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Report of a WHO Study Group

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WHO Study Group on Tobacco Product Regulation

Kobe, Japan, 28–30 June 2006

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1. Introduction

The third meeting of the WHO Study Group on Tobacco Product Regulation (TobReg) was held in Kobe, Japan from 28 to 30 June 2006. The meeting was held in response to Decision 15 of the first session of the Conference of the Parties to the World Health Organization's (WHO) Framework Convention on Tobacco Control (*1*), held in Geneva, Switzerland from 6 to 17 February 2006. During that session, the Parties adopted the templates for the elaboration of guidelines for the implementation of Articles 9 and 10 of the Framework Convention, which relate to the regulation of the contents of tobacco products and of tobacco product disclosures. According to the template, work on the guidelines should be based on the work already done by the Study Group and the WHO Tobacco Free Initiative (TFI), which serves as the Study Group's secretariat and coordinating body.

This report presents the conclusions reached and recommendations made by the members of the Study Group at its third meeting, during which it reviewed four background papers specially commissioned for the meeting and which dealt, respectively, with the following four themes.

1. The contents and design features of tobacco products: their relationship to dependence potential and consumer appeal.
2. Candy-flavoured tobacco products: research needs and regulatory recommendations.
3. Biomarkers of tobacco exposure and of tobacco smoke-induced health effects.
4. Setting maximum limits for toxic constituents in cigarette smoke.

The Study Group's recommendations in relation to each theme are set out at the end of the section dealing with that theme; its overall recommendations are summarized in section 6.

1.1 Background

In contrast to tobacco products, chemical products such as medicines, pesticides and food additives are well regulated. The regulation of these products involves the initial, comprehensive identification and characterization through toxicological and analytical testing of a product's potential health hazards. Such testing aims to establish the potential of the product to induce various types of short-term and long-term toxicological damage of organs and tissues, allergy, carcinogenicity, reproductive toxicity and mutagenicity. Dossiers containing the information derived from toxicological testing are submitted to regulatory authorities, under whose authority scientific experts evaluate the hazard data. Many jurisdictions classify and label chemical consumer products according to their inherent hazardous properties based on toxicological testing. Further, evaluation of toxicological test results, together with exposure assessments related to product use, may lead either to acceptance of specified uses of the product or to prohibition on the placing of the product on the market.

The regulation of tobacco products, however, is in its infancy in many parts of the world. For this reason, one of the aims of the WHO Framework Convention on Tobacco Control (*2*), through the provisions of its Articles 9, 10 and 11, is to lay the groundwork for the future regulation of the contents, emissions, disclosure of ingredients and additives, packaging and labelling of tobacco products. The research and scientific evidence that informed the negotiations of these provisions contributed to the consensus position reached by the Parties that regulation of tobacco products would serve public health goals by providing meaningful oversight of the manufacturing, packaging and labelling, and distribution of tobacco products. The scientific basis for the principles guiding the implementation of Articles 9 and 10 establishes the rationale for the principles guiding the implementation of Article 11. For this reason, and in order to achieve the synergistic effect of these provisions, all three articles should be treated as a single set of interrelated and mutually reinforcing regulations.

The WHO Tobacco Free Initiative was established in July 1998 to focus international attention, resources and action on the global tobacco epidemic. Its mission is to reduce the global burden of disease and death caused by tobacco, thereby protecting present and future generations from the devastating health, social, environmental and economic consequences of tobacco consumption and exposure to tobacco smoke. This mission is aligned with the objective of the Framework Convention, WHO's first and only treaty, which entered into force in February 2005, and which provides a framework for tobacco control measures, to be implemented by the Parties at the national, regional and international levels, in order to reduce continually and substantially the

prevalence of tobacco use and exposure to tobacco smoke. The Tobacco Free Initiative was the WHO department that took the lead in the convening of the intergovernmental negotiations on the Framework Convention, and it has been serving as the interim secretariat to the Convention until such time as the permanent secretariat of the Convention is established within WHO.

In the light of the recommendations of the International Conference on Advancing Knowledge on Regulating Tobacco Products, which was held in Oslo, Norway from 9 to 11 February 2000 (*3*), the WHO Tobacco Free Initiative considers tobacco product regulation to be one of the four pillars of any comprehensive tobacco control programme. The other three are: (i) preventing the uptake of the use of tobacco products; (ii) promoting cessation of their use; and (iii) protecting the public from exposure to second-hand smoke.

However, in general, a laissez-faire attitude towards tobacco product regulation prevails. As a result, tobacco continues to be unregulated or underregulated in many WHO Member States, even though, when used as directed by the manufacturers, it remains the only legal consumer product that kills half of its regular users. Contributing to this attitude is the fear among many tobacco control advocates that promoting the wrong policy might even be worse than the status quo, and hence some argue for continued analysis and debate. This fear is understandable in the light of the unintended consequence of earlier strategies aimed at reducing the adverse effects of tobacco use, which was and continues to be the use by the tobacco industry of misleading labelling of lower tar cigarettes as "light" and "mild". However, in this regulatory vacuum, the ingenious efforts of tobacco companies to make people become dependent on smoking in the interests of increasing their market share continue unbridled. Tobacco product regulation – including the regulation of the contents and emissions of tobacco products through testing and measuring, and the mandated disclosure of those results, and regulation of the packaging and labelling of tobacco products – requires governmental supervision of the manufacture of tobacco products and enforcement of the regulations governing their design, contents and emissions, as well as their distribution, packaging and labelling, with the aim of protecting and promoting public health. Tobacco product regulation needs to be developed and implemented in our lifetime.

Further to the recommendations of the International Conference on Advancing Knowledge on Regulating Tobacco Products (*3*), WHO established the Scientific Advisory Committee on Tobacco Product Regulation (SACTob), which provided sound scientific information on tobacco product regulation, specifically to fill the knowledge gaps that existed at the time in the area of tobacco product regulation, and served as the basis for the negotiations and the subsequent consensus reached on the language of these three articles of the Convention.

In November 2003, in recognition of the critical importance of regulating tobacco products, the WHO Director-General formalized the ad hoc Scientific Advisory Committee on Tobacco Product Regulation by changing its status to that of a study group. Following the status change, the Advisory Committee became the "WHO Study Group on Tobacco Product Regulation" (TobReg). It is composed of national and international scientific experts on product regulation, tobacco-dependence treatment, and the laboratory analysis of tobacco ingredients and emissions. Its work is based on cutting-edge research on tobacco product issues. It conducts research and proposes testing in order to fill regulatory gaps in tobacco control. As a formalized entity of WHO, the Study Group reports to the WHO Executive Board through the Director-General in order to draw the attention of Member States to the Organization's efforts in tobacco product regulation, which is a novel and complex area of tobacco control.

The Study Group hopes that the recommendations contained in this report, as well as its other recommendations and advisory notes, will be useful to the Contracting Parties to the WHO Framework Convention that have been identified as key facilitators (Canada, the European Community and Norway), partners (Brazil, China, Denmark, Finland, Hungary, Jordan, Kenya, Mexico, the Netherlands, Thailand, and the United Kingdom of Great Britain and Northern Ireland) and reviewers (Australia, France and Jamaica) and that have volunteered to assist the interim secretariat to the Framework Convention and the Tobacco Free Initiative to draft guidelines for the implementation of the product regulation provisions of the Framework Convention.

The Study Group also looks forward to the time when the product regulation implementation guidelines, once adopted by the Conference of the Parties, will become the "gold standard" for the implementation of tobacco product regulation at the national and subnational levels. Finally, in formulating their tobacco product regulations, it is essential not only that Member States avoid potential loopholes in their legislation, but also that they make allowance for the regular revision of their regulations to take into account new knowledge about any tobacco product or its modified or re-engineered version.

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1. *Conference of the Parties to the WHO Framework Convention on Tobacco Control, First session, Geneva, 6–17 February 2006*. Geneva, World Health Organization, 2006 (http://www.who.int/gb/fctc/E/E_cop1.htm, accessed 28 February 2007).
2. *WHO Framework Convention on Tobacco Control*. Geneva, World Health Organization, 2003, updated reprint 2005 (http://www.who.int/tobacco/fctc/text/en/fctc_en.pdf, accessed 28 February 2007).
3. *Monograph: advancing knowledge on regulating tobacco products*. Geneva, World Health Organization, 2001 (http://www.who.int/tobacco/media/en/OsloMonograph.pdf, accessed 28 February 2007).

2. Contents and design features of tobacco products: their relationship to dependence potential and consumer appeal

2.1 Background

Historically, cigarettes and other tobacco products have been exempt from health and safety standards governing contents and designs that are typically applied to other consumer products, including foods, beverages and drugs (*1–3*). Although some countries have begun to develop and apply standards for allowable contents, there are no globally accepted principles or guidelines (*2*). Currently, limits on emissions from tobacco products have not been implemented, with the exception of machine-measured yields of tar, nicotine and carbon monoxide (*2*). An important consideration in the regulation of contents and designs is that when the cigarette (or any tobacco product that is combusted or heated) is used as intended, changes in contents and designs can modify emission profiles during the processes of combustion ("burning") and pyrolysis ("modification by heat"). Tobacco, even in its non-combusted or non-heated form, is harmful and has the potential to cause dependence because its naturally occurring constituents include carcinogens and other toxicants, as well as nicotine.

Therefore, the focus of this report is on the importance of evaluating the contents and designs of tobacco products together with the emissions of combusted, heated and non-combusted tobacco products under the conditions in which these products are actually used. The purpose of the report is to provide recommendations to support the development of protocols for assessing the contents, designs and associated emissions of tobacco products. It is expected that, in combination with other elements of comprehensive tobacco control, actions resulting from these recommendations will contribute to the reduced prevalence of tobacco use and disease, although the effects, if any, on disease outcome of any specific element of guidance on tobacco content and design remain uncertain at present.

The toxicity and dependence potential of tobacco products are related to their contents, designs and emissions. The contents and designs affect the consumer appeal of the product and directly relate to initiation and persistence of use. The tobacco industry has a long history of manipulating

contents, designs and other factors related to consumer appeal in order to increase use and dependence, often with the additional result of increased toxicants in the products and emissions (*4–8*).

These observations apply to all tobacco products subject to the WHO Framework Convention on Tobacco Control (*9*), including cigarettes, oral non-combusted and non-heated tobacco products, bidis, kreteks, waterpipes and roll-your-own tobacco materials. However, it is recognized that the available data relate mainly to manufactured cigarettes and smokeless tobacco products, which are the focus of this report.

In 2003, the Scientific Advisory Committee on Tobacco Product Regulation addressed the topics of tobacco product contents and emissions and specifically recommended that upper limits for known toxic chemicals in tobacco product ingredients and emissions should be set (*10*). Progress has been made in implementing this recommendation through the collaborative efforts of the International Agency for Research on Cancer (IARC) of the World Health Organization and the Tobacco Free Initiative. Whereas the collaborative efforts with IARC aim to restrict emissions based on their toxicity, the main focus of this report is on those aspects of contents, emissions and design features that are relevant to dependence potential and consumer appeal. These issues are not mutually exclusive because the contents, designs and emissions of many tobacco products have multiple effects, as discussed in this report.

The recommendations in this report are based on new findings, and they therefore update and supplement many of the conclusions contained in the recommendation made by the Scientific Advisory Committee in 2003 (*10*). The overall purpose of this report is to guide implementation of the WHO Framework Convention on Tobacco Control, and, in particular, the provisions in Articles 9, 10 and 11 relating to the regulation of the contents of tobacco products and of tobacco product disclosures, packaging and labelling.

2.2 Terminology

In accordance with the terminology used in the WHO Framework Convention and the recommendation of 2003 by the Scientific Advisory Committee, the term “contents” is used synonymously with the term “ingredients”. Consequently, “contents”, as used herein, means all product components, the materials used to manufacture those components, residual substances from agricultural practices, storage and processing, substances that can migrate from packaging into the product, as well as what may be termed “additives” and “processing aids” in some countries and regions. The WHO Study Group on Tobacco Product Regulation recognizes that the definition and regulation of what are commonly referred to as “additives” vary according to the different policies of each Member State, and it urges Member States that

currently regulate additives to modify their regulations to accommodate the potentially larger number of product constituents that are more likely than not to contribute to dependence potential and toxicity.

The term "emissions" is used to refer to all substances released from the product when it is used as intended. It is evident that they are responsible for most tobacco-attributable deaths and disease. In the case of cigarettes and other combusted or heated products, "emissions" refer to the constituents of the "smoke" (particulate and gas phases). These include those emissions directly inhaled by the user of the product, when referring to cigarettes, bidis, kreteks, waterpipes, and other combusted or heated products ("mainstream smoke") and those inhaled by non-users and users alike ("second-hand tobacco smoke"). In the case of smokeless and non-heated tobacco products, emissions refer to substances released during the process of oral use, including substances that change as a result of interaction of saliva and the product material (e.g. those substances that alter the relative proportion of free nicotine that was present in the unused product).

"Exposure" refers to those emissions actually taken into the body and absorbed by the user and other exposed persons. Whereas emission potential can be assessed under various conditions by machine testing, human exposure can only be assessed by human studies.

"Attractiveness" or "consumer appeal" refer to 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. Physical product characteristics are often integrated with marketing. For example, a flavour such as "menthol", "mint", or "cherry", which is intended to appeal to a target population, may be incorporated into the product name or descriptors and marketed to reach out to that population.

In this report, the meanings of the terms related to dependence are consistent with those defined by the WHO Expert Committee on Drug Dependence (2003) (*11*) and *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines* (1992) (*12*), which discuss the technical terms in greater detail. The following are brief definitions of these dependence-related terms as used in the present report.

"Addiction" is the commonly used term referring to what is technically known as "dependence" and is widely employed to connote severe substance dependence, as has been demonstrated to occur in tobacco users. As such, it is used to describe tobacco dependence by national organizations such as the Royal College of Physicians of London (2000) (*13*), the Ministry of Health

and Family Welfare of the Government of India (2004) (*14*), the Surgeon-General of the United States (1988) (*15*), and WHO (2004) (*4*). In the present report, the term "dependence" is being used as a synonym for "addiction".

"Addictiveness", or "dependence potential", refers to the pharmacological effects of a drug assessed according to standardized animal and human tests ("dependence potential testing"), which are relied on by WHO and other organizations for evaluating other dependence-causing substances under the international and various national drug control treaties (*11*, *16*, *17*). In the present report, the terms "dependence-causing" and "dependence potential" are being used as synonyms for "addictive" and "addictiveness", respectively.

For any given substance, including nicotine, dependence potential is related to the dose, speed of absorption, and to physical and chemical features of the formulation, in addition to the characteristics of the substance itself. Although the risk of dependence on any substance is partially related to the attractiveness and/or ease of use of the delivery system, these features are not typically evaluated in dependence-potential testing but rather are generally described as factors affecting "consumer appeal" or "attractiveness".

2.3 Relationship between dependence potential and harm

Contents and designs that modify dependence potential and attractiveness can contribute to harm directly (e.g. by increasing toxic emissions) or indirectly (e.g. by increasing the amount and persistence of use). For example, the proliferation of candy-flavoured and exotic-flavoured tobacco products is a major public health concern due to their potential to contribute to initiation by and dependence among youths (see section 3). These flavoured brands and brand extensions are marketed to the youth population and other high-risk populations with colourful and stylish packagings, and flavours that mask the harsh and toxic properties of tobacco smoke.

Cigarettes (and variants such as bidis, kreteks or waterpipes) are associated with the highest levels of disease among tobacco products because their designs and ingredients both facilitate and reinforce powerful dependencies and deep lung exposure of toxins. They deliver mildly acidic smoke that is inhaled more easily than the alkaline smoke of most pipes and cigars. The absorption of nicotine in the lung has a high potential to cause dependence because it very rapidly results in delivery of small doses to the brain, establishing the repetitive and persistent smoke self-administration characteristic of smokers of cigarettes and other smoking products.

2.4 Effect of contents and designs on dependence potential

2.4.1 *Nicotine dose level*

The key determinant of the dependence potential of a tobacco product is its ability to deliver pharmacologically active levels of nicotine (*18*, *19*). Nicotine dosage can be carefully controlled by manufacturers to ensure that nicotine dose levels are sufficient for target populations to produce desired effects such as relaxation and mental acuity, while minimizing the risk of producing undesirable effects such as nausea and intoxication (*20–22*).

2.4.2 *Other contents*

Some contents and designs that may increase dependence potential and/or consumer appeal may also increase toxicant exposure (*23*, *24*). For example, acetaldehyde (*25*), a by-product of the combustion and/or pyrolysis of simple sugars, is a known carcinogen that appears to potentiate the dependence-causing effects of nicotine. Chocolate and its derivatives are added to indirectly facilitate the development of dependence by contributing flavour and mouth sensations, and this appears to increase the carcinogenicity of smoke (*6*, *26*, *27*).

Certain additives (menthol in manufactured cigarettes, eugenol in kreteks) are added specifically to reduce the smoke harshness and enable the smoker to take in more dependence-causing and toxic substances. Many smokers smoke kreteks and menthol cigarettes, which are often marketed as less toxic; and the added ingredients possibly contribute to the perception that the cigarettes are less noxious and harmful. For example, in the South-East Asia Region, punk ash is added to tobacco to make *iq'mik* and lime is added to tobacco to make *khaini*, *naswar* and zarda products. Other substances may alter the attractiveness and/or ease of use of the product, thereby contributing to dependence risk. Menthol, chocolate, licorice, manipulations of appearance, and smoke yields of the product estimated by the International Organization for Standardization/United States Federal Trade Commission (ISO/FTC) also contribute to the risk that the product will be used, and if so, used repeatedly (*4*, *6*, *25*, *27–29*).

2.4.3 *Modifying nicotine delivery speed and efficiency by pH and free nicotine control*

For most dependence-causing drugs, the rate of absorption can influence the dependence-causing and reinforcing effects: more rapid absorption is associated with stronger reinforcing and dependence-causing effects (i.e. "impact") (*17*).

In the case of tobacco products, speed of delivery – and hence impact, dependence-causing and reinforcing effects – is related to the proportion of nicotine in a tobacco product and/or its emissions that is in the unprotonated or "free-base" form (also known as the un-ionized free-base form). Approximately 50% of the nicotine in a moist tobacco product or smoke is in the free form at a pH of 8. Because the pH scale is logarithmic, the proportion increases or decreases sharply with relatively small changes in pH. For example, at a pH of 7 about 7% of the nicotine is free; at a pH of 9 more than 80% of the nicotine is in the free form. By a variety of potential mechanisms discussed elsewhere (*4*, *25*, *27*, *30–32*), free nicotine enhances the speed at which nicotine reaches the sites in the brain and thereby enhances the dependence potential of the product.

