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WHO study group on tobacco product regulation

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

World Health Organization

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Tbilisi, Georgia
13–15 December 2022

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WHO also wishes to acknowledge its Member States, as most of the papers in this report were written in response to their requests to WHO for technical guidance.

Abbreviations and acronyms

AHRR	aryl hydrocarbon receptor repressor
BaP	benzo[*a*]pyrene
BAT	British American Tobacco
CC	conventional cigarette
CC16	club cell 16-kDa protein
CEMA	cyanoethyl mercapturic acid
CI	confidence interval
CO	carbon monoxide
COHb	carboxyhaemoglobin
COP	Conference of the Parties
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CVD	cardiovascular disease
DLCO	diffusing capacity of the lung for carbon monoxide
ENDS	electronic nicotine delivery system
ENNDS	electronic non-nicotine delivery system
EU	European Union
FEV_1	forced expiratory volume in 1 min
FVC	forced vital capacity
GC	gas chromatography
GMR	geometric mean ratio
HDL-C	high-density lipoprotein cholesterol
1-HOP	1-hydroxypyrene
HTP	heated tobacco product
IARC	International Agency for Research on Cancer
IF	inhalation facilitation
ISO	International Organization for Standardization
LC	liquid chromatography
LD_{50}	lethal dose for 50% of animals
MS	mass spectrometry
nAChR	nicotinic acetylcholine receptor
NGL	Next Generation Labs
NHANES	National Health and Nutrition Examination Survey
NMR	nuclear magnetic resonance
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N*′-nitrosonornicotine

NO	nitric oxide
OR	odds ratio
g-OH-Acr-dGuo	(8*R/S)*-3-(2´-deoxyribos-1´-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3*H*)-one
PAH	polycyclic aromatic hydrocarbons
PATH	Population Assessment of Tobacco and Health
PGEM	prostaglandin E2 metabolite
PGF_{2a}	(Z)-7-[1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(E,3S)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid
PheT	phenanthrene tetraol
PMI	Philip Morris International
RIVM	National Institute for Public Health and the Environment) Netherlands [Kingdom of the])
TAPS	tobacco advertising, promotion and sponsorship
TNE	total nicotine equivalents
TNP	tobacco and nicotine products
TobReg	WHO Study Group on Tobacco Product Regulation
TPD	[European] Tobacco Products Directive
TRP	transient receptor potential
US	United States [of America]
USA	United States of America
USFDA	United States Food and Drug Administration
WHO FCTC	WHO Framework Convention on Tobacco Control
WS	Wilkinson Sword

1. Introduction

Tobacco is a global public health threat and kills more than 8 million people a year globally *(1)*, about 1.2 million of those deaths resulting from exposure of non-smokers to second-hand smoke *(2)*. Comprehensive tobacco control is therefore essential to tackle the global tobacco epidemic and prevent needless deaths. Product regulation can play a role in reducing the demand for tobacco, and effective tobacco product regulation is an essential component of a comprehensive tobacco control programme *(3)*. It includes regulation of contents and emissions by mandated testing, disclosure of test results, setting limits as appropriate, disclosure of information on products 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) *(4)* and in the partial guidelines for implementation of Articles 9 and 10 of the WHO FCTC *(5)*. Other WHO resources, including the basic handbook on tobacco product regulation *(3)*, the handbook on building laboratory testing capacity *(6)* and the online modular courses based on the handbooks, are available on the WHO website *(7)*, support Member States in this respect. Additionally, the WHO Study Group on Tobacco Product Regulation (TobReg) has published a number of reports and advisory notes that provide guidance on several aspects of regulating tobacco products and non-therapeutic nicotine products.

The Study Group was formally constituted by the WHO Director-General in 2003 to address gaps in the regulation of tobacco products. Its mandate is to provide evidence-based recommendations on policy for 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 are from countries in all six regions of WHO *(8)*. As a formal entity of WHO, TobReg submits technical reports that provide the scientific basis for tobacco product regulation to the WHO Executive Board, through the Director-General, to draw the attention of Member States to WHO's work in this field. The reports, which are part of the WHO Technical Report Series, include previously unpublished background papers that synthesize published scientific literature and have been discussed, evaluated and reviewed by TobReg. In accordance with Articles 9 and 10 of the WHO FCTC, relevant decisions of the Conference of the Parties (COP) to the WHO FCTC and relevant WHO reports submitted to the COP, the TobReg reports identify evidence-based approaches to regulating all forms of tobacco products and non-therapeutic nicotine products, including new and emerging products such as electronic nicotine delivery systems (ENDS), electronic non-nicotine delivery systems (ENNDS), heated tobacco products (HTPs) and nicotine pouches. These reports,

now considered to be WHO technical products (formally known as WHO global public health goods), respond to World Health Assembly resolutions WHA53.8 (2000), WHA53.17 (2000) and WHA54.18 (2001). WHO technical products or global public health goods are initiatives developed or undertaken by WHO that are of benefit either globally or to many countries in several regions *(9)*. This designation presents a unique opportunity for TobReg to engage directly with Member States and contribute to national, regional and global policy.

The ninth meeting of TobReg took place from 13–15 December 2022 in Tbilisi, Georgia, hosted by the Georgian National Centre for Disease Control and Public Health and organized by the WHO Tobacco Free Initiative Unit of the Health Promotion Department. About 40 participants, including TobReg members, WHO staff, the Secretariat of the WHO FCTC and invited experts, discussed the scientific literature on pertinent topics in product regulation, including emerging issues, according to Member States' requests to WHO and requests of the COP. Topics previously considered by the Global Tobacco Regulators Forum, such as synthetic nicotine and nicotine pouches, which have recently presented regulatory challenges because of the way in which they are marketed and used to exploit regulatory loopholes, are also addressed in the report. In response to repeated requests by Member States to the Secretariat to provide technical assistance and authoritative guidance on emerging issues in tobacco product regulation, the report focuses on newer ways in which non-therapeutic nicotine in nicotine and tobacco products is delivered and promoted to people of different ages, including children and adolescents. The meeting thus provided a platform for discussing six background papers:

- Additives that facilitate inhalation, including cooling agents, nicotine salts and flavourings;
- Synthetic nicotine: science, global legal landscape and regulatory considerations;
- Nicotine pouches: characteristics, use, harmfulness and regulation;
- Biomarkers of exposure, effect and susceptibility for assessing electronic nicotine delivery devices and heated tobacco products, and their possible prioritization;
- Internet, influencer and social media marketing of tobacco and non-therapeutic nicotine products and associated regulatory considerations; and
- The WHO Study Group on Tobacco Product Regulation: two decades of recommendations – translating evidence into policy action.

The sixth background paper was included to inform the future work of the Study Group in translating science into policy and will be considered separately by the Study Group. The report therefore includes the first five background papers. The requests of Members States in all WHO regions, the knowledge of the Secretariat and the Study Group in these areas and relevant literature formed the basis of the content of the five background papers. The information in these background papers updates current knowledge and will advance nicotine and tobacco product regulation and inform national and global policy.

The background papers were prepared by experts according to the terms of reference or an outline drawn up by the WHO secretariat for each paper and were reviewed and revised by TobReg members and by expert reviewers identified by WHO. The period of the literature search is indicated in each paper; for most, this was the second quarter of 2022 or the first quarter of 2023. The papers were subject to several rounds of review before and after the meeting by independent technical experts, the WHO secretariat, people in other relevant WHO departments, colleagues at regional offices and members of the Study Group before compilation into the technical report.

The secretariat, in consultation with the Study Group, invited experts who contributed to discussions and provided the most recent empirical scientific evidence and regulations on the topics under consideration. This ninth report of TobReg on the scientific basis of tobacco product regulation is designed to guide Member States in achieving the most effective evidence-based means to bridge regulatory gaps in tobacco control and to develop coordinated regulatory frameworks for tobacco products. Additionally, it identifies future areas of work, focusing on the regulatory needs of countries, thus providing a strategy for continued technical support to Member States. All experts and other participants in the meeting, including members of the Study Group, were required to complete declarations of interests, which were evaluated by WHO.

The report comprises this introduction to the context of the report, five papers on topics pertinent to tobacco control regulations and civil society organizations, and concludes with a summary of the recommendations in each section. The recommendations, which represent syntheses of complex research and evidence, promote international coordination of regulation and adoption of best practices in product regulation, and capacity-building for product regulation in all WHO regions, represent a ready resource for Member States, based on sound science, for implementation of the WHO FCTC by its Parties. Given the aggressive promotion of nicotine and tobacco products globally, the Study Group urges Member States to continue their focus on evidence-based measures to reduce tobacco use, as outlined in the WHO FCTC, and to avoid distraction by the tobacco and related industries.

This ninth report of the Study Group addresses additives that facilitate inhalation, synthetic nicotine, nicotine pouches, biomarkers for assessing ENDS, ENNDS and HTPs and social media marketing of tobacco and non-therapeutic nicotine products. It does not cover all the emerging issues in nicotine and tobacco product regulation, including flavours and design features such as filters and flavour accessories. The Study Group will continue to cover other aspects of product regulation, including other products of interest (such as waterpipes, cigarettes and smokeless tobacco) and other emerging issues that directly impact tobacco control in subsequent reports, guided by countries' regulatory requirements and pertinent issues in tobacco product regulation. The Group will thus ensure continued, timely technical support to all countries and address non-therapeutic nicotine and tobacco products broadly and factors with regulatory implications for product regulation, especially those that affect the attractiveness, addictiveness and toxicity of these products.

In summary, the outcomes of TobReg's deliberations and its recommendations will improve Member States' understanding of the evidence on the topics considered in the report, including synthetic nicotine, online marketing of tobacco products and nicotine pouches, contribute to the body of knowledge on product regulation, inform WHO's work, especially in providing technical support to Member States, and keep Member States, regulators, civil society organizations, research institutions and other interested parties up to date on product regulation through various platforms. Parties to the WHO FCTC will be updated by a comprehensive report to be submitted to COP10, via the Convention Secretariat, on technical matters related to implementation of Articles 9 and 10 of the WHO FCTC, which will include the messages and recommendations in this report. Thus, the Study Group's activities will contribute to meeting target 3.a of the Sustainable Development Goals: strengthening implementation of the WHO FCTC *(9)*.

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2. Additives that facilitate inhalation, including cooling agents, nicotine salts and flavourings

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Contents

Abstract

Objective: Some additives counteract the harshness and bitterness of the aerosols of tobacco and nicotine products (TNPs), making them easier to inhale. This is a problem for public health, as it may stimulate the uptake and continued use of TNPs, especially by young people. This paper provides a conceptual framework of the processes, mechanisms and methods for assessing inhalation facilitation (IF). Specific additives in TNPs that may promote IF are reviewed and their potential health impact discussed.

Methods: A targeted (non-systematic) search of PubMed and other bibliographic sources with no restrictions on time period, up to September 2022, included terms related to IF processes (e.g. "harshness", "puff duration"), candidate

additives (e.g. "menthol") or candidate mechanisms (e.g. "TRPM8 [transient receptor potential cation channel, family 8] receptor"). Inclusion of studies in the review was agreed by consensus by the two authors.

Results: We defined IF as a modification to a TNP that improves the user's sensory experience of inhaling the product's aerosol (reduced bitterness and harshness) and may alter inhalation behaviour, particularly more intense inhalation (e.g. deeper puffs, faster inhalation, larger puff volume) and also restoration of breathing patterns that are disturbed by inhaled irritants. The review showed that: (a) menthol and synthetic coolants decrease the irritation caused by nicotine and other TNP aerosol constituents by activating TRPM8 and other receptors and may promote dependence in inexperienced users; (b) acid additives and sugars, which yield acids upon combustion, lower the "pH" of TNP aerosol, resulting in higher levels of protonated nicotine, which is perceived as less harsh than free-base nicotine and may increase blood nicotine levels; (c) sweet flavourings in e-cigarettes reduce perceptions of bitterness and may escalate use, although their effects on perceived harshness are inconclusive; (d) sugars in tobacco impart sweet flavour sensations, but limited industry-independent data preclude a strong conclusion for IF; (e) some effects of additives on IF are amplified in non-smokers and younger populations; and (f) studies should be conducted on inhalation behaviour.

Conclusions: Several additives may facilitate inhalation of tobacco smoke and/or e-cigarette aerosol by improving the sensory experience. IF additives may increase nicotine blood levels, dependence and, in some cases, inhalation behaviour, especially in young people and non-smokers. Further research on the effects of TNP additives on sensory attributes and inhalation behaviour may provide useful evidence for regulatory policy.

Keywords: tobacco and nicotine products (TNPs), product attractiveness/appeal, inhalation facilitation, additives, cooling effects, pH lowering, masking bitter taste

2.1 Introduction

Research on internal tobacco industry documents has shown that cigarette manufacturers have manipulated product design, including appearance, flavour and smoke characteristics, to enhance their appeal and consumer acceptance *(1,2)*. The mechanisms included increasing nicotine delivery and facilitating smoke inhalation *(3,4)*. More than 100 cigarette additives have been found that camouflage the odour of environmental tobacco smoke emitted from cigarettes, enhance or maintain nicotine delivery, could increase the addictiveness of cigarettes, and mask undesirable sensory effects associated with smoking, such as irritation *(2)*. The products also include additives that may facilitate inhalation from tobacco and nicotine products (TNPs) such as e-cigarettes, cigars and hookah tobacco water pipes *(5,6)*.

Additives that facilitate inhalation may affect individual and population health in several ways. Especially for people starting use, nicotine and tobacco are aversive because of unpleasant sensory sensations, which can deter regular tobacco use *(7,8)*. Thus, for young people who try inhalable TNPs, additives may increase a product's attractiveness and thereby their odds of becoming a regular user, contributing to a higher prevalence of use. Furthermore, inhalation facilitation (IF) may promote nicotine addiction and the risk of long-term, heavy use *(7)*. Together, they constitute higher abuse liability. Increased inhalability of e-cigarettes may also, however, make them more satisfying nicotine substitutes for some adult smokers and encourage them to quit smoking and switch to vaping. Policy-makers have identified additives that facilitate inhalation of TNPs as key determinants of use and therefore potential targets for regulation *(8,9)*. A science-based framework to guide research and regulatory policy on IF from TNPs is, however, lacking.

This paper describes operationalization of IF in terms of effects, underlying mechanisms and studies addressing IF; reviews and weighs the evidence for a targeted set of additives that plausibly promote IF; and discusses the findings in terms of their potential health impact. First, we describe how nicotine and other sensory irritants decrease the inhalability of smoke, especially for novice users. Next, we propose a definition and conceptual model of IF, including factors other than additives. We also describe study designs in which IF of additives can be assessed. The subsequent section provides an overview of the categories of additives that facilitate inhalation and their effects in TNPs, with a focus on tobacco cigarettes and e-cigarettes (considered to be an inhalable nicotine product). Other inhalable products, such as heated tobacco products, were not included in our search, nor did we include other factors such as physical design (e.g. filter ventilation) that may also affect IF. Finally, we discuss our findings, identify gaps in the evidence and describe the potential impact of banning or setting upper limits on such additives and existing legislation on additives that facilitate inhalation.

2.2 Methods

A search was conducted in the bibliographic database PubMed and other sources (e.g. conference proceedings, general web search), with no restrictions on time period, up to September 2022 (with one exception, a paper in January 2023 with additional evidence on organic acids in e-liquids). A targeted (non-systematic) strategy was used that included search terms related to IF (e.g. "harshness," "puff duration"), candidate additives (e.g. "menthol") and candidate mechanisms (e.g. "TRPM8 receptor"). Papers were also obtained in exploratory "snowball sampling", in which the reference sections of articles were examined and potentially relevant articles were obtained and reviewed. As the review was not exhaustive, studies were included in the review by consensus between the co-authors. Priority was given to studies with stronger designs and greater relevance.

2.3 Nicotine and other sensory irritants that affect inhalability

Often, the first encounter with a tobacco product is unpleasant, as cigarette smoke contains numerous irritants that stimulate chemosensory nerves, leading to unpleasant burning and tingling sensations and reflex responses such as coughing, sneezing and avoidance *(7,10)*. As nicotine is the main irritant in tobacco smoke, many of these effects also occur with use of oral (such as nicotine chewing gum) and inhalable (such as e-cigarettes) nicotine products *(11)*. Nicotine also has rewarding effects in both humans and animals, even at low concentrations *(7,10)*. Nicotine activates brain systems that control reward by binding to nicotinic acetylcholine receptors (nAChRs) located within the mesolimbic dopaminergic pathway and the antinociception (pain reduction) system. Nicotine also elicits aversive sensory effects in the oral cavity and throat, including irritation, pain, a bitter taste, nausea and dizziness *(11,12)*. In response, smokers titrate their nicotine intake in order to experience the rewarding effects while avoiding aversion *(13)* by mixing smoke with air to allow inhalation without too much irritation *(11)*. Although the initial harshness of nicotine and tobacco is aversive, especially to novice users, and therefore can deter the uptake of regular tobacco use *(7,14)*, with repeated use, sensory stimuli that are paired repeatedly with the central effects of nicotine (unconditioned stimuli) can acquire motivational significance and promote smoking-related behaviour due to the association with a pending nicotine reward *(1,15)*. Sensory cues arise from various neural responses, including smell (via the olfactory nerve), irritation (trigeminal nerve) and taste (facial, glossopharyngeal and vagal nerves), and the cues may develop incentive value through a learnt association with the centrally mediated drug reward *(1)*. Other components of cigarette smoke, discussed below, can reinforce this effect.

The irritating properties and aversive bitter taste of nicotine are mediated mainly by activation of nAChRs located in nociceptive nerve endings, such as in the oral or nasal mucosa and lungs *(1,11,13,16)*. The nociceptors excite neurons in the trigeminal subnucleus caudalis and other brainstem regions *(11,12)*. Upon subsequent exposure, these neurons decrease firing, with desensitization of peripheral sensory neurons and progressively decreasing oral irritation *(12)*. Nicotine also elicits an nAChR-mediated bitter taste by excitation of gustatory afferents. In studies in rodents, the animals avoided nicotine solutions, even when sweeteners were added *(12)*.

Transient receptor potential (TRP) cation channels are involved in the local irritation and pain induced by nicotine, in particular the subfamilies TRPV1, TRPA1 and TRPM8, which are widely expressed in the human oropharynx and larynx *(1,11)*. Several compounds that target these TRP channels, such as menthol, can modify the oral irritation and pain elicited by nicotine *(7,11)*. TRPM5, a signal mediator in chemosensory cells and a key component of taste transduction, has been implicated in the bitter taste of nicotine *(17)*.

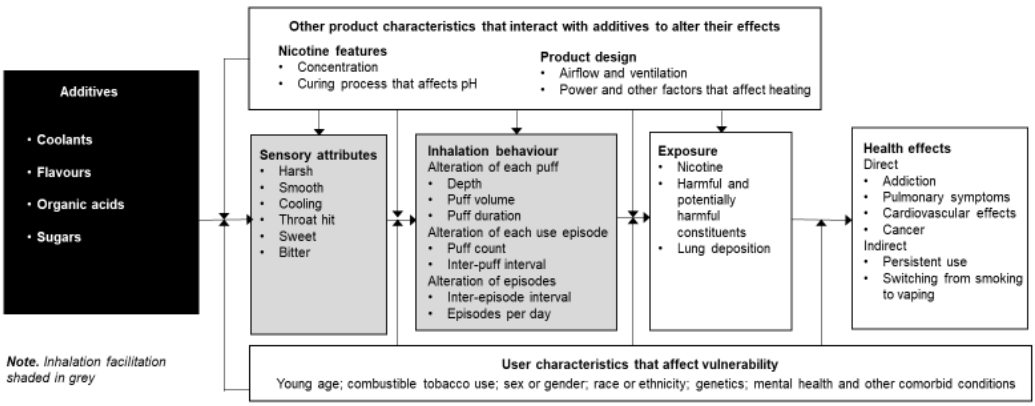
Other compounds in tobacco smoke are also involved in smoke-induced pain and irritation, such as reactive aldehydes (e.g. formaldehyde and acrolein), acids (acetic acid) and volatile organic hydrocarbons (cyclohexanone) *(7)*. For example, acrolein activates chemosensory nerves via the TRPA1 irritant receptor, and acetic acid and cyclohexanone probably act through acid-sensing ion channels, TRPV1 receptors and other classes of sensory receptors *(7,18)*.

2.4 Additives that facilitate inhalation

2.4.1 Definition and conceptual framework

We define IF as a modification to a TNP that improves the user's sensory experience of inhaling the product's aerosol (reduced bitterness and harshness) and may alter their inhalation behaviour (in particular, more intense inhalation [e.g. deeper puffs, faster inhalation, larger puff volume], but also restores breathing patterns that are disturbed by inhalant irritants). A conceptual model of IF is shown in Fig. 1 and described below, with supporting evidence reviewed in section 4.2. It should be noted that evidence is not available for all the factors in the conceptual model for all the additives reviewed. The illustration rather depicts the authors' proposal of the concepts involved in IF.

Fig. 1. Conceptual model of the effects of additives in facilitating inhalation and of the corresponding effects on health



Additives to TNPs (e.g. flavourings, cooling agents, organic acids, sugars) are the focus of this paper. Other factors can affect IF, including extraction of compounds from tobacco, the nicotine concentration and design manipulations (e.g. filter ventilation, airflow, heating element, curing process); however, these factors are not directly addressed.

IF processes

Additives can improve the sensory experience of inhaling TNP aerosol by affecting airway sensations (increased smoothness or coolness, reduced harshness or irritation, and a pleasant "throat hit"), potentially making the aerosol easier to inhale. Olfactory and oro-sensory features (increased sweetness and decreased bitterness) elicited by additives may peripherally promote IF by increasing the appeal of a product, so that more aerosol is inhaled. IF-related increases in inhalation include instantaneous effects, such as greater inhalation depth, volume, velocity and duration per puff. Such effects may be strongest for nicotine-naive users who are not accustomed to inhaling harsh and bitter TNP aerosols. For inexperienced and younger users, TNPs with desirable sensory features may also shorten the inter-puff interval and increase the number of puffs per use episode (e.g. lighting and then putting out a cigarette), because they may need less time to "recover" from sensory irritation between puffs. This could escalate use and dependence. For established daily TNP users with severe nicotine dependence who are used to maintaining nicotine blood levels and avoiding withdrawal symptoms, additives that promote IF may result in inhalation of more nicotine per puff, which could promote faster satisfaction per puff and reduce the number of puffs necessary to achieve nicotine satiation.

Consequences of IF

Altered inhalation behaviour may increase exposure in two ways. First, IF-related changes in inhalation behaviour can directly increase the total quantity of aerosol consumed per puff, per use episode and per day and deeper inhalation. Secondly, IF-related increases in inhalation may alter pulmonary deposition, allowing more nicotine absorption and rendering the exposed parts of the lung more vulnerable. Two reviews of the weight of evidence for a causal relation between filter ventilation and lung adenocarcinoma showed that deeper inhalation of cigarette smoke may increase the rate of adenocarcinoma *(19)*. IF-related increases in exposure can have numerous direct health effects, including on the cardiovascular and pulmonary systems and increased risks of various cancers.

Additionally, higher puff volume, shorter inter-puff intervals and deeper inhalation affect the rate and volume of nicotine delivered to the blood, which corresponds directly to the product's reinforcing effects *(20,21)*. The pleasant sensory attributes of a tobacco product also contribute to its reinforcing effects and increase reinforcement synergistically with nicotine *(22)*. A product's reinforcing effects are directly related to its addiction potential and the likelihood of persistent use. IF is harmful in any inhalable TNP for youth and adults who are not TNP users, as IF may stimulate uptake and continuation of TNP use. IF in e-cigarettes could, however, be useful for adult smokers who wish to switch to e-cigarettes. Additives that promote IF in e-cigarettes may increase their nicotine yield and

reinforcement and thus increase adoption and switching to e-cigarette products and cessation of tobacco smoking. In adults who switch completely from tobacco cigarettes to e-cigarettes, however, additives that promote IF in e-cigarettes might promote sustained vaping and potentially greater exposure to harmful constituents. Additional data on the net effect at population level are necessary.

Interaction with other products and user characteristics

The quality of a product's sensory attributes that promote IF may depend on user characteristics. On the one hand, youth and never-smokers may be deterred by harsh and bitter tastes, while additives that promote IF would suppress the deterrence. On the other hand, long-term adult smokers who wish to switch to e-cigarettes may seek products to replace the sensory attributes of cigarettes and provide a suitable throat hit and robust tastes. Hence, additives that suppress the bitterness and harshness of e-cigarettes may have less effect in promoting IF among smokers who are already accustomed to inhaling harsh, bitter tobacco smoke. Additional user characteristics (e.g. genetics, mental health, other comorbid conditions, race or ethnicity, sex or gender) may also affect their sensitivity to the sensory attributes of TNPs and their vulnerability to the effects of exposure to nicotine or harmful or potentially harmful constituents. Other product characteristics can interact with additives that promote IF by amplifying their effect on inhalation behaviour and on exposure and outcomes. For instance, additives that suppress the harsh, bitter taste of nicotine may have a particularly strong effect in e-cigarettes with a very high nicotine concentration.

2.4.2 Evidence review and integration

The literature on several classes of additives and their role in the IF processes depicted in the model is summarized below. We considered primary evidence of IF as that which demonstrated effects of additives on sensory experience and/or inhalation behaviour. Studies of IF-related mechanisms of action and the consequences of IF (biomarkers of exposure and health outcomes) were reviewed to provide supporting evidence for the biological plausibility and health significance of the IF scientific framework.

Additives and their effects and putative mechanisms are summarized in Table 1, which is based on the following types of evidence: (1) basic mechanistic studies of the effects of additives on the sensory and pain pathways that putatively underlie IF; (2) animal models of exposure to tobacco-product aerosol on IF-related sensory processes, exposure and inhalation behaviour; (3) human clinical laboratory experiments on the effect of self-administration of tobacco product aerosol with various additives on IF-related sensory processes, product appeal, exposure and inhalation behaviour; and (4) observational studies on whether use of products that contain additives is associated with altered inhalation behaviour.

Table 1. Classes of additives implicated in IF: mechanisms and effects

Mechanism	Additive	Reported sensory effects	Comments
TRPM-8 activation	Menthol	Increase cooling, reduce harshness of nicotine, minty flavour	Evidence available for both tobacco products and e-cigarettes Increased inhalation behaviour in rodents but inconclusive effects on inhalation behaviour in humans
	Wilkinson Sword (WS) compounds such as WS-3, WS-5, WS-14 and WS-23	Increase cooling, reduce harshness, reduce bitterness	Evidence predominantly for e-cigarettes Often combined with other flavours in "ice" hybrid flavours in e-cigarettes
pH lowering	Organic acids and nicotine salts in e-cigarettes	Increase mildness, reduce irritation	Higher blood nicotine levels
	Sugars in tobacco	Combust to acids, increase mildness	Mainly industry data
	Organic acids in tobacco	Increase mildness, decrease irritation	Mainly industry data
Olfactory and oro-sensory mechanisms	Flavourings with sweet properties	Increase sweetness, reduce bitterness, partial evidence of increased smoothness and reduced harshness	Predominantly in e-cigarettes, hookahs and cigars
	Sugars	Impart a sweet flavour	Predominantly in cigarettes; mainly industry data

2.5 Additives with cooling effects

2.5.1 Menthol

Menthol is a naturally occurring compound in the mint plant (*Mentha* spp.). It is used as an additive in various food, medicinal and cosmetic products and in TNPs. Menthol has been detected not only in "menthol flavoured" TNPs but also in TNPs that are not explicitly marketed as "menthol-containing" *(23)*. Menthol affects the central nervous system by activating nAChRs in the brain; however, its role in IF is mediated by its anti-irritant, cooling, analgesic properties *(24)*. The sensory effects of menthol are mediated mainly by its interactions with the TRP cation channel melastatin 8 (TRPM8) in cold-sensitive sensory neurons lining the airways and the TRP ankyrin 1 (TRPA1), a sensory irritant receptor *(12)*. Menthol may also have analgesic and cross-desensitizing properties, in which pre-treatment with menthol may reduce the irritating effects of nicotine, even after its acute cooling effect dissipates *(11)*. Evidence from studies in rodent models indicates that the effects of respiratory irritants in TNP aerosol can be suppressed by menthol, resulting in more frequent breathing, shorter inter-breath intervals and faster respiratory flow rate *(7,25)*. Rodent inhalation behaviour is an analogue of increased puff count, shorter inter-puff intervals and faster puff velocity associated with IF.

The US Food and Drug Administration (USFDA) conducted a comprehensive review of the literature on the effects of menthol in tobacco cigarettes, including human clinical experiments and observational studies *(24)*.

The conclusion was that menthol increases the palatability of cigarettes by masking the harsh taste of tobacco smoke and reducing aversive sensory responses associated with initial smoking experiences (e.g. irritation, coughing) and thus promotes continuation of smoking. The conclusion was strongest for the role of menthol in uptake and dependence in youth, difficulty in quitting smoking and a disproportionate impact on Black smokers *(24)*. For example, in one observational cross-sectional study, young adult smokers of menthol and non-menthol cigarettes, particularly African Americans, reported on their positive and negative subjective responses to smoking; greater positive subjective responses were associated with more frequent smoking *(26)*. The USFDA found mixed evidence for an association between menthol and dependence in adults and with measures of smoking topography *(24)*. As adults with an established smoking habit have strong preferences for certain brands of cigarettes and there is a natural selection bias for menthol flavours, it is difficult to draw strong conclusions from human clinical and observational studies of menthol-flavoured cigarettes in this population.

Several clinical laboratory experiments with e-cigarettes have shown that menthol increases perceptions of coolness and a pleasant taste *(27–31)*. Four studies showed that menthol interacts with nicotine to alter some of the sensory attributes or appeal of e-cigarette aerosol *(28–31)*. For example, a study of young adult e-cigarette users showed that menthol flavour interacted with nicotine at a concentration of 6 mg/mL to counteract the aversive sensory features of nicotine *(31)*. The study also showed that the direct, interactive effects of menthol with nicotine in increasing the appeal of e-cigarettes were more pronounced in vapers who had never smoked than in dual users or vapers who had previously smoked *(29)*. In a study of adolescents, however, no evidence was found that menthol e-cigarettes altered the effects of nicotine level on sensory attributes or appeal; menthol increased perceived coolness at two nicotine concentrations *(27)*. None of the studies indicated that menthol affected puffing behaviour or short-term exposure to nicotine *(27,30)*.

2.5.2 Synthetic cooling agents

Synthetic coolants, including compounds such as WS-3, WS-5, WS-14 and WS-23, have been detected in various types of TNPs. Tobacco industry documents show that, in the 1970s and 1980s, major tobacco manufacturers, including RJ Reynolds and Phillip Morris, tested but initially did not widely market tobacco cigarettes containing WS synthetic coolants *(32–34)*. Synthetic coolants have, however, been identified recently in cigarette products in Germany *(35,36)*. Synthetic coolants may be present in tobacco cigarette products with cooling features that are marketed as "non-menthol" in certain US markets in which menthol tobacco cigarette sales have been banned (e.g. California) *(37)*. They

have been detected in e-cigarette products in the past few years *(38,39)*, including products marketed as "ice" hybrid flavours that combine constituents with a fruit, mint or other characterizing flavour with the synthetic coolant (e.g. "raspberry ice"). Recent studies indicate that ice-hybrid-flavoured e-cigarettes that may contain synthetic coolants are commonly used by young people and young adults in the USA *(40–42)*, where sales have recently increased *(43)*.

Several synthetic coolants are based on the *p*-menthane structure of menthol. Like menthol, WS-3 and WS-23 are pharmacologically active at the TRPM8 cold receptors lining the airways and oral cavity *(7,16,25,44)*. Some evidence indicates that WS-3 is more active at TRPM8 receptors than menthol, generates stronger cooling sensations *(45, 46)* and may activate the sensory irritant receptor, TRPA1 *(47–49)*.

In view of the pharmacological properties of these synthetic cooling agents, in TNPs containing these compounds, they may generate cooling sensations that mask the harshness of nicotine without providing a strong minty flavour, unlike products that contain menthol *(41)*. Anecdotal reports by users on social media and online discussions indicate a substantial cooling effect of WS-23 or WS-3 in e-cigarettes, without the strong minty taste of menthol *(41)*. In a human laboratory study, administration of e-cigarettes flavoured with nicotine salt and with WS-23 (vs no cooling agent) to adult users of TNPs increased the e-cigarettes' appeal, smoothness and coolness and reduced their bitterness and harshness *(50)*. Additionally, e-cigarettes flavoured with WS-23 were perceived as smoother, cooler and less harsh than those with menthol. The effects of cooling agent additives did not significantly differ between fruit, tobacco or mint; 2% vs 4% nicotine concentration; or smoking status. The possible IF effects might explain why young adult users of ice-hybrid flavoured e-cigarettes reported more symptoms of nicotine dependence than with other flavours in an observational study *(40)*.

2.6 Additives that lower pH

The tobacco industry has conducted research on the effects of manipulating pH levels on tolerance to cigarette smoking *(3)*. In TNPs, the extent of nicotine absorption across membranes and nicotine-mediated harshness depend on the extent of nicotine protonation *(51)*. The fraction of protonated vs unprotonated (free-base) nicotine depends on the pH of the product and thus can be influenced by adding acidic or basic additives. In its free-base state, nicotine permeates membranes and is then converted to the protonated state, which is the ligand of nAChRs *(52–54)*. At pH > 7–12, above the physiological level, nicotine is present in a free-base form, which is more readily absorbed across membranes and also provides a stronger throat hit and is experienced as harsher. Free-base nicotine can be aversive especially at high concentrations, because it is absorbed preferentially

in the upper respiratory tract, causing irritation, whereas protonated nicotine is less irritating and hence can be inhaled more deeply, resulting in deposition deeper in the respiratory tract *(55)*. This results in greater net absorption of nicotine into the systemic circulation. Cigarette smoke is usually slightly acidic, with a pH of about 6, which makes the smoke less harsh and easier to inhale than smoking products with higher pH, such as cigars *(3,51)*. Once cigarette smoke reaches the pulmonary alveoli, nicotine leaves the smoke and, at the physiological pH of the lungs, is readily absorbed through the pulmonary capillaries into the systemic circulation *(51)* due to the larger absorptive surface of the lung at pH 7.4 and the high local buffering capacity of the lung *(56,57)*. This effect is expected only for inhalable products and not for products in which nicotine is absorbed in the oral cavity, such as nicotine pouches.

