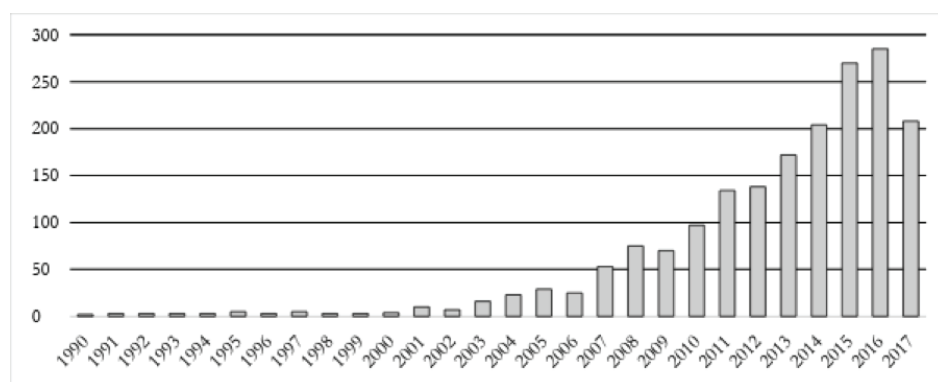
- summarize regional and global patterns of waterpipe tobacco use, including changes in prevalence;
- evaluate the acute and chronic health effects of waterpipe tobacco use;
- describe cultural practices in relation to initiation and maintenance of use;
- explain the influence of flavourings on initiation, 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.

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.

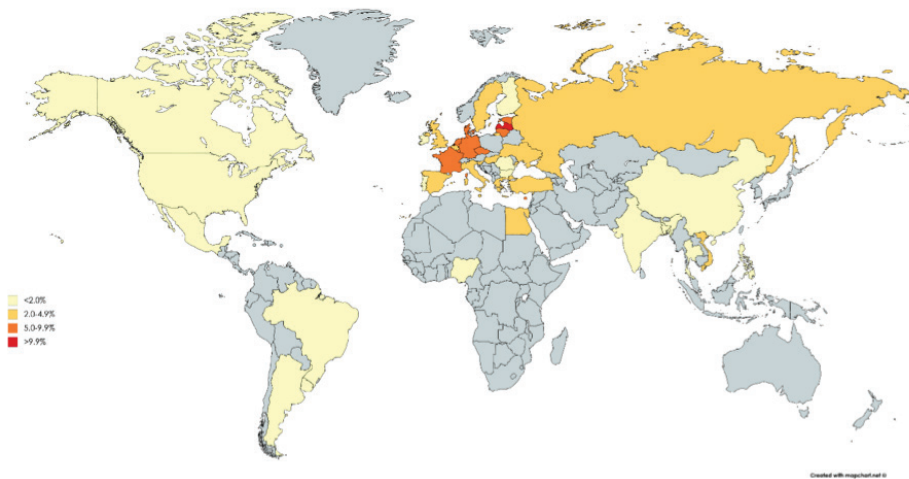
10.2.1 Regional and global patterns of waterpipe tobacco use

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

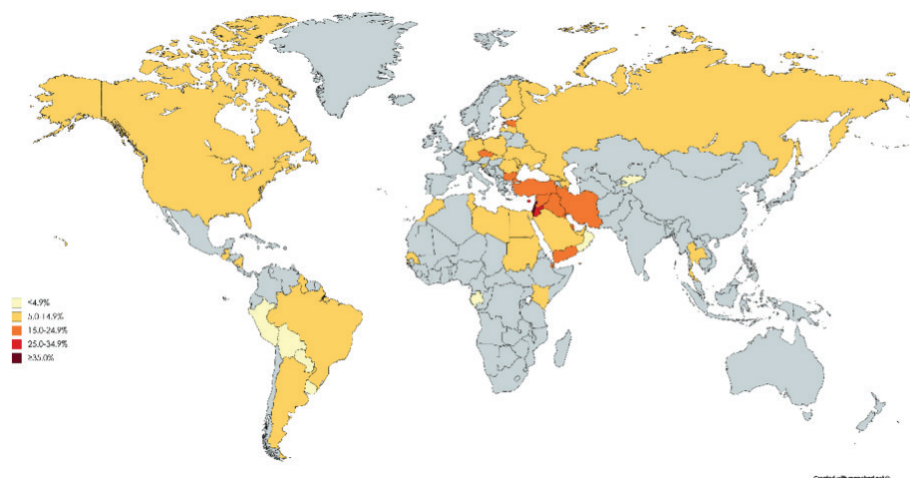


Fig. 10.4. Use of waterpipe tobacco in the past 30 days by young people, Middle East



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% (\pm 0.2) to 1.2% (\pm 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). Alarming, 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 *moassel* 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/COP7/10 (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.

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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.

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