Tobacco and smoke pH appear to be controlled primarily by the use of ammonia compounds and other substances used in tobacco processing and final cigarette production. These constituents serve to optimize the free nicotine levels and subsequent dependence potential (*4, 20, 21, 25, 27, 30, 33*).

Cigarette ventilation designs also modify free nicotine levels in the smoke. For example, when cigarettes are highly ventilated, the proportion of free and un-ionized nicotine is in the order of 30–40%. In cigarettes with low tip ventilation, the proportion of nicotine in the free base form is only a few percent of the total. Thus, when highly ventilated cigarettes are smoked and the ventilation holes are not blocked, the total mass of delivered nicotine is low, but much of that nicotine is delivered in the free, un-ionized state. This is an important design feature, since highly ventilated cigarettes can continue to supply the total amount of free nicotine needed to maintain dependence whether or not the ventilation holes are blocked. Highly ventilated cigarettes are designed with a lower amount of base additives such as ammonia. If ammonia levels equivalent to those used in low ventilation cigarettes (full-flavour) were used in highly ventilated cigarettes, the amount of free nicotine might overwhelm the smoker.

Smoke from combusted cigar and pipe tobacco tends to be mildly alkaline with pH values ranging from about 7.5 to 8.5, but with considerable variation across products and during sequential puffs. This range of alkalinity allows efficient and rapid rates of absorption of nicotine in the mouth, thereby circumventing lung inhalation as a means of causing and sustaining dependence (*34*). Inhalation of alkaline smoke is more noxious than is mildly acidic smoke. In comparison with cigarettes, for example, this discourages inhalation and contributes to the overall lower risk of lung disease in populations of cigar and pipe smokers. When cigar and pipe users do inhale the smoke, as many former and concurrent cigarette smokers tend to do, the risk of lung disease is similar to that of cigarette smokers (*35*).

The effect of tobacco pH on free nicotine levels has been well documented for smokeless tobacco products. For some oral tobacco products, such as shredded or twisted tobacco leaves intended for chewing, the typically low pH means that the products tend to deliver their available nicotine slowly as the product is chewed. For the categories of oral smokeless products known as moist snuff, including snus, the design and method of use of the product require the pH of the product to be controlled with sufficient buffering material to enable nicotine to be free for absorption over the many minutes that the product may be kept in the mouth. In practice, buffering and pH levels are controlled to create products with the desired free nicotine levels for the target populations. For example, "starter" products are lower in free nicotine than those marketed to experienced and dependent users.

Many smokeless tobacco products are also made or modified by local vendors or users with ingredients that may affect free nicotine levels. For example, adding punk ash to tobacco to produce *iq'mik*, slaked lime to tobacco to make *khaini*, *nass*, or *pan masala* or boiling with lime to make zarda increases the pH of the tobacco, the amount of free nicotine available to the user, and the dependence potential of the product. Although these products have been used in this way traditionally, it is important for those who use this modified tobacco to know they are using a product with increased dependence potential, and thus, increased harm.

2.5 Regulatory implications and challenges

All tobacco products have contents and emissions that could potentially be regulated. Because the emissions from non-combusted and non-heated products are primarily the contents themselves, regulation of their contents appears feasible and valid. For combusted or heated products, it appears more practical to focus regulatory effort on their emissions, although certain added ingredients and design features should also be included in regulation (e.g., ammonia, chocolate, glass fibres and cigarette ventilation).

This dual focus is consistent with the emphasis the tobacco industry itself places on the nature and acceptability of emissions in their product development and evaluation (*1*, *2*, *5*, *21*). This includes industry research on the physical nature of smoke ("smoke chemistry" and appearance) and its acceptability to potential consumers (*21*, *36*). The physical design characteristics of the tobacco product interact with its chemical composition to influence its function and effect (*2*, *21*). For example, the size of the cuttings of the tobacco in cigarettes and non-combusted and non-heated tobacco, its level of acidity (measured as pH), and the presence of other substances interact to influence the release of nicotine from the product (*3*, *21*). Similarly, the physical and chemical characteristics of cigarettes interact to alter the size

distribution of the aerosol particles that convey nicotine and other chemicals, and thus influence absorption (*21*).

The emphasis on both contents and emissions recognizes that the health effects of tobacco products depend on their physical nature, their chemical make-up and how they are used (*2*, *3*, *13*, *37*, *38*). For example, more frequent or longer use of a product delivering lower levels of toxins per unit may result in greater risks to health than less frequent use or fewer years of use of a product that is more toxic per unit (*39–41*). Because the tobacco industry has a history of marketing its products on the basis of apparent reductions in toxicity with the intent of increasing consumption of their products, a regulatory strategy to reduce toxins must be accompanied by oversight of marketing and by surveillance of consumer use to detect such adverse effects (*3*, *41*).

It should be recognized that tobacco is a unique consumer product that could not be introduced into the market today under any known consumer regulations if it were not already established worldwide among a variety of substantially dependent populations. Products that prematurely end lives or lead to the death of the consumer when used as intended by the manufacturer have no place in a civilized society. Indeed, for this very reason, the regulatory norms applied to other consumer products such as foods, cosmetics and drugs do not readily translate to tobacco products. Tobacco product regulation, therefore, requires an unconventional approach that acknowledges the unacceptable levels of harm already in play. Given that tobacco product emissions are known to vary greatly and consist of thousands of toxicants, one approach is to establish upper limits for selected constituents, based on toxicity profiles, as a means of progressive toxicant reduction in emissions as part of an effective regulatory strategy. This same approach can be applied to contents and emissions that influence dependence potential and/or appeal (*7*, *42*).

It is important to note that for many products, regulatory limits are established by defining safe levels of exposure. The level of toxicants in tobacco products is so high, however, that no regulatory tactic could be based on either safe levels or product safety. It is acknowledged that standards for upper limits of contents or emissions will not necessarily result in decreased health risks because they do not necessarily render reduced exposure and because the relationship between exposure and disease is not necessarily a simple function of exposure level. Therefore, these recommendations must not form the basis for the development of product descriptors and claims that would imply health benefits or claims about the health effects of the products. To be clear, health effects include all forms of tobacco-related damages and diseases, including dependence.

2.5.1 *Personal and local vendor-made products*

Locally made oral smokeless tobacco products, as well as some non-cigarette smoked tobacco products, pose special challenges for regulation and communication. In India and other countries in the South-East Asia Region, individuals and small informal companies that may be difficult to regulate in the near future make a substantial proportion of oral smokeless tobacco, kreteks, bidis, *gutka* and other products. Such products, even when marketed by registered companies, are often assembled in small household manufacturing units. Furthermore, the makers vary widely in the ways in which they construct the products, and ingredients may vary with the season and local preferences. It is probable that, even if nearly all local commercial manufacturers are eventually effectively regulated (a process that undoubtedly will take many years), there will still be many people using tobacco products manufactured by individuals or family-based, informal "companies", for personal and local use.

2.5.2 *Nicotine levels*

Nicotine is the key dependence-causing pharmacological agent in tobacco, and therefore its elimination would be expected to drastically reduce the dependence potential and use of tobacco products. This goal, however, appears unrealistic in the foreseeable future. Most of the world's more than 1.3 billion tobacco users are dependent on nicotine and it is impractical to consider an abrupt elimination of access to nicotine. Because of this, nicotine reduction strategies would have to involve a long-term disengagement process. Conversely, substantially increasing nicotine levels in proportion to toxicants would be expected to at least slightly lower the daily intake of toxicants. However, whether this would be sufficient to significantly reduce exposure to the toxic and carcinogenic emissions from tobacco is unknown.

2.5.3 *Assessing and regulating dependence potential*

Objective laboratory testing methods involving animal and human models are used by WHO and other organizations for assessing the dependence potential of substances with potential for abuse as well as pharmaceutical products (*11*, *16*, *17*). These methods have also been applied to tobacco products and nicotine-delivering medicines (*15*, *43*). They could be applied to target tobacco product contents, emissions and combinations thereof suspected of contributing to the dependence potential of a product, as was done in one study by tobacco industry researchers (*44*). These methods have been used less extensively to compare physical formulations and complex mixtures than for single-entity chemicals, and therefore their application to some issues concerning tobacco products requires adaptation and validation

of methods, as is currently being undertaken for various pharmaceutical formulations (*17*).

The following conclusions will serve as the foundation for enumerating specific recommendations and topics for research, a process that may, in turn, lead to further recommendations.

2.6 Conclusions

1. The regulation of tobacco plant material needs to be consistent with that of many agricultural products, which are routinely measured and regulated with regard both to their purity, their contaminants and their levels of allowable chemicals used agronomically, and to their processing, manufacturing and packaging. For example, tobacco products in many countries may contain numerous unintentional contaminant by-products of their agronomic, processing and storage practices that introduce non-tobacco materials, including, but not limited to, pesticides (herbicides are pesticides), microorganisms, and animal or insect excrement or parts.
2. Contemporary molecular biology techniques can be and have been applied to commercial tobacco to produce cultivars having transgenically induced the synthesis of chemicals (e.g. pesticides) that confer systemic disease and pest resistance.
3. For tobacco products that are intended to be smoked or heated, the manufactured product needs to be differentiated from the product actually intended for consumption, which is its emission ("smoke"), and the principal focus of regulation should therefore be on the emissions.
4. Highly flavoured tobacco products target young and novice smokers through increasingly sophisticated product design and marketing.
5. Combustion and pyrolysis of contents in tobacco products, such as cigarettes (both manufactured and hand-made), pipes, cigars, waterpipes and bidis, result in compounds delivered to the user that increase the dependence-causing effects of nicotine.
6. Cigarette contents and emissions regulation is intended to support tobacco disease-control efforts, to prevent initiation and to stimulate cessation, as well as to contribute to reduced exposure to toxicants in persons who use tobacco products.
7. Non-combusted and/or non-heated tobacco products also produce emissions that cause dependence and are toxic, and therefore warrant regulation.

8. One of the purposes of such regulation of tobacco products is a progressive reduction in the level of toxic chemicals in tobacco product contents and emissions, through periodic setting of standards. While ingredient and emission regulations are based on the public health principal of reducing toxicants in products, the scientific complexity of linking changes in individual emission constituents to changes in disease risks precludes any expressed or implied harm-reduction claim based on changes resulting from these regulations.

2.7 Research needs

1. Methods to assess the effects of contents and designs on the dependence potential of tobacco products require considerable attention as such methods have not been widely applied to the broad range of tobacco product types.
2. Systematic study is needed of the contribution of tobacco product contents and designs to the consumer appeal of these products (e.g. candy-like or exotically flavoured) to various target populations (e.g. children, young adults, male versus female, racial marketing – menthol-containing cigarettes in the United States – and former tobacco users).
3. With respect to nicotine, it remains ill-defined at this time whether public health would be better served by increased or decreased levels of nicotine per unit (e.g. per cigarette), and further study of this issue is required.
4. Contents and design features that reduce toxicity, consumer appeal and/or dependence potential need to be investigated in order to provide regulatory agencies with the basis for requiring certain features aimed at substantial plausible benefit. Note that, whereas one premise of this recommendation is not to investigate or provide any guidance on the contents and designs of tobacco products, in the same way that regulatory agencies on occasion require certain types of modifications to products (e.g. seat belts in cars), it should be determined whether particular features might merit such consideration.
5. The potential effect on tobacco-use patterns of efforts to control contents, consumer appeal and dependence potential needs to be assessed by population surveillance and research to detect unintended consequences and provide regulators with information to modify guidance.
6. The possibility of reducing the dependence potential and consumer appeal of non-combusted and non-heated tobacco products without eliminating their potential to provide dependence-causing doses of nicotine needs to be investigated.

7. The effect of the proportion of free, unprotonated nicotine to protonated forms of nicotine delivered by a tobacco product on sensory impact, uptake rate, absorption, and dependence potential needs to be investigated.
8. The effect of aerosol particle size and distribution in combusted or heated tobacco products on sensory impact, degree and rate of absorption, toxicity, and dependence potential needs to be investigated.

2.8 Regulatory recommendations

1. The implementation of the relevant articles of the WHO Framework Convention on Tobacco Control and national and regional tobacco regulatory actions may have consequences for tobacco initiation, cessation and health effects. Both surveillance and research efforts addressing these consequences are needed to assess the effects of regulatory efforts on these behaviours and to modify the regulatory process on a regular basis as required.
2. No health claims based on the level of contents or emissions or on whether the products meet regulatory standards for contents and emissions should be permitted.
3. Regulation of non-combusted and/or non-heated products applies to the contents and designs of the products, whereas regulation of combusted and/or heated products applies to the contents, designs and emissions.
4. The contents and designs of combusted and/or heated tobacco products should be altered in ways expected to contribute to reduced dependence potential.
5. Regulators should monitor the proportion of nicotine available in its free-base form in all tobacco products and emissions.
6. Regulations should be developed to prohibit manufacturing and marketing of candy-like and exotically flavoured tobacco products targeting young and novice smokers.
7. Use of genetically modified tobacco for any purpose in commercial blends needs to be communicated to regulatory authorities and consumers.
8. Regulators should monitor and establish standards for non-intentional contaminant by-products of agronomic, processing and storage practices such as, but not limited to, pesticides, microorganisms, and animal or insect excrement or parts in the final product.
9. Regulation of contents and designs that contribute to consumer appeal and palatability is essential because they indirectly contribute to health

impact by their effects on tobacco use initiation, patterns of use, product selection and persistence of use.

10. Efforts to measure and reduce dependence potential should be consistent with approaches used to measure and reduce the dependence potential of pharmaceutical products, including standardized abuse-liability testing procedures relied on by WHO for international drug control.

11. To expedite progress on the understanding and control of the effects of contents, designs and emissions on dependence, consumer appeal and palatability, an expert panel should be formed to review tobacco products and make recommendations for potential targets for control. Its work should be coordinated with the collaborative work of the Tobacco Free Initiative and the International Agency for Research on Cancer, enabling it to make recommendations for potential targets for reducing carcinogens and other toxins.

12. For combusted or heated products, none of the contents should enhance nicotine potency or emission toxicity.

13. For combusted or heated products, no design feature should be included in any tobacco product that enhances nicotine potency or emission toxicity. Further research will be required to guide this recommendation.

14. For non-combusted or non-heated products, no ingredient should be added to the contents of any tobacco product that enhances nicotine potency or emission toxicity.

15. For non-combusted or non-heated products, no design feature should be included in any tobacco product that enhances nicotine potency or emission toxicity.

16. In India and other parts of the South-East Asia Region and other WHO regions where a substantial proportion of the tobacco products are locally made without standardized contents and manufacturing methods, it is essential to provide information to both consumers and makers about the potential impact of contents and designs on toxicity and dependence potential. These communications must be developed to recognize regional and product-specific needs.

17. It is important to educate users of kreteks and menthol cigarettes that additives in these products are masking the harshness of their emissions and allowing them to bypass the body's normal defence mechanisms for preventing exposure to detrimental substances.

18. It is important to educate users of smokeless tobacco products in the African Region, the Region of the Americas (particularly Alaska), the

South-East Asia Region and other WHO regions that some of the ingredients used in traditional products can enhance their dependence-causing effects (for example, buffering agents and flavouring agents, and possibly other substances with psychoactive effects such as beetle nut), and that these products are not a safe alternative to cigarette smoking. Women of childbearing age and parents with children need to be educated about the dangers of using tobacco products that have enhanced dependence potential, even if those products are of traditional use.

19. A timetable for expeditious implementation needs to be developed. This timetable should take into consideration resources and capacity, and should take account of the fact that, as certain targets are achieved, others will need to be revised and new targets will need to be developed.

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3. Candy-flavoured tobacco products: research needs and regulatory recommendations

3.1 Introduction

Tobacco products are manufactured in a vast array of varieties to target as many consumers as possible. One tobacco brand style, which is growing in popularity among young and inexperienced smokers, is flavoured tobacco, especially candy-flavoured tobacco. The application of flavouring agents to tobacco products is a long-standing practice in the tobacco industry; however, new technologies are being introduced to deliver the flavour to the product more effectively. The flavours are added primarily at the terminal step in manufacturing, by the use of an alcohol carrier; microencapsulation; or thermal-activated or filter-embedded additives. Making these highly flavoured products available to youths to encourage initiation raises further questions regarding the practices of the tobacco industry, specifically the targeting of youths as consumers. The current lack of regulation of these products is of considerable concern to the tobacco control community. In view of the little research that has been conducted on flavoured tobacco, the WHO Study Group on Tobacco Product Regulation (TobReg) urges health authorities to consider public health initiatives to reduce the marketing and use of flavoured tobacco products. Basic public health principles dictate that flavours should not be used to adulterate contaminated food or make highly dependence-causing drugs more enticing.

3.2 Purpose of the recommendations

In its continuing effort to combat the deadly effects of tobacco consumption, the WHO Study Group on Tobacco Product Regulation has prepared these recommendations in order to address the growing concerns over the increasing prevalence and potential health effects of flavoured tobacco products, especially among young and inexperienced smokers. The purpose of the recommendations is to provide guidance to WHO and its Member States concerning the potential risks of flavoured tobacco products; to inform regulatory agencies in their efforts to implement the provisions of the WHO Framework Convention on Tobacco Control; and to educate the public about the potential risks of flavoured tobacco products. The recommendations are

also intended to provide guidance to researchers and research agencies interested in facilitating a more thorough understanding of the health effects of flavoured tobacco products, and to those engaged in developing tobacco-smoking prevention and cessation programmes.

The Contracting Parties to the WHO Framework Convention on Tobacco Control are bound by the Convention's provisions concerning tobacco product regulation that are contained in its Articles 9, 10 and 11. Hence, flavoured tobacco products would fall within the group of tobacco products that pose an immense threat to the health of the world population.

3.3 Background

The application of flavour additives to tobacco products is a long-standing industry practice (*1, 2*), dating back to the addition of molasses to burley tobacco in the nineteenth century to create "American" blended tobacco. In recent years, tobacco manufacturers have qualitatively changed this practice by introducing a range of flavoured, brand-specific tobacco products including cigarettes, cigars, smokeless tobacco, kreteks (cloves), bidis and water-pipe (hookah) tobacco. The recent production and promotion of flavoured tobacco products is a major public health concern. These brand extensions are being heavily marketed to youths and minorities, with colourful and stylish packaging and flavours that mask the harsh and toxic properties of tobacco smoke (*3–5*). Flavours could entice youths to experiment with tobacco products by masking the natural harshness of smoke. Flavour additives could also facilitate the development of tobacco dependence by enhancing the sensory rewards of smoking. The role of reducing sugars, predominant in many flavour packages, leads to the production of increased levels of acetaldehyde that enhance dependence as well as toxicity.