Protonated nicotine is thus less harsh and bitter on inhalation than free-base nicotine, so that high amounts of nicotine are more palatable *(5)*. Thus, at lower pH, overall nicotine delivery may be higher *(3)*. As the irritation due to the free base is largely attenuated, protonated nicotine is less aversive at high concentrations, increasing the attractiveness of the product. Furthermore, greater nicotine absorption, with faster, higher peak blood nicotine levels, probably predicts greater abuse liability *(51)*.

This mechanism and its consequences for sensory appeal and smoking behaviour are discussed below for acid additives that lead to nicotine salts (with protonated nicotine) in e-liquids. Other examples are also touched upon, such as laevulinic acid as an example of a tobacco additive and sugars in tobacco that result in increased acid levels in smoke upon combustion.

2.6.1 Organic acids in e-liquids

In the USA, marketing of Juul and similar e-cigarettes led to a rapid increase in e-cigarette use by young non-smokers *(58,59)*. These products contain high levels of aerosol nicotine, and the e-liquids contain protonated nicotine instead of free-base nicotine due to the addition of organic acids *(60)*. Several organic acids have been used in salt-based e-cigarettes, including lactic, salicylic, benzoic, laevulinic, ditartaric and maleic *(61)*. The effects of nicotine protonation on nicotine blood levels have been studied by several groups *(62–65)*. Some studies have shown that e-cigarettes with nicotine salt solutions, unlike e-cigarettes filled with free-base liquid, result in nicotine blood profiles similar to those of smokers of tobacco cigarettes *(62–65)*. Secondary data analysis of a randomized clinical trial of e-cigarettes also showed that smokers who switched to nicotine salt pod-style system e-cigarettes (similar to Juul) maintained their nicotine levels and transferred their dependence, suggesting that these products have a reinforcement potential similar to that of cigarettes and facilitate switching *(66)*. Observational data show that adolescents who

use Juul and other pod-style e-cigarettes that contain nicotine salts experience similar levels of nicotine dependence as adolescent smokers *(67)* but greater dependence than young users of other e-cigarette products that may not contain nicotine salts *(68)*, emphasizing the need to regulate access and marketing to this age group.

Thus, use of nicotine salts increases the addiction potential of TNPs *(64)*, and the effect increases with nicotine dose. A study funded by Juul Labs of liquids containing protonated nicotine showed that higher levels of protonated nicotine give rise to significantly higher plasma nicotine levels and relief from craving than lower levels *(69)*. A version of Juul produced in the European Union, with nicotine at 18 mg/mL, delivered less nicotine and reduced the urge to smoke or vape less strongly than tobacco cigarettes *(70)*. A comparison with the US Juul product, containing 59 mg/mL, gave similar results *(71)*.

Three studies were conducted to compare the sensory effects of nicotine salts with those of free-base nicotine. A randomized clinical trial in the USA showed that formulations containing salt nicotine at 24 mg/mL had significantly higher ratings than free-base nicotine for appeal, sweetness and smoothness and lower ratings for bitterness and harshness. The effects of nicotine salt on enhancing smoothness and reducing harshness were stronger in people who had never smoked cigarettes than in those who had ever smoked cigarettes *(72)*. Nicotine salts improved the sensory experience and thereby the attractiveness of vaping, particularly among never smokers unaccustomed to inhaling free-base nicotine. These findings are in accordance with observational data from England, which indicate that Juul, which contains nicotine salts, is more commonly used by never smokers than by current smokers, whereas tank devices, which typically include free-base nicotine, are more commonly used by current or former smokers, although other confounding factors (e.g. age) could explain the association *(73)*. A clinical laboratory study in the USA found that nicotine lactate and benzoate (protonated) e-liquids had greater appeal, smoothness and sweetness and less harshness and bitterness than free-base nicotine. There was some evidence that e-liquids that are highly protonated had stronger effects than e-liquids that were moderately protonated. The effects of nicotine formulation did not differ by tobacco use status or flavours *(74)*. In Netherlands (Kingdom of the), a study of home use showed no significant difference in scores for appeal, harshness and topography of nicotine salts versus free-base nicotine at a concentration of 12 mg/mL *(75)*. This is the only study of the effects of nicotine protonation state on topography. Apart from the lower nicotine levels, users in the Dutch study could vape freely, with monitoring of puffing parameters, whereas a set puffing protocol was used in the study in the USA.

2.6.2 Laevulinic and other organic acids in cigarettes

Many different acid additives have been used in the production of conventional cigarettes to increase their smoothness and decrease the throat hit *(56,76)*. For example, lactic acid has been used to decrease harshness and bitterness and produce a sweeter flavour. Citric additives have been used not only to reduce harshness and modify flavours but also to modify the pH of smoke and to neutralize the throat hit. Tartaric and lactic acids have also been used to modify the pH of smoke.

A review of internal tobacco industry documents indicated that laevulinic acid was used to increase nicotine yields while enhancing perceptions of smoothness and mildness *(3)*. Laevulinic acid reduces the pH of cigarette smoke and desensitizes the upper respiratory tract, promoting inhalation of cigarette smoke deeper into the lungs. Industry studies also found significantly increased peak plasma nicotine levels in smokers of ultralight cigarettes with added laevulinic acid.

2.6.3 Sugars

The pH of smoke can also be affected by sugars in tobacco. An industry document mentioned that harshness can be reduced by adding a suitable organic acid or by increasing the sugar level in tobacco *(76)*. In cigarettes, 0.5% of the sugars in the tobacco are transferred into mainstream smoke, where most is combusted, pyrolysed or pyrosynthesized *(77–79)*. Addition of sugars to cigarette tobacco has been reported to increase the acidity of smoke *(77,78,80)*; however, combustion of sugar during smoking results in acids that reduce the pH *(81)*, thus decreasing the harshness and irritation of the smoke *(82,83)*, increasing the palatability of the product and facilitating inhalation. Sugars have been referred to by the tobacco industry as "ameliorants", to "… smooth out harshness and bitterness and/or eliminate pungent aromas from tobaccos" *(84)*.

2.7 Additives with flavouring properties that may mask bitter taste

2.7.1 Flavourings with sweet features

Hundreds of flavouring constituents have been identified in various types of inhalable TNPs *(85,86),* many classified in categories that could be considered as having sweet features (e.g. fruit, mint, dessert) *(85,86)*. Given the wide variety of such constituents, it is difficult to identify one or several biological pathways for the effects of sweet flavourings. From a psychosensory perspective, there is some evidence that TNPs with sweet features may exert their effects via olfaction and not by their oro-sensory impact alone *(87,88)*.

Studies of the effects of flavours with sweet properties on the processes of IF provide some evidence of possible effects, but the results for specific outcomes are not consistent. A wide variety of additives with sweet elements identified in

tobacco products (e.g. carob bean extract, liquorice) may facilitate inhalation indirectly by pyrolysis of sugars *(9)*, as reviewed in more detail in section 4.5.2. A systematic review of qualitative studies indicates that sweet flavours in cigars, hookahs, e-cigarettes and cigarettes reduce perceptions of harshness and make the products more tolerable *(89)*. Flavours with sweet properties in e-cigarettes (e.g. fruit flavours) have been shown to reduce perceptions of bitterness in clinical experiments *(17)*. The results for an effect of sweet flavours in reducing harshness and increasing the smoothness of e-cigarettes are inconsistent *(31,90–92)*. There is also evidence that sweet flavours reduce the bitter-enhancing effects of nicotine in e-cigarettes *(31,90)*. In a laboratory clinical study of adolescent e-cigarette users, green apple e-cigarette flavour increased the acute puff count and puff duration to a greater extent than menthol or no flavour *(27)*. A longitudinal observational cohort study of adolescent e-cigarette users showed that sweet or fruit rather than menthol, tobacco or mint flavours were associated with more self-reported puffs per vaping episode 6 months later but not in the number of vaping episodes per day *(93)*; cross-sectional associations were not reported. In a cross-sectional observational population-based study of US residents, vaping of sweet-flavoured e-cigarettes was more common among adolescents and young adults than among older adults *(94)*.

Use of strawberry rather than tobacco flavoured e-cigarettes (19–20 mg/mL nicotine) was assessed in a clinical laboratory study of 14 adult e-cigarette users *(95,96)*. The effects were similar for the amount of nicotine inhaled and systematically retained, but, in the standardized 15-puff protocol, the plasma nicotine level was significantly higher. In an ad-libitum protocol, the average puff duration was significantly longer with the strawberry e-liquid than with the tobacco e-liquid. There were no differences in subjective measures of abuse liability between the two flavours. Although inferences are limited by the small sample size, this study provides some evidence that sweet-flavoured e-liquids may be associated with increased nicotine exposure and inhalation behaviour.

In a population-based observational study of biomarkers of exposure in the USA in 2015–2016 of 211 exclusive e-cigarette users who reported having used their product within the past 24 h, the biomarker for acrylonitrile was higher in users of fruit-only flavoured e-cigarettes than in users of any other non-tobacco flavour (mint, clove, chocolate or other); however, the concentration of acrylonitrile did not differ. Concentrations of biomarkers of exposure to nicotine (cotinine), benzene and acrolein did not differ significantly by flavour group *(97)*. Because this was an observational study, which did not account for differences in user behaviour (e.g. frequency of vaping), device or e-liquid (e.g. nicotine concentration), it is difficult to determine whether the biomarkers of exposure of users of different flavours were influenced by these external factors.

2.7.2 Sugars and sweeteners

In addition to their effect on pH (see 4.4.3), sugars also contribute to the flavour of TNPs *(78,83,98–100)* and e-cigarettes *(101,102)*. In smoked products, the sweet taste of caramel flavours generated by the combustion of sugars improves the taste and smell of the tobacco smoke for both users and bystanders *(78,82,103–105)*. Furthermore, during curing and smoking of tobacco, sugars can participate in Maillard reactions to produce flavouring that gives TNPs a characteristic woody, caramel and baking flavour *(15,106)*. One class of compounds resulting from sugars via Maillard reactions is pyrazines, which are also used as tobacco additives, especially in low-tar cigarettes with cocoa, nutty or popcorn-type flavours *(15,106)*. It has been hypothesized that they may reduce noxious sensations such as irritation in the upper airways or have chemosensory effects that reinforce the learnt behaviour of smoking *(15)*. Sweet flavours probably also lower a smoker's cough threshold. Rinsing the mouth with sucrose solution modulates sensitivity to the cough reflex, and it has been suggested that this is due to release of endogenous opioids in response to a sweet taste *(107)*.

Industry documents indicate that the acceptance of tobacco smoke by smokers is proportional to the sugar level in the tobacco, which could be due to their flavours and their effect on pH (see section 4.3.3) *(78,99)*. When the ratio between sugars and tobacco alkaloids such as nicotine is increased, the impact is decreased and "liking" increased to a certain optimum *(76)*. Addition of sugars to cigarettes to enhance the sensory attributes of cigarette smoke and encourage smoking initiation and maintenance have been discussed by industry as part of their marketing strategy *(108,109)*. In e-cigarettes, addition of sucralose, an artificial sweetener, increased overall flavour and sweetness but had no significant effect on harshness or irritation *(87)*. High-intensity sweeteners like saccharine and glycyrrhizin are also added to the mouthpiece and wrapper of tobacco products such as cigarillos *(110)*.

2.8 Discussion

2.8.1 Main findings

Taken together, the literature reviewed in this paper partially validates the proposed IF framework. We found evidence that several additives facilitate inhalation of tobacco smoke and/or e-cigarette aerosol, for example by providing more desirable sensory attributes. Evidence was also found that some additives that improve the sensory attributes of TNPs result in higher nicotine blood levels or maintenance of nicotine dependence. Few studies were found on the effects of additives on objective measures of puffing topography and inhalation behaviour. We found evidence for the biological plausibility of the framework in studies that showed that several additives impact pathways implicated in sensation and respiration.

These findings indicate that research on the effects of additives in TNPs on sensory attributes and inhalation behaviour may provide useful evidence for regulatory policy. This was particularly the case of studies of harshness and smoothness in humans and the biological pathways of airway irritation in animal models.

Menthol and synthetic cooling agents have been found to reduce aversive sensory responses to both tobacco cigarettes and e-cigarettes. The biological plausibility of their effect on IF is based on studies showing that the sensory effects of cooling agents are mediated mainly by their interactions with TRPM8 in the cold-sensitive sensory neurons lining the airways and by TRPA1. In a study of inhalation by rodents, menthol resulted in deeper inhalation of cigarette smoke and higher blood cotinine levels. The evidence for more intense or otherwise altered puffing behaviour in humans is, however, mixed. Direct experimental evidence of the effect of synthetic coolants was observed in one study of e-cigarettes. The similarity of the effects of synthetic coolants to those of menthol is biologically plausible, as they share an underlying mechanism, although synthetic coolants and menthol differ in potency, with potentially stronger effects of synthetic coolants on coolness and pleasant respiratory sensations. Some evidence indicates that the effect of menthol on IF is more robust in younger populations who are not regular tobacco cigarette smokers and who differ in other population characteristics (e.g. sex, race).

With regard to additives that lower pH, many studies suggest that acid additives in e-cigarettes facilitate inhalation of e-cigarette aerosol. While more research should be conducted, with lower nicotine levels, the available studies show that, at higher nicotine concentrations (> 20 mg/mL), protonation of nicotine (with organic acid additives) in e-liquids increases several IF processes over that with free-base nicotine at the same concentration, including more desirable sensory attributes, which, in turn, result in higher nicotine blood levels and maintenance of nicotine dependence. More information is needed on whether acid additives result in more intense or otherwise altered inhalation behaviour. It has been reported that acid additives and sugars, which yield acids upon combustion, lower the pH of cigarette smoke, and other studies indicate that such compounds decrease the harshness and increase the smoothness of tobacco smoke. Most of the evidence on the effects on human perception has been found in older internal industry documents. Thus, even though it is likely that similar effects as presented in section 4.3.1 for e-cigarettes will also be found in cigarettes, as they share the same mechanism, i.e. lowering the pH, additional and independent research is necessary. Some evidence indicates that the effect of pH on IF may be stronger for non-smokers and younger populations.

Additive flavours with sweet properties in e-cigarettes (e.g. fruit flavours) consistently reduce perceptions of bitterness, although evidence that they reduce perceptions of harshness and increase smoothness is inconclusive, as is evidence

on the relative contribution of olfactory and gustatory effects. Two studies with different designs found that adolescent use of fruit-flavoured e-cigarettes was associated with increased levels of most inhalation behaviour, including puff duration and count. In e-cigarettes, addition of sucralose, an artificial sweetener, increased the overall flavour intensity and sweetness but had no effect on harshness or irritation. Sugars have been reported to impart a sweet or caramel taste to cigarette smoke, but most of the evidence on effects on human perception was in older internal industry documents. Thus, even though it is likely that effects similar to those of sweet flavourings will be found, independent research is necessary. While some effect on desirable sensory attributes has been found for all additives with flavouring properties, data are lacking on effects on nicotine blood levels, maintenance of nicotine dependence and intensity of puffing.

2.8.2 Regulatory mechanisms in the European Union and North America for additives that facilitate inhalation

The European Tobacco Products Directive (TPD), Article 7.6.d, stipulates that European Union Member States shall prohibit the placing on the market of tobacco products for smoking and e-cigarettes containing additives that facilitate inhalation or nicotine uptake *(111)*. The TPD does not, however, provide a definition of IF or nicotine uptake facilitation. Belgium *(112)* and Germany *(113)* already prohibit use of menthol for its IF properties at any level, which is further supported by advice from the European Union Joint Action Tobacco Control *(114)*, which concluded that all menthol analogues, including geraniol, have a TRPM8-dependent cooling effect and may act cumulatively. As this effect is an intrinsic property of the compounds, products containing menthol and its analogues at any level do not comply with Article 7.6.d of the TPD, even if their level of application in tobacco does not induce measurable effects. Belgium has banned all activators of the TRPM8 thermoreceptor, and Germany has also banned other specific TRPM8 activators.

Canada does not permit use of additives with any flavouring properties, sweeteners, colouring agents or several other compounds that increase the attractiveness of tobacco products, although there are a few exceptions (guar gum, alcohol flavours) *(115)*. The USA has planned Federal product standards that would prohibit all characterizing flavours in cigarettes and cigars, including menthol *(8,116)*. Whether these regulations will extend to non-menthol synthetic coolants is unclear. The USA has no other specific product standards that ban other types of additives that may facilitate inhalation. The USFDA decides case by case on legal marketing of e-cigarettes and other novel products for each brand and product line. The US Tobacco Control Act stipulates that any regulatory decision take into consideration the impact on the population as a whole. Thus, regulatory restrictions should be designed to minimize TNP use by young people

and non-users and should, if possible, not deter adult smokers from quitting use of conventional tobacco products. To date, the USA has denied applications for numerous e-cigarette products marketed with characterizing flavours and sweet features on the basis of evidence that they attract young people *(117)*. Decisions on marketing of menthol-flavoured e-cigarettes are pending. The USFDA has authorized the marketing of several e-cigarette products that contain organic acids and protonated nicotine *(118,119)*.

2.9 Recommended research

To provide actionable evidence for regulatory decisions, a wider evidence base on most additives is necessary. In view of gaps in the evidence, we recommend the following:

- clinical studies of the effects of additives on inhalation behaviour, such as those measured by topography devices attached to TNPs;
- prospective longitudinal studies of users to determine whether use of TNPs with additives is associated with more pleasant sensory perceptions and/or increases in measures of inhalation behaviour;
- preclinical research with animal models to address specific questions that cannot be investigated in humans, such as the effect of introducing TNP-naive research subjects to TNPs with or without additives;
- comparisons of the effects of additives in e-cigarettes that promote IF in adult smokers, adult non-smokers and young people; and
- research on the IF of a wider range of products, other than tobacco cigarettes and e-cigarettes, including hookahs, cigars and heated tobacco.

Studies should also be conducted of products that contain possible inhalation facilitating additives, including specific brands and flavours, which could be triangulated with measures of additives in those brands. Survey instruments could be used to ask participants which flavours they use, the sensory attributes of their preferred product (e.g. how harsh it is), and, for e-cigarettes, the device type or nicotine formulation (salt or free base).

Research on potentially less harmful TNPs, in particular e-cigarettes, to determine the effects of additives on IF processes should include comparison of the effects on young non-smoking populations and older adult smokers. For example, additives in e-cigarettes that promote IF and adverse exposure in young non-smokers but do not encourage switching to e-cigarettes by adult smokers would be priorities for regulatory restrictions. For example, additives in e-cigarettes that promote IF and adverse exposure in young non-smokers but do not encourage switching to e-cigarettes by adult smokers would be priorities for regulatory restrictions.

Several fundamental aspects of what constitutes IF merit further research. It is unclear whether additives that promote sweetness or reduce bitterness directly increase inhalation behaviour or simply make products more attractive. Experimental studies in which the sweetness-enhancing or bitterness-reducing properties of additives (e.g. blocking olfaction, bitterness receptor knockout rodent models) on inhalation behaviour might be useful. It is unclear which study design is optimal for assessing whether additives increase inhalation behaviour. Studies of inhalation behaviour in established users are at risk of selection bias, because participants have pre-existing preferences. Animal models of inhalation behaviour in which exposure to TNP is controlled may be especially useful, although not in accordance with the ambition to limit studies in animals. Research should be conducted on whether increased inhalation is necessary or sufficient to increase exposure to nicotine and other harmful constituents and to increase the risk of adverse health outcomes, including addiction. Comparison of the effects of altered puff duration, count, velocity, volume, inter-puff interval and inter-episode interval on exposure and outcomes would be useful. Such research will indicate which sensory and inhalation behaviour outcomes are critical for inclusion in studies of the impact of new additives.

Testing might be conducted by asking research participants to use the product as intended and to report on their sensory experience during use. Studies with unblinded and blinded testing, in which the participant does not know the name of the product or see the marketing materials, to elicit subjective harshness, sweetness, coolness or other sensory attributes during self-administration of the product, might be valuable. Additional outcomes related to inhalation (e.g. puff duration, velocity, volume; inter-puff interval) would also be useful. Such data (with the scientific literature) could be triangulated with lists of ingredients and marketing materials to determine whether a product is in violation of a ban or product standard that restricts additives that promote IF.

2.10 Policy recommendations

We make the following recommendations to policy-makers on all inhalable TNPs.

- Ban ingredients that facilitate inhalation, as they facilitate use of inhaled tobacco products (cigarette, cigars, hookah, heated tobacco products or any other inhaled product containing tobacco). There is no justification for permitting the use of ingredients, such as flavouring agents, which make tobacco products more attractive.

The partial guidelines for implementation of Articles 9 and 10 of the WHO Framework Convention on Tobacco Control *(120)* state that, from the perspective of public health, there is no justification for permitting the use of ingredients

such as flavouring agents that make TNPs more attractive. The partial guidelines therefore recommend that "Parties should regulate all tobacco product design features that increase the attractiveness of tobacco products, in order to decrease the attractiveness of tobacco products". Consequently, given the WHO definition of attractiveness (factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use), policy-makers should ban ingredients that facilitate inhalation, which facilitates use of a product. Such a ban is included in the European TPD for smoked tobacco products and e-cigarettes, in Article 7.6.d *(95)*.

The evidence reviewed here provides support for the definition of IF that we propose for use by policy-makers to regulate additives permitted in TNPs: IF as a modification to a TNP that improves the user's sensory experience of inhaling the product's aerosol (reduced bitterness and harshness) and may alter inhalation behaviour (in particular, more intense [e.g. deeper puffs, faster inhalation, larger puff volume]) and also restoration of breathing patterns that are normally disturbed by inhalant irritants.

In addition to a general ban on ingredients that facilitate inhalation, it is recommended that policy-makers include a non-limited list of such compounds, particularly inhalable tobacco products. It is recommended that this list be included in legislation such that it can easily be adapted when new scientific insights necessitate addition of compounds to the list. A list of specific compounds would facilitate surveillance and enforcement. For example, Belgium *(112)* and Germany *(113)* already prohibit use of menthol for its inhalation facilitating properties at any level. Belgium then banned all activators of the TRPM8 thermoreceptor *(112)*. Another straightforward approach is to provide a list of additives that are permitted in TNPs that do not include any compound with IF effects, which is similar to the policy in Canada *(115)*.

It is recommended that ingredients that facilitate inhalation from conventional cigarettes be banned, as use of conventional cigarettes is not beneficial for any type of user, smoker or non-smoker. Legislation of e-cigarettes and other inhalable products that are potentially less harmful than conventional cigarettes may depend on each country's circumstances and policy aims. Policy-makers may consider effects at population level and weigh the evidence for whether additives that promote IF could make these products more satisfying nicotine substitutes for some adult smokers on the one hand and whether they increase appeal, risk of dependence and other adverse outcomes in young people and non-smokers on the other hand. The aim of the recommendations below is to prevent young people and never smokers from taking up any type of inhaled TNP, including e-cigarettes. Thus, we propose that all additives in all inhaled TNPs be banned when they facilitate inhalation.

- Ban the addition to TNPs of menthol at any level and also of both synthetic (e.g. WS) and natural (e.g. geraniol) coolant chemicals with similar chemical structure or physiological and sensory effects to avoid substitution.

A previous exhaustive review *(24)* provides sufficient evidence that menthol additives facilitate IF in tobacco cigarettes, and the conclusion is reinforced by the evidence in the current paper. We recommend that any regulatory agency that aligns its policies with the TPD and similar legislative frameworks impose a ban on the addition of menthol at any level to all inhalable TNPs. Chemicals with a similar chemical structure or similar physiological and sensory effects should be included in the ban to avoid substitution. These would include synthetic analogues such as WS compounds with cooling properties similar to those of menthol and natural compounds such as geraniol, which have similar properties and may also facilitate inhalation. In view of the evidence that coolants in e-cigarettes facilitate inhalation more strongly in younger populations and non-users of smoked tobacco products, regulatory restrictions on cooling agent additives for inhalable TNPs merit consideration.

- Ban nicotine salts in e-liquids at levels that exceed 20 mg/mL to protect children, adolescents and non-smokers. Setting minimal levels of pH in e-liquids and tobacco products would reduce the bioavailability of nicotine and reduce the addictiveness of products.

By triangulating evidence on additives that lower pH in e-cigarettes and to amplify their impact on IF in younger populations and non-smokers, regulators should consider banning acid additives and nicotine salts in e-liquids at nicotine levels > 20 mg/mL. While sufficient evidence is not available for nicotine levels < 20 mg/mL, regulators might consider banning such additives at any nicotine level as a precaution. Furthermore, setting minimal levels of pH in e-liquids could be a pragmatic application of regulation of these products. Measures to ban acid additives or acid-generating additives in cigarettes should also be considered. Some TNPs may have other additives or modifications that lower pH, such as certain tobacco leaf curing processes or sugar additives. Regulatory restrictions on inhalable tobacco products according to a pH threshold rather than the presence of a particular additive merit consideration.

- Ban all flavourings that impart a sweet taste, including sugars, in all TNPs.

Our findings on additives with flavouring properties that mask the bitter taste indicate that all flavourings that impart a sweet taste be banned, including sugars. In e-liquids, this refers to flavourings that facilitate IF at any level of addition,

including all constituents used in any e-cigarettes with non-tobacco characterizing flavours. In tobacco products, regulators may consider banning such flavourings at levels that impart a characterizing flavour other than tobacco, although flavourings may also have effects at concentrations below the threshold for a clearly noticeable flavour other than tobacco. Assessment of characterizing flavours requires sensory panels for surveillance and enforcement; a less time-consuming approach would be to ban addition of such flavourings at any level. Although the current paper focuses on additives that facilitate IF, natural tobacco leaves may also contain sugars and flavourings. For example, sugar is naturally present in many tobacco types. Regulators could also consider banning sugars and flavourings that are naturally present in tobacco, as they also impart a flavour. For the consumer, it is immaterial whether sugars or flavourings are added or naturally present.

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3. Synthetic nicotine: science, global legal landscape and regulatory considerations

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Contents

Preface: key findings

- Synthetic nicotine products – including nicotine pouches, e-liquids, disposable e-cigarettes, gums, toothpicks and infused combustible products – are marketed and sold throughout the world.
- Synthetic nicotine products are sold with marketing claims (e.g. "tobacco-free") that may suggest they are safer than products containing tobacco-derived nicotine, and some products are sold with flavour concepts (e.g. "chocolate dream," "pink lemonade") that are likely to appeal to young people.
- Synthetic nicotine is added to marketed products in two forms, S- and R-nicotine. S-Nicotine is the primary form of nicotine in tobacco plants. The pharmacological, metabolic and toxicological effects of R-nicotine and of mixtures and R- and S-nicotine, however, are poorly understood.

- No standard methods for the chemical analysis of synthetic nicotine are available, and adulteration of products with tobacco-derived nicotine is a concern.
- Whether synthetic nicotine products are regulated under current regulations for tobacco control depends on how the laws define the products covered by the regulations. Laws that apply only to "tobacco products" or "tobacco-derived" products may not be broad enough to cover synthetic nicotine products, because synthetic nicotine is not derived from tobacco plants.
- Tobacco companies are aware that some tobacco control laws do not cover synthetic nicotine products and have sought to take advantage of such regulatory gaps.
- Some countries have amended their tobacco control laws so that they apply to products containing nicotine that is not made or derived from tobacco, such as synthetic nicotine. The tobacco control laws of many countries do not, however, clearly apply to such products or do not apply to the full range of currently marketed products.

3.1 Introduction

Companies are increasingly marketing a wide range of synthetic nicotine products, which contain or are promoted as containing nicotine that is chemically synthesized rather than derived from tobacco plants. These products have not been shown to pose fewer risks than products containing tobacco-derived nicotine, although their marketing sometimes claims or implies that they do. In many countries, synthetic nicotine products are not clearly subject to current tobacco control regulations, although they may be subject to other laws, such as for consumer protection, in some countries. In other countries, however, tobacco control laws have been updated to cover these products in various ways. Countries should consider legal adjustments that they might make to close regulatory gaps for synthetic nicotine products, covering both the broad range of products currently on the market and those products that might emerge in the future.

3.1.1 Background

The rise of novel and emerging tobacco products, such as electronic nicotine and non-nicotine delivery systems, imitation tobacco products and nicotine pouches, has led to new forms of nicotine use, including by young people. The success of tobacco control measures and the social stigma associated with consuming conventional tobacco products (including cigarettes, cigars, waterpipe tobacco and smokeless tobacco products) contributed to motivating the industry to develop e-cigarettes and other novel products distinct from conventional products. Lately,

companies have begun to sell versions of these novel or unconventional products with claims that they contain synthetic rather than tobacco-derived nicotine *(1)*. These products are sometimes sold with flavours that appeal to young people. Additionally, although there is currently no evidence that products containing synthetic nicotine have different health effects from or are less addictive than products containing tobacco-derived nicotine, synthetic nicotine products are being sold with marketing claims that may suggest that they are safer than tobacco-derived nicotine products *(2)*.

The recent appearance of products promoted as containing synthetic nicotine or "tobacco-free" nicotine on many markets, including products sold worldwide over the Internet, has caused many WHO Member States to consider sharing regulatory information on this topic. Many Member States have requested technical assistance from WHO to address this issue and to provide a synthesis of the available evidence and authoritative advice on addressing products that are claimed to contain synthetic nicotine. This report, commissioned by WHO, was prepared to clarify those issues.

The report covers synthetic nicotine products marketed for recreational use, rather than for medical use, such as smoking cessation. It provides an overview of the types of synthetic nicotine products being sold, the claims with which they are marketed, and the science of synthetic nicotine production, toxicology, pharmacology and detection. It also provides information about the global legal landscape for synthetic nicotine products, focused on tobacco control laws. Specifically, we reviewed and coded the laws of 210 countries and the European Union (EU) Tobacco Products Directive on the Tobacco Control Laws website (www.tobaccocontrollaws.org). Of the 211 jurisdictions, 21 did not have a law or had no English translation. In 52 of the remaining 190 jurisdictions, the laws provided definitions broad enough to cover at least certain synthetic nicotine products (e.g. e-cigarettes but not other synthetic nicotine products), 29 provided definitions that covered a broader range of synthetic nicotine products, 92 had definitions that did not apply to any type of synthetic nicotine product, and in 17 jurisdictions, it was unclear whether the laws cover synthetic nicotine.

3.1.2 Types of synthetic nicotine products

News reports *(3)* suggest that the United States of America (USA) is currently the largest market for synthetic nicotine products, although this may change as a result of an amendment to US law in March 2022 that brings synthetic nicotine products within the purview of tobacco products authorities of the US Food and Drug Administration (USFDA). The second largest market is that of the Republic of Korea *(3)*. Currently, most products marketed as containing synthetic nicotine are either e-cigarettes, e-liquids or nicotine pouches. These are not, however, the only kinds of synthetic nicotine products being sold *(2)*. For example, several

companies sell chewing-gum products described as containing synthetic or "tobacco-free" nicotine *(4,5)*; at least two companies are marketing synthetic nicotine toothpicks *(6,7)*; and a Canadian company, PODA, announced plans in 2021 to launch a "heat-not-burn product" containing "pelletized tea leaves infused with synthetic nicotine" *(8)*. This company has since been purchased by Philip Morris, and it is not clear whether its products will be marketed. At least one company, Outlaw Dip, is offering "100% tobacco free" moist snuff "that does NOT come from tobacco" *(9)*, and at least one other company, Ronin, is selling a combustible cannabidiol cigarette infused with "non-tobacco nicotine" *(10)*. There is, therefore, a wide variety of products sold as containing synthetic rather than tobacco-derived nicotine, and new types of products may continue to emerge.

Additionally, many of these synthetic or "tobacco-free" nicotine products contain flavours that are likely to appeal to young people. For instance, some toothpicks are sold with flavours such as "butterscotch cake" and "strawberry cheesecake" *(7)*, and certain disposable e-cigarettes are sold with flavour concepts such as "banana ice" and "blue razz" *(11)*.

3.1.3 Marketing and promotion of synthetic nicotine products

Many companies that market synthetic nicotine products make claims that may suggest, implicitly or explicitly, that their products are "safer" than products containing tobacco-derived nicotine. These include claims that synthetic nicotine contains fewer impurities than tobacco-derived nicotine and that synthetic nicotine is equivalent to pharmaceutical-grade nicotine. Companies also claim that synthetic nicotine products have other advantages over products with tobacco-derived nicotine, such as that they provide more satisfaction and a better taste experience and that they are more environmentally friendly. Some synthetic nicotine products are marketed as effective aids for smoking cessation or as equivalent to approved nicotine replacement therapy, sometimes with a disclaimer that the product is not a smoking cessation product. Table 1 provides a few examples.

Table 1. Examples of promotional claims about synthetic nicotine products

Product	Owner or manufacturer	Claim
Juice Head pouches	Juice Head (USA)	"…may offer higher nicotine satisfaction with potentially less risk than tobacco-derived nicotine. In addition, while tobacco nicotine often features a strong pungent odor and taste, synthetic nicotine is virtually tasteless and odorless." "…it is important to note that tobacco cultivation (which is commonly very subsidized) can be very damaging to the environment and is often a process that is highly labor-intensive, cumbersome, and wasteful." "It should be noted that tobacco-derived nicotine may come along with more risks of side effects than pouches made without tobacco." (12)

Pacha Mama vape pen	Charlie's Holdings, Inc. (USA)	"increased purity and consistency over traditionally harvested nicotine" (13)
Outlaw Dip	Outlaw Dip Company (USA)	"pharmaceutical grade" (9)
Bidi Pouch	Kaival Brands Innovations Group, Inc. (USA)	"aims to help adult smokers take their first steps in going smokeless" (14)
ZIA gum	Next Generation Labs LLC (USA)	"the Only Nicotine Gum Developed with Synthetic Nicotine" (4) "offers the same nicotine satisfaction as any tobacco-derived product containing nicotine" (4) and "ZIA™ gum is not intended to assist in quitting efforts" (15)
VaporX e-juice and disposable e-cigarettes	Vaporex Co., Ltd (Republic of Korea)	"We are committed to protecting the health of smokers by providing them with a valuable and appropriate vaping experience" (16)

3.2 The science of synthetic nicotine

The rapid, poorly regulated introduction of synthetic nicotine products (electronic cigarettes, oral pouches and other product categories) in the USA and other countries raises questions about their safety and potential differences in the addictive and reinforcing properties of synthetic nicotine. In this section, we review the strategies for chemical synthesis, the different forms of synthetic nicotine in products, the manufacturers and patent landscape, and the toxicological, pharmacological and metabolic properties of synthetic nicotine.