Analyses of internal documents of the tobacco industry have established that the industry uses a range of additives to alter the perception and impact of tobacco smoke delivery (*6–8*) and environmental tobacco smoke (*9*).

Studies based on the tobacco industry's internal documents suggest that flavouring agents may also play an important role in the industry's targeting of young and inexperienced smokers. Menthol has been used to target new smokers across different ethnic groups (*10*), and additives such as chocolate, vanillin and licorice have been part of an intensive industry effort to increase the market share of the Camel brand within the youth market (*6*). Additives have also been shown to promote smoking among youths by masking the negative taste of tobacco smoke with flavours (*8*).

Younger and inexperienced smokers are more inclined to try flavoured cigarettes since the enticing flavouring agents suppress the harsh and toxic properties of tobacco smoke, making it more appealing to novices in smoking.

Figure 3.1.
Examples of flavoured cigarette brands

Source: reproduced, with the permission of the publisher, from reference *15*.

The candy-like flavouring agents not only affect the sensory perception and inhalation, including changes to smoke irritation, smoothness, aroma and smoking topography; their pyrolysis also alters the overall chemistry of smoke and its toxicity.

It has been hypothesized that the recent introduction of flavoured cigarettes and other tobacco products is a means of targeting young smokers (*4*), and tobacco industry documents demonstrate that this targeting has long been associated with younger and more inexperienced consumers (*11*). The consumer research carried out by Brown & Williamson in 1984 revealed notable agreement among respondents that flavoured cigarettes would be much more popular among young and inexperienced smokers.

Internal studies of differences in taste and flavour preferences by age group have confirmed that younger smokers are more open to unique and exotic flavours (*11*). Further, internal industry research suggests that young and inexperienced smokers may also be especially vulnerable to product benefits related to flavoured cigarettes. For example, in 1992, Philip Morris tested several flavours among young adult smokers and identified a number of possible consumer benefits, including increased social acceptance via pleasant aroma and aftertaste, increased excitement (e.g. sharing flavours), smoking enjoyment, and a "high curiosity to try factor"(*12*).

The regulation of these flavoured products is challenging. It is a basic public health principle that toxic consumer products should not be contaminated with substances that hide potential harm from the product's odour or taste, such as the addition of sugar to contaminated food products. Tobacco manufacturers are adding enticing flavour additives to dependence-causing and dangerous products. Regulatory strategies need to focus on outcomes at the population level as well as the individual level. In the pursuance of regulatory actions, research is needed to determine the impact of these products on youth smoking, exposure to second-hand smoke, and cessation.

However, traditional public health principles as well as recent evidence showing the popularity of flavoured products among youths warrant action being taken today. At a minimum, regulators should require the disclosure of flavouring agents in tobacco products, particularly flavoured products, by brand and level; legislation introduced in the Netherlands in 2003 requires such disclosure. As research develops, additional regulatory actions should be taken, including prohibiting the use of flavours in new brands and setting limits for existing products.

Menthol-flavoured cigarettes are a popular category of flavoured tobacco products that have been aggressively marketed for decades by the tobacco industry within particular regions and populations, such as among African-American smokers in the United States of America. To date, proposals to prohibit flavoured cigarettes in the United States have excluded menthol because of the already widespread acceptance of this brand category. Until recently, no other flavour additive had been used in advertising, except for wintergreen snuff, which has been promoted for many years.

3.4 Description of flavoured tobacco products

3.4.1 *Flavoured brands*

Several commercial tobacco products have been developed to deliver flavours to the smoker. Table 3.1 provides a sample of flavoured cigarette brands that were available in April 2006. Table 3.2 presents examples of brands including flavoured smokeless tobacco, cigars, waterpipe tobacco, bidis and kreteks, also available in April 2006. Flavoured tobacco products can be purchased from retail shops and from Internet vendors.

The number of flavoured sub-brands available on the commercial market has grown over the last few years. In the United States, state-level data indicate, for example, that, in 1997, apart from mint, spearmint and wintergreen, Cherry Skoal smokeless tobacco was the only candy-flavoured choice available. By 2004, Skoal was also available in Apple, Berry and Vanilla smokeless tobacco sub-brands. According to the brand's web site, a new peach blend has been introduced (*13, 14*).

Periodic changes to certain flavoured brands create the illusion of "newness" and "festivity". For example, for two years (2003 to 2004), "Bayou Blast" was released to coincide with Mardi Gras, while, in 2003, "Midnight Madness" was a New Year's promotion (*15*). The limited seasonal availability of certain flavoured products such as RJ Reynolds' Exotic Camel Blends provides further evidence of their role as "starter" cigarettes rather than as regular brands intended to create and foster brand loyalty.

Table 3.1
Examples of flavoured cigarette brands

Country	Manufacturer	Brand	Flavours
Belgium	Kretek International Inc.	Sweet Dreams	Vanilla, Chocolate Mocha, Mint, Cherry
Denmark	Mac Baren Tobacco Company	Arango Sportsman (rolling tobacco)	Vanilla
Germany	Von Eicken Group	Harvest (rolling tobacco)	Cherry, Peach, Vanilla, Strawberry, Menthol
Indonesia	P.T. Djarum	Djarum	Cherry, Vanilla, Splash, Bali Hai
Netherlands	Kretek International Inc.	Liquid Zoo	Coconut, Strawberry, Cool Mint
	VCT BV	Passion	Cherry, Honey, Vanilla, Coffee
USA	Brown & Williamson	KOOL[a]	Midnight Berry, Caribbean Chill, Mocha Taboo, Mintrigue
		Smooth Fusions	
	Kretek International Inc.	Natural Dreams	Vanilla, Cherry
	RJ Reynolds Tobacco Company	Exotic Camel	Regular distribution: Mandarin Mint, Twist, Izmir Stinger, Crema, Dark Mint Limited distribution: Mandalay Lime, Beach Breezer, Margarita Mixer, Bayou Blast, Back Alley Blend, Kauai Kolada, Twista Lime, Midnight Madness, Winter MochaMint, Warm Winter Toffee
		Salem Silver[a]	Cool Myst, Fire & Ice, Dark Currents, Deep Mintrigue
	Top Tobacco	Wildfire (rolling tobacco)	Cherries Jubilee, Margarita, Sunrise Strawberry, Velvet Peach

[a]Currently unavailable.

Source: RJ Reynolds Tobacco Company (www.smokerswelcome.com); My cigarettes (www.mycigarettes.com); Rollyourown.com (www.rollyourown.com); Ryocigarette (www.ryocigarette.com); Smoke-Spirits.com (www.smoke-spirits.com); Trinkets and Trash: artifacts of the tobacco epidemic (http://www.trinketsandtrash.org).

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Table 3.2
Examples of other flavoured tobacco products

Type of product	Country	Manufacturer	Brand	Flavours
Bidis	India	Dhanraj Imports	Darshan	Mandarin Orange, Grape, Raspberry, Wild Cherry, Cinnamon, Chocolate
		Sopariwala Exports	Soex	Chocolate, Vanilla, Menthol, Strawberry, Cherry, Mixed Fruit, Coffee, Grape, Aniseed, Raspberry, Lime-Lemon, Coconut, Clove, Cardamom, Black Licorice
	USA	Kretek International Inc.	Om	Strawberry, Vanilla, Cherry, Honey, Chocolate
			White Rhino	Strawberry, Chocolate, Mint, Vanilla, Wild Cherry, Grape
Cigars and cigarillos	Dominican Republic	HBI International	Juicy Blunt Wraps	Fruit Punch, Raspberry, Peach, Strawberry Kiwi, Coconilla, Candela, Cognac, Honey
	Germany	Al Capone	Al Capone	Slims Rum Dipped, Sweets Cognac
	Mexico	Royal Blunts Inc	Royal Blunt Wraps	Watermelon, Wet Mango, Blueberry, Passion Fruit, Strawberry, Sour Apple, Cherry Vanilla, Georgia Peach
	Nicaragua	Nestor Plasencia	Chevere Ice Cream Flavor	Amaretto, Irish Cream, Vanilla, Rum
	USA	CAO International Inc.	Flavors by CAO	Moontrance (exotic fruit and bourbon vanilla), Earth Nectar (Tuscan flavours and infused chianti), Gold Honey, Eileen's Dream (white chocolate truffles and Irish cream), Bella Vanilla (pure Madagascar vanilla)
		Cojimar Cigars	Cojimar	Cherry, Vanilla, Rum, Cognac, Peach, Sambuca, Amaretto, Chocolate, Cinnamon, Mint
		Don Lino Cigars	Tatiana Classic	Vanilla, Rum, Cinnamon, Honey, Chocolate
		Swisher International Inc.	Blackstone	Vanilla, Cherry
			Optimo	Peach, Icy Mint

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			Swisher Sweets	Strawberry, Peach, Honey, Tequila
		True Blunts	True Blunt Wraps	Cherry, Xtreme Berry, Bubble Gum, Candy Apple, Grape, Peach, Cotton Candy
Cloves	Indonesia	P.T. Djarum	Djarum	Cherry, Vanilla
	Malaysia	PT HM Sampoerna Tbk	Dji Sam Soe	Vanilla
Smokeless tobacco/snus	International	Swedish Match	Catch	Eucalyptus, Licorice, Vanilla, Peppermint
			Exalt	Peppermint
			Timberwolf	Wintergreen, Mint, Apple, Peach
	USA	Conwood Company	Kodiak	Wintergreen, Ice
		Swisher International Inc.	Kayak	Wintergreen, Cherry, Apple, Mint
			Redwood	Wintergreen, Cherry, Apple, Mint
			Silverado	Wintergreen, Cherry, Apple, Mint
			Silver Creek	Wintergreen, Cherry, Apple, Mint
		US Smokeless Tobacco Company	Copenhagen	Black Bourbon
			Husky	Wintergreen, Mint
			Red Seal	Wintergreen, Black Cherry, Mint
			Revel (PREP)[b]	Mint, Wintergreen, Cinnamon
			Rooster	Bold Wintergreen, Icy Mint
			Skoal	Berry, Cherry, Mint, Vanilla, Apple, Spearmint, Wintergreen, Peach

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Waterpipes (hookahs)	Egypt	Nakhla Tobacco	Nakhla – Exotic Flavored Tobacco	Apple, Apricot, Banana, Cappuccino, Cherry, Coconut, Cola, Grape, Jasmine, Lemon, Mango, Mint, Mixed Fruit, Orange, Pistachio, Rose, Sweet Melon, Vanilla, Arabic Coffee
	India	Sopariwala Exports	Soex	Apple, Mixed Fruit, Strawberry, Aniseed
	Israel	Abed Elkader	Abed Elkader (Premium)	Lemon, Mint, Rose, Melon, Mixed Fruit, Red Apple, Cherry, Orange, Peach, Grape, Licorice
	Jordan	Al-Ouns	Al-Ouns (Premium)	Grape, Mixed Fruit, Strawberry, Cherry, Double Apple
		Al-Waha Tobacco	Al-Waha	Apple, Apricot, Banana, Double Apple, Grape, Mint, Mixed Fruit, Sweet Melon (Cantaloupe), Cappuccino, Coconut
		Romman Tobacco	Romman Tobacco	Apple, Banana, Cherry, Cocktail, Coconut, Double Apple, Grape, Lemon, Licorice, Mango, Mint, Orange, Peach, Pineapple, Raspberry, Rose, Strawberry, Sweet Melon
		Saet Safa	Saet Safa (Premium)	Rose, Strawberry, Grape, Apple, Watermelon, Mixed Fruit, Candy, Melon, Mint, Orange
	Saudi Arabia	Al-Amir Tobacco	Al-Amir	Vanilla, Raspberry, Pina Colada, Banana Split, Bubble Gum, Blackberry, Caramel Apple, Guava, Kiwi, Kiwi Strawberry, Mocha Latte
	United Arab Emirates	Al-Fakher (now Havana Tobacco)	Al-Fakher	Apricot, Banana, Cherry, Coconut, Cola, Double Apple, Lemon, Mango, Mixed Fruit, Pineapple, Plum, Rose
		Al-Qemah Tobacco	Al-Qemah	Apple, Grape, Rose
		Havana Tobacco Company	Havana	Bahraini Apple, Banana, Cappuccino, Cherry, Coconut, Cola, Lemon, Mango, Mint, Mixed Fruit, Orange Rose, Special Grape, Plum
	USA	Hookah-Hookah Company	Hookah Hookah Tobacco	Green Apple, Cherry, Grape, Hazelnut, Melon, Margarita, Strawberry, Pineapple, Vanilla, Apple, Peach, Berry, Mixed Fruits, Key Lime Pie, Spearmint, Butter Scotch, Mango, Watermelon, Pina Colada, Jamaican Rum, Kiwi, Orange, Honey

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	Tangiers Tobacco	Tangiers	Apricot, Blue Gum Ball, Blueberry, Cinnamon, Cocoa, Cool Strawberry,Orange, Grape, Green Apple, Guava, Juicy Peach, Kashmir Apple, Kashmir Peach, Lemon Blossom, Lemon Lime, Licorice, Marigold, Melon Blend, Mint, Mixed Fruit, Orange Soda, Passion Fruit, Pear, Pineapple, Raspberry, Red Apple, Red Teas, Root Beer, Strawberry, Vanilla, Watermelon

PREP, potential reduced exposure product.

Source: CAO Cigars (http://www.caocigars.com); Connecticut Government (http://www.ct.gov/drs/lib/drs/cigarette/directory_manufacturers.pdf); Conwood (www.conwood.net); Copenhagen (http://www.freshcope.com/products.asp); DIP Time (http://dip-time.tripod.com/smokeless/id1.html); P.T. Djarum (http://www.djarum.co.id/en); flavoured rolling papers (http://www.zensmoke.com/mainframe.html); Hookah Company (http://www.hookahcompany.com); Hookah-Hookah Company (http://www.hookahhookah.com); Hookah-4-Less (http://www.hookah4less.com/shisha); Hookah & Shisha Central (http://www.hookah-shisha.com); “Kretek pages” (http://www.gimonca.com/kretek); Royal Blunts (http://www.royalblunts.com/products/ezroll.htm); Smoke Shop Magazine Online (http://www.smokeshopmag.com/0898/brand.htm); Smoking-Hookah (http://www.smoking-hookah.com/store); snus, The Northerner (http://www.northerner.com); Soex (http://www.soex.biz/bidis.asp); SouthSmoke (http://southsmoke.com/cat_flavored.cfm); Swedish Match (http://www.swedishmatch.com); Swisher (www.swisher.com/main/products.cfm);
True Blunts (http://www.trueblunts.com/index.html); US Smokeless Tobacco Company (www.ussmokelesstobacco.com).

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Additionally, flavoured cigarettes have gained a substantial proportion of overall magazine advertising expenditures. Advertising for "candy" flavoured brands rose sharply from US$ 0.5 million (<1%) in 2000 to US$ 4.1 million (2%) in 2001, and to US$ 14.2 (15%) million in 2005. In 2003 and 2004, the Youth Exposure Index was 104 and 107, respectively, for flavoured cigarette advertising, and 81 and 103, respectively, for non-flavoured advertising, indicating greater exposure to flavoured cigarette advertising after the new products entered the market (*16*).

3.4.2 *Flavour application*

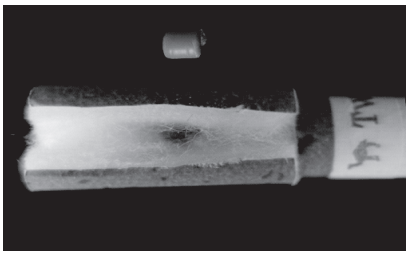
In the case of cigarettes, flavours may be added to tobacco, cigarette paper, the filter or even the foil wrapper, in an attempt to enhance the tobacco flavour, mask unpleasant odour, and deliver a pleasant cigarette-pack aroma. Due to differences in application and volatility of the flavouring agent, some compounds (e.g. cocoa) are burned with the tobacco column and pyrolysed in the smoke, while others (e.g. menthol) are transferred intact in the smoke stream (*2*).

The tobacco industry has pursued many non-conventional flavour technologies to address the goal of unique flavour delivery. For example, internal documents reveal that polymer pellet technology, using a flavoured filter pellet (polyethylene bead), was designed to provide controlled release of flavour for delivery to the smoker (*17*, *18*). Philip Morris also explored flavour release technology using carbon beads (*19*) and various additives (i.e. cinnamaldehyde and gluco-vanillin) designed to flavour mainstream (*20*) and sidestream smoke (*21*) with a sweet, vanillin-type aroma. Additional flavour technologies described in tobacco industry documents include flavour microencapsulation in the paper, packaging technology, polymer-based flavour fibres inserted into the filter, and flavoured tipping (*18, 22–27*).

A recently published study has revealed the development of flavour delivery technologies that are hidden from consumers and public health professionals, including the use of a plastic pellet placed in the cigarette filter of an Exotic Camel brand, Twist (*11*), manufactured by RJ Reynolds (see Figure 3.2). The limited availability of internal testing underscores the need for independent studies to assess the effects of new technologies on the delivery and toxicity of these new products.

An article published in *Tobacco International* (2006), a tobacco industry publication, describes the current expansion of the tobacco flavouring industry. Growing tobacco flavouring trends include the pursuit of products such as: (i) a more acceptable cigarette that would meet consumer health concerns; (ii) better tasting "safer" tobacco products; (iii) innovative flavour application systems; and (iv) the application of flavour to sidestream adhesive. Regarding sidestream smoke, a tobacco technology expert has stated

Figure 3.2.
Plastic pellet found in Camel Exotic Blend, *Twist*



Source: reproduced, with the permission of the publisher, from reference *11*.

that "this method of flavour application is an effective way to maximize flavour/aroma release to the sidestream while minimizing mainstream smoke taste…The result is a more 'socially accepted' smoking article to bystanders while maintaining a traditional mainstream smoke taste"(*28*).

The article describes the recent success of distinctively flavoured cigars produced by the global cigar industry, observing that this success should serve as an example to other tobacco product industries. It notes that "the oral side of tobacco flavoring is currently red hot…. As this market [snus industry] gets more competitive, we believe that manufacturers will introduce new tastes to try and capture the market as it grows"(*28*). Further, the article states: "Bidis, kreteks and waterpipes have experienced huge growth over the last few years as new flavorings have greatly improved their acceptance and increased their product demand. We can expect these changes to continue in 2006" (*28*).

3.5 Regional and global patterns of flavoured tobacco product use

Bidis (hand-rolled cigarettes containing tobacco imported from India) and kreteks (clove-flavoured tobacco cigarettes, often imported from Indonesia) are alternative tobacco products that have higher concentrations of nicotine, tar and carbon monoxide than conventional cigarettes (*29*). Research indicates that bidi smoking is associated with an increased risk of dangerous health outcomes, including oral cancer, and cancer of the lung, stomach and oesophagus (*29*). Kretek smoking is associated with increased risk for lung damage and abnormal lung function (*29*).