3.2.1 Methods

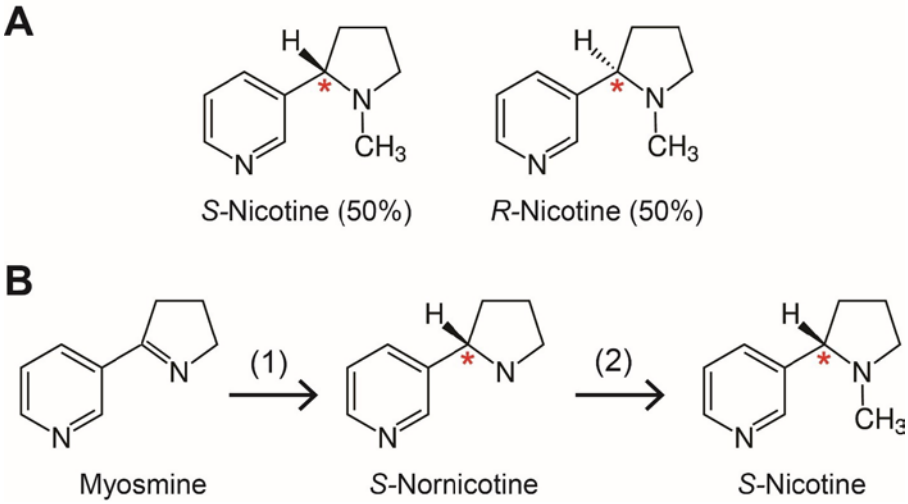
Research databases, including PubMed and Web of Science, were searched with terms such as "synthetic nicotine", "*R*-nicotine", "(+)-nicotine", "L-nicotine", "D-nicotine", "racemic nicotine" and "nicotine synthesis" for journal articles on synthetic nicotine and studies of the effects of nicotine enantiomers (defined below). Patents were sought on patents.google.com with combinations of terms such as "nicotine" "synthesis" and/or "stereoselective", "enantioselective". The tobacco legacy database at www.industrydocuments.ucsf.edu/tobacco/ was searched with terms such as "synthetic nicotine", "nicotine synthesis", "synthesis of nicotine" and "*R*-nicotine".

3.2.2 Results

Synthetic nicotine: what is it and how it differs from tobacco-derived nicotine

Nicotine exists in two chemical forms that are structural mirror images. The two forms, termed enantiomers, are *S*- and *R*-nicotine (Fig. 1A). Nicotine in tobacco plants consists of > 99% *S*-nicotine and only minimal amounts of *R*-nicotine *(17).* Chemists first synthesized nicotine in 1904 *(18),* resulting in a mixture containing both *S*- and *R*-nicotine in a 50:50 ratio *(18,19)*, known as a racemic mixture. This mixture differs from tobacco-derived nicotine in that it has a much higher *R*-nicotine content and a lower *S*-nicotine content.

Fig. 1. Structure and chemistry of synthetic nicotine



(A) Structures of *S*- and *R*-nicotine. The compounds differ in their configuration at the carbon atom labelled with a red asterisk, a chiral centre. In tobacco leaf, > 99% of nicotine is present as *S*-nicotine. Synthetic "tobacco-free nicotine" marketed by Next Generation Labs is racemic, containing 50% *S*-nicotine and 50% *R*-nicotine. Pure synthetic *S*-nicotine is chemically indistinguishable from *S*-nicotine purified from tobacco.
(B) Synthesis of *S*-nicotine as described in a patent assigned to Zanoprima involving a biotechnological step. The starting material is myosmine, which is first converted to *S*-nornicotine with a recombinant enzyme *(1)*, a NADH/NADPH-dependent imine reductase by a stereoselective reaction. *S*-Nornicotine is then converted to *S*-nicotine by methylation *(2)*.

A search in the Truth Tobacco Industry Documents (the database of tobacco industry internal corporate documents compiled during litigation in the USA) with the term "synthetic nicotine" revealed that the industry had already considered use of synthetic nicotine in the 1960s. Employees of British American Tobacco proposed addition of synthetic nicotine to increase the nicotine:tar ratio in combustible cigarettes *(20)*; however, the proposal was not pursued further because of concern that synthetic nicotine was available only as a racemic mixture, with unknown health effects. Furthermore, the price of synthetic nicotine was much higher than that of tobacco-derived nicotine *(20)*. Employees of RJ Reynolds and Liggett & Myers also considered use of synthetic nicotine to adjust nicotine levels in cigarettes; however, the idea was abandoned for the same reasons *(21,22)*. The Truth Tobacco Industry Documents database provides no further evidence after 1978 of consideration of the use of synthetic nicotine use by the major US tobacco companies. Subsequently, chemists developed new strategies for synthesizing nicotine, including methods to produce pure *S*-nicotine, the form of nicotine prevalent in tobacco leaf *(19,23)*.

The synthetic nicotine marketplace: manufacturers, patents and pricing

In 2015, the company Next Generation Labs (NGL) began marketing synthetic nicotine in the USA under the trademarks TFN® (Tobacco Free Nicotine) for consumer products and PHARMANIC® for pharmaceutical products. In the same year, NGL filed an application for US and world-wide patents with the title "Process for the preparation of (RS)-nicotine" *(24)*. The US patent, assigned to NGL in 2017, describes a synthetic pathway with ethyl nicotinate as the starting material. Ethyl nicotinate is derived from nicotinic acid (niacin), a synthetic chemical produced from petrochemical sources. It is reacted with *N*-vinyl-2-pyrrolidinone to form myosmine, a tobacco alkaloid. Myosmine is then converted to nornicotine. Subsequent methylation of nornicotine results in a racemic (50:50) nicotine mixture of *S*- and *R*-nicotine (Fig. 1A) *(24)*. NGL also filed a patent for use of their synthetic nicotine in smoking cessation products *(25)*. In 2016, Hellinghausen et al. analysed the nicotine content of electronic cigarette liquids marketed in the USA and containing TFN-branded synthetic nicotine manufactured by NGL and confirmed that the product is racemic nicotine *(26)*. While vaping products containing synthetic nicotine have been marketed in the USA since 2015, they attracted public attention only in 2021, when the popular vaping company Puff Bar announced a switch to synthetic nicotine in their products *(27)*. Analysis of these products showed that they contained racemic nicotine *(28)*. The source of the synthetic racemic nicotine in Puff Bar products has not been revealed.

At the same time, advances in chemistry resulted in optimization of strategies for manufacturing pure *S*-nicotine. Several companies have filed patent applications for the synthesis of *S*-nicotine. Contraf-Nicotex-Tobacco (Germany), the world's largest supplier of pharmaceutical-grade nicotine, developed a process for synthesizing racemic nicotine from ethyl nicotinate and *n*-vinylpyrrolidone nicotinic acid; subsequent selective purification enriches the compound to produce pure *S*-nicotine *(29,30)*. Vaping products containing Contraf-Nicotex-Tobacco's synthetic *S*-nicotine have been marketed in the USA since 2020 *(31,32)*. Zanoprima Life Sciences Ltd (London, United Kingdom) also manufactures synthetic *S*-nicotine *(33)* and was granted a US patent for a process involving a biotechnological step for the synthesis of *S*-nicotine in 2021 *(34)*. The starting material is myosmine, which is first converted to *S*-nornicotine with a commercially available recombinant enzyme, an NADH/NADPH-dependent imine reductase. *S*-Nornicotine is then converted to *S*-nicotine by methylation (Fig. 1B). This product is currently marketed under the brand name SyNic *(33)*. Hangsen International Group, a major manufacturer of vaping devices and e-liquids, applied for a Chinese and a world patent for a similar process and markets synthetic *S*-nicotine under the brand name "Motivo" *(35,36)*. NJOY, a major Ecigarette manufacturer (soon to be owned by the cigarette-maker Altria *(37)*), was also awarded a patent for nicotine synthesis and purification *(38)*. Some

patents describe the resulting nicotine as "> 99.9% pure", with a chiral purity of > 99.6% *S*-nicotine or more. Wholesale products are listed as having a purity of 99.9% *S*-nicotine *(39)*.

In 2019, a representative of NGL stated that the company's synthetic racemic nicotine product, the racemic mix of *R*- and *S*-nicotine, "is only three to four times the current cost of tobacco-derived nicotine" *(40)*. As of 21 March 2023, the wholesale price of 1 L of NGL TFN racemic synthetic nicotine was quoted as US$ 1800, while the same wholesaler offered 1 L of tobacco-derived nicotine for US$ 229.99–429.99, depending on the brand. Thus, the price of the synthetic version is four to eight times higher than that for tobacco-derived nicotine *(41,42,43)*. Zanoprima's SyNic synthetic *S*-nicotine was marketed at a price of US$ 999.99 per litre, while the same seller quoted a price of US$ 229.99 for tobacco-derived nicotine, a difference of about four times *(39, 44)*. Thus, although the price of synthetic nicotine remains substantially higher than that of tobacco-derived nicotine, synthetic nicotine products continue to be marketed, including electronic cigarette products and oral nicotine pouches, also known as "white snus". These products are often advertised with claims that they are purer and healthier than products containing tobacco-derived nicotine.

Manufacturers of synthetic nicotine (Table 2) have begun to enforce their intellectual property, leading to legal conflicts and market consolidation. NGL's intellectual property was recently confirmed by Chinese authorities, enabling the company to enforce its patents in the country, where the large majority of e-cigarette products are manufactured *(45)*. Zanoprima sued a major ecigarette and liquid manufacturer, Hangsen, for infringement of its patent in a US district court *(46)*. Nicotine manufactured by Hangsen was added to "Geekbar" products marketed in the USA in 2021; however, Hangsen ceased marketing its "Motivo"-brand synthetic *S*-nicotine in the USA after the lawsuit was filed, while continuing sales outside the USA *(35,47)*.

Table 2. Major manufacturers of synthetic nicotine and their synthesis routes

Manufacturer	Starting material	Product	Stereoselective step
Next Generation Labs LLC (NGL)	Ethyl nicotinate	Racemic (50:50) *R*-:*S*-nicotine	Not applicable
Contraf-Nicotex-Tobacco	Ethyl nicotinate	*S*-Nicotine	Stereoselective recrystallization
Zanoprima Lifesciences Ltd	Myosmine	*S*-Nicotine	Enzymatic stereoselective step
Hangsen International Group	Myosmine	*S*-Nicotine	Enzymatic stereoselective step
NJOY LLC	Racemic (50:50) R-:*S*-nicotine	*S*-Nicotine	Stereoselective recrystallization

Health claims by manufacturers of synthetic nicotine

Like the sellers of ENDS, the companies that manufacture synthetic nicotine promote their products with health-related statements. NGL claims that "TFN is devoid of many of the residual impurities that tobacco derived nicotine contains

... TFN is virtually tasteless and odorless … there is no need to mask the off-flavor and aroma of tobacco-based nicotine" *(48)*. NGL also claims that "specific ratios of the 'R' to the 'S' isomers could potentially offer nicotine use at satisfying but non-addictive or less addictive levels". Contraf-Nicotex-Tobacco opposes this notion, claiming that its synthetic *S*-nicotine is superior to the racemic version, stating "If you look at the European and the US pharmacopoeias, the percentage of *S*-isomers in nicotine must be higher than 99 percent" *(40,49)*. Zanoprima claims that its synthetic *S*-nicotine "is free of related tobacco alkaloids, TSNAs [tobacco-specific nitrosamines], odour, and harsh taste" *(33)*. These statements may represent claims that their synthetic nicotine has superior, drug-like properties. Companies also claim that they use a sustainable "green chemistry" approach for production that is environmentally more friendly than agricultural tobacco production, which requires pesticides, fertilizers, extensive land use and hazardous production methods.

Toxicological, pharmacological and metabolic properties of synthetic nicotine

As described above, there are currently two forms of synthetic nicotine in marketed products, *S*-nicotine and racemic nicotine, the latter consisting of 50% *S*-nicotine and 50% *R*-nicotine. As synthetic *S*-nicotine is chemically identical to tobacco-derived *S*-nicotine, its toxicological, metabolic and pharmacological properties should also be identical, especially if they are added at the purity claimed by the major manufacturers of synthetic products (> 99.9%). Nevertheless, even at this high degree of purity, trace amounts of other chemicals remaining from the chemical process might be present, which deserve further attention.

If a consumer uses a product containing synthetic racemic nicotine, 50% of their nicotine intake is *R*-nicotine. Less is known about *R*-nicotine's toxicological, metabolic and pharmacological effects than about those of *S*-nicotine. A study in mice established that the dose necessary to have a lethal effect in 50% of the animals (LD_{50}) 60 min after intravenous injection was 0.33 mg/kg for *S*-nicotine and 6.15 mg/kg for *R*-nicotine, which is > 18 times higher, suggesting that *R*-nicotine is less acutely toxic than *S*-nicotine under those conditions *(50)*. The study also established that a higher dose of *R*-nicotine than of *S*-nicotine is necessary to induce convulsions.

Pharmacological studies have shown that *R*-nicotine is about 10 times less potent as an agonist of nicotine receptors than *S*-nicotine *(51)*. A study of nicotine binding in the brain showed that *S*-nicotine is 10 or more times more potent than *R*-nicotine *(52)*. Long-term administration of either form of nicotine was shown to increase the number of nicotine binding sites in rat brain *(53)*.

In an operant behavioural study of the capability of rats to discriminate injected *R*- or *S*-nicotine from saline, *S*-nicotine was nine times more potent than *R*-nicotine *(54)*. A study to characterize the locomotor stimulant action

of nicotine in rats showed that *S*-nicotine was at least 10 times more potent in stimulating motor activity *(55)*. *S*-Nicotine was four to five times more potent than *R*-nicotine in conditioned taste aversion assays in rats *(56)*. In contrast to *S*-nicotine, *R*-nicotine did not induce weight loss in rats and did not trigger epinephrine release *(51,53)*. Pharmacological studies of the enantiomers in standard experimental paradigms for nicotinic pharmacology showed that *S*-nicotine, the prevalent nicotine enantiomer in tobacco (> 99%), is 4–28 times more potent than *R*-nicotine, which is present at high levels (50%) in synthetic racemic nicotine *(51,52,54,55,57,58,59,60)*.

S- and *R*-nicotine also differ in their metabolism. Studies in guinea pigs showed that *S*-nicotine formed only oxidative metabolites, whereas *R*-nicotine formed both oxidative and *N*-methylated metabolites *(61)*. The degradation kinetics of the resulting *S*- and *R*-cotinine also differed. Studies of metabolism in various laboratory animal species showed strong differences between *S*-nicotine and *R*-nicotine in degradation and excretion and also sex differences in *R*-nicotine metabolism *(60,61,62)*. Species differences were also observed in *N*-methylation of *S*- and *R*-nicotine in human, rat and guinea pig liver cytosol extracts *(63)*. While the human extract catalysed *N*-methylation of both forms of nicotine, rat extract did not form any *N*-methylation products, and guinea pig extract transformed only *R*-nicotine and not *S*-nicotine *(63)*. It is not known whether these *N*-methylation products are bioactive and whether *S*- and *R*-nicotine methylation products act differently. These findings indicate that human metabolism of *R*-nicotine and its behavioural effects should be investigated further, and that predictions of the toxicological outcomes of *R*-nicotine consumption should not be based on animal models alone. The absence of such key data and the observed species differences preclude assessment of the toxicological risk of *R*-nicotine to humans.

In addition to the differences in nicotinic receptor-mediated pharmacological effects, *R*- and *S*-nicotine have differential effects on other pharmacological targets. For example, a tobacco industry-sponsored study on acetylcholinesterase, the enzyme that degrades the neurotransmitter acetylcholine in the synaptic cleft to terminate neurotransmission, revealed that *R*-nicotine is a more potent inhibitor of the enzyme than *S*-nicotine, binding to a different site on the enzyme protein *(64)*. The experiments were performed with acetylcholine esterase isolated from electric eels and at nicotine concentrations much higher than those received by smokers. Whether such effects occur in humans and how they affect acetylcholine levels and neurotransmission should be studied further. Both forms of nicotine interfere with the production of certain lipid mediators involved in regulation of inflammation, with similar potency, showing that some biological processes are equally affected by the two forms of nicotine *(65)*.

Psychophysical studies

Psychophysical studies were conducted to determine whether *R*- and *S*-nicotine elicit different odour or irritant sensations. People perceive nicotine vapour as aversive when they are exposed through the nose. At higher concentrations, nicotine vapour causes nasal irritation, including stinging and burning sensations, mediated by the trigeminal nerve, which transmits pain signals to the brain. Test subjects reported lower thresholds for detection of *S*- than for *R*-nicotine and greater burning and stinging intensity, while olfactory perceptions were elicited at similar levels. In electrical recordings of mucosal potential, *S*-nicotine elicited stronger responses than *R*-nicotine. Smokers perceived *S*-nicotine as more hedonic than non-smokers, probably because of previous experience *(66)*. This appears, so far, to be the only systematic study of human responses to *S*- and *R*-nicotine. The experiments were very short, as individual vapour stimuli were applied for only 250 ms.

Analytical detection of synthetic nicotine

Hellinghausen et al. *(26)* developed a method to validate the presence of synthetic racemic nicotine in vaping products labelled as containing the compound. They used a chiral stationary phase for separation of *R*- and *S*-nicotine by high-pressure liquid chromatography, followed by circular dichroism detection and electrospray ionization mass spectrometry. They reported that one product contained twice more total nicotine (sum of *R*- and *S*-nicotine) than the content stated on the product label, effectively listing only the strength of *S*-nicotine, while the nicotine content listed on other labels was equivalent to that measured, half of which was *S*-nicotine. These observations suggest that uniform product labelling practices should be imposed by regulators, to prevent unknowing users from exposure to higher levels of *R*-nicotine or to lower levels of *S*-nicotine than they are used to. Inappropriate labelling of nicotine content could motivate consumers to purchase products with a higher total nicotine content, potentially resulting in significantly higher *S*-nicotine intake. The authors also detected impurities that require further characterization *(26)*. Analysis of Puff Bar vaping products for the presence of *S*- and *R*-nicotine by ^{1}H-nuclear magnetic resonance spectroscopy, polarimetry and gas chromatography–mass spectrometry (GC/MS) confirmed the presence of both nicotine forms, but a slightly higher content of *S*-nicotine than *R*-nicotine. The authors speculated that the manufacturer might have added tobacco-derived nicotine, although further analysis would be necessary *(28)*.

Several methods have been proposed to differentiate nicotine derived from tobacco from synthetic nicotine. As synthetic *S*-nicotine is now available at high purity, it is difficult to differentiate the two; as the compounds are chemically identical, they cannot be differentiated by standard analytical techniques. Carbon isotope analysis has been proposed as a solution. Carbon has three isotopes,

^{12}C, ^{13}C and ^{14}C. ^{14}C has a half-life of 5700 years, a property that is used in radiocarbon dating of biological materials. ^{14}C is constantly replenished in the atmosphere by the sun's radiation and is then integrated into living plant matter, including tobacco plants and their natural products, such as nicotine. Synthetic nicotine is produced from petrochemical precursors that were formed in the earth millions of years ago and have a much lower ^{14}C content. For example, a ^{14}C analytical method has been developed to differentiate between natural and fossil chemical-derived vanillin, a popular flavour chemical *(67)*. Depending on the metabolic pathways involved, natural products may also contain a higher ratio of ^{13}C:^{12}C. High-temperature liquid chromatography coupled with isotope ratio mass spectrometry ("HT RPLC/IRMS") has become the standard approach for identifying foods adulterated with synthetic additives and can be used to differentiate between natural and synthetic caffeine, ethanol, sugars and other chemicals *(67,68)*. Cheetham et al. *(69)* used a ^{14}C method to compare samples of tobacco-derived and synthetic nicotine and found that the tobacco-derived samples contained 100% "modern" biocarbon, such that their carbon isotope distribution is identical to the current distribution in the earth's atmosphere. The synthetic nicotine samples contained only about 35% biocarbon, indicating that some natural precursors were probably used in their synthesis. The commercial synthetic nicotine preparations tested were found to be of high purity (> 99.9% nicotine content), containing only minor amounts of nicotine derivatives and degradants, fulfilling the US Pharmacopeia criteria for pharmaceutical-grade nicotine *(70)*. The commercial purified tobacco-derived nicotine samples were of similarly high purity, also fulfilling the US Pharmacopeia criteria for pharmaceutical-grade nicotine. The authors also devised methods to identify products containing mixtures of synthetic and tobacco-derived nicotine and a method for purifying nicotine from electronic cigarette liquids, an essential first step in the analysis of marketed products to detect the presence of carbon-based solvents (propylene glycol, glycerol), flavour chemicals and other additives in marketed products.

Qualitative and quantitative methods for analysis of hydrogen isotopes (hydrogen and deuterium) and nitrogen isotopes (^{15}N) in nicotine also revealed substantial differences between tobacco-derived nicotine obtained from various locations and from synthetic nicotine *(71,72)*. Thus, while significant advances have been made in analytical methods to discriminate synthetic from tobacco-derived nicotine, no standard method is yet available. The instrumentation and skills necessary to apply such methods are costly, and few countries have such capability.

3.2.3 Summary and discussion

Manufacturers have developed several methods for more efficient, more economical production of synthetic nicotine. Currently, two forms of synthetic nicotine are added to marketed products – racemic nicotine, consisting of 50% *S*-nicotine and 50% *R*-nicotine, and pure *S*-nicotine. The price of synthetic nicotine remains significantly higher than that of tobacco-derived nicotine. Consumers who use products containing racemic nicotine inhale much higher amounts of *R*-nicotine than users of tobacco-derived nicotine or pure *S*-nicotine, which raises questions about the long-term safety of such products. While *R*-nicotine is significantly less potent than *S*-nicotine in standard pharmacological assays and behavioural tests, the only toxicological studies of the effects of *R*-nicotine are studies of acute effects. There is evidence that *R*-nicotine differentially affects other pharmacological and toxicological targets, raising concern about unexpected toxicological effects. In none of the published pharmacological studies were animals exposed for longer than 1–2 weeks, and in none were subsequent pathological effects examined. None of the published studies addressed the effects of racemic nicotine, in which both *R*- and *S*-nicotine are present, and in none were their effects compared after inhalation and after ingestion, the routes through which consumer products dispense nicotine. Most of the studies of the effects of *R*- and *S*-nicotine were published between the 1970s and the 1990s. Toxicological methods have advanced significantly since then and should be used to examine the long-term effects of *R*-nicotine intake. Chemical analytical methods allow differentiation between synthetic and tobacco-derived nicotine; however, the methods have not been standardized and require substantial investment in advanced equipment and training. Analytical studies raise concern about inaccurate labelling of marketed products and undisclosed addition of tobacco-derived nicotine, probably added to increase addictiveness and increase profits, while health claims for synthetic nicotine are maintained. Tested commercial preparations of both purified tobacco-derived nicotine and synthetic nicotine fulfil US Pharmacopeia criteria for the purity of pharmaceutical-grade nicotine; however, not all currently marketed preparations have been compared. Because of the closely similar purity of synthetic and tobacco-derived preparations, claims of health attributed to synthetic nicotine and to purified tobacco-derived nicotine should be based on strong scientific evidence.

If regulators restrict the use of synthetic nicotine in marketed products, the chemical synthesis methods developed by manufacturers could be modified rapidly to generate nicotine analogues *(19)*. The tobacco industry has a long history of studying the addictive and reinforcing effects of nicotine-related tobacco alkaloids, including anabasine, nornicotine, anatabine, cotinine and myosmine *(19,59,73–76)*. Regulators should be aware that these analogues might be used to replace nicotine in marketed products.

3.3 The legal landscape

An unregulated market of synthetic nicotine products risks undermining public health progress in mitigating the harm of tobacco use *(2,77,78)*. For instance, lawmakers in the USA wrote a letter to the USFDA in November 2021, expressing concern that unregulated sale of synthetic nicotine products was "undermin[ing] efforts to reduce the continued popularity of youth vaping" *(79)*. Additionally, current marketing claims for certain synthetic products may mislead people who use those products by suggesting, for example, that they are safer than tobacco-derived nicotine products, even though such a claim is not supported by evidence.

If synthetic nicotine products remain unregulated, companies are likely to make a business choice to sell products containing synthetic rather than tobacco-derived nicotine (or at least claim to be doing so), undermining comprehensive regulation of novel tobacco and nicotine products *(77)*. Companies are aware that synthetic nicotine products are not covered by tobacco control laws in some countries. Two of the major global suppliers of synthetic nicotine, Hangsen and NGL, have both touted "[f]ewer restrictions for new market introductions" as one of the key benefits of synthetic nicotine *(48)*. Before recent changes to US law, an investment analyst in the USA referred to synthetic nicotine as a potential "golden ticket", as use of synthetic nicotine instead of tobacco-derived nicotine might mean "no FDA regulation, no tobacco taxes, no flavor restrictions, and no restrictions on direct to consumer e-commerce".[1] Puff Bar, which produces disposable e-cigarettes that are popular among young people, took advantage of the former regulatory gap in the USA, when, after a USFDA enforcement action, it relaunched its products in early 2021, claiming that its use of synthetic nicotine exempted it from regulation as a tobacco product *(80)*.

A key question for policy-makers is therefore whether products containing synthetic nicotine (or other, non-tobacco-derived nicotine alternatives) are covered by existing regulatory frameworks for tobacco products. This depends on the definitions of the terms used in relevant laws and whether those definitions are specific to (and limited to) tobacco-derived products. The WHO Framework Convention on Tobacco Control (WHO FCTC) defines "tobacco products" as "products entirely or partly made of the leaf tobacco as raw material which are manufactured to be used for smoking, sucking, chewing or snuffing", which would appear to exclude non-tobacco synthetic nicotine products *(81)*. The WHO FCTC language does not, however, prevent Member States from including synthetic nicotine products in the definition of "tobacco products" in their national laws or from otherwise including products containing synthetic nicotine in national (or subnational) tobacco control laws. Notably, the Conference of the Parties to the WHO FCTC has requested the Secretariat of the WHO FCTC "to advise, as appropriate, on the adequate classification of novel and emerging tobacco

1 Lavery MS. Tobacco synthetic nicotine bursts on to the scene. 2021 (available on request from the authors).

products such as heated tobacco products to support regulatory efforts and the need to define new product categories" *(81)*.

To better understand the global legal landscape for synthetic nicotine products and how Member States might revise existing regulatory definitions, including to comply with international obligations *(81)*, we surveyed the tobacco control laws of 210 countries and the EU to determine whether and how those laws apply to synthetic nicotine products.

3.3.1 Methods

Our review covered tobacco control regulations for market entry requirements (e.g. registration before marketing), sales restrictions (e.g. age restrictions for sales or restrictions on where retailers place tobacco products), packaging and labelling requirements (e.g. requirements for certain warning statements or images), and advertising regulations (e.g. restrictions on television advertisements). We excluded other kinds of tobacco-related laws, such as tax laws, smoke-free laws and regulation of flavours.

Most of the laws were found on the Tobacco Control Laws website *(82)*, which contains the laws of 210 countries and the EU. The amendment to the definition of "tobacco products" by the USA in March 2022 was not yet available on the website and was accessed elsewhere *(83,84,85)*. Accordingly, laws from a total of 211 jurisdictions were included in the analysis.

Of the 211 jurisdictions, 21 either did not have any laws available or did not have a version in English. Three English-speaking individuals with training in US law (two of the authors, MLB and PJZ, and Annamarie Beckmeyer) reviewed the relevant laws for the remaining 190 jurisdictions (189 countries and the EU). The EU directives are not binding law but are instead "legislative act[s] that set … out a goal that all EU countries must achieve …, [leaving] individual countries to devise their own laws on how to reach these goals". We coded the EU as a separate jurisdiction because of the importance of its Tobacco Products Directive to tobacco policy-making in Europe *(86)*.

MonQcle, legal research software *(87)*, was used to code laws for their application to any synthetic nicotine products. If the laws did apply to synthetic nicotine products, they were then coded for whether they applied to any synthetic nicotine product in addition to e-cigarettes and whether the covered synthetic nicotine products subject to market entry requirements, sales restrictions, packaging and labelling requirements and advertising restrictions.

3.3.2 Results

The phrasing of laws in some countries is broad enough to cover certain synthetic nicotine products or to cover such products more broadly. Tobacco control laws in many countries, however, do not clearly apply to such products (Table 3).

Table 3. Applicability of tobacco control laws in 211 jurisdictions to control of products containing synthetic nicotine

Coverage of products containing synthetic nicotine	Number of jurisdictions	Characteristics
No coverage	92	"Tobacco products" defined as products containing elements made from tobacco plants
Clear coverage of certain products	52	E-cigarettes (and other specific product types) defined to include nicotine derived from any source, but "tobacco product" otherwise limited to products made from tobacco plants
Broader coverage	29	"Tobacco products" defined to explicitly include synthetic nicotine or nicotine derived from any source
Unclear coverage	17	Product definitions refer to tobacco plant or smoke without expressly requiring that the products be made or derived from tobacco
Not available	21	

Laws that do not cover synthetic nicotine products

Of the 190 laws coded, 92 did not apply to any type of synthetic nicotine product. Many of the jurisdictions in this category had laws that define the products covered according to their tobacco content. For example, before March 2022, US law defined "tobacco products" (for the purposes of federal regulation) as products "made or derived from tobacco" *(84,85,88)*.

Some laws in this category did not expressly include the relevant terms, but the terms themselves suggested that synthetic nicotine products are probably not covered. For example, in some laws, the term "tobacco product" was used without a definition. Countries in the WHO African Region were the most likely to have laws that did not apply to any type of synthetic nicotine product.

Laws that clearly cover only certain synthetic nicotine products

In 52 jurisdictions, the laws include definitions broad enough to cover certain synthetic nicotine products –usually e-cigarettes – but not other currently marketed synthetic nicotine products such as pouches, toothpicks and chewing-gums. Many of these laws define "tobacco products" according to the tobacco content (as in the category above) but then separately define "electronic cigarettes" or similar terms without specifying the source or content of nicotine.

Other laws in this category do not define the relevant terms but include terms that can encompass e-cigarettes that contain synthetic nicotine. For example, in some laws, terms such as "electronic cigarettes" or "electronic nicotine delivery systems" are used without defining their limits. These terms are therefore probably broad enough to cover e-cigarettes that contain synthetic, rather than tobacco-derived, nicotine.

The laws of some jurisdictions apply to a limited extent to products other than e-cigarettes. For instance, some laws also cover herbal smoking products

that contain no tobacco, which could leave room to include combustible products infused with synthetic nicotine.

Of the laws that cover only certain synthetic nicotine products, a few completely ban the sale and distribution of e-cigarettes. There is no clear geographical pattern of jurisdictions that have implemented laws covering only certain synthetic nicotine products. There does, however, appear to be a temporal trend, as most laws in this category were enacted in 2017 or later.

Laws that cover synthetic nicotine products more broadly

The laws of 29 jurisdictions are drafted broadly enough to cover all or most synthetic nicotine products that are currently marketed and also products that may emerge (Table 4). Only a few of these laws completely ban a broader range of synthetic nicotine products.

Table 4. Examples of national laws that include product definitions that cover all synthetic nicotine products

Country	Date	Comments and definitions
Republic of Moldova	2015	The Republic of Moldova adopted comprehensive revisions to its Tobacco Control Act to comply with its obligations under the WHO FCTC and to align its policies with those of the EU pursuant to the Moldova–EU Association Agreement. In addition to regulating "tobacco products", the revised law regulates "related products", defined as including "products made of plants for smoking *and* products that contain nicotine, including electronic cigarettes" [emphasis added] *(89)*.
Singapore	2010	Singapore's law has included the regulation of "tobacco substitutes" since 2010. Although the definition has been amended over time, it has consistently been used as a catch-all term to regulate products that contain nicotine (regardless of source) but are not included in the other defined categories in the Tobacco Act *(90)*.
USA	2022	In response to the introduction of synthetic nicotine products that claimed to be outside the reach of the US Tobacco Control Act, the USA amended the definition of "tobacco product" in the Act to include "any product…containing nicotine from any source, that is intended for human consumption" *(91)*.

Countries in the WHO European Region are most likely to have laws that cover a broader range of synthetic nicotine products. Most of the laws were passed after 2016.

For example, the law in the Republic of Moldova differentiates "related products" from "tobacco products" to include "products made of plants for smoking and products that contain nicotine, including electronic cigarettes", which provides broad coverage of existing and emerging synthetic nicotine products *(89)*. The law in Singapore includes a definition that provides broad coverage of synthetic nicotine products, "tobacco product" being defined as including "tobacco substitute", which in turn is defined as "any article, object or thing that contains nicotine", with no requirement that nicotine be derived from tobacco. The law expressly excludes from "tobacco substitute" "(a) a cigarette or cigar, or any other form of tobacco; (b) a tobacco derivative; (c) a mixture

containing any form of tobacco or a tobacco derivative; (d) a therapeutic product registered under the Health Products Act" *(90)*.

The USA now regulates but does not explicitly ban synthetic nicotine products *(92)*. In March 2022, the Federal Food, Drug, and Cosmetic Act was amended to include synthetic nicotine products within USFDA tobacco product authorities. The definition of "tobacco product" now covers "any product made or derived from tobacco *or containing nicotine from any source* that is intended for human consumption, including any component, part, or accessory of a tobacco product" [emphasis added]. This definition thus covers all or most currently marketed synthetic nicotine products as well as products that may emerge. Under this law, synthetic nicotine products now require premarketing authorization from the USFDA before they can be legally sold. No synthetic nicotine products have yet received such authorization, but the USFDA reported that > 1 million marketing applications from > 200 companies had been received *(92)*. The USFDA refused 925 000 of the applications and accepted 8600 for further review.