Smokeless tobacco (i.e. chewing tobacco, snuff and snus) is common and has become a leading cause of death in many developing countries (*30*). In the past, transnational manufacturers have promoted new forms of smokeless tobacco in ways that appeal to youths. This marketing effort has been coupled

Figure 3.3.
Advertisement for Kauai Kolada and Twista Lime flavoured cigarettes (Camel Exotic brand)

Source: reproduced, with the permission of the publisher, from reference *15*.

with increases in use, especially among young people (*30*). The recent introduction of flavoured smokeless tobacco products may also have greater appeal among youths.

According to an advisory note of 2005 on waterpipe tobacco smoking by the WHO Study Group on Tobacco Product Regulation, the highest rates of use occur in the African Region, the South-East Asia Region, and the Eastern Mediterranean Region. Waterpipe use has been growing among other populations such as college students and youths in the United States, Brazil and European countries. Waterpipe smoking is associated with many of the same risks as cigarette smoking and possibly has its own unique health risks (*31*).

While more research is needed to assess the extent to which flavoured cigarettes will influence adoption and experimentation among youths, recent surveys have revealed age differences in "past 30-day use" of flavoured brands. Twenty per cent of younger smokers (17 to 19 years old) reported using flavoured cigarettes in the last 30 days, while only 6% of smokers older than 25 reported smoking a flavoured brand. Flavoured cigarette use was highest among younger smokers (17–19 years) and lowest among older smokers (40 years and older) (*32*). More research is needed into the impact of flavoured cigarettes on smoking initiation and patterns of use among new and established smokers (*5, 11*).

3.6 Impact on public health

Aggressive marketing and advertising of flavoured tobacco products targeting youths deserves further investigation. Published research strongly suggests that youth targeting through marketing and product modifications influences youth smoking behaviour (*6, 32–35)*. Flavoured tobacco products may play a crucial role in this process, promoting youth initiation and helping young occasional smokers to become daily smokers by reducing or masking the natural harshness and taste of tobacco smoke. Their potential for increased harm at the individual and population level may go unrecognized without appropriate governmental regulation of the technology used in this new generation of flavoured tobacco products.

Legislation under consideration in the United States at the federal level would prohibit the use of candy flavours in tobacco products. Laws to ban candy-flavoured cigarettes have been proposed in regions of Australia, and a number of states in the United States (*36*). Legislation filed in 2005 in Massachusetts, for example, which attempted to amend the existing General Laws, stated:

> A cigarette or any of its component parts (including the tobacco, filter, or paper) containing, as a constituent (including a smoke constituent) or additive, an artificial or natural flavor (other than tobacco or menthol) or an herb or spice, including strawberry, grape, orange, clove, cinnamon, pineapple, vanilla, coconut, licorice, cocoa, chocolate, cherry, or coffee, that is a characterizing flavor of the tobacco product or tobacco smoke shall be prohibited from being sold or marketed in the Commonwealth (*37*).

Legislation introduced in the United States and elsewhere should address cigarettes as well as other flavoured tobacco products.

Research that identified toxic flavour-related compounds (i.e. alkenylbenzenes) in cigarette brands in the United States suggests that the toxic properties of these flavour-related compounds may introduce additional smoking-related health risks. Studies on these compounds call for additional investigation to better understand the inhalation toxicology and potential health effects of inhaling these compounds (*38, 39*). Differences in delivery characteristics between conventional and new flavoured tobacco products are not known, and thus require further study.

The use of new flavour technologies raises further questions regarding the practices of the tobacco industry, particularly given the current lack of regulation. Technologies such as the pellets employed in the Camel Exotic filter raise serious concerns regarding unknown delivery characteristics and possible health risks associated with smoking new flavoured cigarettes. The use of new flavour technologies has been concealed from smokers and public health officials. In the case of the flavoured pellet, the device is completely

hidden from the consumer, unless the pellet is dislodged from the filter and exposed (*40*).

3.7 Science base and conclusions

Flavoured tobacco products have not been studied extensively. However, the introduction of new flavoured tobacco products raises serious public health concerns. Preliminary research on patterns of flavoured cigarette use shows that younger smokers are more likely than older smokers to try flavoured cigarettes. Further, other flavoured tobacco products may be associated with increased use and interest among younger smokers. Little is known regarding the delivery characteristics or possible health risks associated with these products. The use of flavour technologies has not been disclosed to public health officials, and in the case of the flavoured pellet found in certain flavoured cigarettes, the device is concealed from the consumer. The limited availability of internal industry testing clearly underscores the need for independent studies to assess the effects of new technologies on the delivery and toxicity of these new products.

The science base supports the following conclusions:

1. The tobacco industry maintains that it supports youth smoking prevention and that it has put a stop to the targeting of young smokers. However, the evidence suggests that tobacco manufacturers continue to target young and inexperienced smokers with increasingly sophisticated products and marketing, in particular with flavoured tobacco products.
2. Youth targeting through marketing and product modifications influences youth smoking behaviour.
3. The use of new flavour technologies raises further questions regarding the practices of the tobacco industry, particularly given the current lack of regulation.

3.8 Research needs

Little research has been carried out, either at the individual or the population level, on the effects of flavours. Vigorous efforts should be made to meet the following research needs in the light of the global tobacco industry's promotion of these manufactured and traditional tobacco products.

1. National and global trends in the use of tobacco products with flavours, with particular attention paid to youth.
2. National and global trends in the manufacturing and marketing of flavours in tobacco products by tobacco companies.

3. The impact of the pyrolysis of flavouring agents on overall smoke chemistry.
4. The impact of flavours on the attractiveness and dependence potential of tobacco products.
5. The role that flavours play in youth initiation and transition from an occasional to a daily tobacco user.
6. The effects of flavouring agents on sensory perception and inhalation, including effects on smoke irritation, smoothness, aroma and smoking topography.
7. Consumer perception of advertisements for flavoured tobacco products, including perceptions of health risks and dependence.
8. The use of flavoured tobacco products among high-risk groups, including minorities, women, children and persons from developing countries.
9. The impact of flavours on smoking topography, and intake of toxicants including nicotine, carbon monoxide and other toxins.
10. The use of flavours in "starter" tobacco products such as smokeless tobacco products with low nicotine yield, cigarettes, waterpipes, kreteks and bidis.

3.9 Regulatory recommendations

The WHO Study Group on Tobacco Product Regulation urges consideration of the following public health initiatives to reduce the marketing and use of flavoured tobacco products. Basic public health principles dictate that flavours should not be used to adulterate contaminated food or to make drugs having a high potential for dependence more enticing. The popularity of these products with youths, combined with the need for strict adherence to these principles, warrants action.

1. Tobacco manufacturers should be required to disclose flavouring agents in tobacco products by brand and level, as legislation recently introduced by the Government of the Netherlands requires. The disclosure of these flavouring agents should be part of the contents and ingredients testing and disclosure requirements under the WHO Framework Convention on Tobacco Control.
2. Claims that imply reduced health risks should be prohibited.
3. Manufacturers should be prohibited from using flavouring agents in new tobacco brands.

4. For existing brands, consideration should be given to setting limits on flavouring agents that contribute to dependence or initiation, and that increase second-hand smoke exposure or deter cessation.

5. Strategies to regulate flavouring agents should be part of overall strategies to regulate tobacco product design, function and disease reduction.

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4. Biomarkers of tobacco exposure and of tobacco smoke-induced health effects

4.1 Introduction

The WHO Study Group on Tobacco Product Regulation (TobReg) has reviewed the evidence on the use of biomarkers, particularly for purposes of tobacco regulation. This report presents the uses and limitations of biomarkers, and the Study Group's recommendations on the role of biomarkers in tobacco regulation. The report focuses on the existing scientific evidence on biomarkers in order to define the available scientific foundation for the use of biomarkers by regulatory authorities. The substantially greater utility of biomarkers for research on tobacco products and their risks are outside the scope of this report and have been discussed by others (*1*, *2*). An effort has been made, however, to identify those areas where additional research could substantially enhance the regulatory utility of biomarkers. Most prominent among these research issues is validating that changes in levels of specific biomarkers reliably predict changes in disease outcomes.

4.2 Background

Tobacco use status and the intensity of cigarette exposure have traditionally been assessed using self-report of whether an individual uses tobacco and the frequency and amount of use that the individual reports. These measures have been established in epidemiological studies as valid predictors of increased disease risks.

Biochemical validation of tobacco use status is discrepant with self-reported status for a modest fraction of former users, and smaller fractions of current and never-users. In addition, while a clear association exists between the number of cigarettes smoked per day and levels of smoke constituents or their metabolites measured in bodily fluids, there is substantial variation in the amount of smoke constituent present for any specific number of cigarettes smoked per day (*3–6*). The discrepancy in smoking status and the individual variability of constituent/metabolite levels among smokers of similar numbers of cigarettes smoked per day call into question the accuracy of self-reported smoking status and number of cigarettes smoked per day as measures

of smoke exposure for individual smokers. While less fully documented, similar concerns also exist for self-reported status and intensity of use in relation to other forms of tobacco.

Measurement of tobacco or tobacco smoke constituents or their metabolites in bodily fluids, particularly those constituents specific to tobacco (e.g. nicotine, and tobacco-specific nitrosamines), has been used to improve the accuracy of self-reported tobacco use status in intervention and epidemiological studies, and has also been used to quantify the amount of tobacco exposure in experimental and other investigational settings. Biochemical verification of tobacco use status is now standard in settings where the accuracy of individual-level smoking status is critical, such as in studies of smoking cessation therapies or in determining the eligibility of applicants for lower life insurance rates as non-smokers. However, to date, only a few epidemiological studies of disease outcomes have been conducted to demonstrate that quantitative estimates of smoke exposure using biochemical measures improve the accuracy of disease risk prediction compared with self-reported data (*7*, *8*).

The current standard for assessing smoking status and the amount of smoke exposure in the general population remains self-report, in part due to the expense and difficulty of collecting biological samples for large populations and in part due to the unresolved research questions as to how to use these data to estimate population exposure.

The reality that more accurate definition of tobacco use status is possible, and the prospect that more accurate quantification of exposure is attainable, supports the use of biomarkers of tobacco exposure in those settings where the question being asked necessitates greater accuracy of use status or quantitative exposure than can be achieved by self-reported data.

An additional concern for scientists and regulators has been the long durations of exposure required to demonstrate the effects of tobacco exposure on many types of disease risks using traditional epidemiological approaches with disease manifestation as the outcome. Evaluating the risks resulting from changes in tobacco product designs using epidemiological approaches could take decades of exposure, making this approach of very limited value for regulatory assessment of the risks or claims for newer tobacco products and designs. Measures of cellular or organ changes consistent with tobacco-related injury and disease clearly precede disease manifestation, and they offer the potential for more rapid demonstration of differences in risk resulting from changes in the design or use of tobacco products (*9*). A number of these cellular and organ changes have been suggested as potential biomarkers of tobacco-related injury, but to date none has been validated since it has not been demonstrated that a change in the biomarker reliably predicts a difference in disease risk (*2*).

4.3 Biomarkers: definition and description

A recent comprehensive review of biomarkers by the National Cancer Institute of the United States (*2*) defined and classified biomarkers as follows.

> Biomarkers can be classified as a measure of (a) chemical exposure, that is, a direct or indirect measure of a tobacco-derived constituent or metabolite, that ideally can provide a quantitative estimate of tobacco exposure; (b) toxicity, including biologically effective dose, that is, "the amount that a tobacco constituent or metabolite binds to or alters a macromolecule either in target or surrogate tissue" (*9*) ; (c) injury or potential harm, that is, "a measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure or function, and clinical symptoms consistent with harm" (*9*); and (d) direct measures of health outcome. Genetic biomarkers for disease susceptibility also exist that may play a significant role in whether or not a smoker develops a disease (*2*).

Within this framing, biomarkers can be used for two purposes: assessing and quantifying exposure and assessing and quantifying injury and disease from tobacco use.

4.4 Measuring exposure

Biomarkers of exposure provide evidence of the presence of a tobacco toxicant and/or its metabolites in the body. The most straightforward biomarkers directly measure the concentration of the toxicant or its metabolites in exhaled breath, blood, saliva, urine or hair. Ideal characteristics for a biomarker of exposure include tobacco or tobacco smoke being the only source of the biomarker, with other sources of exposure being minor or non-existent; the marker should be easily detectable; the analysis methods should be reproducible across laboratories; and the marker should reflect a specific toxic exposure or be a reliable surrogate of tobacco smoke toxicant exposure. Other issues of importance in using biomarkers as measures of exposure include: how well the biomarker reflects long-term exposure to tobacco (the half-life of the biomarker, $t_{1/2}$, indicates the period of time for which the biomarker reflects exposure, and may vary from several hours to several weeks), what additional information is obtained by adding a particular biomarker to existing indicators, and how applicable the biomarker is to studying large populations in epidemiological studies.

The simplest use of biomarkers of exposure is to define tobacco use status. This is usually achieved by setting a value of the biomarker above which the individual is presumed to be a current user. Since current users include those who are very light or non-daily users, and since some non-smokers are exposed to very high concentrations of second-hand smoke, there will be some overlap between light current users and heavily exposed non-smokers in the constituent levels present in biological fluids, even for those constituents that

are only present in tobacco or tobacco smoke (e.g. nicotine or tobacco-specific nitrosamines (TSNAs)). This overlap is greater for constituents with other sources of exposure (e.g. carbon monoxide (CO)), and some studies have used combinations of biomarkers to define tobacco use status. Nevertheless, it is generally accepted that biochemical verification of tobacco use status leads to a substantially more accurate definition of who is a current user.

A second use of biomarkers of exposure is to quantify the amount of exposure experienced by the individual user. This quantification may be specific to the constituent and the constituent's consequences, for example, quantifying nicotine levels in studies of dependence. The level of an individual constituent biomarker may also be used as a proxy to quantify whole smoke or total smokeless tobacco exposure. The relationship between biomarker levels of a single constituent and the total smoke or tobacco exposure may be influenced by individual characteristics, genetic and metabolic differences, patterns of use and the presence of other sources of the constituent in the environment affecting the individual. When levels of a specific biomarker are used for whole exposure comparisons between products, differences in the composition of the emissions of the different products also need to be considered. For example, the toxicant burden from using smokeless tobacco may be well estimated by cotinine levels among users of a single smokeless product, but the toxicant burden at the same cotinine level will be very different among users of smokeless tobacco products in India when compared with smokeless tobacco users in Sweden because of the much higher concentrations of many toxic constituents present in the products sold in India.

A final form of exposure biomarker is one that measures the biologically effective dose of a single constituent or groups of constituents. These biomarkers attempt to quantify the exposure that has reached the tissue in ways that can result in injury and cellular or organ damage. Measurement of carcinogen-DNA adducts in lung tissue is one example of this effort to measure the biologically effective dose. The concept of a biologically effective dose is based on an understanding of the mechanism(s) by which constituents cause disease and attempts to quantify with precision the dose of the agent present in that mechanistic pathway. A limiting corollary of that mechanistic precision is that the biomarker may have less validity for organs or disease processes other than the one measured. For example, carcinogen-DNA adducts in lung tissue may define a biologically effective dose for the carcinogen(s) in the lung, but may have less relevance to estimating the biologically effective dose for heart disease.

4.5 Measuring injury and disease

A substantial body of evidence exists on the mechanisms by which tobacco use causes various diseases; and a number of biochemical, cellular and organ system measures exist that define the various mechanistic pathways either qualitatively or quantitatively. Similarly, a number of measures exists that can predict qualitatively or quantitatively the rate of disease occurring in a population and that are accepted as independent risk factors for disease. This is particularly true for cardiovascular disease where a substantial number of risk factors have been identified. Measuring changes early in the mechanistic pathway of disease occurrence offers the promise of more rapid characterization of the risks that can result from use of different tobacco products, and this promise has stimulated great interest in defining biomarkers where a change in level of the biomarker would accurately predict a change in disease risks.

Unfortunately, our understanding of the mechanisms by which smoking causes disease is not complete enough to identify with confidence the rate-limiting steps in the mechanistic pathways and therefore the changes that will reliably predict risk. We also are unsure which changes are markers of tobacco use, and therefore their presence is associated with increased risk but not part of the pathway by which disease occurs and therefore, if altered, will not alter risk. These limitations mean that acceptance of a given biological change as a biomarker of injury and risk requires validation that a change in the biomarker independently predicts a change in the frequency of disease occurrence.

Biomarkers do exist that can measure the presence and extent of various systemic processes, including inflammation, which may play a mechanistic role in disease occurrence. However, the diseases caused by cigarette smoking involve multiple processes and it remains unproven whether alteration of a single process (e.g. reduced inflammation) will reduce disease frequency.

4.6 Existing evidence on biomarkers

A comprehensive review has recently been published of the evidence establishing the utility of biomarkers for study of tobacco exposure and disease risk, representing the deliberations of four working groups at a conference sponsored by the National Cancer Institute of the United States (*2*). While primarily focusing on the value of biomarkers in an investigational setting, the groups examined the existing evidence for biomarkers related to cancer, cardiovascular disease, lung disease and fetal toxicity. The criteria used to evaluate the biomarkers were evidence showing: (i) differences in the biomarker level between tobacco users and non-users; (ii) change in biomarker level as a consequence of cessation; (iii) a dose-response relationship between

extent of exposure and level of the biomarker; and (iv) change in level of the biomarker as a result of tobacco use reduction. Table 4.1 lists the biomarkers the groups assessed as having sufficient current evidence to recommend them for studies of tobacco use and harm, and to suggest that they may be useful to assess constituent exposure with the use of potential reduced exposure products (PREPs) in a research setting. The groups concluded that the listed biomarkers "by no means describe biomarkers that can be used to assess disease risk for PREPs". In addition, they highlighted the judgement that "to date, we have no valid biomarkers that serve as proxies for tobacco-related disease to test potential reduced exposure products" (*2*).