Laws with unclear coverage of synthetic nicotine products

The laws of 17 jurisdictions were not clear about whether the definitions cover synthetic nicotine products. For example, some of the laws referred to tobacco when defining nicotine products but did not state whether application of the law was limited to nicotine derived from tobacco. The Tobacco Control Laws website did not have sufficient information in English on the laws in some countries for the authors to be able to determine whether the laws applied to products containing synthetic nicotine.

3.3.3 Discussion

Various legal adjustments could include synthetic nicotine products in the scope of tobacco control regulations. The approaches that some countries have adopted cover only e-cigarette or e-liquid synthetic nicotine products. These approaches do not include potential regulation of other kinds of synthetic nicotine products that are currently marketed or that may emerge, which will undermine comprehensive regulation of novel tobacco and nicotine products. As approaches in countries such as the Republic of Moldova, Singapore and, most recently, the USA show, however, legal adjustments could include the full range of currently marketed synthetic nicotine products and products that may emerge under tobacco control regulations.

Although our analysis was limited to synthetic nicotine, it provides an example of how the tobacco industry may seek to exploit gaps or uncertainty in laws to market new products or to evade tobacco-related regulations. Further work on the legal landscape of nicotine analogues may be useful to help countries in developing appropriate regulatory approaches *(73)*.

The description of the global legal landscape for synthetic nicotine products has several limitations. Only laws available in English and only tobacco control laws that cover market entry, sales restrictions, packaging and labelling and advertising were coded. The laws that were not available in English and other types of tobacco laws, such as tax laws, may include synthetic nicotine products. The coded laws generally did not include subnational jurisdictions, where the laws may define terms differently. The Tobacco Control Laws website may not be complete, as it may not include the most recently adopted laws or court decisions that affect the interpretation or enforceability of laws. Furthermore, the English versions of laws may not faithfully reflect the original versions.

Importantly, the laws we coded did not include laws to regulate products other than tobacco products. Even if synthetic nicotine products are not covered by a country's regulatory scheme for tobacco products, they may be subject to regulation as drugs (or drug–device combination products) or to other laws for consumer protection. Such laws may provide countries with opportunities to regulate synthetic nicotine products without changing their tobacco control laws *(76)*. For example, before the US law was amended in March 2022 to bring synthetic nicotine products under the law, public health groups urged the USFDA to regulate synthetic nicotine products as drugs *(93)*. Synthetic nicotine manufacturers such as Hangsen and NGL boast that their products "provide the same satisfaction smokers are seeking from their nicotine", which implicitly acknowledges that the products they sell are used for their effects as addictive drugs *(48)*. Most product websites include warnings or disclaimers, acknowledging that the nicotine in their products is addictive and may be hazardous. Additionally, the Australian Therapeutic Goods Administration requires a prescription for buying e-cigarettes containing nicotine *(94)*. Although this requirement appears to cover synthetic nicotine products, it is not imposed through Australia's tobacco control laws and is thus outside the scope of this analysis.

Finally, this analysis did not allow assessment of whether the requirements imposed through tobacco control laws or other kinds of law are enforced. Enforcement may vary within and between countries.

3.4 Recommendations for consideration by policy-makers

1. Countries in which there is a regulatory gap for synthetic nicotine products (as compared to products containing nicotine derived from tobacco) should consider amending their tobacco control laws to ensure that they include synthetic nicotine products.
2. Countries that choose to amend their tobacco control laws to cover synthetic nicotine products should consider legal adjustments that extend the coverage of the laws to the full range of synthetic nicotine

products that are currently marketed as well as products that may emerge. These may include products that contain synthetic nicotine analogues, other chemicals with similar properties or chemical systems that generate nicotine or analogues in situ.

3. Countries are advised to enforce standards for the purity of synthetic nicotine in products, preferably those of the European and US pharmacopoeias. Regulators should consider implementation of product standards to ban the mixing of tobacco-derived nicotine with synthetic nicotine in marketed products.
4. Policy-makers are advised to enforce uniform labelling rules for products containing nicotine, either natural or synthetic, and to declare the content of *S*-nicotine and, separately, the content of *R*-nicotine and any other nicotine analogue or any other chemical with similar properties.
5. Countries should consider banning synthetic nicotine products that contain *R*-nicotine, or any nicotine analogue apart from *S*-nicotine, at levels that exceed those in tobacco-based products, until the safety of consumption of these chemicals in such products is established.
6. Regulators should consider restricting marketing practices for promotion of synthetic nicotine as generally "tasteless and odourless", "purer" or "healthier" than purified tobacco-derived nicotine, unless scientific evidence to support such claims is provided.

3.5 Conclusions

Companies are marketing an increasingly wide range of synthetic nicotine products, which, if not regulated, may undermine work to reduce use of tobacco and nicotine addiction and the work of WHO Member States to regulate tobacco and nicotine products comprehensively. Knowledge about the effects on human health of synthetically derived nicotine in different types of consumer products is still incomplete. Although synthetic nicotine products are not clearly regulated under current tobacco control legislation in many countries, the laws in some countries have been updated to cover these products. The information presented above shows that countries could make various legal adjustments to close regulatory gaps for synthetic nicotine products, including adjustments that cover both the broad range of products currently on the market and those that might emerge.

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4. Nicotine pouches: characteristics, use, harmfulness and regulation

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Contents

Key findings, challenges and regulatory implications

- Nicotine pouches have recently become available in many markets worldwide, and their sales are growing rapidly.
- Nicotine pouches deliver sufficient nicotine to induce and sustain nicotine addiction.
- Nicotine pouches have attractive properties, such as appealing flavours, and can be used discretely without the stigma of smoking.
- Nicotine is harmful to health, including to the nervous and cardiac systems.

- There are few data on nicotine pouches because they have been on the market for only a short time. A cautionary approach is warranted, given their similarities to conventional oral tobacco products, in particular snus.
- Nicotine pouches are not regulated or not specifically regulated in several jurisdictions. Some countries had already made their regulations and laws "future-proof" and resilient, so that nicotine pouches are regulated under existing laws. Others have recently updated their laws, while some retain definitions that refer only to conventional tobacco products.

Keywords: nicotine products, nicotine pouches, characteristics, harmfulness, regulation, regulatory mechanisms

4.1 Introduction

In the past decade, novel and emerging nicotine and tobacco products, such as electronic nicotine delivery systems (ENDS), non-nicotine delivery systems (ENNDS) and heated tobacco products (HTPs) have proliferated on markets globally. Some of these products, such as ENDS, have also been marketed and promoted to children and adolescents by tobacco and related industries *(1,2)*. Since about 2018, another category of products, commonly known as nicotine pouches, has been introduced onto several markets, as the tobacco industry continues to expand its portfolio of novel and emerging nicotine and tobacco products *(3)*. Other names used to describe these products include "tobacco-free nicotine pouches", "tobacco leaf-free pouches" and "tobacco-derived nicotine pouches"; in this paper, they are referred to as "nicotine pouches". In some jurisdictions, such as the United States of America (USA), they are referred to as "white pouches".

Synthetic nicotine is becoming increasingly popular, although most nicotine-containing products on the market in the USA reportedly contain tobacco-derived nicotine *(4)*. Nicotine pouches are pre-portioned pouches that contain nicotine. They are similar to conventional smokeless tobacco products such as snus in some respects, including appearance, inclusion of nicotine and manner of use (placing them between the gum and lip); however, unlike snus, which contains tobacco, nicotine pouches reportedly do not contain tobacco but rather cellulose powder and some other ingredients. The nicotine may, however, have been extracted from tobacco and may therefore contain substances originally present in the tobacco, as in ENDS. They have been promoted as "tobacco-free", which could be misleading if the nicotine was extracted from tobacco.

The pouches are available in flavours similar to those in, for example, ENDS, ENNDS and conventional smokeless tobacco products. These flavours can

enhance the effects of nicotine by sustaining use and can improve palatability and increase their appeal to adults and especially to young people, including nicotine naive adolescents. Some ingredients in nicotine pouches, such as alkaline agents which increase pH, may increase the delivery of nicotine *(5)*. Some nicotine pouches are marketed as containing synthetic nicotine, usually a racemic mixture of *S*- and *R*- nicotine isomers; a few are stereoselective, containing more of the more potent *S*-isomer, which predominates in the tobacco plant. (See also Paper 2.) Little is known about the pharmacological and metabolic effects of *R*-nicotine in humans *(6)*. Until 2022, the definition of tobacco products of the US Food and Drug Administration (USFDA) included tobacco-derived nicotine, and products containing synthetic nicotine were not legally considered tobacco products. The definition was changed in 2022, since when the USFDA has regulatory authority over tobacco products containing nicotine from any source that are not used for therapeutic purposes *(7)*. In some other jurisdictions, nicotine products are not considered tobacco products unless they are explicitly included in the tobacco law. (See e.g. section 6.2.)

Nicotine pouches were first introduced in Europe but are now available in other countries, such as Indonesia, Kenya, Pakistan and the USA and some countries in the WHO Western Pacific Region. Some of these countries have sought technical assistance from WHO to address these products. Sales of nicotine pouches are increasing rapidly in many parts of the world *(5,8)*, including Denmark, Norway, Sweden and the USA. For example, the sales of nicotine pouches increased from US$ 642 000 in 2016 to US$ 52 million in 2018 in the USA *(5)*, and sales are expected to increase in European countries such as Austria, Croatia, Germany and the United Kingdom *(8)*. Euromonitor International reported an estimate that, globally, 6.8 billion units of nicotine pouches had been sold in 2021, representing more than a 2000% increase over its estimated retail volume in 2018. It was further estimated that, by the end of 2023, projected global retail volume sales will amount to more than 11 billion units *(9)*.

Introduction of new products that closely resemble traditional tobacco and nicotine products poses serious regulatory challenges in all WHO regions. Many manufacturers and retailers promote them as "healthy alternatives", and these products are often advertised with themes that appeal to young people *(10,11)*. A large collection of nicotine pouch advertisements is available online *(11)*.

Manufacturers have attempted to persuade regulators to classify nicotine pouches as non-tobacco products, as it is sometimes unclear whether these products are included in tobacco regulations or whether they occupy a regulatory "grey area" *(6)*. For example, they are often promoted as "non-tobacco" products, "white pouches" and "tobacco-free products". In some countries, especially low- and middle-income countries, manufacturers of these products claim that the nicotine contained in them is not derived from tobacco and therefore claim

that the products fall outside the scope of tobacco control law *(6)*. Tobacco manufacturers also seek regulatory exemption for newer nicotine and tobacco products, including nicotine pouches, as in the case of Lyft in Kenya. The Lyft product was approved for sale in Kenya by the country's drug regulatory authority and has been available since July 2019 *(12)*. Several health advocacy groups in Kenya submitted a petition to the national Cabinet Secretary for Health, urging him to ban the Lyft nicotine pouches, arguing that they had been allowed onto the Kenyan market illegally. Sales were suspended once the rationale for approval of the pouches as a drug was questioned by the Cabinet Secretary, who subsequently informed British American Tobacco (BAT) that Lyft had to adhere to Kenya's requirements for tobacco products. Health advocacy groups now insist these products should not be available at all *(12)*.

Member States have sought technical assistance from WHO on defining nicotine pouch products and the knowledge and evidence available on these products, which includes the potential and actual risks associated with the products, their characteristics and how they are regulated in countries. This paper summarizes the known characteristics of nicotine pouches, the users of the product, the potential risks of their use and mechanisms for regulating nicotine pouches. This information, from the scientific literature, internet searches, the web pages of manufacturers and market data on nicotine products, will improve regulators' understanding of these products, country experience and regulatory challenges. The paper also provides guidance on regulatory options for nicotine pouches and some recommendations for consideration by countries.

4.2 Methods section

A search was conducted in the bibliographic database PubMed and other sources (e.g. general web search, specialized search on Euromonitor International, ECigIntelligence and Tobacco Intelligence, web pages of manufacturers and market data on nicotine products). Peer reviewed publications up to March 2023 were included. These were initially screened on title and abstract, and then further considered for full review. Keywords that were searched included "nicotine products", "nicotine pouches", "tobacco leaf-free pouches", "tobacco-derived nicotine pouches", "non-tobacco products", "white pouches", "tobacco-free products", "characteristics", "harmfulness", "regulation" and "regulator mechanisms".

Further, in 2020, WHO distributed a questionnaire to WHO regional advisors in all six regions to elicit country experiences with nicotine pouches, the regulatory mechanisms in place and difficulties found in regulation. A further questionnaire was disseminated to the WHO Global Tobacco Regulators Forum in 2021, who were given three weeks to complete the questionnaire and return to WHO. A subsequent questionnaire was formulated, and data collection done

in 2022. Information was informally sought from regulators in European Union Member States via email and a review was conducted on tobacco control laws in the Tobacco Control Laws Databases of WHO and the Campaign for Tobacco Free Kids. 124 national laws were reviewed in total.

4.3 Characteristics of the products

Different brands of nicotine pouches and different products within a brand differ in weight, nicotine concentration and bioavailability, flavour and pouch size. Like traditional tobacco-containing snus, 20–25 nicotine pouches are typically packed in a pocket-sized tin (Fig. 1). In some countries, the tins may have a compartment for discarding used pouches (e.g. Zyn, Ace) *(13)*. The brand names, flavours and pouch sizes (e.g. "slim") are often listed on the lid. In some countries, the nicotine content is described as "strength" on a dot or a numerical scale (e.g. 4 out of 5; Fig. 1, right). The nicotine content varies from brand to brand and may be expressed in mg or mg/pouch. The lack of a requirement for standardized labelling and the resulting variety of expression of nicotine concentration probably confuses consumers. A warning is often placed on the lid or the bottom of the tin. Although such warnings are not required if the product is not regulated as a tobacco product, it may give the false impression that the company is abiding by the provisions under tobacco product regulations.

Fig. 1. Bottom and lid of a nicotine pouch tin of Thunder Cool Mint, with the content or "strength" on a numerical scale.

Photo credit: WHO

A single nicotine pouch weighs 149–800 mg *(5,14)* and generally contains nicotine at 3–50 mg/g, equal to a nicotine dose of 2–32.5 mg per pouch *(3,15,16)*, whereas a traditional portion of snus weighs 0.3–1.13 g, with a nicotine dose of 6.81–20.6 mg/g wet tobacco *(17)*. Some nicotine pouches with exceptionally high nicotine levels have been reported, e.g. pouches with up to 120 mg/g nicotine have been reported on the Estonian market, the strongest products coming from the Russian Federation *(8)*. A nicotine content of 1.29–6.11 mg per pouch was measured in 37 brands (2–6 mg/pouch) from six manufacturers, with 1.12–47.2% moisture content, pH 6.9–10.1 and 7.7–99.2% free-base nicotine, which is more bio-available than protonated nicotine (see section 5.2) *(5)*. The nicotine in these products is either derived from tobacco or synthesized. The brands offer a variety of flavours, such as fruity and sweet, but also coffee and menthol. In some cases, the flavours are combined and described for example as "a balanced combination of sweet and tart pineapple with creamy coconut and a nutty undertone" (Lyft) and given "concept" names, such as "tropic breeze" (Velo). Mint flavours appear to be used most widely in the USA, representing 54.6% of the total US nicotine pouch market in 2019 *(18)*. An increase in sales of fruit-flavoured nicotine pouches was observed between January 2019 and June 2020 *(19)*. Cooling and fruit categories dominate the market, representing almost 70% of the flavours in these products *(20)*. Nicotine pouches are available in several sizes, the smaller ones promoted as "slim" or "mini" on the package.

The pouch itself is made of water-insoluble material, similar to tea bags, made predominantly of cellulose fibres but permeable to saliva and nicotine *(21)*. The pouch contains an off-white or white powder containing either salts consisting of nicotine and an acid or free-base nicotine (Fig. 2). Other ingredients in addition to nicotine include cellulose, water, salt and other additives, such as pH-adjusting agents, filler, noncaloric sweeteners, a stabilizer (hydroxypropyl cellulose) and flavourings *(3,22)*.

The user places one nicotine pouch under the upper lip, where nicotine and flavours are released. Brand websites and web shops advise retaining the pouch for a minimum of 5 min to up to 1 h *(23)*. Shortly after the pouch is placed in the mouth, a tingling sensation is felt (due to the nicotine) that can last for up to 15 min *(22)*. Data from surveys and websites indicate that Zyn users consume 10–12 portions *(22)* daily *(24)*.

Fig. 2. Nicotine pouch package labelled "slim", a nicotine pouch and its content

Photo credit: WHO

4.4 Marketing

The nicotine pouches currently on the market are produced mainly by large tobacco manufacturers such as BAT (Lyft, Velo, Zonnic) *(25)*, Altria Group Inc. (On!), Swedish Match (Zyn, G.4) *(21)*, Imperial Brands (Skruf, Knox, ZoneX) *(26,27)*, Philip Morris International (Shiro, Sirius), Swisher (Rogue), and Japan Tobacco International (Nordic Spirit) *(3,28)*. In the USA, the Federal Trade Commission reported on nicotine pouches in 2021 for the first time *(29)*, when the companies sold 140.7 million units of such products in the USA, for US$ 420.5 million. Nicotine pouch sales increased from 163 178 packages of 15–20 pouches (US$ 709 635) in July 2016 to 45 965 455 units (US$ 216 886 819) in June 2020 *(19)*. The highest US market share in 2020 was that of Swedish Match (78.7%), followed by Altria (10%) and BAT (7.6%). Small companies also manufacture nicotine pouches, such as Ace Superwhite by the Ministry of Snus (Denmark) or N!Xs by Microzero AB (Sweden and other European countries) *(30)*.

Nicotine pouches are marketed online and by tobacconists as smoke- and tobacco leaf-free alternatives to tobacco and nicotine products that are "less harmful" than traditional snus and conventional cigarettes *(14)*. These are also some of the reasons reported by users (see below) *(22,31)*. Smokeless-tobacco users also perceive snus as less risky than conventional cigarettes *(32,33)*. Although nicotine pouch containers often bear a warning about addiction to nicotine, they usually do not bear the warnings required for smokeless tobacco in many countries about the risks of oral cancer and gum disease. Nicotine pouches are also called "white snus", because of their white powder filler, instead of the traditional brown tobacco snus *(34)*. "White snus" is marketed as "milder, slimmer, flavoured, and more visually appealing" *(26)*. Different sizes of nicotine pouches are available, the smaller pouches being marketed as discreet ("Since the pouch is thin and small no one will see that you have it under your lip") *(35)*. Online marketing stresses that nicotine pouches can be used "anywhere, anytime", including where smoking is prohibited *(23,34)*. Cross-over advertising was observed on websites of leading brands of conventional cigarettes when the parent companies also owned the pouch brand. Examples include Altria's co-promotion of On! pouches with Marlboro cigarettes, and On! and Camel consumers received an e-mail invitation to "explore the nicotine options from our friends at Velo" *(37)*. E-mail advertising included the claim that the product could be used anywhere (84% of e-mails), that nicotine pouches are an alternative to other tobacco products (69%), do not contain tobacco leaf (55%) and are "spit-free" (52%) or "smoke-free" (31%) *(38)*. Nicotine pouches are not more expensive than cigarettes, because a container of nicotine pouches is slightly cheaper or comparable in price to a package of conventional cigarettes *(3)*. It has also been claimed by companies that, in contrast to e-cigarettes, batteries and charging devices are not necessary *(35,39)* and that, in contrast to traditional snus, the white pouches look cleaner and do not stain the teeth *(34,36)*.

According to a Euromonitor report *(40)*, "use of influencer marketing and social media platforms, such as Instagram, has been embraced by modern oral manufacturers". An article by the Bureau of Investigative Journalism summarizes marketing tactics for new BAT products, including heated tobacco and oral nicotine, presenting nicotine products as "cool" and "aspirational" in a glossy youth-focused advertising campaign; paying social media influencers to promote nicotine pouches; sponsoring music and sporting events; and an international offer of free samples of nicotine pouches, which appears to have attracted underage people and non-smokers *(10)*. Nicotine pouches are promoted on the social media accounts of musicians, football players and influencers in many countries *(10)*.

4.5 User profile

A few studies have reported the prevalence of nicotine pouch use. The overall prevalence among 10 296 adult current cigarette smokers or recent ex-smokers in Australia (0.1%), Canada (0.9%), England (1.1%) and the USA (0.7%) was 0.8%.

Among the few current and ex-smokers, more males (1.1%) than females (0.5%) used nicotine pouches. In all the countries studied, the prevalence was highest among those aged 18–24 years (2.3%) (25–39 years, 1.4%; 40–54 years, 0.4%; ≥ 55 years, 0.1%) *(41)*.

In a survey of 3883 smokers, vapers, dual users and recent ex-users in the United Kingdom in 2019, 15.9% had heard of nicotine pouches, and 3.1% had seen them for sale; 4.4% had ever used nicotine pouches, and 2.7% were current users *(42)*. In a survey of a sample of 5805 people representative of the Dutch population, only 6.9% were aware of nicotine pouches, mainly because they knew someone who used them (33%) *(31)*. Of the respondents, 0.6% had ever used a nicotine pouch, and 0.06% were current users. Current smokers had higher than average ever use (1.91%), especially those who preferred menthol cigarettes (6.26%). Awareness among adolescents (13–17 years) was relatively high (9.1%), but only 0.3% had ever used a nicotine pouch, and none reported current use.

In an online repeat cross-sectional survey in 2019 of 11 714 young people aged 16–19 years in Canada, 11 170 in England and 11 838 in the USA, 1% in Canada, 1.3% in England and 1.5% in the USA had used nicotine pouches in the previous 30 days *(43)*. Data from the 2021 National Youth Tobacco Survey in the USA indicated that 1.9% of middle- and high-school students (age 11–18 years) had ever used nicotine pouches *(44)*. Further, 0.8% of the students reported current use (past 30 days) of nicotine pouches. Of the students who reported current use of nicotine pouches, most (63.5%) reported having used them on 1–5 days in the past 30 days, and 17.2% reported use on 20–30 days in the past 30 days. Additionally, 61.6% of current users reported having used flavoured nicotine pouches in the past 30 days, mint and menthol being the most commonly reported flavours.

A study conducted in the USA in early 2021 from a web-based survey of US adults who were current, established smokers (had smoked at least 100 cigarettes in their lifetime and now smoked every day or on some days) found that 29.2% had ever seen or heard of nicotine pouches, 5.6% had ever used them, and 16.8% expressed interest in using them in the next 6 months *(45)*. Younger adult smokers were more likely to have ever seen or heard of nicotine pouches than older adult smokers. Among adults who smoked, those with more education had lower odds of ever using nicotine pouches, while those who had attempted to quit before using traditional methods or had ever used smokeless tobacco had higher odds of ever use.

The demographics of Zyn users and the patterns and reasons for use were investigated in a study based on data from Swedish Match North America *(22)*. The average Zyn user was about 33 years old, male, white, had finished high school and earned more than US$ 50 000 per year (i.e. middle income). The majority were current smokeless tobacco users and former tobacco users (mostly former dual cigarette–smokeless tobacco users). Two other studies reported similar profiles of nicotine pouch users: male, 25–34 or 44 years and had formerly smoked and/or vaped *(31,42)*. Zyn users found nicotine pouches moderately to extremely

appealing. The reasons for use were "less harmful to my health than other tobacco products" (62%), "ease of use" (53%), "no one can tell when using it" (50%), "less harmful to my health than cigarettes" (49%), and "no smell like smoke/tobacco and to avoid spitting" (48%). Interestingly, 40% of the never users were "curious to see what it was like" *(22)*. Among the Dutch respondents, nicotine pouches were used mainly "at a party" (38%), "with friends" (38%) or "at home" (26%) *(31)*. The main reasons for using nicotine pouches were "out of curiosity" (72%) and "it is tasty and/or pleasant" (23%), but also because they considered "it is less unhealthy than cigarettes" (23%). Only 8% indicated the "availability of different flavours" an important reason for use.

4.6 Evaluation of potential harmfulness of the products

4.6.1 Attractiveness

The Partial Guidelines on Articles 9 and 10 of the WHO Framework Convention on Tobacco Control (WHO FCTC) *(46)* recommend regulation of attractive product characteristics, in particular to decrease uptake by young never users. Nicotine pouches have many attractive features, as mentioned above. For example, they are available in a variety of fruit, mint and other flavours (e.g. cinnamon, liquorice and coffee) *(3,5,22)* and contain sweeteners *(22)*. The cost of the product in the USA is slightly lower than or comparable to that of a pack of conventional cigarettes *(3,47)*, which might be a barrier to use for some but not all potential users. Further attractive features are, for example, the perception that the product is effective for quitting smoking, less harmful than other tobacco products and easy and discreet to use, in places where smoking is banned *(22)*.

4.6.2 Addictiveness

Nicotine pouches contain sufficient nicotine to sustain addiction *(3,47)*: Zonnic 4-mg delivers 2 mg of nicotine *(47)*, similar to the levels delivered by cigarettes. Release of nicotine from On! pouches into artificial saliva released equivalent levels of nicotine with all flavours *(48)*.

Two studies have addressed the pharmacokinetics of nicotine pouches *(16,49)*. In a study by Lunell et al. *(16)*, which was funded and designed by Swedish Match, pouches with a concentration of 3 mg, 6 mg or 8 mg nicotine were tested. After 1 h of use, 1.6 mg (56%), 3.5 mg (60%) and 3.8 mg (50%) nicotine respectively were released from the bags, respectively, and the amount of nicotine in the users' blood gradually increased during use, with peak concentrations of 7.7 ng/mL, 14.7 ng/mL and 18.5 ng/mL. The authors reported that Zyn (6 and 8 mg) delivered nicotine as quickly and to a similar extent as smokeless tobacco products. In a study by Rensch et al. *(49)*, funded by Altria Client Services LLC, nicotine pouches with various flavours and 4 mg nicotine were tested. The amount of nicotine in venous blood from participants increased gradually during the 30

min of use and for 10 min afterwards, to a peak concentration of 9.6–12.1 ng/mL. Use of the pouches reduced the urge to smoke or craving for a cigarette. All the nicotine pouches were considered pleasant but not as much as one's own brand of cigarette. Flavour did not appear to influence the pharmacokinetics of nicotine or the subjective responses.

In comparison, 1–2 mg of nicotine are inhaled from one tobacco cigarette over about 5 min *(50)*. The peak plasma nicotine concentration in venous blood after smoking one cigarette is 10–30 ng/mL and is reached within 5–8 min of the first puff of the cigarette *(50,51)*. Thus, the amount of nicotine to which users of nicotine pouches are exposed, especially from pouches with ≥ 4 mg per pouch), is in the same range as that to which smokers are exposed. One difference is that peak concentrations are reached in a very short time during smoking, while there is a slower, more gradual increase with use of nicotine pouches. A similar observation was made for snus, nicotine plasma levels rising less rapidly than during smoking a cigarette *(51)*.

The slower release of nicotine is an important difference, because it is precisely the fast peak that makes smoking so addictive. The faster a drug is absorbed and reaches the brain, the greater the "rush" it causes and the stronger the rewarding effect. In addition, a short interval between the act and the "reward" provides strong conditioning of behaviour *(52)*. Nicotine replacement therapy products, such as nicotine chewing-gum, which is absorbed in the stomach and intestines, and patches, which are absorbed through the skin, result in very slow absorption of nicotine and are therefore much less addictive *(50)*. The rate at which nicotine is absorbed from nicotine pouches appears to be closer to that of nicotine chewing-gum than that of inhalable nicotine-containing products *(16,50)*.

Nicotine pouches such as Zyn contain pH adjusters *(22)*, which probably increase the addictive potential of the product, since a higher pH results in more so-called "free" nicotine *(5)*, which makes the products harsher but is more easily absorbed in the mouth than other forms of nicotine. Tobacco snus products with a higher pH deliver more nicotine to the user *(53)*. Nicotine pouch products vary in pouch content mass, moisture content (1.12–47.2%), alkalinity (pH 6.86–10.1), and percentage of free nicotine (7.7–99.2%). The total nicotine content ranges from 1.29 to 6.11 mg/pouch and that of free nicotine from 0.166 to 6.07 mg/pouch *(5)*.

4.6.3 Toxicity

Nicotine pouches do not have the chemical by-products of burning or smouldering tobacco leaf, and they are not inhaled. The ground tobacco leaves of conventional smokeless brands emanate toxic chemicals that are not present in nicotine pouches. Indeed, tobacco-free nicotine pouches may have the fewest harmful constituents of all tobacco and nicotine products *(3,54)*. The term "tobacco-free" may, however, be misleading, as many pouches contain nicotine extracted from tobacco and may

thus be not entirely free from tobacco residues. Product constituents, exposure and biomarkers of harm have not been investigated independently *(3,55)*. The main risk factor is nicotine, a known toxicant *(56)* registered under the European Union REACH regulations *(57)*. It is classified as acutely toxic (category 2) after oral, dermal or inhalation exposure and is subject to hazard statements H300: fatal if swallowed, H310: fatal in contact with skin, and H330: fatal if inhaled *(55)*. The higher the nicotine dose of tobacco-containing snus, the larger the increase in heart rate and systolic blood pressure when used by never-tobacco users *(58)*. A concern associated with synthetic nicotine is that the pharmacological and metabolic effects of *R*-nicotine are largely unknown *(6)*. Nicotine extracted from tobacco may be contaminated with tobacco-specific nitrosamines, which are carcinogenic. Most of the other ingredients are also used in food and can therefore be assumed to be relatively safe by the oral route *(212)*, although this has not been addressed, and these ingredients should be studied in the context of nicotine pouches, particularly for local effects. A study by BAT on toxicants such as metals, aldehydes and tobacco-specific nitrosamines in nicotine pouches showed low levels of chromium and formaldehyde in some but not all samples *(59)*.

Dentists have warned of the harmful effects of nicotine pouches *(60,61)*, while a tobacco industry publication shows minimal enamel staining *(62)*. Chaffee et al. *(63)*, reviewed the literature on oral and periodontal effects and concluded that evidence was lacking.

A study of screening assays in vitro by BAT showed toxicological responses to reference cigarette extract in most, while a snus extract had minimal-to-moderate effects and a nicotine pouch extract gave little or no response in all the assays *(64)*.

4.7 Population effects and related factors

For tobacco product users, nicotine pouches might be perceived as a less harmful alternative to conventional cigarettes, heated or smokeless tobacco products, and it would be best to refrain from use of tobacco and nicotine completely. Uptake by never-tobacco or -nicotine users, however, results in exposure to nicotine, which may cause addiction and may even be a gateway to use of other nicotine and tobacco products. Unfortunately, limited data are available on these effects. Data from Swedish Match showed that most Zyn users were former tobacco users (43%), and only a few were never users (4%); most used Zyn every day *(22)*. Zyn appealed most to dual cigarette-smokeless tobacco users (76%), smokeless tobacco users (52%) and smokers (36%), while never and former tobacco users showed much less but still some interest (11–12%) *(22)*. Novel nicotine products can, however, be taken up rapidly by adolescents and young adults, as seen, for example, with Juul e-cigarettes in the USA *(3)*. Uptake by never smokers, in particular young people, can be stimulated by several factors, such as marketing and design, the variety of flavours and discretion *(3)*. The likelihood of progression to the use of tobacco products, as has been reported for e-cigarettes *(65,66)*, is not known, but

the nicotine levels are sufficiently high to sustain addiction, which is generally ≤ 50 mg/g nicotine per pouch, although nicotine pouches with up to 120 mg/g of nicotine have been reported on certain markets.

Nicotine pouches may undermine tobacco control policies such as bans on flavours in tobacco products, including conventional cigarettes. Nicotine pouches can be used discreetly in places where smoking is not allowed and may lead to dual use with conventional cigarettes, which would undermine the beneficial effects of tobacco-free policies. Discontinuation of nicotine exposure imposed by non-smoking rules (e.g. in workplaces, on transport systems, in restaurants and bars) helps tobacco users to quit. Thus, sustaining nicotine dosing in places where smoking is not allowed exacerbates addiction and makes quitting less likely.

Another concern is that nicotine pouches blur the distinction between nicotine replacement therapy and smokeless tobacco products *(47),* as some manufacturers promote these products as nicotine replacement therapy or tools for stopping use of smokeless and smoked tobacco products. Pouch advertisements make both explicit and implicit promises of their usefulness in tobacco cessation: "Designed with smokers in mind" (On!), "I can breathe again" (Zyn) and "never going back" (Zyn).

A study funded by both the manufacturer of Zonnic pouches and the New Zealand National Heart Foundation showed that, for smokers, a Zonnic pouch is as effective as nicotine chewing-gum in relieving craving but subjectively more attractive *(67)*. Overall, nicotine pouches are not proven tools for cessation, and it is unknown how their availability will affect overall smoking cessation rates; there may be competition with proven cessation tools *(47)*.

4.8 Regulation and regulatory mechanisms

According to ECigIntelligence *(68)* and WHO Member States, nicotine pouches are currently available in more than 30 countries, and the market is set to expand in coming years. Between 2020 and 2022, WHO disseminated questionnaires and elicited information from various tracks to capture country experiences with nicotine pouches, the regulatory mechanisms in place and challenges found in regulation. In total, 71 countries provided information on nicotine pouches, and 124 national laws were reviewed. The majority of Member States that provided information reported that nicotine pouches had entered their market between 2018 and 2020, and many reported that the sale of these products was becoming an issue in their countries.

Various regulatory approaches have been adopted, including regulation of nicotine pouches as consumer products, poisons, medical or pharmaceutical products, nicotine pouches (in their own class), nicotine-containing products and tobacco products. These classifications have resulted in bans on nicotine pouches in 12 countries, including Australia and the Russian Federation, regulation in some other countries, and application of existing tobacco control regulations in others.

While the WHO review identified 22 countries in which nicotine pouches are regulated, these products appear not to be regulated in 161 countries, albeit with general consumer laws applying. Very few tobacco control laws cover nicotine pouches, and very few countries regulate or ban nicotine pouches specifically. The majority of countries that regulate nicotine pouches do so through non-tobacco control laws, such as laws on pharmaceutical products, poisons, food and general consumer protection. Table 1 presents examples of regulation of nicotine pouches through various approaches.

Table 1. Examples of approaches taken by countries to regulate nicotine pouches

Regulatory approach	Countries	Law or regulation	Description
Consumer product	Austria, Bulgaria, Croatia, Cyprus, Dominican Republic, Greece, Iceland, Luxembourg, Malta, Poland, Portugal	Consumer laws apply	In Austria, nicotine pouches are classified as both "consumer products" and "medicines". As long as no claims are made about smoking cessation aids, they are classified as "consumer products". In other situations, nicotine pouches are classified as "medicines".
Food	Germany, Netherlands (Kingdom of the)	Commodity law and Article 14 Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 (Netherlands, Kingdom of the)	In Germany, nicotine pouches are banned because they contain nicotine, an unauthorized novel food ingredient. In Netherlands (Kingdom of the), nicotine pouches containing ≥ 0.035 mg of nicotine per pouch may no longer be sold or traded, as they are classified as harmful foods.
Poison	Brunei Darussalam, Ireland	Poisons Act	In Brunei Darussalam, nicotine pouches are classified as both a "poison" and an "imitation tobacco product". They are listed as a "poison" under the Poisons Act; importation and sale of poisons require a license. (See note on "imitation tobacco product" below).
Medicine or pharmaceutical product	Austria, Canada, Chile, Finland, Hungary, Japan, Malaysia, South Africa	Canadian Food and Drugs Act Finnish Medicinal Products Act (section 3)	In Austria, nicotine pouches are classified as "medicines" if smoking cessation claims are made. Otherwise, they are classified as a "consumer product". Pouches that deliver < 4 mg of nicotine per dose are exempt from prescription, are regulated as "natural health products" and are subject to the Natural Health Products Regulations in Canada and as a licensed self-medication product in Finland. Pouches that deliver > 4 mg per dose are considered a prescription drug and subject to the requirements of the Food and Drug Regulations (Canada) and the Medicinal Products Act (Finland). The pouch that delivers the drug is considered a Class I medical device. No nicotine pouch has yet been granted authorization for sale as a drug in Canada.

Nicotine pouch Nicotine-containing product Tobacco-free products Tobacco alternatives Imitation tobacco	Belgium, Brunei Darussalam, Estonia, New Zealand, Republic of Moldova	Tobacco Order 2005 (Brunei Darussalam) Smoke free Environments and Regulated Products Amendment Act 2020 (New Zealand) Law No. 278-XVI on Tobacco and Tobacco Products, as amended in 2015 (Republic of Moldova)	In Belgium, nicotine pouches are classified as "similar to tobacco products". In Brunei Darussalam, nicotine pouches are classified as both a "poison" and as an "imitation tobacco product". Nicotine pouches may be considered an "imitation tobacco product" and are therefore prohibited. Nicotine pouches are not currently sold in the country. See note on "poisons". In Estonia, nicotine pouches are considered "snus imitation products" and taxed as "alternative tobacco products". See Table 2. In New Zealand, the Government prohibits the import for sale, packaging and distribution of oral nicotine products (unless approved as medicines). A significant change to the legislation by amending the definition of "tobacco product" was avoided; instead, oral nicotine pouches are directly prohibited, consistent with regulation of snus and chewing tobacco under New Zealand law. See Table 2 for more details on regulation in the Republic of Moldova.
Tobacco product	USA	Code of Federal Regulations – Title 21, Volume 8	See Table 2.

As these products are relatively new on many markets, they are unregulated in several countries. In some, current tobacco control or other laws include no measures that could be applied to this product. In other countries, the products are not specifically regulated, although general consumer protections laws apply. Some countries are exploring ways in which to address nicotine pouches, such as considering them as nicotine chewing gum and imposing excise duties. The definitions applied to nicotine pouches under existing tobacco control laws and their legal interpretation are listed in Table 2.

Table 2. Examples of legal definitions in tobacco control laws applied to nicotine pouches, and legal interpretations

Country	Relevant regulations or law	Relevant definition(s)	Interpretation
Estonia	Tobacco Act (RT I 2005, 29, 210), as amended in 2018	"Products related to tobacco products" are defined as "products used similarly to tobacco products which imitate consumption of tobacco products and products used to replace tobacco products, including electronic cigarette, herbal products for smoking, different materials to replace waterpipe tobacco and tobacco-free snus, regardless of the nicotine yield of such products".	The provisions of the Act apply to tobacco products and products related to tobacco products. As such, nicotine pouches are considered snus imitation products and therefore regulated under the Tobacco Act; bans on advertising, sales to minors and point-of-sale display apply, and pouches are taxed as alternative tobacco products
Republic of Moldova	Law No. 278-XVI on Tobacco and Tobacco Products, as amended in 2015	"Nicotine-containing products" are defined as "any product consumed by inhalation, ingestion or otherwise, to which nicotine is added during the production process or is added by the consumer himself before or during consumption"; "tobacco-related products" are defined as "products made of plants for smoking and products which contain nicotine, including electronic cigarettes".	Under the Law, nicotine-containing products are regulated and Articles 23a and 23e specifically apply to nicotine pouches, stipulating that "a) the nicotine content does not exceed 2 mg per unit or product" and "e) the product does not contain additives specified in paragraph (3) of Article 11". This includes nicotine that is added by the consumer before or during consumption and tobacco-related products that meet the specified definition.

Russian Federation	Federal Law No. 303-FZ of July 31, 2020 "On Amendments to Certain Legislative Acts of the Russian Federation on the Protection of Citizens' Health from the Consequences of Consuming Nicotine-containing Products	Nicotine-containing products are defined as "products that contain nicotine (including those obtained by synthesis) or its derivatives, including nicotine salts, intended for the consumption of nicotine and its delivery by sucking, chewing, sniffing or inhaling, including products with heated tobacco, solutions, liquids or gels containing liquid nicotine in a volume of at least 0.1 mg/mL, nicotine-containing liquid, powders, mixtures for sucking, chewing, sniffing, and are not intended for consumption (except for medical products registered in accordance with the legislation of the Russian Federation, food products containing nicotine in natural form, and tobacco products)".	The law prohibits the wholesale and retail trade of nicotine-containing products intended for chewing and sucking, effectively banning nicotine pouches. This applies to any form of nicotine, including synthetic nicotine.
USA	Code of Federal Regulations – Title 21, Volume 8	Tobacco products are defined as "any product made or derived from tobacco that is intended for human consumption, including any component, part, or accessory of a tobacco product (except for raw materials other than tobacco used in manufacturing a component, part, or accessory of a tobacco product".	All tobacco products, including nicotine pouches that meet the definition of a "tobacco product", are subject to the USFDA's regulatory authority. Tobacco products are covered under the Federal Food, Drug and Cosmetic Act and its implementing regulations. Under the Act, the USFDA's regulatory authority covers the manufacture, sales, distribution, labelling, advertising, promotion and marketing of cigarettes, cigarette tobacco, roll-your-own tobacco, smokeless tobacco and other tobacco products that the Agency, through regulation, deems subject to the law.

A complementary but independent study was conducted by the Johns Hopkins Institute for Tobacco Control as part of its biannual survey to collect information from countries on tobacco and nicotine product regulation in 2021 *(69)*. A total of 67 countries in all six WHO regions in various income categories provided information. The policy scan identified 34 countries that regulate nicotine pouches, of which 23 regulate both tobacco-derived and synthetic nicotine, while the other 11 regulate only tobacco-derived nicotine pouches. Representatives of 38 countries that do not regulate synthetic nicotine pouches cited the wording of their legislation as the main barrier to regulating synthetic nicotine. Of 33 countries that reported that nicotine pouches were sold on their markets, 20 had regulations, while 14 countries that reported that nicotine pouches were not sold on their markets nevertheless had regulatory policies in place.

4.8.1 Regulatory considerations

Countries that are interested in taking regulatory action with respect to nicotine pouches have two possible regulatory pathways: ban or regulate. In banning or regulating these products, countries that are Parties to the WHO FCTC should

take into account their obligations under the Convention in formulating or adopting policies with regard to these products. Countries should also consider existing relevant national laws (on food, consumers, drugs and tobacco), trade classification and classification according to product constituents or characteristics. The Harmonized Commodity Description and Coding System, administered by the World Customs Organization *(70)*, is a standardized system adopted by many countries for classifying traded products. The classification may affect application of domestic laws, and countries may consider whether the Harmonized System codes apply to nicotine pouches.

In regulating nicotine pouches, Parties may introduce measures to prevent nicotine addiction, in line with Article 5 (2b) of the WHO FCTC. This provides that Parties shall, in accordance with their capabilities,

> adopt and implement effective legislative, executive, administrative and/or other measures and cooperate, as appropriate, with other Parties in developing appropriate policies for preventing and reducing tobacco consumption, nicotine addiction and exposure to tobacco smoke.

Countries may also consider banning these products, in line with WHO FCTC Article 2.1, which encourages Parties to implement measures beyond those required by the WHO FCTC. If a country opts to regulate rather than imposing a ban, it may also consider prohibiting or restricting ingredients that may be used to increase the palatability of these products (such as flavours), as recommended in paragraph 3.1.2.2 of the Partial Guidelines on Articles 9 and 10 of the WHO FCTC *(46)*, in order to reduce uptake by young people or never users.

In regulating nicotine pouches, their classification is an important consideration, as it determines to a large extent how a product is regulated. In some jurisdictions, such as the European Union, nicotine is classified among chemicals; however, nicotine pouches are not regulated in a harmonized manner, as they are currently not covered by the Tobacco Products Directive. Countries may also consider whether domestic laws can be applied to these products, including consumer, food and tobacco control laws.

4.8.2 Country case study: Netherlands (Kingdom of the)

A report from the National Institute for Public Health and the Environment (RIVM) *(14)* described nicotine products available on the Dutch market and reported that nicotine pouches were becoming increasingly popular. The report included information that nicotine is addictive and harmful to health, such as to the nervous system and can cause cardiac arrhythmia, particularly at high dosages. The RIVM therefore advised the Dutch Ministry of Health, Welfare and Sport to discourage use of nicotine pouches by imposing stricter regulations and organizing public information campaigns. Nicotine pouches currently fall under

the Commodities Act. The RIVM considered the question of which existing legislation could apply to nicotine products without tobacco. According to the Ministry of Health, these products are not presently within the scope of the Tobacco Act, as they do not contain any tobacco. Policy-makers could consider adding nicotine products without tobacco to the list of products covered by the legislation, for example, by broadening the definition of tobacco and related products. In view of the harmful and addictive effects of nicotine pouches, people, particularly young people, should be prevented from starting their use. On 9 November 2021, the Dutch State Secretary of Health declared that he intended to include nicotine products without tobacco in the law on tobacco and smoking products and to prohibit nicotine pouches in particular *(71)*. Until that is done, these products remain under the Commodities Act. RIVM also proposed that nicotine pouches containing ≥ 0.035 mg of nicotine per bag be considered harmful foods *(72)*, and these products were prohibited under the Commodities Act in November 2021.

4.9 Discussion

Although nicotine pouches are relatively new on many markets, tobacco manufacturers appear to be expanding their markets and are lobbying governments to classify and license nicotine pouches as non-tobacco products. Manufacturers are also seeking to ensure that more lenient regulations are applied to these products than to conventional tobacco products. A key strategy is conflation of product categories (i.e. blurring the line between different product categories) to create confusion in order to penetrate global markets, maximize profits and "get a seat at the table" with regulators. Regulators sometimes lack information on the harm caused by these products and on the regulatory options for addressing the challenges they pose. One of the challenges faced by some regulators is the claim by some manufacturers that, as the products do not contain tobacco and/or that the nicotine contained in the products is not derived from tobacco, they should not be regulated under tobacco laws. Some manufacturers also attempt to bypass ministries of health and have the products registered by other ministries in order to evade strict regulations. Further, these products are pitched as "less harmful" or "smoke free" alternatives to conventional products and sold with a variety of flavours, which could undermine tobacco control policies, such as bans on flavours and smoke-free laws.

Nicotine pouches contain significantly fewer ingredients and toxicants than conventional cigarettes and are being marketed as and perceived by consumers as "less harmful". While the pouches may present fewer risks than conventional tobacco products, manufacturers should not make such claims, unless they are proven and authorized by regulators. Governments can use their policies and regulatory frameworks to decide to educate their populations according to the

available evidence. Regulations that distinguish between nicotine and tobacco products and between nicotine derived from tobacco and synthetic nicotine open the possibility for discussions on whether a product is "tobacco-free" or not and should be regulated more leniently. From the perspective of public health, such a distinction is not fruitful, and regulators should therefore consider widening their regulatory frameworks to include non-therapeutic nicotine products in general, irrespective of whether they contain tobacco or whether the nicotine is derived from tobacco.

While there are limited data on the prevalence of use, the available evidence suggests that strategies similar to those used to market conventional tobacco products are used to market nicotine pouches. These products are similar in appearance to conventional smokeless tobacco products, such as snus, contain nicotine and are used similarly. The attractiveness of these products, including the flavours, suggests that they could sustain use through improved palatability. This is a public health concern, especially in relation to young people and non-users of tobacco. The nicotine content of some of these products, which may be as high as or higher than that of conventional tobacco products, suggests reasonable concern about nicotine addiction. Although limited data are available on these products because of their recent introduction, a cautionary approach is warranted in view of their similarities to conventional products. These products are sold online and by tobacconists, and their sale is largely uncontrolled or unrestricted in many countries, especially on the Internet, including sales to the USA of very high-strength nicotine pouches from Europe. Some of these products are difficult to distinguish from conventional smokeless tobacco products *(8)*. In view of their intense marketing and use of flavours that are attractive to young people, countries are encouraged to protect their existing policies or formulate new policies, as appropriate. In addition, they should broaden their regulatory requirements to cover the wide range of nicotine and tobacco products that are appearing on several markets around the world.

Our preliminary analysis of national laws and the results of the survey indicate that nicotine pouches are unregulated or not specifically regulated in several jurisdictions, and manufacturers have exploited the regulatory vacuum. Other countries have, however, previously made their regulations and laws "future-proof" and resilient to ensure that nicotine pouches are regulated under existing laws. Some have recently updated their laws, whereas some still use the definitions that cover conventional products. The industry might use the latter case to its advantage, using strategies to "get a seat at the table" and present themselves as part of the solution to reducing tobacco use, despite fuelling widespread use of nicotine. A few countries have nevertheless designated nicotine pouches as tobacco products, and other countries may consider acting similarly. Parties to the WHO FCTC interested in banning or regulating nicotine pouches can use certain provisions of the Convention to protect their populations. Some

countries in the WHO European Region that have made legislative amendments to include these products have met opposition from tobacco manufacturers. It is urgent to harmonize regulation of new tobacco and nicotine products to ensure strong protection of health, as required in the Treaty on the Functioning of the European Union *(8)*. It is particularly important to regulate access and promotion to young people.

4.10 Research gaps, priorities and questions

Currently, limited information is available on nicotine pouches, including on their abuse potential, harm, user profiles and population effects. Furthermore, there is no information on long-term dependence of these products, given the short time they have been on the market. More data, preferably studies independent of the tobacco industry, are required on:

- prevalence of use and user profiles, including tobacco use status;
- whether nicotine pouches can help tobacco users to quit tobacco use;
- whether these products are used in addition to cigarettes or other nicotine and tobacco products (dual use);
- monitoring of product use to ensure that nicotine pouches do not promote nicotine addiction among non-smokers, especially young people;
- the possibility that these products are a gateway to use of conventional tobacco products and addiction, especially for young people;
- the potential for increasing attractive features, such as flavour profiles, and the effect of factors such as marketing on perception and use;
- the precise content of nicotine, flavourings, other additives and contaminants;
- short and long-term health effects of nicotine and other substances in nicotine pouches, including synthetic nicotine;
- the effects of switching completely from use of tobacco products to nicotine pouches on exposure and health; and
- the actual outcomes of people who smoke, smokeless tobacco users, never and ex-users who initiate use of nicotine pouches.

4.11 Policy recommendations for product regulation and information dissemination

Policy-makers should adopt common regulatory principles that have been applied successfully to tobacco and related products in many jurisdictions to:

- minimize product appeal and uptake by young people,
- increase product safety and
- minimize false health beliefs.

According to the legal definition of tobacco products or definitions in other relevant laws, countries could explore use of existing tobacco control or other relevant laws to regulate nicotine pouches. Any decision should be in accordance with the country's domestic regulatory context and should ensure maximum protection of the health of its citizens, especially children and young people.

Recommendations for policy-makers, particularly to protect young people and non-users, are as follow.

- Establish or extend surveillance of the product and of users, including their demographics; use of other tobacco and related products; the brands and types; and the flavours used in nicotine pouches in order to assess prevalence and user profiles.
- Regulate all forms of marketing of nicotine pouches and take all other action necessary to minimize access, appeal and initiation by young people.
- Inform the general public about the risks for toxicity and addiction associated with the nicotine in these pouches.
- Require health warning on packages of nicotine pouches, for example on the effects of nicotine, which could include effects on users, the detrimental effects on fetal development in pregnant women, and the damaging effects on brain development in young people, including on learning.
- Prohibit health-related claims by manufacturers, including their potential effectiveness as cessation products, unless the products are licensed and approved as such by regulators.
- Set an upper limit on nicotine to reduce the addictiveness of the products and harm from inadvertent ingestion.
- Protect existing and formulate new policies, as appropriate, to broaden the regulatory requirements to cover the wide range of nicotine and tobacco products appearing on several markets around the world.
- Regulate nicotine pouches in the same manner as products of similar appearance, content and use.
- Ensure that nicotine pouches are not classified as pharmaceutical products unless they are proven to act as nicotine replacement therapy and undergo stringent pharmaceutical registration for licensing as such by the appropriate national regulatory authority.

- Regulate nicotine pouches to prevent all forms of marketing, and take all other action necessary to minimize access and appeal to and initiation by young people.
- Protect tobacco control activities from all commercial and other vested interests related to nicotine pouches, including the direct and indirect interests of the tobacco industry, and ban all forms of marketing and promotional activities.
- Fully implement Article 5.3 of the WHO FCTC to protect policies against undue influence by the tobacco and related industries.

4.12 Conclusions

Nicotine pouches have recently become available on many markets worldwide. They contain sufficient nicotine to induce and sustain nicotine addiction and have many attractive properties, such as appealing flavours and packaging and discreet use. They contain fewer toxicants and therefore expose users to fewer harmful and potentially harmful constituents than conventional tobacco products; however, no use of non-therapeutic nicotine and of tobacco products is recommended for maximum protection of health, as the benefits of quitting tobacco use are apparent almost immediately. Uptake of nicotine pouches results in exposure to toxic nicotine, which may cause nicotine addiction and subsequently lead to use of other nicotine and tobacco products. Nicotine pouches are not regulated or not specifically regulated in several jurisdictions, whereas other countries have made their regulations and laws "future-proof" and resilient such that nicotine pouches are regulated under existing laws. Other countries maintain definitions that refer only to traditional products.

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5. Biomarkers of exposure, effect and susceptibility for assessing electronic nicotine delivery devices and heated tobacco products, and their possible prioritization

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Contents

Abstract

Biomarkers have been used extensively in studies of cigarettes and other conventional tobacco products, providing valuable data on harmful exposures, biological effects, and the disease susceptibility of users and non-users exposed to second-hand smoke. This report provides an evaluation of the published literature on use of such biomarkers in studies of electronic nicotine delivery systems (ENDS) and heated tobacco products (HTPs) and an assessment of the potential utility and limitations of biomarkers in tobacco control. The reviewed evidence indicates that switching from smoking conventional cigarettes to exclusive ENDS use is associated with reductions in biomarkers of exposure

to several toxicants and carcinogens that play key roles in smoking-induced diseases. The levels of many such biomarkers are, however, higher in dual users (people who continue to use cigarettes and ENDS at the same time), which is much more common than switching completely. In addition, the health effects of the changes in exposure are not yet well understood, and biomarkers of biological effects suggest that ENDS pose certain risks to users – particularly dual users and when compared with non-use of any tobacco or nicotine product. The review of the published literature underscores the lack of independent, non-industry research on exposure and effects resulting from HTP use. The report proposes a panel of priority biomarkers for tobacco control, identifies relevant research gaps, notes the need for industry-independent research, and recommends regulatory priorities.

Keywords*:* biomarker, exposure, biological effect, toxicity, electronic cigarette, electronic nicotine delivery system, heated tobacco product, health effect

5.1 Background

This section was commissioned to provide evidence-based recommendations on the use of biomarkers for assessing the nicotine and tobacco products that have emerged in the 21st century, in particular electronic nicotine delivery devices (ENDS) and heated tobacco products (HTPs), and to propose policy options to achieve the objectives and measures outlined in the relevant decision (FCTC/COP8(22)). This document serves as a background paper for the ninth technical report of the WHO Study Group on Tobacco Product Regulation (TobReg).

Biomarkers are powerful tools for objective assessment of human exposure to chemical toxicants and carcinogens in tobacco products, ENDS and HTPs and the resulting disease-related biological effects. Such objective assessment is crucial, because product analysis alone is insufficiently informative for predicting constituent uptake, which is significantly affected by users´ behaviour *(1,2)*. Furthermore, chronic diseases associated with use of nicotine and tobacco products, such as cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular disease, take a long time to develop, and their monitoring – while essential for long-term tobacco control policies – is not suitable for regulatory decisions when new products are introduced onto the market. Therefore, biomarkers can serve as surrogate indicators for assessing such health risks. Nevertheless, use of biomarkers for regulatory purposes has been limited. A contributing factor is that countries with limited resources for tobacco control must prioritize their allocation effectively to reduce the public health harm that results from tobacco use, and, in many cases, other activities are prioritized before measuring biomarkers. Furthermore, the challenge of distinguishing differences in the levels of biomarkers due to variations among

products from the differences due to user behaviour was the basis for TobReg´s conclusion in 2008 that measurement of biomarkers is not a suitable regulatory strategy for monitoring differences among cigarette products *(3)*. Substantial new research has, however, been conducted during the past 15 years, with new biomarkers, new technologies and new evidence. Further, manufacturers continue to introduce a constant stream of new nicotine and tobacco product types onto markets worldwide, some of which differ significantly in their chemical composition not only from traditional products such as cigarettes but also from other relatively recent products. For example, HTPs and, more recently, tobacco-free nicotine pouches are significantly different from e-cigarettes in their chemical composition and mode of use; therefore, knowledge on the health effects associated with e-cigarettes cannot be used directly to guide regulatory decisions on such emerging products. Therefore, advances in biomarker research must be summarized in the context of the current product landscape, in order to reassess their potential use as proxies in tobacco control.

In this paper, we review the current literature on biomarkers of exposure, biomarkers of biological effects, including those associated with specific diseases, and biomarkers of susceptibility that have been used in studies of electronic cigarettes and other ENDS and HTPs. In particular, it covers:

- biomarkers of exposure used for ENDS and HTPs;
- biomarkers of biological effects that are part of the pathophysiology of various relevant diseases;
- biomarkers of susceptibility;
- a discussion of the state of biomarker research and implications for tobacco control, including research gaps and limitations of biomarkers;
- recommendations on possible prioritization of biomarkers for tobacco control;
- recommendations on addressing research gaps and priorities; and
- relevant policy recommendations.

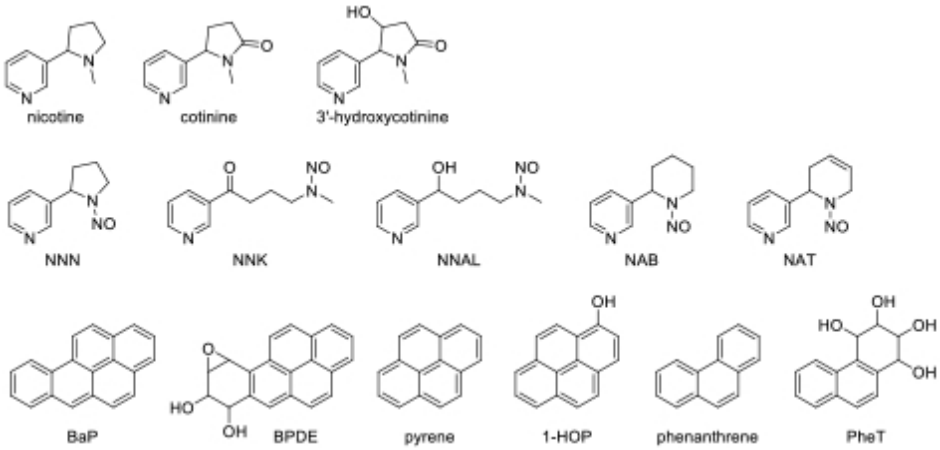
The literature search was conducted primarily in the PubMed database and the SciFinder search tool, which retrieves data from the Medline and CAplus databases. Important relevant articles cited in publications obtained in the database research were also included. In addition, the websites of the US Centers for Disease Control and Prevention, the US Food and Drug Administration and other relevant websites that contain information on exposures and effects associated with ENDS and HTPs were used.

5.2 Biomarkers of exposure

5.2.1 Definition and overview of biomarkers of exposure commonly used in studies of tobacco and nicotine products

A biomarker is defined by Oxford Languages as a measurable substance in an organism the presence of which is indicative of some phenomenon such as environmental exposure. Within this general definition, a biomarker of exposure is an entity that can be reliably quantified and is related to a specific exposure. In the context of this report, a biomarker of exposure can confirm use of, or exposure to, specific nicotine or tobacco products, or indicate changes in exposure to specific chemical compounds when individuals switch between products. The structures of the biomarkers discussed in this report and their sources are presented in Fig. 1.

Fig. 1. Structures of some constituents and biomarkers discussed in this report



Carbon monoxide (CO). CO is a product of incomplete combustion of organic matter. Exhaled CO is a useful, widely applied biomarker of exposure to all tobacco products the use of which involves combustion; these include cigarettes, cigars, pipes and hookah, but can also include HTPs because of the evidence of some level of combustion when such products are used. Marijuana smoking can also increase exhaled CO. CO is not produced in significant amounts during use of ENDS, HTPs or smokeless tobacco if no combustion is involved. Exposure to CO is associated with blood carboxyhaemoglobin (COHb) but is more commonly measured as CO in exhaled breath, as this test can be performed easily with commercially available devices. Various cut-off points of exhaled CO have been proposed to distinguish smokers from non-smokers, as other factors, such as high levels of environmental pollution, can affect measurements. A cut-off point of 5–6 parts per million (ppm) CO in exhaled breath has been suggested to distinguish

users of smoked tobacco products from users of other tobacco products or non-users of any tobacco product *(4)*. CO binds rapidly to haemoglobin in the blood, which can lead to various health effects by diminishing its oxygen-carrying ability. Such effects can be a particular problem for people with underlying cardiovascular or pulmonary disease. Cigar smoking is an especially rich source of CO exposure *(5)*.

Nicotine and its metabolites. Addiction to nicotine is the single most important reason why people continue to use products that efficiently deliver this substance, despite the known adverse health effects of tobacco product use. All tobacco and nicotine products deliver nicotine, with varying pharmacokinetics, resulting in binding to nicotinic cholinergic receptors in the brain and the release of dopamine, which mediates the pleasurable sensations associated with use of these products *(4,6)*. The time between inhaling tobacco smoke and the release of dopamine is only a few seconds, which helps to explain smokers´ addiction *(4,6)*. As the half-life of nicotine in the body is only about 2 h, it is not a very useful quantitative biomarker of nicotine exposure. The major metabolite of nicotine – cotinine – has been widely used as a biomarker of nicotine uptake due to its longer half-life of approximately 16 h (range, 8–30 h, depending on individual characteristics). Thus, cotinine has been quantified in serum, plasma, whole blood, saliva and urine as a biomarker of nicotine uptake. While cotinine is a good general biomarker of nicotine exposure, individual differences in enzymes involved in its formation and further metabolism, including CYP2A6 and UGT2B10, can affect cotinine measurements. Thus, the gold standard biomarker of nicotine exposure is "total nicotine equivalents", which comprise urinary nicotine, cotinine and 3´-hydroxycotinine and their glucuronides. This biomarker is strongly correlated with urinary metabolite measurements that include these compounds and also with several minor nicotine metabolites such as nicotine *N*-oxide *(4,6)*.

Tobacco-specific nitrosamines and metabolites. Tobacco-specific nitrosamines are a group of carcinogens formed during tobacco curing and processing by reactions of tobacco alkaloids such as nicotine, nornicotine, anabasine and anatabine with nitrite in tobacco *(7–10)*. All tobacco-containing products contain tobacco-specific nitrosamines, including *N´*-nitrosonornicotine (NNN), *N´*-nitrosoanabasine, *N´*-nitrosoanatabine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), as well as some minor products *(11)*. Tobacco-specific nitrosamines are present at lower levels in HTP emissions than in the smoke of conventional cigarettes but are generally not found, or are present in very low quantities, in the emissions from ENDS, as discussed below. As the name implies, the occurrence of these carcinogens is specific to tobacco products, including smoked and smokeless tobacco products *(12)*. NNN and NNK are powerful carcinogens, inducing tumours at relevant sites in laboratory animals, such as the oral mucosa, oesophagus, and lung *(9)*. Tumours are observed in animals

treated chronically with low doses of these compounds *(13,14)*. It has been clearly demonstrated that users of tobacco products take up NNN and NNK *(11)*. Thus, NNN and NNK are widely regarded as important causes of cancer in people who use smokeless tobacco or smoked products; they were classified as "carcinogenic to humans" by the International Agency for Research on Cancer (IARC) *(11)*. NNK is metabolized in laboratory animals and humans to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which has carcinogenic activity similar to that of NNK *(9)*. Urinary NNAL has been widely used as a biomarker of exposure to NNK *(10)*. Its tobacco specificity and carcinogenic activity combined make it an important biomarker of exposure to tobacco carcinogens and of cancer risk. Prospective epidemiological studies of cigarette smokers have demonstrated a significant association between relatively high levels of urinary NNAL and lung cancer risk *(15)*. Urinary NNN has similarly been used as a biomarker of NNN exposure and carcinogenicity and was significantly related to the incidence of oesophageal cancer in a prospective study of cigarette smokers *(16)*. Thus, NNAL and NNN are considered potentially useful biomarkers of relevant cancer risks in people who use tobacco-containing products and may be useful in predicting cancer risk; however, further studies are required. NNN has also been identified in the saliva of e-cigarette users as a result of endogenous formation in the oral cavity from nicotine and/or its metabolite nornicotine *(17)*.

Polycyclic aromatic hydrocarbon (PAH) metabolites. PAH, like CO, are products of incomplete combustion of organic matter. Thus, mixtures of PAH are found in the smoke of cigarettes, cigars, pipes and hookah but in far lower quantities in smokeless tobacco that does not contain fire-cured tobacco, where their presence is due in part to environmental pollution *(18–20)*. Similarly, the levels of PAH are consistently lower than in conventional smoked products or not detected at all in ENDS or HTPs *(21)*. PAH have been known since the 1970s to contribute significantly to tobacco-smoke carcinogenesis from studies in many animal models of the carcinogenic activity of selected subfractions and individual compounds in tobacco smoke condensate, including benzo[*a*]pyrene (BaP), chrysene, methylchrysenes, benzofluoranthenes, benz[*a*]anthracenes and others *(22)*. More than 500 PAH have been at least partially identified in tobacco smoke, and BaP as a representative PAH is classified as "carcinogenic to humans" by IARC *(18,23)*. 1-Hydroxypyrene (1-HOP) and hydroxyphenanthrenes, urinary metabolites of the non-carcinogenic PAH pyrene and phenanthrene, which are components of all PAH mixtures, have been widely used as biomarkers of exposure to PAH *(14)*. The population-based National Health and Nutrition Examination Survey (NHANES) showed significantly higher levels of these metabolites in cigarette smokers than non-smokers in the USA, the exposure of the latter group resulting from inhaling polluted air or consuming charbroiled food *(24)*. Cigarette smoking has consistently been shown to be a major source of exposure to PAH.

A pathway of metabolism of PAH that leads to carcinogenesis is formation of diol epoxides *(25)*. This important metabolic pathway can be quantified by analysis of a urinary BaP-tetraol, an end-product of metabolic BaP-diol epoxide hydrolysis; however, a more practical approach is use of phenanthrene tetraol (PheT), as its concentration in urine is more than 1000 times higher than that of BaP tetraol *(10,26)*. The levels of PheT in cigarette smokers were significantly associated with lung cancer in the Shanghai Cohort study *(15)*. There is no doubt that PAH contribute to cancer risk in cigarette smokers, although the relative extent of their contribution to the etiology of specific cancers versus those of other toxicants and carcinogens discussed here is presently unknown.

Volatile toxicants and carcinogens and their metabolites. Numerous volatile toxicants and carcinogens are produced during the combustion of tobacco. These include the IARC Group 1 (carcinogenic to humans) compounds formaldehyde, ethylene oxide, benzene and 1,3-butadiene; Group 2A (probably carcinogenic to humans) compounds acrolein, acrylamide, dimethylformamide and styrene; and Group 2B (possibly carcinogenic to humans) compounds propylene oxide, acrylonitrile, crotonaldehyde, ethyl benzene and propylene oxide, among others *(27)*. Other volatile compounds, such as methacrolein and methyl vinyl ketone, have well-established toxic effects similar to those of acrolein *(28–30)*. Acrolein is considered one of the most toxic compounds in tobacco smoke, and its non-cancer hazard index is the highest among common smoke constituents *(31)*. Acrolein and related compounds are implicated as causes of COPD *(32)*. Most of these compounds or their metabolites, mainly mercapturic acids, are detectable in blood or urine of all humans due to endogenous processes, inflammation and environmental or dietary exposure, but cigarette, cigar, pipe, hookah and marijuana smoking usually result in significantly higher levels than in non-smokers *(33–46)*. Cyanoethyl mercapturic acid (CEMA), a metabolite of acrylonitrile, which is not an endogenous compound and is seldom encountered in significant quantities in the general environment except in tobacco smoke, is a particularly useful biomarker for distinguishing users of smoked tobacco products from non-smokers. Thus, a cut-off point of 27 pmol/mL urine of CEMA differentiated cigarette smokers from nonsmokers with a sensitivity and specificity greater than 99% *(47)*. ENDS and HTPs also generate volatile toxicants and carcinogens but generally at much lower levels than conventional cigarettes *(48,49)*.