Table 4.1.
Biomarkers useful in evaluating tobacco use

Biomarkers	Measurement of
NNAL and NNAL-glucuronide in urine	Carcinogen (NNK) uptake[b]
3-Aminobiphenyl-, 4-aminobiphenyl, and other aromatic amine-Hb adducts	Carcinogen (aromatic amines) uptake plus metabolic activation[c]
Urine mutagenicity	Mutagen uptake[b]
Sister chromatid exchange in peripheral lymphocytes	DNA damage[c]
Macrophages	Inflammation[d]
Carbon monoxide[a]	Chemical uptake[b]
Nicotine/cotinine[a]	Chemical uptake and metabolism[b]
Flow-mediated dilation	Endothelial function[d]
Circulating endothelial precursor cells	Endothelial function[d]
Fibrinogen	Hypercoagulable state[d]
Homocysteine	Hypercoagulable state[d]
White blood cell count	Inflammation[d]
C-reactive protein	Inflammation[d]
slCAM1	Inflammation[d]
Glucose-clamping studies	Insulin resistance[d]

[a]Should be included in all studies as general measures of tobacco constituent uptake.
[b]Biomarker for exposure.
[c]Biomarker for toxicity including biologically effective dose.
[d]Biomarker for injury or potential harm.
Source: adapted, with the permission of the publisher, from reference *2*.

4.7 Specific biomarkers

There are no biomarkers currently available that fulfil all the requirements of an ideal biomarker for exposure to tobacco and/or tobacco smoke. All biomarkers reported in the literature have their limitations. Therefore, the

choice to use a particular biomarker should be made on the basis of the study goals and on the characteristics of the biomarker.

4.7.1 *Tobacco alkaloids*

Nicotine and metabolites

Nicotine is a chemical found in all tobacco products, and in potential harm-reduction products. It is also found in many medications used to treat tobacco dependence. Nicotine is the main chemical responsible for tobacco dependence. Nicotine may also contribute to cardiovascular disease and reproductive toxicity from tobacco, although its contributions to disease are thought to be small compared with the contributions of other tobacco smoke toxicants.

Nicotine is absorbed rapidly from tobacco smoke, smokeless tobacco or medicinal nicotine products, and it is distributed rapidly to various body tissues. Nicotine can be measured in blood, urine or saliva, but because its half-life is only about two hours, concentrations vary considerably according to when the last cigarette was smoked or the time of the last nicotine exposure. Urine nicotine levels are also strongly influenced by urine pH and flow rate. When very accurate quantitative estimates of nicotine exposure are required, another approach to determining nicotine exposure is to measure the concentrations of nicotine and all of its major metabolites in a 24-hour urine sample (*10*). Nicotine is extensively metabolized (*11*); the major metabolites include cotinine, cotinine N-oxide, cotinine glucuronide, 3'-hydroxycotinine, 3'-hydroxycotinine glucuronide, nicotine N-oxide and nicotine glucuronide. The sum of these metabolites accounts for 90% or more of the dose of nicotine. However, a serious limitation of this approach is the difficulty in collecting a complete 24-hour urine sample, and the technical demands and cost of analysing all of the nicotine metabolites.

Cotinine as a biomarker of nicotine intake

The presence of cotinine in biological fluids indicates exposure to nicotine. Cotinine is the major proximate metabolite of nicotine. Cotinine is, in turn, extensively metabolized, 3'-hydroxycotinine being the major metabolite of nicotine found in the urine. Cotinine has an average elimination half-life of 16 hours, which results in much less fluctuation in cotinine concentration compared with nicotine blood levels throughout the day with regular tobacco use. Cotinine can also be measured in amniotic fluid, cervical lavage fluid, seminal fluid, breast milk, sweat, saliva, meconium, hair, and finger and toe nails.

Cotinine is the most widely used biomarker of exposure to nicotine from tobacco smoke from both active and passive smoking (*12*). Cotinine

measured in plasma, saliva and urine has a high degree of intercorrelation. Plasma cotinine concentration is likely to be a more accurate measure of nicotine intake, and possibly of other tobacco smoke toxicants, than the self-reported number of cigarettes smoked per day (*12*). There is, however, individual variability in the quantitative relationship between steady-state cotinine levels and intake of nicotine. This is because different individuals convert different percentages of nicotine to cotinine (usual range 50–90%), and because different individuals metabolize cotinine at different rates (usual plasma clearance range 20–75 ml/min) (*13*). The relationship between nicotine intake and steady-state cotinine blood levels can be expressed as follows, based on steady-state exposure conditions: $D_{nic} \times f = CL_{COT} \times C_{COT}$, where D_{nic} is the daily intake (dose) of nicotine, f is the fraction of nicotine converted to cotinine, CL_{COT} is the clearance of cotinine, and C_{COT} is the steady-state blood concentration of cotinine. On rearranging the equation, $D_{nic} = (CL_{COT} \div f) \times C_{COT} = K \times C_{COT}$, where K is a constant that converts a given blood level of cotinine to daily intake of nicotine. On average, K = 0.08 mg/24 hours/ng/ml (range 0.05–1.1, CV=21.9%) (*14*). Thus a cotinine level of 300 ng/ml in blood corresponds on average to a nicotine intake of 24 mg per day. The metabolism of nicotine and/or cotinine is also affected by factors such as race, sex, age, genetic variation in the liver enzyme CYP2A6, and/or by the presence of pregnancy, or liver or kidney disease (*15*).

Cotinine as a measure of tobacco exposure or risk

In contrast to the value of cotinine as a measure of acute nicotine intake, an average half-life of 16 hours means that cotinine levels are not measures of long-term exposure to nicotine or other toxicants, and it is the chronic intensity of tobacco use and the duration of that use that are the most important determinants of harm. Cotinine levels may serve as proxies for chronic levels of exposure, just as the current number of cigarettes smoked per day does, particularly among more intense smokers where smoking behaviour is substantively driven by the need to seek a certain level of nicotine intake. It is reasonable to expect that cotinine levels would be at least as accurate, and probably more accurate, than a single self-reported number of cigarettes smoked per day in estimating levels of chronic exposure.

Serum cotinine levels predict lung cancer risks in prospective epidemiological analyses (*8*) and the dose response relationship does not appear to have the plateau at higher levels evident with self-reported cigarettes per day (CPD) (*16*), suggesting that it may be a better marker of the intensity of tobacco smoke exposure than the number of cigarettes smoked. Epidemiological data quantifying the contribution of cotinine levels to disease prediction, independent of its relationship with the number of cigarettes smoked per day, may be helpful in clarifying the value of cotinine in predicting risk.

Cotinine levels in non-smokers are used to assess second-hand smoke exposure and predict cardiac disease risk in prospective epidemiological evaluations (*7*).

Cotinine levels cannot provide estimates of the duration of tobacco use or the intensities of past exposure when those intensities are different from the level of intake at the time of cotinine measurement.

Nicotine and cotinine in hair

The use of hair as a material to measure nicotine and cotinine has been proposed as a way to assess longer-term exposure to nicotine from tobacco products (*17–19*). Nicotine and cotinine are incorporated into hair as it grows over time. The average rate of hair growth is 1 cm/month. Thus, measurements of levels of cotinine may provide a way of assessing exposure of a person to nicotine over several months.

Potential problems with the use of hair include a strong influence of hair pigmentation on nicotine and cotinine binding and uptake (*20, 21*). Nicotine and cotinine are bound to melanin. As a result, dark hair binds much more nicotine than blond or white hair. This makes comparison across individuals from different ethnic groups, or of different ages, difficult. Also, hair is exposed to nicotine and cotinine from sweat and from sebaceous gland secretions, and to nicotine from second-hand smoke exposure. Washing the hair before analysis may reduce this problem of environmental contamination, but it may not remove all environmental nicotine.

Dietary sources of nicotine

Dietary sources of nicotine have been alleged to be a potential confounder of cotinine levels used in measurement of second-hand smoke exposure. Several foods contain small amounts of nicotine (*22*). However, the levels of nicotine in these foods are quite low. Based on nicotine levels in foods and the usual daily consumption of various nicotine-containing foods, the levels of cotinine produced by even a diet high in nicotine-containing foods is lower than that seen in individuals exposed to moderate levels of second-hand smoke (*13*).

4.7.2 *Minor tobacco alkaloids*

The primary alkaloid in tobacco is nicotine, but tobacco also contains small amounts of minor alkaloids such as anabasine, anatabine, and others. The minor alkaloids are absorbed systemically and can be measured in the urine of smokers and users of smokeless tobacco (*23*). The measurement of minor alkaloids is a way to quantify tobacco use and establish tobacco-use status when a person is also taking in pure nicotine from a nicotine medication, a

non-tobacco nicotine delivery system. This method has been used to assess tobacco abstinence in clinical trials of smoking cessation with treatment by nicotine medications (*24*).

4.7.3 *Other particulate phase components*

Tobacco-specific nitrosamines

Cured tobacco leaf, as well as cigarette smoke, contains many carcinogens; and measurement of carcinogen exposure is an important aspect of assessing potential harm. The most specific tobacco carcinogens are the tobacco- or nicotine-derived carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1 -butanone (NNK) and its butanol metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). NNAL, and its metabolite NNAL-glucuronide, can be measured in the urine of smokers and smokeless tobacco users, as well as in passively exposed non-smokers (*25*). The analytical methods are somewhat expensive, but highly sensitive and specific.

The rate of elimination of NNAL from the body is relatively slow. The distribution half-life for NNAL and NNAL-glucuronide was found to be 3–4 days, whereas the elimination half-life was 40–45 days (*26*). The explanation for the long half-life is believed to be extensive tissue distribution, with slow release from body tissues over time. The long half-life means NNAL can be detected for several weeks after discontinuing tobacco use. Conversely, when a particular level of exposure to tobacco-specific nitrosamines is changed, such as might occur when a person reduces cigarette consumption, NNAL levels and excretion will take several weeks to reach a new steady-state level. As seen with cotinine, there is considerable variability in metabolic rates and conversion efficiencies of tobacco-specific nitrosamines among people (*6*). The sum of NNAL plus NNAL-glucuronides, termed total NNAL, is the most specific known biomarker of NNK (a lung-specific carcinogen) uptake from tobacco products. It is also consistently elevated in non-smokers with substantial exposure to second-hand smoke. Moreover, total NNAL levels can be used to distinguish tobacco product users from non-users exposed to second-hand smoke, although there may be some overlap between very light or occasional tobacco users (e.g. adolescents still experimenting with smoking, infrequent adult smokers, or users of low nitrosamine smokeless products such as snus) and those with heavy second-hand smoke exposure.

Polycyclic aromatic hydrocarbons

Another important class of chemical carcinogens in tobacco smoke that can be employed as biomarkers of exposure is the polycyclic aromatic hydrocarbons (PAHs) (*25*). PAHs are generated by incomplete combustion of organic

materials and are also present as environmental contaminants in foods (such as charcoal-broiled meat), as well as in other environmental sources of combustion such as diesel and gasoline exhaust and biomass fuel used for cooking or heating. The PAH most extensively studied as a carcinogen is benzo[*a*]pyrene. Benzo[*a*]pyrene levels in tobacco smoke are low, and it is difficult to quantify human exposure to this chemical; however, the metabolites of several other PAHs can be measured in the urine. These include 1-hydroxypyrene and various hydroxylated metabolites of phenanthrene, naphthol and fluorene. The best studied PAH metabolite is 1-hydroxypyrene, which has been shown to be present at considerably higher levels in the urine of smokers than of non-smokers, and levels have been shown to change when cigarette smoke exposure is altered (*25*).

As an important class of chemical carcinogens, PAHs should be used as complementary biomarkers of tobacco smoke exposure where possible. Levels of PAHs may not change in similar proportion to levels of other carcinogens in smoke requiring independent measurement of different classes of carcinogens. For example, urinary levels of 1-hydroxypyrene were measured in the study of Omni cigarette smoking, and they did not demonstrate a reduction in levels, even though there was a statistically significant reduction in nitrosamine levels (*27*). Due to the many other sources of PAHs in the environment, one must control for PAH exposure from occupational sources (e.g. coke ovens, asphalt, aluminium smelters), food, especially grilled meat, traffic exhaust, and biomass combustion (wood, coal) in the home. PAHs may also be found in some smokeless tobacco products, particularly those that are made from fire-cured tobacco.

4.7.4 *Gas phase components*

Carbon monoxide

Carbon monoxide (CO) has been used for many years to assess exposure to combustion gases from cigarette smoking. It is easy to measure, either in expired air or in blood as carboxyhaemoglobin (COHb). The use of CO is limited by the fact that it is a short-term measure (half-life about 4 hours) and because there are many common sources of CO other than cigarette smoking. Since CO is predominantly absorbed at the alveolar level, the fraction absorbed is more dependent on depth of inhalation than is nicotine. Endogenous generation of CO or modest environmental pollution can result in COHb levels of 1–2%. Levels can be as high as 5% or more with heavy environmental exposure. The use of CO to assess tobacco smoke exposure is problematic in individuals who smoke few cigarettes per day or where environmental contributions are substantial. CO has been used to evaluate second-hand smoke exposure, but the low levels of CO in second-hand smoke

and the many other sources of CO make its use for this purpose difficult. Because CO is a potential cardiovascular toxicant and since it is easy to measure, many studies have included CO as a biomarker of exposure; and it may be particularly important in evaluating waterpipes where the burning charcoal itself contributes significant amounts of CO. It has no value for evaluating products where the tobacco is not heated or burned.

Thiocyanate

Cigarette smoke contains hydrogen cyanide, which is metabolized in the body to thiocyanate. Thiocyanate can be measured in serum, saliva or urine, and fairly simple colorimetric assays are available for its measurement. Thiocyanate is eliminated slowly from the body by renal excretion and its half-life is long, estimated at 7–14 days. This makes thiocyanate attractive as a potential marker for longer-term exposure to cigarette smoke. The major limitation of the use of thiocyanate is that there are many dietary sources of this chemical. Blood levels of thiocyanate are substantial even in the absence of cigarette smoke exposure. As with CO, thiocyanate is relatively insensitive to low-level cigarette smoking or as a biomarker of tobacco smoke exposure in non-smokers.

Benzene

Benzene is a gas phase component of cigarette smoke, and is an established human carcinogen. The benzene metabolites *trans,trans*-muconic acid and S-phenylmercapturic acid have been measured in human urine and found to be higher in smokers than non-smokers (*25*). However, due to occupational and environmental sources, benzene and its metabolites are more appropriate for research settings.

4.7.5 *DNA and protein adducts*

DNA adducts

Many carcinogens are metabolized to form reactive intermediates that covalently bind to DNA and/or proteins (*25*, *28*); the result is the formation of DNA or protein adducts. Such binding may interfere with replication of the DNA coding, leading to an increased incidence of point mutations and chromosomal instability, as well as other changes thought to be involved in carcinogenesis. Assays of levels of DNA adducts of tobacco carcinogens are measures of the "biologically effective dose" of tobacco carcinogens present in the tissue in which they are measured. Some DNA adducts are quite stable and therefore provide a long-term indicator of carcinogen exposure.

The major problem with measurement of DNA adducts, however, is that their levels in human DNA are generally quite low, present once in every 10^6 to 10^8 normal bases. The amounts of DNA that are routinely available for analysis are also generally low. Therefore, detection methods must be extremely sensitive. We know little about the half-lives of specific DNA adducts in human tissues, but, on the basis of animal studies, it is clear that these could be quite variable and structure dependent, because some adducts are efficiently removed by cellular repair systems, while others persist.

Two widely used techniques for measuring DNA adducts are the ^{32}P-postlabelling method and immunoassays (*29*), but the former of these methods especially is non-specific for particular carcinogens. ^{32}P-postlabelling measures "hydrophobic DNA adducts", which probably includes some polycyclic aromatic hydrocarbon (PAH)-DNA adducts in smokers. However, few, if any, of the adducts detected by this method in human tissues have been positively identified. Immunoassays predominantly use antibodies raised against benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE)-DNA adducts. These antibodies cross-react with various PAH-DNA adducts and possibly with other substances.

Many, but certainly not all, studies carried out using these methods have reported elevated levels of DNA adduct formation in tissues of smokers, compared with non-smokers (*30–32*). A recent meta-analysis demonstrated that adduct levels were significantly higher in cancer tissue samples (lung, oral and bladder cancer) than controls (*33*). One prospective study found that DNA adduct levels measured in white blood cells predicted increased lung cancer risk in current smokers (*34*).

More specific methods for the quantification of BPDE-DNA adducts, using HPLC-fluorescence and mass spectrometry, have been described (*35*). Reliable data have been obtained using these methods, but one limitation of such approaches is that there is a high frequency of samples in which no adducts can be detected (55% in smokers). Inconsistent data have been obtained for measurements of 7-methylguanine, a DNA adduct that results from *N*-methylnitroso compounds such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or N-nitrosodimethylamine (NDMA), although some studies show elevated levels in smokers (*36*). Levels of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing DNA adducts (HPB-DNA), formed by interaction of the tobacco smoke carcinogens NNK or *N'*-nitrosonornicotine (NNN) with DNA, are higher in lung tissue DNA isolated from lung cancer patients than from controls (*37*, *38*).

Protein adducts

Carcinogen-haemoglobin adducts have been proposed as proxies for the level of DNA adducts present in tissue, since most carcinogen metabolites that react with DNA also react with proteins (*39*, *40*). Advantages of haemoglobin adduct measurement include the ready availability of large quantities of haemoglobin in the blood and the relatively long lifetime of erythrocytes in humans (approximately 120 days), which provides an opportunity for adducts to accumulate. Levels of serum albumin adducts have also been quantified.

In addition, haemoglobin adducts of aromatic amines have been reported as carcinogen biomarkers (*41*). Their levels are consistently higher in smokers than in non-smokers (*30*). In a recent study, for example, the relative risk of bladder cancer in women who smoked was significantly higher than in men who smoked a comparable number of cigarettes. Consistent with this sex difference, levels of 3- and 4-aminobiphenyl-haemoglobin adducts in relation to cigarettes smoked per day was statistically higher in women than in men (*42*). These adducts have also been shown to be increased with second-hand smoke exposure (*43*).

Other adducts that react with the N-terminal valine of haemoglobin have also been informative with respect to estimating carcinogen dose in smokers (*44*, *45*). Prominent examples include adducts derived from ethylene oxide, acrylonitrile and acrylamide (*28*, *46*, *47*).

4.7.6 *Mutagenic activity of the urine*

The urine of smokers is mutagenic in the standard Salmonella mutagenicity test. This test assesses the activity of a urine extract to revert selected strains of the bacterium *S. typhimurium* in the absence and presence of a metabolic activation system. Mutagenicity in urine is believed to reflect exposure to potentially carcinogenic chemicals. In within-subject studies, urine mutagenicity has been shown to vary in relation to cigarette consumption and to decrease when smokers are switched from regular to ultra-low-yield cigarettes (*48*). However, other environmental sources contribute to urinary mutagenicity, and exposure to these must be controlled in any study of cigarette smokers.

4.8 Measuring biological changes

Cigarette smoke contains more than 4000 individual constituents and damages most of the organs in the body (*49*). Our understanding of the mechanistic relationships between exposure to these individual constituents and the diseases produced by inhalation of whole smoke is incomplete, and efforts to estimate individual disease risks from known constituent levels and toxicity

under-predict the level of risk that occurs with smoking (*50*). Furthermore, reduction of any one chemical or class of chemicals will not necessarily lead to a reduction in disease risk, and the magnitude of chemical reduction needed to produce a substantive change in disease risk remains uncertain.