Metals. The occurrence of various metals, including arsenic, beryllium, cadmium, hexavalent chromium, cobalt, lead, nickel and radioactive polonium, has been reported in tobacco *(20)*. The highest mean concentrations in total particulate matter of cigarette smoke were those of cadmium and lead, at 40.2 ± 5.4 and 11.0 ± 1.1 ng/cigarette (ISO conditions), respectively *(50)*. These results were consistent with those obtained in other studies *(20)*. In agreement with these data, the NHANES study demonstrated that blood and urinary cadmium levels and blood

lead levels were higher in smokers than in non-smokers *(51)*. Similar results were found for cadmium in blood and urine of a German population *(52)*. Cadmium and its compounds are carcinogenic to humans, causing lung cancer and possibly kidney and prostate cancers *(53)*. Lead is toxic to the neurological, renal, cardiovascular, haematological, immunological, reproductive and developmental systems *(54)*.

Some studies have reported the presence of metals in ENDS aerosols. Chromium and lead were reliably measured in e-cigarette aerosol *(55,56)*. Other studies have reported the presence of cadmium, copper, nickel, manganese, aluminium and tin and shown that product design is an important factor in the levels of metals *(57,58)*. Because they remain for a long time in exposed people, cadmium and lead may serve as long-term markers of cumulative exposure *(59)*.

5.2.2 Application of biomarkers of exposure in studies of ENDS and HTPs

Carbon monoxide (CO)

Several investigations, including randomized clinical trials and cross-sectional studies, have included quantification of exhaled CO or blood COHb and demonstrated significantly lower levels in ENDS users than in cigarette smokers, and most studies did not find elevated CO or COHb in exclusive ENDS users (reviewed in *21* and *60*). Some examples are cited here. Oliveri et al. *(61)* reported a 47% lower concentration of COHb in adult exclusive ENDS users than in cigarette smokers. Hatsukami et al. *(62)* observed a significant 60% reduction in expired CO when cigarette smokers switched to ENDS for 8 weeks, although not all study participants switched to exclusive ENDS use. McRobbie et al. *(63)* reported a significant 80% decrease in expired CO when cigarette smokers switched to ENDS for 4 weeks. Czoli et al. *(64)* found a significant 41% reduction in expired CO when subjects switched from 7 days of dual use of cigarettes and ENDS to 7 days of exclusive ENDS use. O´Connell et al. *(65)* found a significant 88–89% reduction in expired CO and an 84–86% reduction in COHb when cigarette smokers switched to ENDS for 5 days. Cravo et al. *(66)* reported rapid decreases in expired CO and blood COHb in subjects who switched from conventional cigarettes to ENDS. Expired CO decreased from 20.3 ppm to 7.4 ppm after 1 week of ENDS use and was 7.6–9.0 ppm from week 2 until the end of the study (12 weeks), and COHb decreased from 6.79% to 4.06–4.37% after 1 week of ENDS use until the end of the study. Morris et al. *(67)* reported a 79% reduction in COHb when subjects switched from conventional cigarettes to ENDS use for 9–14 days.

A review by Akiyama and Sherwood (tobacco industry researchers) *(60)* provides comparative biomarker results after cigarette smoking and in 30 clinical trials of HTPs, with a median intervention period of 8 days. Reductions of 80–90% in expired CO and of 50–90% in COHb were observed within 1 week in most studies. These results are consistent with significantly less combustion in HTPs than during cigarette smoking. For example, in one study in which

subjects switched from conventional cigarettes to an HTP, expired CO decreased by approximately 80% within 6–7 days, reaching levels similar to those achieved after smoking cessation *(68)*. In a comparison of a menthol HTP with menthol cigarette smoking, average COHb was reduced by 62% within 5 days of switching from smoking to the HTP, similar to that achieved in 5 days of abstinence *(69)*.

Nicotine and its metabolites

Randomized clinical trials and cross-sectional studies of the levels of biomarkers of nicotine and its metabolites in cigarette smokers and ENDS users were reviewed by Akiyama and Sherwood *(60)* and by Scherer et al. *(21)*. Some studies indicated lower levels of urinary total nicotine equivalents in ENDS users than in cigarette smokers, while others reported no difference. For example, Round et al. *(70)* conducted a randomized, parallel-group clinical study of smokers who switched to an ENDS product for 5 days. Total nicotine equivalents measured in 24-h urine samples decreased by 38.3% ($P < 0.05$), and plasma cotinine and nicotine were similarly statistically significantly decreased. Shahab et al. *(71)* conducted a cross-sectional study and found that urinary total nicotine equivalents were not significantly different in cigarette smokers and ENDS users. In theory, the levels of total nicotine equivalents should be similar in smokers and ENDS users, as both products are designed to deliver nicotine efficiently, and there is likely to be some self-titration, although differences in ENDS product characteristics and use patterns may lead to the different results. It is important to note that use of ENDS with non-salt liquids, in which most of the nicotine is present in unprotonated form, leads to predominantly oral absorption of nicotine. This results in slower nicotine pharmacokinetics and may therefore have lower abuse liability than conventional cigarettes. Many currently marketed ENDS contain nicotine in the form of salts, however, which makes ENDS aerosols easy to inhale and results in faster nicotine absorption.

In the review by Akiyama and Sherwood *(60)*, the levels of total nicotine equivalents in HTP users were similar to those in cigarette smokers in most studies, not differing by more than 20%. For example, in a three-arm, parallel-group study, 160 Japanese adult smokers were randomized to a menthol HTP ($n = 78$) or a menthol cigarette ($n = 42$) for 5 days in a confined setting and 85 days in ambulatory settings. No significant differences in the levels of total nicotine equivalents were found between the HTP and conventional cigarette users in either setting *(72)*, although substantial differences in the characteristics of different HTPs may be related to differences in nicotine delivery.

Tobacco-specific nitrosamines and metabolites

Significant reductions in urinary NNAL were reported in all the randomized clinical trials in which cigarette smokers switched to ENDS or HTPs, and its levels were also significantly lower in ENDS users than in cigarette smokers in cross-sectional

studies, including the Population Assessment of Tobacco and Health (PATH) study *(60,73,74)*. NNAL is barely detected in the urine of ENDS users because it is a metabolite of NNK, which occurs only in tobacco-containing products. The low levels that are occasionally detected in ENDS users may be due in part to carryover from use of tobacco products (due to the long half-life of NNAL) or exposure to second-hand tobacco smoke *(75–77)*. NNN levels were either extremely low or not detected in the urine of ENDS users *(60,78)*. Bustamante et al. *(17)* presented evidence for the presence of NNN in the saliva of ENDS users (14.6 ± 23.1 pg/mL) and concluded that it was formed endogenously, as it was not detected above trace amounts in ENDS liquids. Scherer et al. *(79)* did not find statistically significantly higher levels of tobacco-specific nitrosamines or their metabolites in the urine or saliva of ENDS or HTP users than in non-users of tobacco products.

PAH metabolites

A randomized clinical trial of cigarette smokers who switched to ENDS for 5 days showed significant 63.5% and 63.8% reductions in urinary 1-HOP and 3-hydroxyBaP, respectively, as well as significant reductions in fluorene and naphthalene metabolites *(70)*. The results were similar when mentholated products were used. A similar 5-day switching trial showed a 70.5% reduction in urinary 1-HOP *(65)*. In another trial in which cigarette smokers switched to ENDS for 8 weeks, significant 20% reductions were found in PheT *(62)*. A comparison of urinary 1-HOP levels in ENDS users with those reported in three studies of cigarette smokers showed significant 57–61% reductions in ENDS users *(80)*. As reviewed by Akiyama and Sherwood *(60)*, many randomized clinical trials have shown a reduction in 1-HOP after switching from conventional cigarettes to HTPs. The reductions were frequently greater than 60%, although some trials reported 15–30% reductions. Similar results were observed in the PATH study *(74)*. The results summarized here are consistent with substantial decreases in exposure to combustion products in users of both ENDS and HTPs.

Volatile toxicants and carcinogens and their metabolites

Consistently, randomized clinical trials of cigarette smokers who switched to ENDS found significant decreases in biomarkers of exposure to volatile toxicants and carcinogens, including acrolein, acrylamide, acrylonitrile, benzene, 1,3-butadiene, crotonaldehyde, and ethylene oxide *(60)*. In a study in which participants were randomized to 8 weeks of instructions for complete substitution of cigarettes with e-cigarettes, significant decreases in urinary biomarkers of acrylamide (32%), acrolein (47%), acrylonitrile (66%) and crotonaldehyde (47%) were observed *(62)*. In a study in which smokers were randomized to 5 days of ENDS use, significant decreases were found in the levels of mercapturic acids of acrolein (70.5%), acrylonitrile (85.9%), benzene (89.7%), 1,3-butadiene (55.5%),

crotonaldehyde (77.5%) and ethylene oxide (62.3%) *(71)*. Cross-sectional studies, including the PATH study *(60,74)* and a recent study in which cigarette smokers or ENDS users were confined for 3 days *(81)*, gave similar results.

A number of studies, most of which were based on one or two times, have shown higher levels of urinary biomarkers of exposure to volatile agents such as acrylonitrile, acrolein, crotonaldehyde and propylene oxide in ENDS users than non-users of any tobacco or nicotine product (reviewed in *82*). One study in which urine samples were obtained monthly for 4–6 months found significantly higher levels of 3-hydroxypropyl mercapturic acid, a major metabolite of acrolein, in the urine of ENDS users than in non-users of any tobacco or nicotine product *(82)*.

Several randomized clinical trials of cigarette smokers who switched to HTPs, conducted by industry researchers, also showed large decreases in the mercapturic acids of volatiles. The results were consistent across all the published industry trials *(60)*.

A clinical study in which 10 subjects per group were confined for 3 days and used only their specified product (cigarettes, ENDS, HTPs, oral tobacco, nicotine replacement therapy or non-users of any tobacco or nicotine product) showed slight increases in mercapturic acids related to acrolein, acrylamide, and crotonaldehyde in HTP users than in users of other non-cigarette products *(81)*.

Metals

Cadmium and lead are the toxic metals to which cigarette smokers are exposed at the highest levels, as noted above. Data from Wave 1 of the PATH study (2013–2014) also indicated significantly higher levels of urinary cadmium and lead in ENDS users than in never users of any tobacco product or ENDS, by 23% and 19%, respectively *(73)*. The authors noted that the long half-lives of biomarkers of metal exposure were possible confounding factors, as some ENDS users may have been former smokers or were exposed in other ways. Prokopowicz et al. *(83)* reported that the blood levels of cadmium decreased significantly in cigarette smokers who switched to ENDS, while there was no significant difference in blood lead levels. Smokers had significantly higher levels of both biomarkers than non-smokers. A cross-sectional study of urinary elements including chromium, nickel, cobalt, silver, indium, manganese, barium, strontium, vanadium and antimony, in addition to cadmium and lead, showed no differences in the levels of these elements in ENDS users and non-smokers *(84)*. A review found inconsistent results with respect to biomarkers of lead, chromium, nickel, selenium and strontium in ENDS users as compared with non-users *(85)*.

No biomarkers of exposure to metals have been reported in HTP users. A search in PubMed for "metal exposures heated tobacco products" and a similar Google search produced only unrelated articles or monographs.

Salivary propylene glycol as a novel biomarker for ENDS

Propylene glycol is a major constituent of e-cigarette aerosol. An assay was developed recently for measuring propylene glycol in saliva,[2] showing that the average concentrations of propylene glycol in the saliva of ENDS users were approximately 100 times higher than those in non-smokers and 30 times higher than those in smokers. Therefore, salivary propylene glycol could be used as a novel biomarker to validate ENDS use.

Biomarkers of exposure and dual use

A significant number of smokers who adopt ENDS continue to smoke conventional cigarettes (referred to as "dual users"), with varying degrees of substitution *(86)*. Studies have shown that dual users generally have similar or higher levels of many biomarkers of exposure compared to those of exclusive smokers and that complete switching to ENDS is necessary to achieve meaningful reductions in exposure *(87–90)*. In a recent study, Anic et al. *(90)* used biomarker data from 2475 adults in the PATH Study who were smokers in Wave 1 (2013–2014) and who transitioned to exclusive or dual ENDS use or quit tobacco products in Wave 2 (2015). Cigarette smokers who became dual users of cigarettes and ENDS did not have significant reductions in most of the assessed biomarkers. Table 1 gives examples of data from that study, with the levels of some of the biomarkers discussed above for smokers who continued exclusive smoking, became dual users, or quit any tobacco or nicotine use.

Table 1. Biomarker levels in PATH study participants in Wave 2, by product use status

	Product use status			
Biomarker (source)	**Exclusive smoking**	**Dual smoking and ENDS use**	**Exclusive ENDS use**	**No use of tobacco or nicotine**
TNE, µmol/g creatinine (nicotine)	31.2 [28.0 ; 34.8]	38.5 [30.3 ; 48.9]	9.1 [3.6 ; 22.9]	0.1 [0.0 ; 0.1]
NNAL, ng/g creatinine (NNK)	218.1 [199.2 ; 238.8]	231.9 [187.0 ; 287.5]	12.5 [5.7 ; 27.3]	5.0 [3.6 ; 7.0]
1-HOP, ng/g creatinine (pyrene)	316.8 [298.3 ; 336.4]	308.1 [277.4 ; 342.2]	113.4 [93.0 ; 138.4]	167.9 [148.9 ; 189.4]
CEMA, µg/g creatinine (acrylonitrile)	131.7 [121.1 ; 143.2]	128.1 [104.7 ; 156.6]	8.6 [4.9 ; 14.9]	3.9 [2.9 ; 5.3]
3HPMA, µg/g creatinine (acrolein)	1342.2 [1247.8 ; 1443.7]	1531.6 [1321.6 ; 1774.9]	303.8 [228.7 ; 403.7]	299.9 [255.8 ; 351.5]
4HBMA, µg/g creatinine (1,3-butadiene)	31.8 [29.6 ; 34.2]	33.9 [28.9 ; 39.8]	5.1 [3.9 ; 6.8]	5.4 [4.6 ; 6.4]

Source: Anic et al. *(90)*
Each cell shows the geometric mean and [95% CI]. All participants included in these analyses were exclusive smokers at wave 1.

2 Tang MK, Carmella SG, unpublished data; 2022.

5.3 Biomarkers of biological effects (harm or disease)

5.3.1 Definitions and overview of biomarkers of biological effects commonly used in studies of tobacco and nicotine products

Various definitions of biomarkers of biological effect have been used in the literature *(91)*. These biomarkers are commonly referred to as "biomarkers of potential harm", which have been defined as "the measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm, including preclinical changes." *(92–94)*. It should be noted that this definition encompasses (i) a continuum of biological effects and (ii) a spectrum of relevant diseases. In the context of tobacco and nicotine product use, the predominant health outcomes of relevance include cancer and cardiovascular and respiratory diseases. Time is also an important variable. Interpretation of the implications of an acute change as opposed to a long-term change may depend on the biomarker. A definition that is tailored to 'combustible' tobacco product use was proposed by tobacco industry researchers, which specifies that a biomarker of biological effect is "a significant, objective, measurable alteration in a biological sample, after smoking a tobacco product, … which is altered in a proportion of smokers and is reversible on cessation of smoking" *(95)*. This definition includes the notion of reversibility, which is relevant for studies of potential changes in biological effects when users of traditional tobacco products (e.g. conventional cigarettes) switch to tobacco or nicotine products with different harmful constituent yields (e.g. ENDS). The consequences of biomarker reversibility should be further investigated in studies of changes in health effects.

DNA adducts. DNA **add**ition prod**ucts**, commonly called adducts, are produced by reactions with DNA of certain organic or inorganic intermediates formed during cellular metabolism of inhaled toxicants or carcinogens as well as by reactions of intermediates formed from some endogenous compounds. Adducts to DNA bases or phosphates are central to the carcinogenic process because they can cause miscoding in DNA and the consequent mutations observed in many critical growth control genes involved in cancer. Cells have DNA repair systems to mend the damage, but, when the repair systems are inefficient or error prone, mutations can occur in DNA when adducted bases are misread and the wrong base is inserted by DNA polymerases. The result is a permanent mutation, which may occur in critical genes involved in growth control, leading to cancer initiation. Many mutations are produced in the DNA of various tissues during the metabolism of tobacco carcinogens *(96–101)*. Numerous studies with a variety of methods, including ^{32}P-postlabelling, immunoassays and mass spectrometry, have examined specific types of DNA adducts in various tissues of cigarette smokers and non-smokers *(102–108)*. Many of the studies show higher levels of certain DNA adducts in tissues of

smokers than in those of non-smokers, but interpretation was complicated as, in some cases, the numbers of subjects were small or the methods lacked appropriate validation.

Cytokines, chemokines and reactive proteins. Inflammation and oxidative damage are significant factors in various diseases caused by cigarette smoking, including cancer, cardiovascular disease and COPD. The processes involve infiltration of lymphocytes, macrophages and neutrophils into tissues under stress and secretion of pro- and anti-inflammatory cytokines and other factors. Such biomarkers are commonly measured in plasma or serum, and some – such as interleukin (IL)-6 and C-reactive protein (CRP) – are significantly higher in smokers than in nonsmokers *(107)*. The levels of these biomarkers have been directly linked to various relevant diseases. For example, CRP, BCA-1/CXCL13, MDC/CCL22 and IL-1RA have been prospectively associated with the risk of lung cancer *(108)*. In relation to cardiovascular disease (CVD), oxidized LDL is a representative indicator of lipid profiles *(109)*, and CRP, IL-6, fibrinogen and soluble ICAM-1 are indicators of thrombosis and endothelial dysfunction, which play a basic role in the initiation and progression of atherosclerosis, vasoconstriction and coronary heart disease *(110–114)*. COPD is associated with elevated IL-8, TNF-α, IL-6 and RANTES ("regulated on activation, normal T cell expressed and secreted"), reflecting a predominance of macrophages and T cells, which are correlated with the degree of airflow obstruction and emphysema and appear to play a predominant role in apoptosis, leading to lung destruction *(115–124)*. COPD is also characterized by increases in fibrinogen, which is associated with reduced lung function *(118–121,125–131)*.

***Urinary prostaglandin metabolite*s**. Two urinary biomarkers, prostaglandin E_2 metabolite (PGEM) and (*Z*)-7-[1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*,3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid (8-*iso*-$PGF_{2\alpha}$) are considered to be biomarkers of inflammation and oxidative damage, respectively. PGEM is a metabolite of prostaglandin E_2, while 8-*iso*-$PGF_{2\alpha}$ is a product of lipid peroxidation *(132)*. 8-*iso*-$PGF_{2\alpha}$ has also been quantified in blood. Inflammation and oxidative damage are clearly associated with cigarette smoking, and they play a significant role in cancer induction by enhancing the activity of cigarette smoke carcinogens through mechanisms involving co-carcinogenesis or tumour promotion *(102)*. They also have established roles in cardiovascular disease and COPD *(133)*. The Shanghai Cohort Study found an independent association between urinary levels of 8-*iso*-$PGF_{2\alpha}$ in cigarette smokers and the risk of lung cancer, after adjustment for smoking intensity and duration and other possible confounding factors *(134)*. A significant association was also observed in former smokers but not in never smokers, indicating a probable interaction between tobacco smoke carcinogens and oxidative damage *(134)*. The levels of urinary 8-*iso*-$PGF_{2\alpha}$ decrease more slowly than biomarkers

of exposure after cessation of cigarette smoking. The PATH study found that more than 6 months were required for the geometric mean levels of 8-*iso*-$PGF_{2\alpha}$ to return to non-smoker levels, while another study reported a 27% decrease after 12 weeks of cessation *(135,136)*.

5.3.2 Application of biomarkers of biological effect in studies of ENDS and HTPs

DNA adducts

Few studies have been published on DNA damage by ENDS in oral cells (reviewed in *137)*. Mixed results were obtained in a variety of in-vitro studies in which cultured oral cells were exposed to ENDS aerosol or liquid, some studies indicating possible DNA damage while others did not.

A clinical study was conducted of the acrolein–DNA adduct (8*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3*H*)-one (γ-OH-Acr-dGuo) in ENDS users and non-users of any tobacco product in oral cells of 20 people per group who visited the clinic once a month for 3 months. The levels of γ-OH-Acr-dGuo were significantly nine times higher in ENDS users than in non-users and lower than in cigarette smokers *(106,138)*. These results demonstrate specific DNA adduct formation in the oral mucosa of ENDS users rather than non-users, signalling a possible carcinogenic effect. In a study of apurinic/apyrimidinic sites in DNA, a type of endogenous DNA damage common in all human tissues, the levels in ENDS users were significantly 45% and 42% lower than in non-smokers and smokers, respectively, based on data from a single clinic visit (30–35 subjects per group). The direct relation between apurinic/apyrimidinic sites and ENDS use or cigarette smoking is unclear *(139)*.

No studies were found on the effects of HTP use on DNA damage.

Cytokines, chemokines and reactive proteins

Table 2 summarizes data on some circulating and urinary biomarkers of biological effect commonly measured in studies of tobacco and nicotine product use, with geometric mean ratios for users of various product types *(135,140)*. In reviewing data on such biomarkers, it is important to note that they are not specific to exposure to a particular tobacco or nicotine product and are likely to be influenced by pre-existing sub-clinical disease from previous smoking.

Table 2. Geometric mean ratio (GMR) and range, by product use status, for commonly measured biomarkers of biological effects in studies of tobacco and nicotine product use

Biomarker	Matrix	Indicative of	GMR in ENDS vs smokers	GMR in ENDS vs nonsmokers	Population[a]
IL-6	Serum	Inflammation	0.84 (0.71–0.98)	0.98 (0.82–1.18)	PATH
hs-CRP	Serum or plasma	Inflammation, cardiovascular risk	0.73 (0.57–0.93)	0.86 (0.66–1.11)	PATH
Fibrinogen	Plasma	Inflammation, coagulation, cardiovascular risk	0.96 (0.92–1.01)	0.99 (0.94–1.04)	PATH
sICAM	Serum	Inflammation, cardiovascular risk	0.82 (0.75–0.89)	1.02 (0.95–1.1)	PATH
LDL	Plasma	Cardiovascular risk	0.52 (0.24, 1.14)	0.60 (0.31, 1.16)	NHANES[b]
HDL-C	Plasma	Cardiovascular risk	1.00 (0.50, 2.00)	1.82 (0.95, 3.49)[c]	NHANES[b]
TGL	Plasma	Cardiovascular risk	0.26 (0.06, 1.02)	0.42 (0.12, 1.51)	NHANES[b]
8-iso-PGF2a	Urine	Oxidative stress	0.75 (0.68–0.83)	1.10 (0.98–1.22)	PATH

[a] US cohorts. NHANES: National Health and Nutrition Examination Survey; PATH: Population Assessment of Tobacco and Health Study.
[b] Data for exclusive ENDS users with no prior history of smoking
[c] Lower levels of high-density lipoprotein cholesterol (HDL-C) are associated with higher cardiovascular risk.

ENDS users. Analysis of data from Wave 1 of the PATH study shows that the levels of IL-6, hs-CRP, and sICAM-1 in former smokers who switched to exclusive ENDS use are significantly lower than those in current exclusive cigarette users and comparable to those in former smokers who did not use any tobacco or nicotine product *(135)*. Fibrinogen levels were, however, similar in ENDS users and smokers (GMR, 0.96; 95% CI, 0.92 ; 1.01). The analysis also showed that the levels of these biomarkers did not differ by frequency of ENDS use by current exclusive users, and there was no association with the time since smoking cessation. A recent study of data on HDL-C, low-density lipoprotein cholesterol, triglycerides and fasting blood glucose in 8688 adults in two National Health and Nutrition Examination Survey cycles (2015–2016 and 2017–2018) found no statistically significant effect of exclusive ENDS use on these measures *(140)*. Despite common reports of lower levels of cytokines and other circulating inflammatory biomarkers in exclusive ENDS users than in smokers, the results are not consistent across studies, biomarkers or device types *(135,141–144)*. The availability of definitive data on these biomarkers in ENDS users is important, because many studies in vitro, in vivo and in humans indicate that ENDS aerosols may induce inflammation and cause respiratory and cardiovascular effects *(145–148)*. For example, in a study by Mohammadi et al. *(149)*, endothelial function was measured in chronic ENDS users, chronic cigarette smokers and nonusers by assessing the effects of participants' sera on release of nitric oxide (NO) and hydrogen peroxide and cell permeability in cultured endothelial cells. Sera from ENDS users had effects similar to those in smokers in reducing vascular

endothelial growth factor-induced NO secretion by endothelial cells, release of hydrogen peroxide, greater permeability and changes in circulating biomarkers of inflammation, thrombosis and cell adhesion. These results suggest that ENDS use may induce changes in endothelial function. A study of salivary inflammatory biomarkers conducted in India showed that the levels of salivary CRP, TNF-α and IL-1b were significantly higher in ENDS users than in non-users and similar to those in smokers *(150)*.

HTP users. Data on circulating inflammatory biomarkers in HTP users are primarily from tobacco industry-conducted studies. For example, Philip Morris International (PMI) published several reports on such biomarkers in study participants recruited in various countries who switched from smoking to a prototype HTP *(151)*. A study in Japanese smokers who switched to an HTP for 6 days included measurement of serum club cell 16-kDa protein (CC16), which is an indicator of lung epithelial injury; no change was observed *(152)*. Another PMI study, in 316 Polish smokers randomized to an HTP or continued smoking condition for 1 month, included assessment of a broad panel of biomarkers associated with cardiovascular risk *(153)*. An increase (i.e. an improvement) in HDL-C was reported in the HTP group; however, reductions in red blood cell count, haemoglobin and haematocrit were observed. Most other biomarkers did not change after a switch from smoking to HTP use for 1 month. A longer switching study was conducted by PMI in the USA, in which 984 adult smokers were randomized to an HTP device or continued smoking for 6 months *(154)*. Reductions were reported in four biomarkers of effect (HDL-C, white blood cell count, forced expiratory volume in 1 min ($FEV1\%_{pred}$) and COHb) in smokers who switched to an HTP as compared with those randomized to continued smoking. In all these studies, statistically significant decreases in exposure to smoke constituents and in urinary mutagenicity were reported in participants randomized to HTP use.

British American Tobacco researchers reported on a longer (12 months) ambulatory clinical study in which smokers were randomized to an HTP, continued smoking or abstinence *(155)*. Statistically significant positive changes were observed in white blood cell count (reduction) and FeNO (increase) after 6 months of HTP use as compared with continuous smoking. The levels of 11-dTX B2 were also reduced after 6 months of HTP use, but the difference between HTP use and continuous smoking did not reach statistical significance. Further, no substantial effect of switching on sICAM-1 or HDL was observed (only descriptive statistics were provided). In an updated report from this study, the levels of most biomarkers at 12 months were similar to those at 6 months *(156)*. While some biomarkers of biological effect changed in a positive direction after switching to HTP (suggesting less harm than smoking), the outcomes were worse than those of participants who quit.

A real-world, post-marketing study of HTP conducted by researchers from Japan Tobacco *(157)* involved measurement of a panel of inflammatory markers, including HDL-C, triglycerides, sICAM-1, white blood cell count, 11-DHTXB2 and 2,3-d-TXB2 (biomarkers of platelet activation). Reductions were reported in biomarkers of effect in users of HTP (average, 1.2 years of use), although urinary 2,3-d-TXB2 was worse than in non-smokers.

Prostaglandin metabolites and other related urinary biomarkers

Prostaglandin metabolites. Analysis of data from the PATH study indicated that former smokers who currently exclusively used ENDS products had levels of urinary 8-*iso*-$PGF_{2\alpha}$ similar to those of former smokers who did not use ENDS products and to those of participants who had never used tobacco *(135)*. It was not clear, however, whether current ENDS users who were not former smokers also had elevated levels of 8-*iso*-$PGF_{2\alpha}$, and the relatively slow decrease in urinary 8-*iso*-$PGF_{2\alpha}$ upon smoking cessation probably compounds the lack of clarity *(135,136)*. It is important to note, however, that 8-*iso*-$PGF_{2\alpha}$ was significantly higher among dual users of smoking and e-cigarettes than in exclusive smokers in that study (GMR, 1.09; 95% CI 1.03 ; 1.15). Minimal, non-significant changes in urinary isoprostanes, including 8-*iso*-$PGF_{2\alpha}$ were observed in a study in which cigarette smokers switched to an HTP *(158)*. When 20 smokers switched to ENDS or HTPs after 1 week of not-using any tobacco product, significant increases in blood 8-*iso*-$PGF_{2\alpha}$ were reported *(159)*. Several industry-sponsored clinical trials addressed the effects of HTP use on urinary 8-*iso*-$PGF_{2\alpha}$. For example, when healthy adult smokers were randomized to a menthol HTP or a smoking abstinence group for 91 days, the levels of urinary 8-*iso*-$PGF_{2\alpha}$ decreased by 13% ($P < 0.05$) and were similar to those in the smoking abstinence group *(160)*. In the PMI study of 984 US cigarette smokers, a 6.8% reduction in urinary 8-*iso*-$PGF_{2\alpha}$ was observed in those who switched to HTP use for 6 months as compared with those who continued to smoke cigarettes *(154)*. In the post-marketing study of real-world HTP use in Japan, the level of this biomarker was somewhat higher in HTP users than in non-users, albeit at borderline significance (P=0.0646) *(157)*.

Indicators of preclinical changes and symptoms

Other urinary biomarkers of oxidative stress. In DNA, guanine is the major target for direct oxidation by inflammation-induced radicals. The most abundant product of such oxidation is 8-oxo-7,8-dihydro-2′-deoxyguanosine, which can cause chromosomal aberrations and induce mutations and is widely used as a biomarker of oxidative stress *(115)*. Some studies have found significantly higher levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine in ENDS users than in nonsmokers *(161,162)*. Sakamaki-Ching et al. *(162)* found no difference in the levels of this biomarker between ENDS users and smokers.

Direct assessment of chronic disease outcomes due to the use of ENDS and HTPs requires prospective cohort studies, which last many years and involve large numbers of participants. As this is not feasible in most studies and not suitable for time-sensitive regulatory decisions, indicators of preclinical changes and symptoms have commonly been used as surrogate measures of respiratory and cardiovascular risk in users of tobacco and nicotine products. Preclinical indicators and symptoms of cardiovascular disease may include such measures as blood pressure, heart rate, arterial stiffness, platelet reactivity and other cardiovascular outcomes. For respiratory diseases such as COPD, commonly measured preclinical indicators and symptoms include respiratory function (FEV1), forced vital capacity (FVC), FEV1/FVC and diffusing capacity of the lung for CO (DLCO), as well as coughing, wheezing, shortness of breath and other symptoms *(163–166)*. Interpretation of cardiopulmonary preclinical indicators is often based on lipid profile, fibrinogen, D-dimer and hs-CRP. There are no robust preclinical indicators of cancer. Reports of large population-based epidemiological studies of the association between ENDS use and disease outcomes are becoming available, most of which have been published since 2020. Such emerging data will play a key role in future assessments of the predictive value of biomarkers of exposure and biological effects in studies of novel and emerging tobacco products.

ENDS users. Several studies have shown that ENDS use increases blood pressure, heart rate, arterial stiffness, platelet reactivity and other cardiovascular outcomes as compared with no tobacco or nicotine product use *(141,147,148,167,168)*. Respiratory effects and symptoms (e.g. resistance to air flow, accumulation of lipid-laden macrophages in lungs) have been also reported in ENDS users, and a longitudinal study of Waves 1 and 2 of the PATH study showed that people who were exclusive ENDS users at baseline had a higher prevalence of subsequent respiratory symptoms than nonusers (33.6% vs 21.7%, respectively) *(168–170)*. Another report based on Waves 1–4 of the PATH study showed that ENDS use was associated with higher risks for respiratory disease (COPD, emphysema, chronic bronchitis and asthma) than in non-users *(171)*. Switching from smoking to ENDS may, however, result in improvements in some of these indicators and outcomes. A systematic review of six population-based studies with samples ranging in size from 19 475 to 161 529 found a lower odds ratio for respiratory outcomes (COPD, chronic bronchitis, emphysema, asthma and wheezing) but no change in cardiovascular outcomes (stroke, myocardial infarction and coronary heart disease) in former smokers who used ENDS as compared with current smokers *(172)*. A randomized crossover study of hookah users showed increased arterial stiffness and higher levels of inflammatory markers with use of e-hookah than with use of conventional tobacco hookah *(173)*. Differences between preclinical indicators and symptoms (i.e. between

COPD and CVD) are not surprising; they could be due to a complex interaction between exposure and specific biological effects elicited by the exposure. For example, the reduction in risk for lung cancer occurs over 20–25 years after smoking cessation and never reaches that of a never smoker *(174)*. In contrast, the CVD risk falls to that of a never smoker in only 1–3 years *(174)*; however, even low levels of exposure, such as fewer than three cigarettes per day or even second-hand smoke in nonsmokers, increase the risk for CVD *(175–177)*.

HTP users. There are few independent reports on indicators of health effects in HTP users. A cross-sectional study of 58 336 students aged 12–18 years in the 2018 Korea Youth Risk Behavior Survey study found an association between HTP use and asthma, allergic rhinitis and atopic dermatitis *(178)*. Two cases of acute eosinophilic supplementation associated with HTP use were reported in Japan. One case involved a 20-year-old man who had used 20 HTP sticks per day for 6 months and had doubled his consumption 2 weeks before hospitalization *(179)*. The second case was in a 16-year-old boy with bronchial asthma who developed cough, shortness of breath and fatigue immediately after smoking an HTP, the symptoms worsening over the course of 2 weeks of HTP use *(180)*. Most tobacco industry reports on preclinical indicators in HTP users are limited to respiratory measures, namely FEV_1. These studies show either no change or a modest improvement in this measure after switching from smoking to HTP use *(155,157)*.

Notable emerging biomarkers of biological effects for studies of ENDS and HTPs

DNA methylation profile. Extensive literature supports use of epigenetic modifications as a measure of the effect of smoking *(181,182)*. Studies of DNA methylation in saliva and bronchoalveolar lavage fluid found that the epigenetic profiles for ENDS use were similar to those of non-users *(183,184)* and that ENDS use did not affect cg05575921, a highly hypomethylated aryl hydrocarbon receptor repressor (AHRR) site in smokers and a sensitive, specific marker of smoking status *(185,186)*; however, hypomethylation of LINE-1 repeat elements and global loss of DNA hydroxymethylation were reported in leukocytes of ENDS users, suggesting systemic effects *(187)*.

The effect of HTP use on DNA methylation was assessed in the Tsuruoka Metabolome Cohort Study in Japan *(188)*, which found that 10 of 17 smoking-associated genes were significantly hypomethylated, and GPR15 expression was markedly upregulated in HTP users as compared with non-smokers, although AHRR expression was significantly lower than in cigarette smokers. These results suggest that HTP use may result in distinct DNA methylation and transcriptome profiles. The implications of such effects should be investigated.

Gene expression. Changes in gene expression induced by smoking and other harmful exposures can indicate disturbances in cellular metabolic pathways,

and such changes could serve as biomarkers of biological effects linked to specific health outcomes, including lung cancer *(189–191)*. Cross-sectional studies of ENDS users, smokers and nonsmokers found differential gene expression among the groups. For example, Martin et al. *(192)* found that the nasal epithelium of smokers showed differential downregulation of 53 genes, while that of ENDS users showed differential downregulation of 358 genes as compared with nonsmokers. Upregulation of only one gene – growth response 1 ("*EGR1*") – was the same in smokers and ENDS users, while the remaining overexpressed genes were specific to the two products. In the second cross-sectional study, oral cells were used to assess gene expression in the same two groups, with different results: smokers had more differentially expressed genes than ENDS users *(193)*. The most deregulated pathways in smokers and ENDS users were associated with carcinogenic pathways. Studies of gene expression after an acute exposure to ENDS showed significant changes in oral, blood and respiratory cells in response to the exposure *(194,195)*. More research is necessary to understand the pathophysiological consequences of such findings.

The oral microbiome. The oral microbiome is a complex receptor medium for chemical exposures in the oral cavity. Changes in the oral chemical environment create conditions that may be either detrimental or beneficial to certain bacterial populations, leading to changes in the composition and function of the oral microbiome. The oral cavity is also the gateway for bacteria that colonize the respiratory tract *(196–198)*, and there is accumulating information on the association of the oral microbiome with a variety of chronic diseases, including cancer, CVD and COPD *(196,199–207)*. Cigarette smoking affects the oral microbiome *(208)* through immunosuppressive effects *(209)*, favouring biofilm formation *(210)*, altering oral O_2 tension and pH *(211)* and modifying the chemical environment of the oral cavity *(212)*. The type of tobacco used, frequency of use, and smoking history have been reported to influence the degree and the nature of such changes *(213,214)*.

Recent studies suggest that the oral microbiome signatures in ENDS users are distinct from those in cigarette smokers and former or never smokers, including altered taxonomic composition, increased microbial diversity, a significant increase in the abundance of microbial pathways involved in carbohydrate and amino acid metabolism, and diverse virulence factors *(215–217)*. Some of these traits are favourable (e.g. greater diversity than in smokers), while others suggest inflammatory processes. As in smokers, the relative abundance of *Veillonella* in buccal cells and saliva of ENDS users is significantly higher than in non-users *(215,216)*. *Veillonella* are associated with various infections, including in the mouth, lungs and heart *(218–220)*, and some *Veillonella* species may play a role in endogenous nitrosation through their capacity to reduce nitrate to nitrite *(216,221)*, which might be the main reason for the comparable levels of NNN found in the oral cavities of ENDS users and of smokers found by Bustamante et al. *(17)*.

Only one study was found of the oral microbiome in HTP users *(222)*. The study was conducted in 65 adolescents, aged 14–18, in Ukraine who were HTP or ENDS users or non-users of any tobacco product (control group). The composition of the oral microbiomes of participants who used HTPs was different from that of ENDS users. The findings suggest that both products reduce the number of resident plaque microflora, which leads to the emergence of opportunistic transient streptococci such as *Streptococcus pneumoniae* and *S. pyogenes*.

5.4 Biomarkers of susceptibility

5.4.1 Definition and overview of biomarkers of susceptibility used in studies of tobacco and nicotine products

Individual and population differences in the uptake and/or metabolism of toxicants and carcinogens present in tobacco and nicotine products can contribute to differences in susceptibility to adverse biological effects and the subsequent health outcomes. Biomarkers of susceptibility are predictive indicators of individual characteristics (e.g. gene polymorphisms) that drive such differences. In the context of tobacco control, these biomarkers are useful for interpreting and predicting potential population differences in the levels of biomarkers of exposure or of biological effect among users of the same product type. Furthermore, such biomarkers can potentially be used to identify susceptible populations for targeted cessation interventions.

Nicotine metabolite ratio (NMR). The most commonly used biomarker of susceptibility in studies of tobacco use and disease risk is the ratio of two nicotine metabolites, *trans*-3′-hydroxycotinine and cotinine, referred to as the NMR. This biomarker reflects the activity of CYP2A6, the enzyme primarily responsible for nicotine metabolism, which is mainly defined by the presence or absence of functional polymorphisms in the *CYP2A6* gene *(223)*. Inter-individual differences in the NMR have been associated with smoking behaviour and dose *(224)*, smoking abstinence *(225)*, and the risk of lung cancer *(6)*. Representative values for the NMR in daily tobacco users in the USA, overall and by age, sex and race or ethnicity are available from a recent analysis of Wave 1 of the PATH Study *(226)*.

Urinary metabolites of carcinogens and toxicants. As discussed above, urinary metabolites of tobacco smoke constituents such as nicotine, tobacco-specific nitrosamines, PAH and volatile organic compounds are well established biomarkers of exposure. In addition, analysis of data from the Shanghai Cohort Study and the Singapore Chinese Health Study, two large prospective epidemiological studies, showed that total nicotine equivalents, total NNAL, total NNN and PheT were independently associated with significantly higher cancer risks among smokers (Table 3) *(10,15,227)*. These biomarkers can therefore also be considered biomarkers of susceptibility to disease, namely cancer. In

these studies, total nicotine equivalents served as dose monitors for all other constituents of tobacco smoke. Although the effects of total NNAL, total NNN and PheT were still apparent after correction for total nicotine equivalents, this was not the case for mercapturic acid biomarkers of 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde *(229)*. The relevance of use of urinary carcinogen and toxicant biomarkers to assess risk in users of ENDS and HTPs requires further research, as users of these products appear to have limited exposure to the relevant parent compounds (NNK, NNN and PAH). As noted above, Bustamante et al. *(17)* presented evidence of the presence of increased levels of NNN in the saliva of ENDS users.

Table 3. Urinary carcinogen and toxicant metabolites that have been prospectively associated with lung cancer risk in smokers

Constituent	Biomarker	Odds ratio	Study population	Reference nos
Nicotine	cotinine	0.85–3.52	Shanghai, Singapore, USA	*229–231*
NNK	Total NNAL	1.57–2.64	Shanghai, Singapore, USA	*229–231*
PAH	PheT	1.23–2.34	Shanghai, USA	*229,231*
Volatile organic compunds	Mercapturic acids	0.97–1.20	Shanghai	*228*

Examples of other potential biomarkers of susceptibility. Certain biomarkers described above, such as DNA methylation, gene expression and the microbiome, are also important individual characteristics that can affect the metabolism of tobacco constituents and/or the protective mechanisms (e.g. immune responses, DNA repair) against their harmful effects. Therefore, DNA methylation, gene expression and the microbiome could serve as biomarkers of susceptibility in studies of tobacco or nicotine products.

5.4.2 Application of biomarkers of susceptibility in studies of ENDS and HTPs

Use of biomarkers of susceptibility in studies of ENDS and HTPs has been limited.

NMR

It is not known whether NMR is predictive of ENDS or HTP use behaviour or of any health outcome resulting from use of these products. The relatively short time since these products have been on the market, their diversity and continuous evolution, and the variation in nicotine delivery are probably the main reasons for lack of data. A study of PATH data (Waves 1 and 2) of the association between NMR and transitions in cigarette smoking and ENDS use *(232)* found a significant two-way interaction, women with higher NMR (i.e. faster nicotine metabolism) being 10 times less likely to quit ENDS use than women with lower NMR. These results indicate that NMR could potentially be used as a biomarker of quitting ENDS use in women.

Other potential biomarkers of susceptibility

Urinary metabolites of carcinogens and toxicants. There is no consistent evidence for substantial increases in metabolites of NNK or PAH in the urine of ENDS or HTP users as compared with non-users of any tobacco or nicotine product *(60,233)*.

DNA methylation, gene expression and the microbiome. Use of these biomarkers in studies of tobacco and nicotine products is relatively recent and limited. The potential associations of these biomarkers with the metabolism of ENDS or HTP constituents or with biological effects in ENDS or HTP users have not been studied.

5.5 Established and validated methods for measuring biomarkers

Well-characterized methods are available for most commonly used biomarkers of exposure and biological effects, particularly those used for large cohorts. Liquid chromatography (LC) or gas chromatography (GC) coupled with mass-spectrometry (MS) are highly sensitive and selective methods of choice for these measurements. Examples of methods, along with their analytical parameters, are illustrated in Table 4. For circulating biomarkers of biological effects, such as cytokines and reactive proteins, immunoassay methods with commercially available kits are typically used. The performance of the kits is validated for quality and specificity by their manufacturers.

Table 4. Examples of validated methods for some biomarkers of exposure and biological effects

Biomarker	Method description	Method characteristics	Reference no(s)
Biomarkers of exposure			
Urinary total nicotine equivalents (TNEs)	LC–MS/MS analysis of nicotine, cotinine, 3′-hydroxycotinine and their glucuronides after enzymatic treatment of urine (to release these biomarkers from their glucuronide conjugates) and solid-phase extraction	Accuracy: 93–96% Intra-day CV: 4.2–7.1% Inter-day CV: 0.4–5%	*234*
Urinary total NNAL	LC–MS/MS analysis of NNAL and its *O*- and *N*-glucuronides after enzymatic treatment of urine and two extraction steps	Accuracy: 94% Intra-day CV: 3.0% Inter-day CV: 5.7%	*235*
Urinary CEMA	LC–MS/MS analysis after a purification step with mixed mode anion exchange on a 96-well plate	Accuracy: 98% Intra-day CV: 6.4% Inter-day CV: 6.6%	*236*
Urinary PheT	GC–NICI–MS/MS analysis after treatment with β-glucuronidase and arylsulfatase and purification on styrene-divinylbenzene plates in a 96-well format	Accuracy: 95% Intra-day CV: 2.9% Inter-day CV: 3.7%	*235*

Biomarkers of biological effect			
Urinary 8-iso-PGF_{2a}	LC–MS/MS analysis after a single purification step	Accuracy: 103% Intra-day CV: 4.0% Inter-day CV: 5.5%	*237*
DNA adducts of acrolein in oral cells	DNA is isolated, and, after hydrolysis and solid-phase extraction, the adducts are quantified by LC-MS/MS.	Accuracy: 96% Intra-day CV: 1.6% Inter-day CV: 3.4%	*238*

CV, coefficient of variation; NICI, negative ion chemical ionization

5.6 Summary of evidence on biomarkers for ENDS and HTPs and implications for public health

Research on biomarkers for evaluating tobacco and nicotine products has proliferated in the past 15 years, extending application of known biomarkers, providing new biomarkers and generating new evidence on their levels in users. The national longitudinal PATH study in the USA and other large, longitudinal cohorts were instrumental platforms for using a broad panel of biomarkers to assess exposure and effects in users of various tobacco product types. A substantial body of published literature supports use of biomarkers of exposure for evaluating ENDS, and new support has become available for use of DNA adducts in oral cells and certain cardiopulmonary biomarkers of biological effects for this purpose. The same biomarkers of exposure and effect are likely to be useful for assessing HTPs; however, most of the research on biomarkers for HTPs to date has been conducted by the tobacco industry. Little information is available on the potential role of NMR, a biomarker of susceptibility, in measuring exposure and effects in ENDS or HTP users.

5.6.1 Summary of available data and implications for public health

The main conclusions of this review of data on biomarkers for ENDS and HTPs and implications for health are outlined below.

Switching from conventional cigarette smoking to exclusive ENDS use is associated with reduced exposure to several toxicants and carcinogens that play key roles in smoking-induced diseases. This conclusion is supported by extensive literature, including the most recent analyses based on Waves 1 and 2 of the PATH study *(74,90)* and a secondary analysis of a Cochrane systematic review of trials of use of ENDS for smoking cessation *(239)*.

Public health implications:

- The extent and nature of the effects on health of these reductions are not yet well understood. Biomarkers of exposure do not account for the potential combined effects of numerous individual constituents

and, therefore, have limited capacity to predict changes in disease risk. For example, the levels of many biomarkers of exposure, including those of volatile toxicants such as acrylonitrile and acrolein, are higher in exclusive ENDS users than in non-users of nicotine or tobacco products *(88,142,170,240–242)*, and the effects of these low-level exposures are not well understood. In addition, ENDS users may be exposed to certain organophosphate flame retardants (probable contaminants in ENDS devices) *(243)*, and a study of untargeted chemical profiling showed that ENDS aerosols may contain more than 2000 chemical constituents, many of which are yet to be characterized *(244)*.

- While the long-term effects of ENDS use are poorly understood, smokers who switch completely to ENDS may benefit from reductions in exposure to many tobacco toxicants and carcinogens.

A panel of biomarkers of exposure can be used to determine or validate product use (Table 5). A cut-off point of 5–6 ppm CO will distinguish users of combustible cigarettes from users of ENDS and non-users. ENDS users have urinary TNE (at least 2000 pmol/mg creatinine) *(242)* and lower (< 27 pmol/mL urine) CEMA concentrations than non-users of any tobacco or nicotine product, who will have minimal (essentially zero) TNE *(242)* and CEMA (< 27 pmol/mL urine) *(245,246)*. If the nicotine use status of participants is ambiguous, NNAL (1–2 pmol/mL urine) can be measured as a biomarker of tobacco-specific NNK, which occurs at low levels in ENDS users (0.023 pmol/mL urine) *(242,247)*. Salivary propylene glycol (3.5 μmol/mL in ENDS users and 0.004 μmol/mL in non-users, our unpublished data) can be used to confirm ENDS use. The biomarker half-life should be considered when using these cut-off points, particularly in studies of recent switching from smoking to ENDS use. Anatabine, a minor tobacco alkaloid (which should not be present in exclusive ENDS users), has been used in a few studies.

Table 5. Expected relative values for biomarkers in users of various tobacco and nicotine products

Category of use	Urinary biomarker				Propylene glycol in saliva
	CO	TNE	Total NNAL	CEMA	
Exclusive cigarette smoking	High	High	High	High	Low or ND
Exclusive ENDS use	Low	High	Low or ND	Low or ND	High
Dual use of ENDS and smoking	Variable	High	High	High	Variable
No use of any tobacco or nicotine	Low	Low or ND	Low or ND	Low or ND	Low or ND

ND, not detectable

Public health implications:

- Use of the proposed panel of biomarkers is important for advancing research on exposure and effects in users of ENDS.

Cigarette smokers who become dual users of ENDS and conventional cigarettes do not experience meaningful reductions in most biomarkers of exposure. The amount of smoking appears to be the primary determinant of exposures in dual users.

Public health implications:

- Dual users are not likely to experience improvements in biological effects over those seen with exclusive smoking.
- Dual use exposes users to the same levels of tobacco toxicants and carcinogens as cigarette smoking and also to emissions of ENDS. The health consequences of such mixed exposure are unknown; however, a systematic review suggests that dual use may be associated with the same or a significantly higher risk of self-reported symptoms or disease as exclusive cigarette smoking (248).

DNA adducts in oral cells are useful biomarkers of carcinogen dose and biological effects and can be used to compare the effects of ENDS with those of conventional cigarettes. Such biomarkers should be used more widely, when possible.

Public health implications:

- As stated above, biomarkers of exposure have limited capacity to predict changes in disease risk. As formation of DNA adducts is a key step in chemical carcinogenesis process, these biomarkers might indicate cancer risk.
- In addition, given the reactivity of aldehydes and other inflammatory agents present in ENDS and ENNDS aerosols, urinary biomarkers of exposure may not fully capture important exposures and biological effects at the place of immediate contact of the aerosols with human tissues, such as the oral cavity.

The results of studies of circulating inflammatory cytokines associated with cardiovascular and respiratory effects after ENDS use are inconsistent. One challenge in interpreting the findings is that different types and panels of such biomarkers have been used in different studies.

Public health implications:

- Definitive data on these biomarkers in ENDS users are necessary, as a substantial body of research suggests that ENDS may be a source of inflammatory exposure and thus contribute to respiratory and cardiovascular effects in users.

Studies with indicators of preclinical changes and symptoms suggest that ENDS increase the risks for respiratory and (potentially) cardiovascular effects over that with non-use of any product.

Public health implications:

- While some improvements in respiratory symptoms have been reported in smokers who switch to ENDS, prolonged use of ENDS by former smokers should not be encouraged. Innovative cessation interventions are necessary to help users to quit both smoking and ENDS use.
- ENDS use by never smokers is likely to increase their risks for disease.

Independent studies of biomarkers in HTP users are critically lacking.

Public health implications:

- Although tobacco industry reports indicate significant reductions in biomarkers of exposure and some biomarkers of biological effects in smokers who switch to HTPs, independent academic research is necessary to confirm these findings.
- HTPs are likely to expose users to higher levels of toxicants than ENDS (249); however, no studies with biomarkers are available.

5.6.2 Limitations of biomarkers

The limitations of biomarkers are associated mainly with their specificity, stability and feasibility of measurement.

- Many biomarkers of exposure and biological effect are not specific to a particular tobacco or nicotine product and can be influenced by factors including dietary, environmental and occupational exposures, health status and physical activity. No single biomarker or set of biomarkers can capture all the exposures or effects associated with a tobacco or nicotine product.

- Many biomarkers of biological effect are not specific to one disease. For example, oxidative stress and inflammation are common underlying mechanisms in the pathophysiology of cancer and of cardiovascular and respiratory diseases. Therefore, it is challenging to use such biomarkers to distinguish between the risks for individual diseases.
- Biomarkers have limited, variable half-lives. For example, the half-life of exhaled CO is approximately 8 h or less, the half-life of urinary total NNAL is approximately 3.5 weeks, and the half-life of urinary cadmium can be up to 38 years. Biological stability should be considered when designing and interpreting biomarker studies, particularly in the context of previous smoking and/or the duration of ENDS or HTP product use.
- Measurement of some biomarkers requires highly specialized expertise and instrumentation, which may limit their broad application.

5.6.3 Research gaps

This review of the evidence on use of biomarkers of exposure, effect and susceptibility in studies of ENDS and HTP use indicates the following research gaps and priorities:

- independent (non-tobacco industry) research on ENDS, HTPs and other new and emerging tobacco and nicotine products, such as nicotine pouches;
- research, including untargeted profiling of product emissions and biospecimens, to identify biomarkers specific to ENDS and HTPs, as most biomarkers in current use are based on exposure to cigarette smoke;
- evaluation of biomarkers resulting from use of ENDS and HTPs of different designs and with different ingredients;
- research on biomarkers of biological effects that are specific to exposure to tobacco and nicotine products or to individual health effects (e.g. cardiovascular or respiratory diseases);
- cross-sectional and longitudinal studies to assess and compare ENDS and HTP exposures among populations (including comparisons with non-users of any product) in various countries;
- systematic studies of exposures, biological effects and clinical disease-specific manifestations in ENDS and HTP users, to better characterize the associations with various types of biomarkers and associations of levels of biomarkers with specific disease outcomes; and
- studies to better characterize and communicate the effect of dual and poly-product use, especially with different levels of smoking.

5.7 Recommendations for possible prioritization of biomarkers for tobacco control

Biomarkers are objective measures of harmful exposures and biological effects relevant to disease pathophysiology in users of various tobacco and nicotine products. Therefore, biomarkers of exposure and effects could be useful tools for tobacco control.

Biomarkers of exposure. The advantage of biomarkers of exposure is that they account for the effect of product use patterns on the delivery of harmful constituents to users. Such effects might not be captured by standardized, machine-based product testing in a laboratory. Therefore, biomarkers can be used to more accurately characterize product toxicity and abuse liability. On the basis of the evidence reviewed, the following biomarker panel is recommended for assessing the level of exposure of users of ENDS and HTPs to constituents implicated in smoking-related harm: urinary TNEs (addictiveness), NNAL (exposure to tobacco-derived carcinogens) and CEMA (exposure to combustion). Measurement of exhaled CO and salivary propylene glycol could be added to this panel; however, exhaled CO has a short half-life, and the method for measuring salivary propylene glycol requires validation. Other biomarkers of exposure reviewed in this report can be also used; however, the proposed, limited panel can provide sufficient information on exposure to key classes of harmful constituents and allow classification by product use status.

Biomarkers of biological effects. Biomarkers of biological effects account for interactions among several harmful exposures and potentially for unique exposures that may not be captured by product testing or by urinary biomarkers of exposure. On the basis of the evidence reviewed, the following biological effects are recommended for priority monitoring.

- *DNA adducts in oral cells,* formed by acrolein and potentially by other volatile toxicants, such as formaldehyde. There are clear, striking differences in the levels of these biomarkers between smokers, ENDS users and non-users of tobacco. Given the direct relevance of DNA adduct formation to cancer risk, monitoring of these biomarkers would be valuable for assessing the potential risks of novel products relative not only to conventional cigarettes but also to non-use of any tobacco or nicotine product. DNA adducts in oral cells can also serve as biomarkers of exposure to low but biologically relevant levels of volatile toxicants.
- *Indicators of preclinical changes and symptoms.* Indicators such as blood pressure, heart rate, arterial stiffness, platelet reactivity, respiratory symptoms (coughing, wheezing, shortness of breath) and respiratory function measures (FEV1, FVC, FEV1/FVC and DLCO)

are commonly assessed in general clinical practice and are easy to measure. It is recommended that such measures be included in cross-sectional and longitudinal studies of ENDS and HTP product use, as surrogate measures of respiratory and cardiovascular risk.

Urinary isoprostanes and selected cytokines, for which consistent results have been obtained in comparisons of users of ENDS or HTPs with smokers, could also be used. The absence of differences in the levels of these biomarkers among user groups and the increased levels as compared with non-users of tobacco and nicotine indicate continued systemic oxidative stress and inflammation in ENDS and HTP users. Interpretation of these biomarkers should, however, include recognition that oxidative stress and inflammation are not specific to exposure to these products.

Biomarkers of susceptibility. Anyone exposed to the harmful constituents present in product emissions may be at risk (for addiction or disease). The value of biomarkers of susceptibility is their use for identifying certain population subgroups who are particularly vulnerable to harmful effects. Research on these biomarkers is evolving, and the existing data are insufficient to recommend their direct application in tobacco control. It is recommended, however, that NMR measurements be incorporated into programmes and studies for monitoring TNEs and total NNAL in users of ENDS and HTPs.

Direct epidemiological assessment of disease risk. Emerging epidemiological studies of health outcomes generally show higher risks for ENDS than would be expected from the levels of biomarkers of exposure relevant to cigarette smoke. This could be due in part to the high prevalence of dual use, which may not be captured in such studies, and the yet not well-characterized unique biological effects of ENDS use. Therefore, direct monitoring of health effects resulting from the use of ENDS, HTPs and any new and emerging nicotine or tobacco products should be documented.

5.8 Recommendations for addressing research gaps and priorities

The following strategies are recommended for independent research to address the gaps and priorities that have been identified.

- Conduct research to develop and use biomarkers of exposure, biological effect and susceptibility to better characterize the public health impact of ENDS, HTPs and other new and emerging products.
- Conduct research to compare the relative risks (exposure and biological effects) of use of HTPs and ENDS and comparisons with no tobacco or nicotine product.
- Establish cross-sectional and longitudinal cohorts for real-time monitoring of exposure and biological effects in users of ENDS, HTPs

and other emerging products, to provide regulators with the relative risks of such products and the potential effects of evolving product characteristics.

- Conduct systematic monitoring of various types of biomarkers and indicators to better understand how differences in exposure with various product types translate into biological effects, preclinical symptoms and disease risk.
- Conduct research on communication strategies to inform populations about the exposures and effects of ENDS and HTPs; prevent their dual use with cigarettes; and prevent misperceptions of product risks.

5.9 Relevant policy recommendations

The following regulatory recommendations are proposed for consideration by policy-makers, researchers and the public health community, as appropriate.

- Take into account biomarker-based findings (from all countries) when making policy decisions on ENDS, HTPs and other new and emerging tobacco and nicotine products, relying on data obtained independently of the tobacco or ENDS industry and considering the limitations of biomarkers.
- Prioritize and support independent research, including building capacity for measuring biomarkers and for epidemiological studies to address the research gaps and priorities related to the public health impact of ENDS, HTPs and other new and emerging tobacco and nicotine products.
- In countries with the necessary capacity, monitor the recommended panel of biomarkers in users of ENDS, HTPs and other new and emerging tobacco and nicotine products.
- Clearly communicate to health-care professionals and the general public the current absence of evidence that use of HTPs reduces harm.
- Given the rapid pace at which new products are introduced and the time lag in scientific research on exposure and effect factors, Member States are strongly encouraged to consider requiring that the following information be provided by manufacturers before allowing marketing of any of these products in their country: (i) levels of emission of selected harmful chemicals and (ii) levels of the recommended panel of biomarkers in users.

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6. Internet, influencer and social media marketing of tobacco and non-therapeutic nicotine products and associated regulatory considerations[3]

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Contents

Abstract

Bans on tobacco advertising, promotion and sponsorship (TAPS) are a cornerstone of comprehensive tobacco control laws. Global progress in implementing TAPS bans has been facilitated by adoption of the WHO Framework Convention on Tobacco Control (WHO FCTC). Enforcement of bans on TAPS is, however, over-reliant on self-regulation by producers of entertainment and digital content and online platforms. TAPS laws must maintain pace with the changing media landscape, which includes monitoring and reporting TAPS that cross international borders, primarily through online digital media platforms. TAPS laws must also keep pace with rapid changes in newer non-therapeutic nicotine devices, as well

3 This paper draws on and includes sections from recently published works by the authors ***(1,2)***. The paper also draws on reports and findings from the WHO Expert Advisory Committee on Cross-border Tobacco Advertising and Promotion ***(3)***, chaired by Becky Freeman, and the report and findings of the WHO FCTC Article 13 Working Group on Tobacco Advertising, Promotion and Sponsorship ***(4)***.

as new tobacco products. These include electronic nicotine delivery systems (ENDS), electronic non-nicotine delivery systems (ENNDS), personal vaporizers, heated tobacco products, nicotine salt, other nicotine products resembling nicotine replacement therapy, and various vitamin and cannabis products with the same delivery devices or marketing channels as tobacco products. Many of these products are not regulated, as the manufacturers exploit loopholes in the definition of nicotine and/or tobacco products or are in a regulatory grey area where authority is unclear. Policies are required that anticipate changes in tobacco, nicotine and related products and also in marketing and evolving online and digital media.

Keywords: tobacco advertising, social media, online digital media, marketing, regulation

6.1 Background

In most parts of the world, there have long been bans on direct tobacco advertising in traditional mass media – broadcast television, radio and print – and channels such as billboards. Laws on tobacco advertising, promotion and sponsorship (TAPS) must, however, continue to progress to address the seemingly endless ways in which the tobacco industry attempts to promote its products, maintain current customers, lure back those that quit smoking, and entice new users *(1)*. When only some forms of TAPS are regulated, the industry redirects its promotional efforts and budget to promotions that are exempt from regulation *(5)*. A TAPS ban that is heralded as comprehensive and progressive can quickly be outdated if it is not updated to cover innovations in both promotional opportunities and product offerings. The rapid change to a predominantly digital media environment, including the explosive rise and dominance of online social media, has also enabled the tobacco industry to exploit and develop new forms of promotion *(6)*. The combination of significant circumvention of TAPS laws, new forms of media and new products *(2)* is not intractable: although challenging, it is possible to adopt novel policy approaches to further limit TAPS and exposure.

The WHO FCTC definition of tobacco advertising and promotion is "any form of commercial communication, recommendation or action with the aim, effect or likely effect of promoting a tobacco product or tobacco use either directly or indirectly", and tobacco sponsorship is defined as "any form of contribution to any event, activity or individual with the aim, effect or likely effect of promoting a tobacco product or tobacco use either directly or indirectly" *(7)*. Parties are also encouraged to consider including ENDS and ENNDS in any TAPS regulatory approaches. The definitions are intentionally broad to ensure that they encompass the myriad ways in which the tobacco industry promotes its products. Parties to the WHO FCTC are required to ensure that their TAPS laws are comprehensive and, barring any constitutional impediments, ban all forms of TAPS. This paper

focuses on how manufacturers of tobacco and other nicotine delivery products that are non-therapeutic are using online digital media sharing platforms, particularly social media, to market their products, although we acknowledge that TAPS extends beyond online environments. Additionally, the eighth report of the WHO study group on tobacco product regulation *(8)* included "Global marketing and promotion of novel and emerging nicotine and tobacco products and their impacts". This paper does not duplicate but complements and builds on that work.

The WHO FCTC guidelines for implementation of Article 13 state that the depiction of tobacco in entertainment media, such as films, online videos and computer games, is a form of TAPS *(9)*. It is the commercial nature of these forms of entertainment media that defines the tobacco depictions they contain as TAPS, regardless of any tobacco industry involvement in the creation or funding of the content. Much of this entertainment media content is accessed through social media and streaming platforms on personal Internet-enabled devices, such as smartphones. This type of content can also be created, uploaded or broadcast in one country and then viewed and shared in another. Cross-border digital media consumption provides more channels through which the tobacco industry can circumvent TAPS bans.

Some forms of online pro-tobacco messaging might be considered "legitimate expression" if there is no associated commercial link to the message. For example, a user who posts an image of themselves using a tobacco product on social media would not be considered to be making a "commercial communication" if they did not receive any financial or other benefit (such as free products) for posting the image. Most content on social media platforms is not commercial in nature, but social media platforms rely on commercial content in order to generate revenue. Commercial, paid content is then placed in social media feeds, often targeting specific types of users according to their demographics and interests. Commercial entities also post so-called "unpaid" content onto their social media platform accounts, which are often referred to as "organic" posts *(10)*. The account owner does not pay for "organic" posts to appear in user social media feeds but, instead, crafts posts that are likely to appeal to users. Such "organic" posts readily meet the definition of a commercial communication, as they are posted as part of strategic marketing plans on behalf of manufacturers. Illustrations of these concepts are provided below.

6.2 Impact of online and social media marketing on tobacco and ENDS use

Both exposure to and interactions with social media tobacco content have a significant impact on the patterns of ENDS and tobacco use by adolescents. Due to the amount of time adolescents spend engaging with online content, social media may be a critical place in which to intervene, possibly with anti-tobacco or tobacco prevention messages *(11)*.

A systematic review and meta-analysis of the association between exposure to tobacco content on social media and lifetime tobacco use, use of tobacco in the past 30 days and susceptibility to use of tobacco by never users found that participants who were exposed to tobacco content on social media had higher odds of reporting lifetime tobacco use than those who were not exposed (odds raio [OR], 2.18; 95% CI, 1.54 ; 3.08; $I^2 = 94\%$), past 30-day tobacco use (OR, 2.19; 95% CI, 1.79 ; 2.67; $I^2 = 84\%$), and susceptibility to use of tobacco by never users (OR, 2.08; 95% CI, 1.65 ; 2.63; $I^2 = 73\%$). Subgroup analyses showed similar associations for tobacco promotion, active engagement, passive engagement, lifetime exposure to tobacco content, exposure to tobacco content on more than two platforms, and exposure of adolescents and young adults to tobacco content *(12)*.

6.2.1 ENDS use by young adults

Tobacco product advertising has long been established as a cause of young people starting to use tobacco products *(13)*. Much of the current advertising of ENDS and other nicotine products is based on approaches and themes similar to those used in the past to promote conventional tobacco products *(14)*.

Exposure of young adults to marketing of both tobacco and ENDS and engagement with pro-tobacco and ENDS information increases their likelihood of using ENDS products *(15)*. The increased likelihood of use remains even after adjustment for baseline e-cigarette use and the feedback loop from e-cigarette use to exposure to information and engagement. In contrast, engagement in anti-tobacco and anti-ENDS information reduced their probability of e-cigarette use. These findings not only stress the importance of regulating promotional and marketing information about nicotine and tobacco products on social media but also suggest that social media could be used as a cost-efficient platform for disseminating anti-tobacco, anti-ENDS campaign messages to prevent young adults from using ENDS products *(15)*.

6.2.2 Illustrative examples of TAPS in online and digital media

Several online libraries of examples of digital media TAPS include both tobacco and nicotine products. The site of Stanford Research into the Impact of Tobacco Advertising has an extensive collection (60 000 examples) of all types of TAPS that cover all forms of tobacco and nicotine products, including not only traditional cigarettes but also ENDS, nicotine pouches, waterpipes, smokeless tobacco and cigars *(16)*.

Direct, paid tobacco advertisements on online media are the easiest form of online TAPS to recognize, monitor and enforce. It may, however, be difficult to distinguish between direct, paid tobacco promotion and content with no commercial connection. For example, an investigation in 2018 found that tobacco companies were engaging popular social media influencers to promote tobacco

products through their highly viewed social media profiles *(17)*. The influencers' posts did not disclose that they were advertising tobacco or that they had received tobacco industry incentives to post tobacco depictions and branding on their profile feeds and pages. Influencers are also heavily involved in the promotion of electronic cigarettes and other novel nicotine devices *(18)*.

In addition to TAPS and tobacco depictions in entertainment and online media, tobacco industry corporate communication campaigns are a well-documented source of pro-tobacco messaging. These promotions sidestep TAPS laws and TAPS definitions. Examples include corporate social responsibility messaging *(19)*, industry-funded "Foundation" campaigns *(20)*, industry funding of science and research, political and lobbying activities and promotions, including paid editorials (advertorials) in news media *(21)*, and unpaid posts on social media from both company branded accounts and employees *(22)*. These corporate communications, which often fall under "legitimate expression" exemptions *(7)*, nonetheless have the same media platforms and serve the same purpose as direct advertising. Regulators should consider intervening when companies use the "legitimate expression" exemption inappropriately, such as when their public relations communications that appear in mass media channels (e.g. newspaper advertorials) consist of thinly veiled product promotion.