These limitations of tobacco chemistry and toxicology for estimating disease risks related to cigarette smoking have led to the development of measures of the cellular and physiological responses of subjects who are exposed to the integrated tobacco emissions of whole smoke. Such markers of biological changes do describe differences in biological response to the complex mixtures of tobacco emissions and can be used to characterize differences in that biological response with the use of different tobacco products and PREPs. Additional research will be required to demonstrate which of these biomarkers are valid predictors of disease risks and the levels of change in the validated biomarkers that are required to predict a meaningful difference in subsequent disease manifestation.

To date, none of the markers of biological change discussed in this report has been validated through the establishment of their independent predictive validity. Many of them are established as risk factors for disease in epidemiological studies, and some have been shown to decline with cessation or reduction in tobacco use, demonstrating a dose-response relationship with smoke exposure. The uncertainty that remains relates to whether individuals who have a change in exposure and who have a larger change in the biomarker have less subsequent disease risk than those who have the same change in exposure and a lesser change in the biomarker. It also remains to be established which of these biomarkers simply reflect biological responses to tobacco exposure and which reflect biological changes that are part of the critical pathways by which exposure progresses to disease. The research goal being pursued is the development of biomarkers where a change in level of the biomarker can be reliably inferred to define a change in the likelihood of future disease. Many of the biomarkers discussed in this report offer considerable promise for achieving that goal.

Markers of biological changes are not specific to tobacco use. However, the biomarkers presented in Table 4.1 and discussed below are ones where significant differences have been demonstrated between smokers and nonsmokers, and where changes in smoke exposure through cessation or reduction in use have been associated with changes in the level of the biomarker, suggesting their potential value as biomarkers of injury and risk (*2*).

4.8.1 *Assessing oxidative stress*

Tobacco smoke contains high concentrations of oxidant chemicals in both gaseous and particle phases, which are believed to contribute to tissue injury, inflammation, endothelial dysfunction, thrombosis and other effects involved in the pathophysiology of cardiovascular disease, pulmonary disease and cancer (*51*). The nature of the oxidant chemicals in tobacco products is complex and includes oxides of nitrogen, free radicals and other reactive species.

As oxidant chemicals are highly reactive, it is difficult to measure exposure to these chemicals in the body directly. Several biological markers of oxidative stress as potential indicators of oxidant chemical exposure have been examined in research settings. Oxidants increase lipid peroxidation in membranes, resulting in the release and excretion of F_2-isoprostanes. These can be measured in plasma and urine and represent both an index of oxidative stress and a marker of biological effects on membrane lipids. Oxidative stress also leads to higher plasma levels of oxidized low-density lipoproteins and oxidized fibrinogen. In addition, oxidants result in the formation of 8-oxoguanine or 8-oxodeoxyguanosine adducts in DNA, which may be measured as degradation products in the urine. Oxidative stress can also be measured as thiobarbituric acid-reactive substances.

4.8.2 *Measures of inflammation*

Inflammatory responses in the lung are a significant component of clinically manifest chronic obstructive pulmonary disease, and they are postulated to play an important role in the mechanisms by which that disease develops (*49*). Inflammation has also been implicated in the mechanisms for cardiovascular disease and cancer, making biomarkers of inflammation attractive candidates as potential biomarkers of injury and disease risk. A number of biomarkers are used in research settings to assess inflammatory states (*52*). Those discussed in the review by Hatsukami and co-workers (*2*) include "total leukocyte and neutrophil counts, C-reactive protein, fibrinogen, and interleukin-6. In addition, a number of cell surface adhesion molecules are increased in inflammatory states, including soluble intracellular adhesion molecule (sICAM), soluble vascular cell adhesion molecule (sVCAM-1), and monocyte chemoattractant protein-1 (MCP-1)". Broncho-alveolar lavage and sputum tests for macrophages and neutrophils were also identified as providing useful information on the inflammatory cellular response of the airways and lung to smoke exposure. Smokers have higher numbers of neutrophils in sputum, and macrophages in broncho-alveolar lavage fluid.

4.8.3 *Measures of endothelial dysfunction*

Smoking has been associated with several dysfunctions of the vascular endothelium that may contribute to atherosclerosis and are implicated in the mechanisms of cardiovascular disease development. Although many of these measures are affected by other factors, e.g. diabetes, hypertension, hyperlipidaemia, and certain drugs, they may be useful to assess responses to tobacco use in controlled settings.

Both active and passive smoking affect the most widely used functional measure of endothelial dysfunction, flow-mediated dilation (*53*). The brachial artery is imaged using Doppler ultrasound techniques before and after release of a blood pressure cuff inflated to occlude arterial blood flow. The increase in brachial artery diameter following release of the cuff is mediated by the release of nitric oxide and prostacyclin by endothelial cells. Impairment of flow-mediated dilation has been demonstrated in populations of active and passive smokers, although considerable overlap exists with estimates of these parameters obtained in non-smokers (*2*). Flow-mediated dilation is not easy to measure. It requires highly skilled people to get accurate measurements, and so may be most useful in research settings.

Other potential markers of endothelial dysfunction measured in the blood include asymmetric dimethylarginine, von Willebrand factor, tissue plasminogen activator (t-PA), E-selectin, and P-selectin, as well as prostacyclin metabolites in the urine (*2*, *54*).

4.8.4 *Measures of clotting*

Coagulation plays an important role in the manifestation of cardiovascular disease and smokers have been reported to be hypercoaguable in comparison to non-smokers (*2*). Activation of platelets is associated with damage to the lining of coronary arteries, and with the synthesis and secretion of thromboxanes, which in turn promote constriction of blood vessels and platelet aggregation. Both active and passive smoking are associated with activation of platelets. Markers of a hypercoagulable state include increased urine concentrations of thromboxane A2 metabolites. Thromboxane A2 is released when platelets aggregate in vivo (*55*). Other relevant biomarkers of a hypercoagulable state include fibrinogen, red blood cell mass, blood viscosity, t-PA, plasminogen activator inhibitor (PAI-1), homocysteine, and P-selectin (*56*). Selectins are adhesion molecules released by endothelial cells and by platelets (*57*).

4.8.5 *Insulin resistance*

Insulin resistance is a risk factor for diabetes and cardiovascular disease. The ratio of insulin to glucose after glucose load is useful as an index of insulin sensitivity. The most definitive studies are glucose clamping studies, in which insulin levels are measured when a constant concentration of glucose is present or vice versa.

4.8.6 *Circulating endothelial precursor cells*

Levels of circulating endothelial precursor cells may be useful in research settings, but such measures are too technically difficult to be used for regulatory purposes.

4.8.7 *Femoral and internal carotid artery intima-media thickness*

It has been demonstrated that femoral and internal carotid artery intima-media thickness is increased in smokers and those exposed to second-hand smoke (*58*), and it reflects the extent of early disease in these vessels. Data documenting changes in these measures with cessation are not yet available (*2*).

4.8.8 *Sister chromatid exchanges in peripheral lymphocytes*

Sister chromatid exchanges (SCE) in peripheral lymphocytes are indicators of DNA damage. Smokers have elevated levels of SCE in peripheral lymphocytes compared with levels observed in non-smokers (*59*).

4.9 Summary of existing biomarkers

The WHO Study Group on Tobacco Product Regulation recognizes that effective regulation of tobacco products, particularly products offered as reduced exposure or reduced risk products, can be greatly facilitated by development of validated biomarkers of individual constituent exposure, biomarkers of exposure that are useful proxies for total tobacco emissions exposure, and biomarkers that can reliably predict differences in disease outcome. WHO has previously described the limitations of some of the present methods for making these assessments (*60*, *61*). A large number of potential biomarkers of exposure and effect have been identified in various research settings. A recent review (*2*) of these potential biomarkers concluded that validated biomarkers exist for exposure to some tobacco emissions and for some biological processes such as inflammation and endothelial injury; but the review also came to the conclusion that "we have no valid biomarkers that serve as proxies for tobacco-related disease" (*2*).

Biomarkers of exposure or effect have potential value in the experimental, investigational, evaluation, surveillance and regulatory environments. This

report aims to focus on the role biomarkers can currently play in the regulation of tobacco products and tries to differentiate that role from the much broader utility that a larger range of biomarkers have in the investigational setting.

Self-reported status and intensity of tobacco use are the most widely used tools for assessing tobacco exposures for the general population, and this situation is likely to remain so for the near future for reasons of cost and difficulty of obtaining biomarkers on a representative sample of the general population. Biomarkers of exposure can improve the accuracy of tobacco-use status ascertainment from self-report and can add to the information on intensity of exposure obtained from the self-reported number of cigarettes smoked (or other measures of intensity of tobacco use) per day. Biomarkers of exposure measure recent use (days to weeks, or in the case of hair and nail clippings, months) and therefore are not useful for establishing duration of use or past intensity of use in a longer perspective.

Machine testing of cigarette smoke emissions with existing standardized protocols does not provide reliable estimates of human exposure to tobacco toxicants, and there are no standardized methods for testing the toxic yields of other tobacco products such as bidis, waterpipes and smokeless tobacco. Measure of smoke constituents or their metabolites (nicotine, cotinine, carbon monoxide, thiocyanate, NNAL) can reproducibly be made in biological fluids as measures of individual exposure to these constituents, and such measures have been validated as increasing with increasing intensity of tobacco use, demonstrating their value as biomarkers of exposure.

These biomarkers of specific exposures are used both as measures of exposure to the specific constituent and as proxies for exposure to the full range of constituents present in tobacco smoke or smokeless tobacco. The biomarkers of specific exposures identified by Hatsukami and colleagues (*2*) are valid measures of the intensity of exposure to the specific constituent measured.

Constituent-specific biomarkers can be useful as proxies for total tobacco exposure when assessing or comparing the intensity of tobacco use in populations where the mix of tobacco products used can be assumed to be similar. For example, epidemiological comparisons of disease risks using cotinine levels as a better proxy for intensity of total cigarette smoke exposure than self-reported CPD (*8*), demonstrate the value of biomarkers for assessing total smoke exposures.

However, the use of a single biomarker of exposure as a proxy for other toxicant exposures or for total tobacco exposure requires an assumption that the exposure, intake and metabolism of all toxicants, and of total smoke, have a constant relationship to the biomarker being measured; and that the relationship remains constant across a variety of patterns of use. Stated more

explicitly, this assumption requires that the tobacco products being compared have similar mixes of constituents in their emissions, that the intake of different constituents is constant across individuals with different genetic and metabolic characteristics, and that the relative quantities of different constituents absorbed and metabolized by smokers are similar with different patterns of use. While concerns do not limit the use of biomarkers as proxies for intensity of tobacco exposure in epidemiological evaluations where the need is simply to define groups of more intense users from less intense users, they do limit comparisons of different brands of cigarettes where ratios of toxicants per mg of nicotine can vary widely, and where cotinine values have been demonstrated to vary substantially based on individual characteristics such as race and genotype.

The failure of these assumptions is one reason for the different dose-response relationships of CO, cotinine, NNAL and I-HOP levels to CPD demonstrated by Joseph and colleagues (*6*). When the comparison being made is not between more and less intense users within a population, but rather between users of similar intensities who are using different products, then the differences between the products used, among individuals and across patterns of use, are all likely to limit the utility of a single biomarker for predicting exposures to other toxicants at the level of the individual user. For the same reasons, they limit the use of a single biomarker as a proxy for total toxicant or tobacco exposure when making comparisons between products. These limitations need to be considered carefully when selecting individual biomarkers for use in examining differences between tobacco products, and it is probably unwise to assume that any single biomarker can reflect exposure to all of the other toxicants in smoke when making comparisons across different products.

With comparisons across forms of tobacco use (e.g. smoking and smokeless), or with comparisons of cigarettes and new PREPs, the ability of a single exposure biomarker, such as cotinine, to reflect total toxicant exposure is even more limited; and such comparisons should currently be restricted to statements about individual constituent exposures rather than global toxicant burden.

Only a small fraction of the identified toxicants in tobacco or tobacco smoke, or their metabolites, can be reliably and reproducibly quantified in biological fluids, again limiting the understanding of total toxicant exposure that can be derived from existing biomarkers of exposure.

The exposure biomarker cotinine is of limited value in assessing the efficacy of tobacco-use cessation programmes that use nicotine replacement therapy, because the subjects who have stopped using tobacco will frequently continue to use medicinal nicotine products. Urinary levels of the minor tobacco

alkaloids anabasine and anatabine, or total NNAL, may allow monitoring of compliance with cessation in these circumstances since they are not present in medicinal nicotine products.

The ascertainment of exposure to second-hand smoke by self-report is difficult, with the exception of measures such as marriage to a smoking spouse or employment in a heavily smoke-filled environment. Some biomarkers of exposure, notably cotinine and total NNAL, have established validity in quantifying second-hand smoke exposure.

Intensity of tobacco use is a clearly established predictor for numerous disease outcomes (*49*, *59*) using CPD as a marker for intensity of use. CO, cotinine, NNAL and I-HOP have clear positive correlations with self-reported CPD (*6*) and are correspondingly likely to be proxies for intensity of exposure. Cotinine levels predict lung cancer risk with a dose-response pattern, suggesting that it may be a more quantitatively precise proxy for intensity of tobacco use in epidemiological evaluations than CPD. Additional research using a range of biomarkers will be required to establish how best to assess total toxicant exposure when comparing smokers of similar intensities who use different tobacco products.

A number of biomarkers that measure biological processes such as inflammation are present at higher levels in smokers than in non-smokers, show dose-response relationships, and some make an independent contribution in statistical models of cardiovascular risk prediction. These findings raise the exciting prospect that changes in these measures following changes in tobacco use may allow rapid assessment of the likely differences in long-term disease outcomes. In contrast to exposure biomarkers, which measure the intensity of exposure, these biological process biomarkers offer the opportunity to identify those individual smokers who are at the beginning of or are progressing down the pathophysiological pathways that lead to disease. They can identify individual smokers who are experiencing abnormal levels of these biological processes, and who are at increased risk for subsequent disease, and this capacity raises the possibility that these markers can be used by regulators to assess the toxicity or risk of tobacco products. In addition, they provide insight into the changes occurring in smokers that improve our knowledge of disease mechanisms.

A series of important questions need to be answered to enable regulatory use of these measures of biological processes. They include demonstration that changes in biomarker levels following switching to different tobacco products predict differences in subsequent rates of disease. In addition, it will be necessary to identify the magnitude of the change in a biomarker level that can reliably predict a change in risk. Biomarkers that predict risk may do so because they are a critical part of the causal mechanism by which disease

occurs or they may simply be associated with the exposure or the consequences of exposure, rather than the causation of the organ injury that ultimately results in disease. For example, biomarkers of inflammation are increased in smokers compared with non-smokers, and inflammation clearly plays a role in chronic lung disease produced by smoking. However, the mechanisms by which chronic lung disease develops include processes other than inflammation, and it is not clear that a change in level of inflammation, or in a biomarker of inflammation, independent of concomitant smoking cessation, will result in less chronic disease subsequently becoming manifest.

Smoke contains several thousand constituents and can damage almost every organ system. This suggests that additional research to validate the utility of biomarkers as predictors of harm should consider a broader range of disease outcomes than a single disease process. The current absence of outcome validation for process biomarkers is a formidable research challenge that is currently limiting the use of biomarkers as a regulatory tool.

Regulators are faced with new or modified tobacco products and must make decisions on these products well before the results of long-term studies based on disease outcome measures are available. In the presence of this scientific uncertainty, regulators may seek the advice of panels of experts who can review all of the evidence on a given product (emissions, toxicology, validated biomarkers of exposure and effect, as well as research biomarkers) in order to make assessments of likely relative exposures, risks or harm from different products.

4.10 Recommended uses for biomarkers of exposure and effect

Biomarkers can be useful tools to regulators in their efforts to understand tobacco products and reduce the public health harm of tobacco, and thus biomarkers may be useful in several contexts.

4.10.1 *Improving the accuracy of the definition of current tobacco use status*

For many purposes, self-report of tobacco use status provides information with sufficient accuracy to make judgments about the effects of tobacco control interventions or trends in tobacco use. However, with recent former smokers (*62*), those who may have a disproportionate incentive to misrepresent smoking status, or where the precision of definition of smoking status is critical (such as in clinical trials), biomarkers of exposure can play an important role. In the absence of nicotine replacement therapy or other tobacco use, cotinine levels are the best biomarker to define whether an individual is a current smoker. Cotinine levels are less useful for definition of smoking status among adolescent smokers (where the definition of current smoking is any cigarette use in the past 30 days) (*63*) and in populations where there are

large numbers of non-daily cigarette smokers. In addition, while uncommon, very heavy second-hand smoke exposure can result in cotinine levels that overlap with those of light active smokers, particularly occasional smokers. When nicotine replacement therapy is being used, cotinine levels are not useful for defining smoking status, and other biomarkers such as levels of minor tobacco alkaloids or total NNAL in urine can be used. Similar utilities and constraints exist for using biomarkers of exposure to improve the accuracy of defining smokeless tobacco use status.

Biomarkers of exposure may also be useful to insurance and regulatory authorities in monitoring the success rates of clinical cessation programmes and other funded interventions in order to establish relative effectiveness of cessation programmes and their cost-effectiveness.

The prevalence of tobacco use at the population level can be investigated by population-based surveys that measure tobacco sales or self-reported consumption. The costs and response burden of obtaining biomarkers for representative samples of the population often make impractical the use of biomarkers to improve the accuracy of self-response measures of tobacco use status in surveillance tools. The intrusive nature of collection of many types of biomarkers may potentially decrease participation rates and the increased cost of collection and analysis of biomarkers needs to be balanced against the information that could be gained by investing those costs in expanding the size of the population sample surveyed. Self-response data remain adequate for most indications, including examination of general population effects of tobacco control interventions and describing tobacco use over time.

Improved accuracy in the definition of tobacco use status may be useful when monitoring or evaluating the effectiveness of smoking cessation interventions in health care settings and other locations. Use for this purpose is complicated by the inability to use cotinine levels for establishing tobacco use status when former users are using nicotine replacement products. The cost of using minor alkaloids in tobacco or total NNAL may be justified in this setting by the improved confidence with which the results can be stated and by the potential reduction in the number of individuals who have to be examined to define a statistically significant result.

The periodic collection of population-based sets of biomarkers would be an invaluable research tool. These data can be used to identify those settings where tobacco use status is less accurate with self-report and to describe individual genetic and metabolic characteristics and their influences on the relationships between exposure and biomarkers in biological samples of that exposure.

The use of biomarkers to improve the accuracy of the definition of tobacco use status is recognized as an essential component of studies submitted to regulatory authorities seeking approval for smoking cessation therapies; and it is also the standard by which decisions about differential insurance rates or employment opportunities are related to tobacco use status. The use of biomarkers is also highly recommended as a component of studies evaluating or monitoring the effectiveness of tobacco control interventions for purposes of making public policy decisions about inclusion in programmatic efforts or funding support. In these settings, larger public policy decisions will hinge on the results of the evaluations, making accuracy in defining tobacco use status a substantive value.

4.10.2 *Evaluating the intensity of exposure to specific constituents*

There is no question that levels of nicotine or cotinine in biological fluids or hair and nails are more accurate measures of the amount of recent nicotine intake for an individual than the number of cigarettes smoked per day or other self-reported measures of intensity of tobacco use. This is also true for the other biomarkers of carcinogen/mutagen intake presented in Table 4.1 relative to the specific carcinogen or family of carcinogens being measured.

This improved accuracy in quantifying exposure to individual constituents is of great value in experimental studies examining the mechanisms by which tobacco use causes dependence and disease. Improved accuracy in the measurement of intensity of exposure to nicotine or other constituents can also be of use to regulatory authorities for validating claims of reduced exposure with different products and assessing exposures that occur in different settings.

A number of PREPs have been developed and marketed by tobacco manufacturers. Some, such as the "low tar" cigarette, do not result in reductions in exposure or risk (*9*, *64*, *65*). For other tobacco products making reduced exposure claims, reductions in some carcinogens were verified, but there were not reductions in others (*27*). The variability in these results makes it clear that verification of reduced exposure claims through experimental studies using biomarkers, rather than measurements of emissions, is essential for the regulation of these claims.

The assessment of exposure-reduction claims is complicated by the need to separate the differences due to individual characteristics of users and the factors that define self-selection of the product used from the differences due to changes in product design. At present, exposure-reduction claims can only be reliably examined in experimental investigations where groups of users are randomly assigned to the use of different products. Examining levels of biomarkers of exposure in self-selected users in the general population leads

to confounding of the characteristics of the individual with the characteristics of the product, both of which can influence exposure levels. Therefore, differences in levels of biomarkers of exposure between users of different products found in the general population cannot be reliably ascribed to differences in the product.

Even with an experimental design, careful attention to selection of the control population is necessary (*66*). Smokers who are experimentally switched to a different tobacco product often reduce the intensity of use of that product, particularly if they find it very different from their usual brand or unsatisfactory. Since individuals who find the product unsatisfactory would be unlikely to use it in a non-experimental setting, including these individuals in a comparison of the exposures that occur with switching to a new product is likely to result in an underestimate of the exposure that would occur with self-selected use of the product by the general population. Comparing their exposure before and after switching without a control group could then result in a reduced exposure level that is due to the unacceptability of the product to a fraction of the population studied, rather than to differences in exposure due to differences in product design. For this reason, the recommended experimental approach is to have a control group and to have both the test and the control groups of users switch to different products. The challenge for this experimental design is to find a product that the control group can switch to that is substantively different in design from the product being tested, but that has a similar level of unacceptability. Differences in satisfaction with the control and test products need to be considered when assessing differences in exposure observed in these experimental evaluations (*1*).

The biomarkers of exposure presented can reliably quantify the level of exposure to individual constituents for individual users, and levels of cotinine in general population studies have been used to examine several important public policy questions. Biomarkers of exposure (cotinine) collected in conjunction with representative population surveys such as the National Health and Nutrition Examination Survey and the Health Survey for England have allowed population-based statements about the changing level of secondhand smoke exposure in the United States (*67–69*) and the consistency of nicotine exposure in smokers of cigarettes with different machine-measured yields in the United Kingdom (*5*). Both of these analyses have substantial regulatory significance and demonstrate the value of collecting biomarkers on representative samples of the population to support research on issues of regulatory importance. In particular, atmospheric measurements of smoke constituents in conjunction with biomarkers of exposure are useful tools in demonstrating changes in exposure following implementation of restrictions on where smoking is allowed. The clear demonstration of substantial

differences in second-hand smoke exposure following implementation of restrictions on smoking in various locations has also helped to justify and build support for implementing and extending these restrictions.

Biomarker levels are influenced by individual characteristics including race, metabolic status and genotype; and trends in tobacco use behaviour may also vary according to some of these characteristics. For example, cotinine levels can vary according to individual characteristics such as ethnicity, and genetic and metabolic status even for the same level of nicotine intake. Additional research using population-based surveys of biomarkers and smoking behaviours will help to identify the individual characteristics of tobacco users that influence the relationship between actual level of exposure and level of the biomarker in the biological fluids. A better understanding of these individual determinants of biomarker variability is needed before population-based surveys of biomarkers can reliably be substituted for self-reported behaviour and per capita consumption data in evaluating trends in tobacco use at the general population level.

4.10.3 *Evaluating the intensity of exposure as a proxy for total tobacco exposure*

Biomarkers of individual tobacco smoke constituents are often also used as quantitative estimates of total smoke exposure. This use is based on the assumption that there is a fixed proportionality to the ratio of nicotine or other biomarker exposure and exposure to other smoke constituents or total smoke exposure. Similarly, single measures of tobacco emissions in smokeless tobacco users have been used as estimates of total smokeless tobacco exposure. This assumption has some general validity. For example, smokers with high cotinine levels have higher levels of several other biomarkers including CO and those measuring nitrosamine and PAH exposures (*6*), as well as having a higher self-reported number of cigarettes smoked per day. However, even when examined within individuals not changing brands, differences in levels of one biomarker do not reliably predict quantitative differences in other biomarkers (*6*). Comparisons across brands are also complicated by the differences in composition of the smoke generated, and presumably the exposures that result, with different brands of cigarettes (*70*). As an example, an experimental switching study to a PREP demonstrated that reductions in one biomarker do not necessarily predict that other biomarkers will also be reduced (*27*). These constraints limit the regulatory use of individual biomarkers as proxies for total tobacco exposure, and the very limited number of existing validated biomarkers of individual toxic constituents limits the regulatory use of groups of biomarkers as proxies for total toxicant exposure.

As with active smoking, use of a single biomarker of exposure as a proxy for exposure to all of the constituents of second-hand smoke depends on an

assumption that there are fixed ratios between the many different constituents in smoke. If the question being asked is a general one about the relative levels of second-hand smoke exposure in different populations exposed to conventional cigarettes in a single country, this assumption is sufficiently valid to allow the use of biomarkers to assess the impact of changes in policy on levels of second-hand smoke exposure. However, if the question being asked relates to exposures to specific constituents, to exposures with PREPs, or to comparisons between countries, consideration of the emission characteristics of the products needs to be included in the judgement about differences in exposure levels.

4.10.4 *Measuring reduced injury or harm*

Tobacco harm reduction is defined for this report as "minimizing harms and decreasing total morbidity and mortality, without completely eliminating tobacco and nicotine use" (*9*). Modifications of existing cigarettes, devices that heat rather than burn tobacco, oral delivery of nicotine through a variety of products and devices that allow inhalation of nicotine, have all been offered as harm-reduction products and collectively are known as PREPs (*9*, *60*, *71*). Past efforts at such "reduced exposure products" include filtered cigarettes, and "light" and "mild" cigarettes. Both of these product types are now known not to reduce either exposure or risk. This experience contributes to the need for regulators to evaluate PREPs carefully before allowing harm-reduction claims.

Biomarkers of exposure can be used in an experimental setting to evaluate the exposure-reduction claims made for these PREPs, but the absence of validated biomarkers for harm or risk makes it currently impossible to establish harm-reduction claims in the absence of outcome measures of actual disease frequencies (*1*, *2*, *72*). In addition, the available biomarkers of exposure do not offer a comprehensive or reliable estimate of total toxicant exposure and cannot be used as summary estimates of total exposure or risk.

The limited number of biomarkers that measure early biological effects, alterations in morphology, structure or function, and clinical symptoms consistent with harm, do not offer scientifically valid estimates of disease risk for cancer or any of the other diseases caused by smoking (*2*). Unfortunately, this absence of scientifically validated measures prevents regulatory authorities at the present time from being able to adequately evaluate harm-reduction claims based on these biomarkers alone, and suggests that exposure (rather than risk or harm) reduction may be the limit of the claims that can be supported by biomarkers using existing science. The WHO Study Group on Tobacco Product Regulation has previously recommended that regulatory authorities should not allow harm-reduction claims in the absence of evidence

demonstrating actual reductions in disease risks (*61*). For some products, for example, smokeless tobacco and nicotine replacement therapy, a substantial body of outcome data exists from epidemiological and clinical studies; and this information can be used to supplement the evidence from biomarkers of exposure in evaluating the risks of these products.

Effect biomarkers can be used to characterize differences in biological response with use of different types of tobacco products including cigarettes, waterpipes, smokeless tobacco and PREPs. Effect biomarkers may also be useful for regulators in characterizing the biological response that results from changes in product composition. These differences in biological response, coupled with measures of product emissions and exposure biomarkers, can provide useful inputs for expert panels tasked with advising regulators on the development of regulatory controls intended to reduce the harm of tobacco use.

The Study Group recognizes the obligation for regulators to act, and that actions may need to be implemented even with limitations on or absence of scientific certainty. The description of biomarkers presented in this report is intended to present the current level of scientific evidence supporting the use of biomarkers so that regulators can distinguish those questions that can be answered with scientific certainty from those where qualified extrapolation of existing science by expert panels is needed.

4.11 Summary of biomarker recommendations

Biomarkers of exposure should be required in studies submitted for regulatory approval of tobacco-use cessation interventions, in support of exposure-reduction claims, in studies defining the dependence potential of different products, and when evaluating or monitoring the effectiveness of individual-level tobacco cessation interventions. In addition, biomarkers of exposure have great utility in evaluating specific public policy questions about the effect of specific regulatory changes on exposures in the general population, notably whether restrictions on smoking in general or in specific locations reduce exposure among non-smokers.

The biomarkers currently most useful for these purposes are measures of cotinine in blood, saliva, urine, hair and nails. In settings where individuals may be using nicotine replacement therapy, combinations of CO and thiocyanate have been used, and the minor tobacco alkaloids anabasine and anatabine or NNAL in the urine are highly specific for tobacco use if laboratory capacity for their accurate measurement exists.

Quantitative levels of biomarkers of exposure to nicotine can be used to differentiate more and less intense users of tobacco products; but, when they are

used to compare exposures from different tobacco products, they do not accurately define either levels of other toxicants or the total toxicant burden from tobacco use. Differences in nicotine biomarkers are not sufficient by themselves to support exposure-reduction claims for constituents other than nicotine. Validated biomarkers of a limited number of other tobacco toxicants, largely carcinogens, are available to assess differences in exposure to those constituents; but the existing scientific understanding of tobacco smoke and the mechanisms by which it causes disease is not sufficient to allow a battery of existing biomarkers of exposure to serve as a reliable measure of total toxicant burden or of the risk that will result from that toxicant burden.

Self-reported data on tobacco use status and frequency of use remain the currently recommended measure for estimating and evaluating trends in overall tobacco use behaviours in the general population. Nevertheless, the improvement in accuracy of smoking status ascertainment and quantitative exposures provided by biomarkers offers substantive value in the investigational assessment of changes in tobacco use status or intensity of use in response to public policy changes.

Biomarkers of exposure and biomarkers of biological effects can be used in controlled experimental studies to examine exposure and biological responses that result from use of different tobacco products, including smokeless tobacco products, PREPs and products making exposure-reduction claims.

Changes in existing biomarkers of biological effects have not been validated as predicting differences in tobacco-related injury or disease risk, either as individual measures or as panels of measures. No currently existing biomarkers, or panels of biomarkers, are sufficiently robust to support a risk- or harm-reduction claim in a regulatory setting. Validated biomarkers of some processes, notably inflammation, oxidative stress and endothelial dysfunction, do exist and can provide information to guide regulatory authorities in examining the biological responses to different tobacco products that may be part of disease mechanisms. Information from these biological process biomarkers should be combined with chemical measurement of emissions, exposure biomarkers, design characteristics, and existing epidemiological and clinical data in forming assessments of the toxicities of different tobacco products. Such overall assessments will aid in the regulation of tobacco products with the aim of reducing tobacco-related injury and disease.

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5. Setting maximum limits for toxic constituents in cigarette smoke

5.1 Introduction

There is currently a scientific consensus that yields per cigarette of tar, nicotine and other smoke constituents derived from machine smoking using the International Organization for Standardization/United States Federal Trade Commission (ISO/FTC) protocols do not provide valid estimates of human exposure or of relative human exposure when different brands of cigarettes are smoked (*1–3*). Other more intense smoking protocols, such as those developed by the State of Massachusetts and the Canadian Government, generally produce higher yields and reduce the differences between brands in the yields. Nevertheless, they continue to maintain a ranking of brands for tar and nicotine yields; and these rankings do not provide valid estimates of human exposure or of the relative exposure experienced by smokers when they smoke different brands of cigarettes.

Biomarkers in blood, urine, and saliva that can accurately measure individual human exposure to specific constituents of cigarette smoke do exist; and these biomarkers of exposure are influenced by characteristics of the individual and characteristics of the individual's smoking behaviour, as well as by characteristics of the product smoked (*2*, *3*). The multiplicity of brands on the market (e.g. 1294 varieties of brands in the United States in 1998, according to a report of the FTC issued in 2000 (*4*)), self-selection of smokers who use different products, and differences in how smokers of different products use them, make the current use of biomarkers of exposure as regulatory tools to monitor cigarette product differences problematic. The large sample sizes needed to reduce variability due to individual characteristics and the difficulty of accounting for differences due to self-selection of tobacco products do not make it realistic at present to identify meaningful differences for consumers between products and, therefore, make biomarkers of exposure tools currently better suited to the research laboratory than the regulatory environment.

Markers of biologically effective dose (levels of toxicants in critical target organs or tissues) are likely to be developed and validated in the future, and they are expected to offer more precise measures of smoke uptake and better

predictions of smoke toxicity (*1*). Measures of injury or validated biomarkers of disease risk are also likely to be developed in the near future. These advances may allow assessment of harm reduction or differences in risk between tobacco products. Nevertheless, at the moment, assessing the relative harm of, or consumer exposure to, different levels of specific compounds from different tobacco products using biomarkers from individuals who use different products remains a future hope rather than a current reality (*5*, *6*).

These concerns about using biomarkers of exposure suggest that, for the near future, examination of the consequences of design differences between brands may be limited to assessments of the toxicity of the smoke generated under machine-smoking conditions. Chemical measurements of the smoke produced by machine and use of these measurements as inputs for product hazard assessment may be the limit of current scientific assessment of differences between brands. Measurements based on machine-generated smoke can be made simply and consistently, and they provide information about the effect of design characteristics on toxic compounds of cigarette smoke which is of value to regulators and others interested in examining relationships between design characteristics and emissions. What machine-generated smoke measurements do not provide is an understanding of how smokers respond to those design changes, actual or relative consumer exposure, or an assessment of the consequences of those smokers' responses for exposure or risk.

In considering this evidence, the WHO Study Group on Tobacco Product Regulation (TobReg), at its meeting in Montebello, Canada on 26–28 October 2004 (*7*), recognized that the existing methods for evaluating tobacco products using machine-measured tar, nicotine and carbon monoxide (CO) yields were misleading smokers and most regulators. The WHO Study Group also recognized that abandoning measurements of tar, nicotine and CO using the ISO method as regulatory tools prior to the development of validated biomarkers of risk would leave a regulatory and informational void that would not be in the interest of WHO Member States.

As an *interim* step in the regulation of tobacco products, prior to the development of approaches that could actually assess differences in exposure, harm or risk from different cigarette brands, the WHO Study Group recommended a strategy based on measures of the toxic constituents in the smoke per milligram (mg) of nicotine for a limited set of purposes. The recommendation is to quantify levels of specific toxicants per mg of nicotine in the smoke by the testing methodology discussed below. Standardizing the levels of toxicants to a per mg of nicotine basis was identified as a way to reduce the misleading effect of differences between levels of toxicants when they are expressed per cigarette. These proposed measures also permit researchers to learn more about the relationship between cigarette design and the composition of

cigarette smoke, and provide regulators with a mechanism for reducing the level of identified toxins in tobacco smoke. This recommendation allows regulatory action while the science of assessing harm from tobacco smoke constituents develops further and avoids the current misleading use of machine measurements as estimates of human exposure or risk. It also allows regulators to reduce the level of toxic compounds in smoke emissions rather than focusing regulators on the content or design of the product.

Specifically, the WHO Study Group on Tobacco Product Regulation identified the purpose of testing levels of constituents in machine-generated smoke as follows: "the purpose of these measurements is to enable regulators to set maximum limits for the nominated priority compounds on a per mg of tar or a per mg of nicotine basis. The maximum limits could be based on the values measured for the lowest quintile of brands among a commissioned sample of existing international brands" (*7*).

In order to initiate this effort and to develop scientific guidance on how best to implement the objectives defined by the Study Group, the WHO Tobacco Free Initiative and the International Agency for Research on Cancer (IARC) have established a working group to define maximum limits for tobacco smoke toxicants for submission to the Study Group. The working group, which met in Lyon, France on 10–11 April 2006, was initially charged with establishing maximum limits for tobacco-specific *N'*-nitrosamines.

The Study Group also recommended that tobacco manufacturers should report per mg of tar the results of measurements of the yields of the toxic constituents listed below for each of their cigarette brands based on the toxicity of the constituents and the amounts present in cigarette smoke. Tobacco manufacturers should report the amounts of these constituents present in cigarette smoke for each brand and sub-brand on the market, including changes in constituent yields that occur when a manufacturer alters a cigarette to comply with the limits established pursuant to this proposal.

The Study Group recommended, in its recommendation 1, that the results of measurements of the yields of the toxic constituents listed below should be reported per mg of tar.

- Nicotine/free nicotine
- Tar
- Carbon monoxide
- Ratio of nicotine-free dry particulate matter to nicotine yield
- Polynuclear aromatic hydrocarbons: benzo[*a*]pyrene

- Volatiles: benzene, 1,3-butadiene, formaldehyde, acetaldehyde
- Nitrosamines: NNN, NNK, NAT, NAB
- Metals: arsenic, cadmium, chromium, lead, mercury, nickel, selenium
- Gas-phase compounds: nitrogen oxide, hydrogen cyanide (*7*).