1. Digital media-sharing platforms

– *Direct product promotion through paid advertisements.* Such direct promotion is often signalled by inclusion of the words "paid sponsorship", "paid partnership" or "#ad".

– *Influencer promotions.* The tobacco industry and those working to further its interests incentivize or sponsor individuals to post content online featuring products or brands. Social media influencers, who have thousands or even millions of followers, are compensated by the brands and are coached by influencer marketing companies about when to post for maximum exposure and how to avoid posting content that looks like a staged advertisement. Strategies may also include organizing parties and contests with brand sponsorships and encouraging participants to post on their own social media accounts. Influencers may be instructed to amplify their promotional social media posts via hashtags, both related and unrelated to the brand but with enormous viewership (e.g. #love, #art, #fashion).

  Example: TakeAPart has conducted in-depth reporting on tobacco influencer marketing, including the images posted and the messages associated with different brands and products *(17)*.

– *Commercial promotions of posts by consumers of their own tobacco use.* Consumers who use tobacco products may share content that depicts tobacco use and may also comment directly on content that advocates tobacco consumption or recommends particular brands or products. Depending on the context, this may constitute legitimate expression, such as unpaid personal communication. Other parties working in the interests of the tobacco industry can then choose to increase the reach of this content by paying digital media communication platforms to broadcast it to other audiences, turning these personal, legitimate expression posts into commercial promotions.

– *Event promotion.* Participants or teams in an event are sponsored by tobacco companies and social media, and audiovisual sharing platforms broadcast the event and/or images from the event. In the case of major sporting events such as motor racing, the reach can be global, as these events are widely broadcast, including in traditional media.

  Example: see reference *22*.

– *Corporate and campaign promotions.* Tobacco companies, or those working to further their interests, promote a corporate or campaign brand rather than a tobacco product brand and operate social media accounts that promote the corporate or campaign brand. Corporate promotion campaigns and actions often portray tobacco companies as innovative performers and socially responsible actors and advance novel tobacco products as "less harmful alternatives" to traditional cigarettes, often despite lack of independent scientific evidence to support such claims.

  Examples includes the Philip Morris "Unsmoke" campaign *(24)*.

– Propaganda crusades by Philip Morris International and Altria: "Smoke-free Future" and "Moving Beyond Smoke" campaigns exposing the hypocrisy of the claim: "A tobacco company that actually cares about health" *(25)*.

– *Tobacco use depictions embedded in commercial content in which those depictions are not legitimate expression.* While the bulk of the content on social media is not commercial in nature, commercial content draws a large amount of user traffic (for example, music videos, short films, web series) or is linked to a content creator that generates revenue from user traffic and users purchasing the products featured or reviewed. Music videos, for example, are widely viewed and shared,

and popular content on audiovisual sharing sites are also a major global source of exposure to tobacco depictions.

Example: Cranwell et al. *(26)*.

– *Product integration.* Tobacco companies, or those working to further their interests, work with producers, production companies and screenwriters to build storylines involving their products and integrate them seamlessly into their productions.

– *Sponsored news or "infotainment" content.* The tobacco industry, or those working to further its interests, offers facility visits to news or current affairs journalists or editors, pitch story ideas, or sponsor news stories on related or unrelated topics.

Example: Meade *(21)*.

– *Device advertising promotion and sponsorship.* Advertising or promoting a device or devices for consumption of tobacco products may directly or indirectly advertise or promote tobacco products themselves.

2. Tobacco companies and those working to further their interests operate social media accounts and websites with content that is broadcast across borders. These sites are frequently used not only for legitimate expression but also to promote the corporate brands of a company, to promote specific products or disseminate brand messaging under the guise of providing information to consumers, or as an exercise in so-called corporate social responsibility. Social networking sites and corporate websites are used by the tobacco industry to reinvent itself as a modern, socially responsible, sustainable industry and to dissociate itself from the harm caused by its products. Multiple transnational tobacco companies are using paid full page "public relations" announcements to resume brand promotion in prestigious newspapers and magazines that have long banned tobacco advertising from their pages.

Examples: Foundation for a Smoke Free World *(27)* and Freeman et al. *(28)*

3. *Films, television and streaming content* are significant sources of tobacco depictions. Content that is appealing to young people, such as reality television programming, has been found to contain high amounts of tobacco depictions.

Example: Barker et al. *(29)*

The online database of films https://smokefreemedia.ucsf.edu/sfm-media gives the incidence of tobacco use in cinema.

4. *Streaming television programmes.* With viewership of traditional television decreasing and online streaming and paid subscription increasing, streamed content is a growing source of tobacco promotion. Globally, young people (aged 18–34 years) are much more likely to be users of the Internet and smartphones than those aged 35 and older in both high- and lower-income countries. Tobacco depictions in popular streamed content are more prevalent than in traditional broadcast or cable programming. Many countries have long banned tobacco advertising via "mass media", typically defined as broadcast distribution (television and radio). Today, social media is a potent new mass media distribution channel, which is skewed notably to youthful audiences.

   Examples: Reference *30* provides a detailed report and analysis showing that a global streaming giant's programmes depicted more smoking imagery than broadcast shows. See also Barker et al. *(31)*.

5. *Video and computer games.* Both packaged and online video games are popular among young people, and very few controls are in place to protect or prevent users from exposure to tobacco depictions embedded within games or in-game or in-app purchases. Age restrictions may not take tobacco use into account and are easily avoided by younger players.

   Example of games featuring tobacco use are described in reference *32*.

6. *Smartphone applications.* Some smartphone applications, or "apps" as they are popularly known, show images of cigarette brands or images that resemble existing brands. Pro-smoking apps include approaches such as a cartoon game and an opportunity to simulate a high-quality smoking experience, free apps or apps that facilitate the sale of tobacco products, as well as novel and emerging tobacco products, including devices designed for consuming such products.

   Example: BinDhim et al. *(33)*.

6.2.3 Social media platform tobacco advertising policies

Popular social media platforms, including Facebook *(34)*, Instagram *(35)* and Twitter *(36)*, have adopted policies that prohibit paid tobacco advertising. These policies do not, however, apply to political and corporate messaging ads sponsored by the tobacco industry and do not restrict tobacco companies from using hashtags to attract social media post attention *(37)*, nor do they prevent tobacco companies from operating unpaid "organic" accounts on these platforms, which serve as popular conduits for brand advertisements. PMI, for example, operates a Facebook page that has more than one million followers *(38)*. Google also has an advertising policy on dangerous products or services and prohibits tobacco or any products containing tobacco; products that form a component of a tobacco product, as well as products and services that directly facilitate or promote tobacco consumption; and products designed to simulate tobacco smoking *(39)*. Google searches for tobacco retailers, however, provide localized results and direct links to sales outlets.

A study of e-cigarette and e-cigarette use-related posts on TikTok, a social media platform that has a large adolescent user base, in 2020 showed that the majority of posts positively framed e-cigarettes and use of these products *(40)*. In 2022, TikTok updated its community guidelines and claimed that it banned content that offers the purchase, sale, trade or solicitation of drugs or other controlled substances, alcohol or tobacco products (including e-cigarettes), smokeless or conventional tobacco products, synthetic nicotine products, e-cigarettes, and other ENDS. The new policy further specifies that

> content depicting the use of tobacco products by adults, or mentioning controlled substances, is not eligible for recommendation. Please remember that content which suggests, depicts, imitates, or promotes the possession or consumption of alcoholic beverages, tobacco, or drugs by a minor is prohibited. Content that offers instruction targeting minors on how to buy, sell, or trade alcohol, tobacco, or controlled substances is prohibited per our Community Guidelines as well. *(41)*

An evaluation of social media policies related to tobacco product promotion and sales on 11 sites in the USA that are popular with young people was conducted in May 2021 *(42)*. Nine of the 11 sites prohibited "paid advertising" for tobacco products; however, only three of them prohibited "sponsored content" that promotes tobacco. Six platforms restricted content that "sells tobacco products", and three claimed to "prohibit underage access" to content that promotes or sells tobacco products. Although most platform policies prohibited paid tobacco advertising, few addressed less direct strategies, such as sponsored or influencer content, and few had age-gating to prevent access by young people.

There is no evidence that these voluntary policies lead to reduced exposure to TAPS. This rapidly evolving media environment, coupled with lax regulation of social media communication platforms, including the over-reliance on platform self-regulation *(43)*, complicates extension of comprehensive TAPS bans to truly include online media. A mandate that all social media platforms ban all forms of tobacco, e-cigarettes and novel product advertising, both paid and organic, and prohibit the use of influencers, is crucial. Implementation of these policies should specify mechanisms for reporting noncompliance, provide for periodic audits, and require platforms to report on how they are ensuring that the law is being enforced on their sites. Currently, it is largely tobacco control stakeholders that are monitoring the amount and type of TAPS on social media platforms *(44)*. More of that burden should be shifted to the social media companies themselves. Social media companies could largely automate identification of tobacco promotion via sophisticated artificial intelligence systems for overseeing content.

For example, in an analysis of 4526 unique Instagram users who had created 19 951 IQOS-related posts, nearly half of the users (42.1%) were business accounts authorized by Instagram, of which 59.0% belonged to personal goods and general merchandise stores and 18.1% to creators and celebrities. Most active accounts in the network were directly associated with IQOS (e.g. containing "IQOS" in the user name) or related to the tobacco business as self-identified in the account biography description. These results show clearly that current self-regulation by social media platforms is far from enough *(45)*.

6.3 Global status of tobacco advertising laws

Article 13 of the WHO FCTC recognizes the crucial role of TAPS bans in effective tobacco control and includes banning cross-border TAPS as part of a comprehensive approach *(9)*. Parties to the WHO FCTC recognize the continuing difficulty of monitoring and enforcing cross-border TAPS bans and are preparing an addendum to the Article 13 guidelines to reflect the dramatic changes in the media landscape since the guidelines were adopted in 2008 *(3)*. Parties have also called for a mechanism for more effective global cooperation in managing cross-border TAPS *(3)*. Countries can more easily ban online TAPS that originate in their own countries, but, without international co-operation, it is more difficult to ban those that originate from another country and then "leak" across digital borders. The European Union, for example, requires all its Member States to ban cross-border tobacco advertising and sponsorship and actively monitors and enforces those provisions *(46)*.

6.3 Discussion

About half (91/180, 50.6%) of countries that report to the WHO FCTC on TAPS regulations stated that their TAPS ban included the domestic Internet *(47)*. The cross-border nature of online TAPS presents an additional challenge to regulators. Its nature is cross-border whenever content created, uploaded or broadcast in one country may be consumed or shared in another, thereby crossing geographical borders. The service providers may also be located in different countries from the country in which the service is provided. Content may also cross "digital" borders, as access is not always effectively limited to one geographical location. Cross-border digital media consumption provides new and emerging channels through which the tobacco industry and those acting to further its interests can circumvent controls on TAPS.

Entertainment media may cross borders through Internet-enabled devices (computers, smartphones, tablets, smart televisions) that:

- facilitate online streaming of films, television series or shows, video games, music videos, sporting, news, music, dance and other entertainment events;
- enable access to electronic versions of international and domestic newspapers and magazines;
- facilitate access to social media posts, including commercial and user-generated content and website pages;
- provide opportunities for engagement between consumers and commercial entities through social media; and
- may contain tobacco depictions or deliver embedded advertising content.

While the tobacco industry may not sponsor depictions of tobacco in films in countries with comprehensive TAPS bans, the policies rarely extend to unsponsored depictions in entertainment media. To escape such limitations, tobacco companies could provide free product samples to the prop masters of production companies in the hope that they will appear in the hands of actors. Since 2012, India has required that films depicting smoking should be accompanied by a 10-s Government-issued anti-smoking advertisement and that a static health warning at the bottom of the screen be visible for the duration of the tobacco depiction *(48)*. Any tobacco product brand names that appear on screen must be blurred out. Other countries, including China and Thailand, regulate the smoking and tobacco content permissible in television and films. Although the association between smoking depictions in films and the increased risk of smoking uptake by young people has been replicated in several studies *(49)*, there has been no research or evaluation on the impact of policy interventions to reduce tobacco depiction *(48)*.

Global media content producers and streaming services such as Disney *(50)* and Netflix *(51)* have made public commitments to reduce the frequency of tobacco depictions in new content, particularly that aimed at younger audiences. The move came only after it was revealed that the number of tobacco depictions on Netflix shows popular with young people had increased over time *(30)*. An online database of smoking depictions in media maintained by the University of California San Francisco (USA) documents the continued promotion of tobacco use in both Disney and Netflix content, among all other major media companies *(52)*.

6.3.1 Cross-border advertising

Countries that are committed to ending the promotion of tobacco products must not only strengthen their domestic TAPS bans but work with and support other nations in reducing cross-border TAPS. This will require more effective global cooperation and a commitment by all countries to update TAPS regulations regularly in response to new media and communications platforms and consumption patterns and also the evolving industry tactics that merge political interference, advertising and product development. In order to meet the highest global standards, improving and updating TAPS laws must be continuous, coupled with leadership for regulatory innovations, such as a complete end to the retail sale, including online, of tobacco products *(53)*.

Legal experts have proposed possible ways in which WHO FCTC Parties could act together to reduce cross-border TAPS *(54)*. They include:

- establishing mechanisms through which Parties can report to other Parties instances of tobacco advertising that originate on the other Parties' territory (either directly through specified contact persons or through a central public health body);
- agreeing to take appropriate action upon receiving reports from other Parties of cross-border tobacco advertising originating in their territory and to inform the reporting Party about the action taken;
- agreeing to provide assistance to other Parties in the investigation, preparation and prosecution of offences or possible offences, such as by facilitating access to relevant evidence and witnesses;
- agreeing to enforce, against individuals or organizations residing or with assets in their territory, judgements under FCTC-implementing laws made in the territory of another Party or to give reasons for refusing to do so;
- establishing mechanisms through which Parties report to one another on their experiences in respect of cross-border advertising;

- establish mechanisms through which Parties can discuss the effectiveness of cooperative measures adopted to meet jurisdictional and enforcement challenges and enter into new arrangements, as required; and
- establish mechanisms for sharing experiences and expertise in respect of relevant developments in technology.

6.3.2 Regulation of online marketing of other harmful products

Other commercial determinants of health, such as alcohol, food and non-alcoholic beverages, and gambling face challenges similar to those posed by tobacco in effective regulation of online marketing. As for tobacco, social media platforms have their own ill-defined, poorly enforced polices on the promotion of gambling and alcohol *(55,56)*. Food and non-alcoholic beverages are subject to even weaker restrictions, and social media sites are flooded with promotions of unhealthy processed food and energy and soft drinks *(57)*. Government action on the many forms of unhealthy advertising varies widely.

6.3.3 Challenges to regulation of tobacco advertising

One limitation in assessing the global state of TAPS bans is the limited body of work on implementation and enforcement of TAPS laws and regulations *(58)*. While countries that are Parties to the WHO FCTC Convention report on the scope of their TAPS laws, the reports do not include details of enforcement activities, and exemptions and the limits of policy reach are not well described.

Other challenges to effective regulation of TAPS in entertainment and online media are the following *(4)*.

- The popularity of content-sharing platforms, including social media, allows users to create and share content. People can view and share digital media freely, easily and quickly. This situation has blurred the lines between consumer and brand owner and poses a challenge to controlling cross-border TAPS.
- The changed media landscape and types of TAPS mean that regulations might have to be updated and made "future-proof" against emerging TAPS.
- Countries ban only cross-border TAPS that originate in their own countries and not those that are broadcast into the country from outside.
- It is difficult to distinguish between paid and unpaid depictions of tobacco use and brands.
- It is difficult to identify the origin, both country and creator or owner, of TAPS content, particularly online.

- Systematic documentation and capture of both tobacco industry promotional activities and tobacco depictions in entertainment media is difficult.
- Countries that have not ratified the WHO FCTC might be sources of cross-border TAPS.
- Young adults and adolescents are a highly desirable target population for this type of TAPS; however, only limited research, resources and policy action have been directed to protect this age group from exposure.

6.3.4 New products and associated challenges to online marketing

Enforcement of regulations and advertising control policies is a global challenge. For example, although the sale and import of all ENDS products are banned in Thailand *(59)*, they are sold illegally online *(60)*. The same is true in Brazil, where the marketing, advertising and importation of ENDS are not allowed, but they are sold illegally at e-cigarette shops, tobacco shops, on the Internet and through delivery apps *(61)*. In South Africa, ENDS are licensed to be sold only by prescription, but they are widely advertised as smoking cessation products and sold without prescription *(62)*.

In the Republic of Korea, heated tobacco product devices were considered to be electronics rather than tobacco products *(63)*; therefore, they have been advertised with lifestyle appeal, including a social media campaign by British American Tobacco in 2019 featuring hip-hop musicians popular among young people *(64,65)*. The music video was not subject to age restrictions because it contained images only of the heating devices and not the tobacco pods; it accrued more than a million views *(64)*.

New tobacco, nicotine and other aerosolized products are continuing to enter markets, with aggressive promotion both in high-income countries, where the prevalence of cigarette smoking is decreasing and where consumers can afford expensive new products, and in lower- and middle-income countries, circumventing bans on tobacco advertising *(2)*.

6.4 Conclusions

A wide range of media outlets, including social media, expose users to TAPS. The global media landscape has changed substantially since adoption of the "Guidelines for implementation of Article 13" in 2008 and continues to change and develop. Entertainment media content is increasingly available at regional and global level, including through the Internet, which can result in cross-border exposure to TAPS. The consequence of this shift in technology is that current approaches to controlling TAPS are insufficient.

The new, rapidly evolving media environment coupled with lax regulation of social media communication platforms, including over-reliance on self-regulation, means that extending comprehensive tobacco bans to effectively include cross-border TAPS, while challenging, is a high priority. In addition to strong domestic regulatory action, international action, both regionally and globally, including through cooperation between countries, will be required to reduce TAPS *(58)*. As media platforms are rapidly evolving in ways that may create regulatory loopholes that allow resumption of tobacco brand promotion, regulatory limits must be forward-looking to anticipate likely technological evolution, and regulators should be empowered to act swiftly in response to changing circumstances.

The current self-regulation led by social media platforms is not sufficient. More refined, well-enforced regulatory action, especially to limit marketing by official accounts, online retailers and celebrities, is necessary to restrict the proliferation of promotional content for tobacco products.

6.5 Research gaps and priorities

Published research tends to focus on why laws on TAPS are necessary *(66)*; the impact of exposure to TAPS *(67)*; how TAPS laws affect exposure to TAPS *(68,69)* and the potential impact on smoking attitudes, beliefs and behaviour *(70)*; and how the tobacco industry acts in the face of newly adopted TAPS laws *(71)* and subverts existing TAPS laws *(72)*. There are few data on how TAPS bans are implemented and then monitored and enforced after implementation. Evaluation and assessment of how countries can most effectively collaborate to control cross-border TAPS are also necessary. While the types and modes of online TAPS are increasingly being monitored systematically, data from monitoring the tobacco industry should be collected continuously, especially in light of new tobacco and nicotine product development and the continuously evolving media landscape. Assessment of the comprehensiveness of TAPS bans, particularly in terms of capturing online forms of TAPS and monitoring how loopholes in regulations are exploited are essential.

6.6 Policy recommendations

Regulators should develop a comprehensive strategy to reduce the amount of advertising, promotion and sponsorship of tobacco and nicotine products on social media platforms and online digital entertainment media. Such a strategy could reduce the exposure of adolescents and young adults to tobacco content and, ultimately, tobacco use.

- Ensure that TAPS laws are comprehensive, cover online digital media platforms, including social media, and are sufficiently flexible to encompass new media and platforms.

- The cross-border nature of online digital TAPS requires international cooperation for effective monitoring and enforcement.
- Require the tobacco industry to disclose all TAPS activities, including any activities on online digital media platforms, to government authorities in order to strengthen monitoring and enforcement.
- Include novel and emerging nicotine and tobacco products in comprehensive laws to ban tobacco and non-therapeutic nicotine products advertising promotion and sponsorship.
- Conduct ongoing surveillance of the evolution of both online digital media platforms and novel and emerging nicotine and tobacco products to ensure that TAPS laws remain comprehensive, including prohibition of advertising themes such as lifestyle, fashion, creativity, identity, pleasure and socializing.

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7. Overall recommendations

The WHO Study Group on Tobacco Product Regulation publishes a series of reports to provide a scientific basis for tobacco product regulation. They are a WHO technical product (formerly known as a global public health good); these, as noted above, are goods (or resources) developed by WHO that are of benefit globally or to many countries in many regions *(1)*. The TobReg reports, in line with Articles 9 and 10 of the WHO Framework Convention on Tobacco Control (FCTC) *(2)*, provide evidence-based approaches to regulation of the contents, emissions and design features of nicotine and tobacco products. The previous report *(3)*, on the deliberations of the tenth meeting of the Study Group, provides recommendations substantiated by sound science on novel and emerging nicotine and tobacco products. The recommendations of the Study Group are relevant in various contexts according to the national regulatory environment, the prevalence of use of tobacco and non-therapeutic nicotine products and other relevant factors that have implications for regulation of these products, such as policy goals and capacity for regulation, including bans.

The deliberations, outcomes and recommendations of the ninth meeting of TobReg reported here addressed new ways in which non-therapeutic nicotine, particularly in nicotine products, is promoted and delivered to people of different ages, including children and adolescents. The WHO FCTC *(2)*, which is the first international public health treaty, negotiated under the auspices of WHO to combat the tobacco epidemic, has saved many lives over the past 20 years. Countries are increasingly introducing strong tobacco control policy measures, including supply-and-demand measures, in line with the FCTC, to protect their citizens and reduce the prevalence of tobacco product use. This has led to decreasing sales of cigarettes and other tobacco products globally. Therefore, the tobacco industry is leveraging technology and using innovative means to boost its profits by introducing new ways of marketing and promoting nicotine and tobacco products *(3,4)*, including strategies and tactics to make tobacco and non-therapeutic nicotine products attractive, especially to children and adolescents *(5,6)* to sustain use of the products. Given its mandate, TobReg, assisted by subject matter experts, synthesizes comprehensive evidence from the published literature to develop evidence-based recommendations on product regulation. These are made available to countries, through the WHO Director-General, to assist countries in addressing regulatory challenges in tobacco control, which remains a global priority.

Regulators are reminded that tobacco kills more than 8 million people a year *(7,8)*. More than 7 million of those deaths are attributed directly to tobacco use and about 1.3 million to exposure of non-smokers to second-hand smoke *(9,10)*. Tobacco also eventually kills up to half of its users and remains a global health

emergency *(7)*. A further complication for tobacco control, posing challenges to regulators, is use of synthetic nicotine, which is not necessarily be covered under some tobacco control laws. Online marketing of nicotine and tobacco products and the introduction and promotion, including to children and adolescents, of nicotine pouches adds further complications. Thus, the recommendations of this report, should not be considered in isolation but seen in the context of wider tobacco control to complement the recommendations of the Study Group in other reports on tobacco product regulation *(11–18)*, which address cigarettes, smokeless tobacco, waterpipe tobacco, design features, flavours, as well as novel and emerging products.

The tobacco control community is well aware of the deliberate efforts by the tobacco industry to undermine tobacco control and slow implementation of the WHO FCTC. The industry aggressively markets and promotes novel nicotine and tobacco products, which pose a serious threat to tobacco control, and uses covert strategies to advertise and promote its products online. The Study Group, having examined the requests by Member States for technical assistance on topics of interest to regulators, as considered in this report, made several recommendations. TobReg nevertheless reiterates its conclusion in its previous report *(18)* that regulators should maintain a focus on wider tobacco control and should not allow themselves to be distracted by the tactics of tobacco and related industries, nor the aggressive promotional strategies used by those industries to sustain the use of tobacco and non-therapeutic nicotine products.

This report highlights the importance of:

- continued focus on tobacco control to decrease the prevalence of tobacco use;
- comprehensive tobacco control laws that apply to all tobacco products and all forms of tobacco and non-therapeutic nicotine products, without exception;
- international cooperation to address cross-border marketing of nicotine and tobacco products;
- comprehensive laws to regulate tobacco advertising, promotion and sponsorship laws, including new ways of promoting tobacco and non-therapeutic nicotine products;
- strengthen monitoring and enforcement of regulations on nicotine and tobacco products, including the activities of the tobacco and related industries;
- close regulatory gaps that could be exploited by the tobacco and related industries; and
- implement the recommendations of the Study Group.

Sections 2–6 of the report provide scientific information, evidence on online marketing and policy recommendations and guidance to bridge regulatory gaps in tobacco control. The report also identifies areas for further work and research, with a focus on the regulatory needs of countries, while accounting for regional differences, thus providing a strategy for continued, targeted technical support to all countries, especially WHO Member States. The main recommendations of the Study Group are outlined below.

7.1 Main recommendations

The main recommendations to policy-makers and all other interested parties are the following:

- noting the aggressive promotion of both tobacco and nicotine products globally, the Study Group urges Member States to ensure continuing focus on evidence-based measures to reduce tobacco use, as outlined in the WHO Framework Convention on Tobacco Control, and not to be distracted by the tobacco industry or other vested interests;
- to ensure that regulations on tobacco products are extended and applied to all forms of nicotine and tobacco products and not restricted to conventional cigarettes;
- to require manufacturers to disclose information on these products regarding:
  - emission levels of selected harmful chemicals and
  - levels of biomarkers in the panel used in pre-marketing evaluation;
- to ensure that laws on tobacco advertising, promotion and sponsorship are comprehensive and in line with the WHO Framework Convention on Tobacco Control as a minimum and that they encompass online digital media platforms, including social media and any other forms of direct or indirect marketing;
- to strengthen monitoring and enforcement and cooperate internationally to address cross-border practices of the tobacco and related industries, including online digital tobacco advertising, promotion and sponsorship;
- to require the tobacco and related industries to disclose to government authorities all advertising, promotion and sponsorship activities, including those on online digital media platforms;
- to address the content and emissions of tobacco products and support product evaluation, monitoring and disclosure, in keeping with Articles 9 and 10 of the WHO Framework Convention on Tobacco Control, when formulating, adopting or updating tobacco product regulations;

- to ban the addition of menthol and other ingredients that facilitate inhalation in non-therapeutic nicotine products and all tobacco products, including synthetic coolants with a chemical structure or physiological and sensory effects similar to those of menthol;
- to amend national tobacco control laws to fill any regulatory gap for synthetic nicotine products, to ensure that the full range of synthetic nicotine products fall within their scope, including pharmacologically similar analogues that are currently marketed and any products that may emerge in the future;
- to require uniform labelling rules for manufacturers of products containing synthetic nicotine or mixtures of nicotine from various sources, either natural or synthetic, so that the contents of different nicotine forms or analogues are declared separately;
- to establish or extend surveillance of products and their users, including demographics, use of other tobacco and related products, brand, type and flavour used in nicotine pouches to acquire knowledge and assess the prevalence of use and user profiles;
- to regulate nicotine pouches to prevent all forms of marketing and take all other action necessary to minimize: young people's access to them, their appeal to young people and initiation of use by young people;
- to regulate non-therapeutic nicotine products in the same manner as products of similar appearance, content and use;
- to ensure that nicotine pouches are not classified as pharmaceutical products unless they are proven to be nicotine replacement therapies by following stringent pharmaceutical pathways for licensing as nicotine replacement therapies, as prescribed by the appropriate national regulatory authority;
- to use industry-independent data on biomarkers and country experiences in making policy decisions on electronic nicotine delivery systems, heated tobacco products and other novel and emerging nicotine and tobacco products; and
- to implement the recommendations of the Study Group on specific challenges posed in regulating non-therapeutic nicotine and all forms of tobacco products.

Countries are urged to implement the above recommendations, as enough information is available about the topics under consideration for countries to act to protect the health of their populations, especially the younger generation. While the report acknowledges that still more is to be learnt about some topics,

including synthetic nicotine and biomarkers for assessing ENDS, ENNDS and HTPs, continued independent research is necessary to build further information on these topics. The data required include the prevalence of use of nicotine pouches, the characteristics of those products, the use of synthetic nicotine in nicotine products and their availability, the science of synthetic nicotine, and promotional strategies of the tobacco and related industries. Given that 1.3 billion people use tobacco globally, the tobacco control community should continue to accelerate use of evidence-based policies and recommendations, such as those in the WHO Framework Convention on Tobacco Control, measures outline in WHO MPOWER and the relevant reports of the Conference of the Parties to the WHO FCTC. Countries should thus implement proven policy measures and, in addition, consider implementing the recommendations in this report. Specific recommendations on each of the topics considered are available in sections 2.9, 2.10, 3.4, 4.11, 5.7, 5.8, 5.9 and 6.7.

7.2 Significance for public health policies

The Study Group's report provides guidance for understanding research and evidence on the scientific basis of the regulation of nicotine and tobacco products, including cigarettes, smokeless tobacco and waterpipe tobacco. The Eighth report of the Study Group *(18)* addressed novel and emerging nicotine and tobacco products, in particular electronic nicotine delivery systems, electronic non-nicotine delivery systems, and heated tobacco products. This ninth report highlights the effects of additives that facilitate inhalation; the public health implications of social and digital marketing; the challenges associated with the marketing of nicotine pouches and synthetic nicotine and the regulatory implications of marketing of these products; and current evidence on biomarkers of exposure, effect and susceptibility for assessing electronic nicotine delivery systems, electronic non-nicotine delivery systems and heated tobacco products. The report also considers the potential impact of introduction of these products on tobacco control, identifies research gaps and makes recommendations. The recommendations directly address some of the unique regulatory challenges faced by Member States by direct and indirect product market advertising and by penetration of several global markets of products such as nicotine pouches and products with synthetic nicotine. In addition, the report updates Member States' knowledge and provides guidance for formulation of effective strategies for regulating nicotine and tobacco products.

The Study Group, though its unique composition of regulatory, technical and scientific experts, navigates and distils complex data and research and synthesizes them into policy recommendations to inform policy development at country, regional and global levels. This report, by scientists with expertise in various disciplines relevant to the regulation of tobacco products, addresses the

challenges faced by governments for effective regulation of tobacco and non-therapeutic nicotine products. Regulators, governments and other interested parties can rely on the science and evidence presented to formulate policies to strengthen tobacco control and close regulatory loopholes, as appropriate. The identification of gaps in policy and research on the topics considered, including on nicotine pouches, synthetic nicotine, online and digital marketing of nicotine and tobacco products, indicates areas in which there is insufficient information. In formulating their research agendas, countries could focus on areas pertinent to their policy goals, objectives and country context and regulatory environment. This is a critical role of the Study Group, especially for governments with inadequate resources or capacity to navigate technical information on tobacco product regulation.

The recommendations of the Study Group promote international coordination of regulatory work and adoption of best practices in regulating tobacco and non-therapeutic nicotine products, strengthen capacity for product regulation all six WHO regions, provide a ready resource to Member States based on sound science and support implementation of the WHO FCTC by its Parties. Given the aggressive promotion of nicotine and tobacco products globally, the Study Group urges Member States to ensure continued focus on evidence-based measures to reduce tobacco use as outlined in the WHO FCTC without distraction from the tobacco and related industries.

Tobacco product regulation complements other provisions of the WHO FCTC for reducing the demand for tobacco. The recommendations of the Study Group, if effectively implemented, would contribute to reducing the prevalence of tobacco use and improving health.

7.3 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,[4] which 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 main recommendations will improve Member States' understanding of conventional and newer products and the promotional strategies used by manufacturers.

The report's contribution to knowledge on regulating tobacco and non-therapeutic nicotine products will play a critical role in informing the work of the Secretariat, especially in providing technical support to Member States. It will also contribute to updating regulators, through the Global Tobacco Regulators Forum,

4 In November 2003, the Director-General formalized the status of the former Scientific Advisory Committee on Tobacco Product Regulation from a scientific advisory committee to a study group.

and Parties to the WHO FCTC through WHO's reports to the tenth session of the Conference of the Parties in November 2023, including on technical matters related to Articles 9 and 10 of the WHO FCTC. The report will include the key messages and recommendations of this ninth report of the Study Group. All these actions will contribute to meeting target 3.a of the Sustainable Development Goals (Strengthen implementation of the World Health Organization Framework Convention on Tobacco Control in all countries), as appropriate, and the triple billion targets of WHO's Thirteenth Global Programme of Work.

The report, a WHO technical product (a WHO global public health good *(1)*), is available to all countries to help drive impact nationally and globally to reducing tobacco use and improve overall public health.

7.4 References

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WHO study group on tobacco product regulation

This 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. This report presents the outcomes, deliberations and recommendations of the Study Group at its eleventh meeting on five background papers, which addressed emerging issues in tobacco product regulation, such as newer ways in which non-therapeutic nicotine, particularly in nicotine products is promoted and delivered to people in different age groups, including children and adolescents. The five topics addressed in the report are enumerated below:

1. Additives that facilitate inhalation, including cooling agents, nicotine salts and flavourings (Section 2);
2. Synthetic nicotine: science, global legal landscape and regulatory considerations (Section 3);
3. Nicotine pouches: characteristics, use, harmfulness and regulation (Section 4);
4. Biomarkers of exposure, effect and susceptibility for assessing electronic nicotine delivery devices and heated tobacco products, and their possible prioritization (Section 5);
5. Internet, influencer and social media marketing of tobacco and non-therapeutic nicotine products and associated regulatory considerations (Section 6); and

The report considers the potential impact of the key considerations in the background papers on tobacco control efforts, identifies research gaps and makes some recommendations. The recommendations on each of the topics considered in the report are set out at the end of the relevant section and the overall recommendations of the Study Group are summarized in the final section of the report. These recommendations directly address some of the unique regulatory challenges faced by Member States due to direct and indirect product market advertising and penetration of products, such as nicotine pouches and products with synthetic nicotine, to several markets globally. In addition, the report will update Member States' knowledge and aid the formulation of effective regulatory strategies for nicotine and tobacco products.

The report, which is a WHO technical product (i.e. an initiative developed or undertaken by WHO that is of benefit either globally or to many countries in many regions), is available to all countries to help drive impact at country level and globally, towards reducing tobacco use and improving overall public health.

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