In considering this list, the working group decided that acrolein, the aromatic amines 4-aminobiphenyl and 2-naphthylamine, and ethylene oxide should be added to the above list of compounds presented by the Study Group and that the values should be expressed primarily per mg of nicotine. Subsequently, at its third meeting in Kobe, Japan, the Study Group adopted the recommendation of the working group to include acrolein, the aromatic amines 4-aminobiphenyl and 2-naphthylamine, and ethylene oxide in the above list of compounds.

5.2 Regulatory strategy

The limitations of a single machine-testing protocol for estimating human exposure are due both to the variation in individual smoking patterns and to systematic differences in smoking patterns that result when cigarettes with different designs are smoked. As a result, machine testing using protocols currently in widespread use cannot estimate human exposure and should not be used to support claims of reduced exposure or risk.

The Technical Committee 126 (TC 126) of the ISO, which creates measurement methods for the regulation of tobacco and tobacco products, has recently recognized this misuse of machine-measured yields and submitted for a formal vote a resolution adopting as a rationale for all machine-smoking testing standards the following statement.

- No machine smoking regime can represent all human smoking behaviours.
- Methods are recommended which test the product under conditions of different intensities of machine-smoking testing in order to collect mainstream smoke.
- Machine smoking testing is useful to characterize cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstanding about differences in exposure and risk across brands.
- Smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid measures of human exposure or risks. Communicating differences

between products in machine measurements as differences in exposure or risk is a misuse of testing using ISO standards (*8*).

Even with the limitations of machine smoke yields as measures of human exposure, machine measurement of individual smoke constituents known to be toxicants may permit regulators to reduce the levels of known toxicants in the smoke. Smokers use cigarettes to deliver desired amounts of nicotine; therefore, expressing the level of toxicants in smoke per unit amount of nicotine allows quantification of the levels of toxicants that accompany a specified amount of nicotine in the smoke of different brands, at least under the conditions of smoke generation used with the machine-testing protocol. Regulation based on these measures provides adequate information to reduce the level of identified toxic compounds in the smoke produced by different brands of cigarettes and is a useful interim approach for this limited purpose prior to development of validated measures of exposure, harm or risk.

This regulatory approach taken by the WHO Study Group on Tobacco Product Regulation is based on the well-accepted precautionary approaches used in public health. Wherever possible, this approach moves towards a general reduction of known harmful constituents of any product to the extent technically feasible, as part of good manufacturing processes. It does not require that, for the substance under consideration, there be proof of a specific linkage between a lower level (amount) of any individual constituent and a lower level of human disease (response). It merely requires that the substance be known to be harmful and that processes exist for its diminution or removal. Evidence of actual reduced harm is not required for this approach; and correspondingly, compliance with these regulations does not support a claim that a given brand is safe or less hazardous than other brands.

In addition, given the limitations of machine testing, differences between different tobacco products generated by the proposed machine-testing approach should not be communicated directly or indirectly to consumers. It is unknown whether reducing the levels of even those compounds that have been identified as high priority compounds will actually reduce harm or exposure to harmful compounds. Therefore, it is an essential part of this proposal that regulators assume a responsibility to ensure that consumers are not informed directly or indirectly or are led to believe that cigarettes that meet the toxic limits established pursuant to this proposal are less hazardous, have been approved by the government or meet government-established health or safety standards.

The proposed regulatory approach mandates maximum limits for specific toxic constituents per mg of nicotine and excludes from the market those brands with levels in the smoke that exceed these limits. The existing variation in constituent levels across the brands currently on the market is used to

demonstrate that reducing levels of toxicants is technically achievable. Setting a limit that excludes from the market those brands with high levels of a toxic smoke constituent per mg of nicotine would result in a lowering of the mean level of that machine-measured constituent per mg of nicotine among those brands remaining on the market. A progressive reduction in the amount of toxicants in smoke over time can also be achieved by progressively lowering the maximum limit as technology to further reduce toxicants advances.

Prohibiting any health or exposure claims based on this machine testing is necessary until scientifically validated measures of exposure and harm are developed to allow regulators to determine that differences between brands do reduce risk. This strategy limits the risk that the new standards will become marketing tools that are used to misinform consumers.

The use of the variation in constituent levels for existing brands in order to set the maximum limits ensures that manufacturing approaches exist that allow production of cigarettes acceptable to the market that can achieve the regulatory limit. In addition, this approach may encourage manufacturers of cigarettes to voluntarily decrease toxic emissions to the lowest levels achievable, even for products below the established maximum limits.

The initial maximum limits recommended are the lower of the median values for a sample of international brands or the median for the brands for the country implementing the regulation. The median value of smoke constituent per mg of nicotine for the brands marketed in a country can be obtained from the mandated reporting by the manufacturers of values for the brands on the market of that country. The median values of the brands from a selected set of international brands are derived from published data (*9*) and form the basis of the recommendations on the quantitative maximum levels contained in this report.

It is expected that the measurements of constituent levels by brand will be the responsibility of the cigarette manufacturers and will be funded by them. The results will be reported to the regulatory authority, and an independent laboratory will verify a sample of those results. Enforcement of these maximum limits can follow a period of mandatory or voluntary reporting by the tobacco manufacturers of the smoke constituent yields for each of their brands on the market within a jurisdiction. The variation in levels of smoke constituent per mg of nicotine within a jurisdiction in this reported data can be examined to determine whether the maximum limit should be set based on values for brands sold locally or using the international sample presented by the WHO Study Group on Tobacco Product Regulation in this report. As the capacity of the tobacco manufacturers to achieve lower levels of toxicants increases, the maximum levels should be progressively lowered to ensure the transition of cigarettes to minimal toxicant yields.

As this regulatory approach expands to encompass multiple constituents, methods will need to be developed to balance or weigh the toxins present in the smoke of different brands. The intent is to remove brands with high levels of multiple constituents to ensure that the brands remaining on the market produce, on average, lower levels of most smoke toxicants. Appropriate strategies need to be developed to address the possibility that lowering levels of individual nominated toxins may result in an increase in other known toxicants not identified for regulatory limit setting. In the absence of such strategies, removal of brands with a high level of a single constituent, but low levels of most other constituents, could result in a net increase in the smoke toxicity of the brand mix remaining on the market.

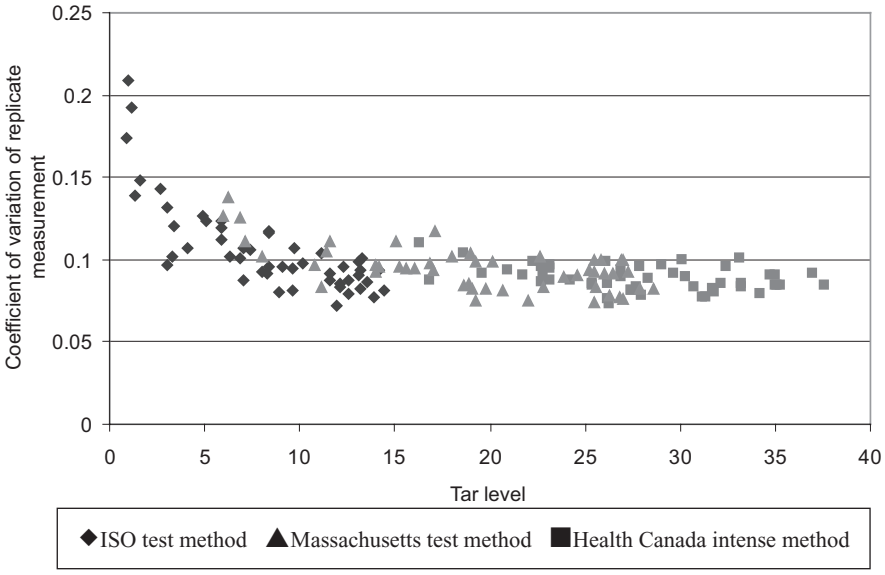
5.3 Selection of the machine-testing method

Measurement of smoke constituents requires machine-generated smoke. Different testing protocols using cigarette-smoking machines result in different levels of constituents per mg of nicotine, and the relative ranking of brands by constituent per mg of nicotine also varies with the testing protocol used. Where possible, it is useful to make measurements using more than one protocol in order to examine how the results, and rankings by brand, vary with the measurement approach.

Three standardized approaches to machine testing where data on machine testing of multiple brands are available were examined: the test method specified by the ISO/FTC; the testing method specified by the Massachusetts Department of Public Health; and the Canadian intense testing regimen, adopted by Health Canada, which is also referred to as the Health Canada intense method. Each of these methods offers advantages and disadvantages; however, the Health Canada intense method was selected as the method with the best fit for measuring constituents for use in the proposed regulatory strategy.

This selection was made based on several criteria. First, the larger quantity of smoke generated by the Canadian intense testing regimen reduces the coefficient of variation (CV) of the replicate measurements for the tobacco-specific nitrosamines that are the initial set of constituents recommended for regulatory consideration. Figure 5.1 presents the mean of the CV for four tobacco-specific nitrosamines, or TSNAs, (NAB, NAT, NNN and NNK) for each of the international brands of cigarettes measured by Counts and colleagues (*9*) and for each brand tested with each of the three testing protocols. The results for all three machine-testing protocols presented in the same figure and the individual values for each brand using each testing protocol are plotted against the level of tar yield for the brand resulting from the machine-testing method.

Figure 5.1.
Mean of the coefficients of variation of replicate measurement for four nitrosamines plotted by the tar level for individual brands measured by each of three machine-testing protocols



Source: adapted, with the permission of the publisher, from reference *9*.

It is evident from the graph that the variability of replicate measurements for the TSNAs increases when the testing protocol generates less than approximately 10 mg of tar. The measurements using both the ISO and the Massachusetts methods include substantial numbers of these international brands that are below 10 mg of tar and correspondingly have larger variation in replicate measurements. Only the Canadian intense method yielded a stable variation in replicate measurements across brands with different tar levels.

A second reason for selecting the Health Canada intense method was to better match patterns of smoking reflecting intense human use that, with certain cigarette design features, may yield levels of individual smoke constituents substantially above those that would result when ISO smoking conditions are used.

Third, in selecting a machine-testing protocol, the WHO Study Group considered that it was important to select a protocol that could accurately characterize those cigarette design changes, other than filter ventilation, where correction of constituent yields by expressing them per mg of nicotine alone was not sufficient to characterize the yields produced under conditions of more intense puffing.

The use of charcoal in cigarette filters is an example of a design change with an impact that is not well characterized by nicotine normalization. Using the ISO smoking regimen (35 ml puff volume, 60 seconds puff interval, 2 seconds puff, no vent blocking) to test the delivery of volatile components (e.g. benzene, 1,3-butadiene and acrylonitrile) in smoke from cigarettes with charcoal filters shows that the levels of these compounds are significantly reduced relative to other smoke constituents, including nicotine.

As long as enough charcoal is included in the filter, these reductions are present even when an intense puffing regimen like the Canadian intense testing regimen (55 ml puff volume, 30 seconds puff interval, 2 seconds puff, 100% vent blocking) is used. The newly introduced Marlboro UltraSmooth that is being marketed in Salt Lake City, Utah in the United States is an example of sufficient charcoal present in the filter to maintain reductions in yields of volatile compounds even under the Canadian intense conditions. However, the Marlboro UltraSmooth cigarette marketed in Atlanta, Georgia, United States and the modified charcoal filtered Marlboro Ultralight marketed in North Dakota, United States have less charcoal, and there is breakthrough of the volatiles with the more intense puffing regimens, resulting in higher yields. For these two products, smoking using the ISO regimen indicates that the levels of smoke volatiles are significantly lower than other cigarettes of similar delivery that are not charcoal filtered, and the difference persists even when presented per mg of nicotine. However, when these products are smoked under the Canadian intense testing regimen, the proportionate increase in the levels of volatile components is much larger than the increase in nicotine. Normalization per mg of nicotine would not correct for the increased smoke-constituent yield of these intermediate-level charcoal filter brands, which results from more intense smoking patterns.

Regulatory authorities need to receive correct information about the products for sale in their jurisdiction, and the regulatory smoking regimen selected should be able to characterize design changes that result in significantly higher smoke-constituent delivery relative to nicotine delivery with more intense smoking. While no one machine-smoking protocol perfectly characterizes cigarette constituent yields, the WHO Study Group on Tobacco Product Regulation concluded that the Canadian intense protocol offered significant advantages over the other two protocols.

5.4 Criteria for selecting constituents for regulating maximum limits

In its initial report, the working group made recommendations relating to TSNAs, but its task is also to consider how other constituents might be identified for regulatory consideration. The major criteria for selecting compounds are their toxicity index (concentration times potency), the variability

of the constituent per mg of nicotine among the brands on the market, and the existence of methods by which the constituent can be altered in the smoke.

Tobacco smoke contains more than 4800 individual chemical constituents (*10*). In order to characterize the inherent hazard of such constituents, it is necessary to know both the level of the particular constituent in smoke and the toxic potency (strength) of that component, as well as its interactions with other components in the smoke. Our understanding of these complex relationships remains incomplete, since the known toxic potency of smoke explains only a part of the observed disease effects in humans (*11*).

Major toxic effects that have been extensively evaluated for individual constituents include carcinogenicity as well as cardiovascular and respiratory effects.

Traditionally, direct genotoxic carcinogens are assumed not to exhibit a response threshold, which means that any dose is associated with some degree of hazard. The toxic potency of such carcinogens may be assessed by determining a value – the benchmark dose level (BMDL) – of the 95% lower confidence limit on the dose, giving a 10% incidence of response (the benchmark dose 10%, or BMDL10) by modelling dose-response data from the United States Environmental Protection Agency's emission inventory or by calculating a T25 value (*12*).[1] Carcinogenic potency may then be assigned by normalizing the BMDL10 or T25 values per unit constituent, assuming a linear relationship between dose and hazard. A measure of the hazard associated with a specific constituent in the smoke may then be expressed by the level in the smoke multiplied by its unit carcinogenic potency value, and this measure, termed a "cancer hazard index", can be used to identify those constituents in smoke where the setting of limits should be prioritized.

Non-genotoxic chemicals are assumed to exhibit dose thresholds for their hazardous effects, below which one would not expect to measure any adverse effects after repeated exposure (*13*). One may assign tolerable levels (reference levels) incorporating uncertainty factors to provide a margin of safety to account for variability in human response and uncertainties in extrapolating from animal studies to humans. A measure of the hazard associated with the constituent in the smoke may be expressed by the concentration in smoke divided by its tolerable level. Such a measure may be termed a "non-cancer hazard index". An underlying assumption, which creates uncertainty for such an index, is that the toxicity of each individual chemical in smoke is additive with other chemicals that affect the same target tissue or organ system.

[1] The T25 carcinogenic potency index is that dose in mg per kg of body weight per day that will give a tumour incidence of 25%, after subtracting the incidence in the control group.

These approaches may be used for selecting smoke constituents for which the setting of maximum limits needs to be considered. Those constituents in smoke with higher indices should represent the chemicals contributing more to the toxicity of the product emission than the constituents having lower indices.

In addition to a smoke constituent's toxicity, other factors may also be important in selecting constituents for regulation. First, the constituent must have substantial variability in its yield per mg of nicotine across the brands on the market or little will be gained by removing the higher-yielding brands. Secondly, and this point is related to the first, the variation across brands should be substantially greater than the variation in repeat measurement for the constituent for a single brand. If this is not true, then larger numbers of measurements would be required for each constituent for each brand in order to tighten the estimation of the mean value, and the cost of testing would increase proportionally.

A final consideration in selecting smoke constituents for regulation is the availability of technology, or other approaches, that can reduce the level of specific constituents per mg of nicotine in the smoke. To the extent that readily available alterations in tobacco processing, or cigarette design and manufacture, are known to reduce the level of toxicants in smoke, then setting limits on these toxicants becomes feasible and therefore of higher priority.

Data representing brands from the United States, Canada, and Australia, in addition to an international sample, were examined to identify a preliminary list of smoke constituents that meet the above criteria. Constituents are ranked by the ratio of the CV for the mean measurements of constituents per mg of nicotine across brands to the mean CV for the replicate measurements of that constituent for individual brands. Higher ratios identify those constituents where the greatest potential exists for regulatory lowering of constituents using their variability across brands on the market. The data for a set of international brands, with the ratios of the CVs for constituents per mg of nicotine and per mg of tar, are presented in Table 5.1.

Data are calculated for smoke constituents both per mg of nicotine and per mg of tar, but the principal value used for regulation should be the level of emissions per mg of nicotine. The level set needs to be specific to the machine-testing protocol utilized.

Table 5.1
For specific toxic constituents, the ratio of the CV for constituent levels across brands to the CV for replicate measurements using the Canadian intense testing regimen

Constituent per mg of nicotine		Constituent per mg of tar	
Constituent	Ratio of brand CV to replicate CV	Constituent	Ratio of brand CV to replicate CV
N-Nitrosonornicotine (NNN)	4.89	*N*-Nitrosonornicotine (NNN)	4.92
Carbon monoxide	4.83	*N*-Nitrosoanatabine (NAT)	4.75
N-Nitrosoanatabine (NAT)	4.72	Cadmium	4.40
Cadmium	4.19	Phenol	4.18
Phenol	3.93	Carbon monoxide	3.70
Nitric oxide	3.84	Nitric oxide	3.65
Nitrogen oxides	3.74	*m*+*p*-Cresol	3.57
m+*p*-Cresol	3.65	Nitrogen oxides	3.52
Hydrogen cyanide total	3.55	Hydroquinone	3.35
Hydroquinone	3.50	Quinoline	3.16
Ammonia	3.41	Lead	3.13
Lead	3.05	Formaldehyde	3.12
Hydrogen cyanide, impinger	3.03	Ammonia	3.11
Quinoline	3.01	Hydrogen cyanide total	2.96
Styrene	2.98	*o*-Cresol	2.93
Formaldehyde	2.97	*N*-Nitrosoanabasine (NAB)	2.93
Hydrogen cyanide, pad	2.93	4-(*N*-Nitrosomethyl-amino)-1-(3-pyridyl)-1-butanone (NNK)	2.90
o-Cresol	2.88	Catechol	2.84
4-(*N*-Nitrosomethyl-amino)-1-(3-pyridyl)-1-butanone (NNK)	2.86	Styrene	2.78
N-Nitrosoanabasine (NAB)	2.86	Hydrogen cyanide, impinger	2.63
4-Aminobiphenyl	2.62	Isoprene	2.43
Propionaldehyde	2.53	4-Aminobiphenyl	2.37
Acetaldehyde	2.52	Hydrogen cyanide, pad	2.25
Acrolein	2.51	Acetone	2.22
Acetone	2.50	Butyraldehyde	2.21
Butyraldehyde	2.49	Acrolein	2.21
Isoprene	2.47	Acetaldehyde	2.16
Catechol	2.44	Propionaldehyde	2.13
Pyridine	2.36	Benzo[*a*]pyrene	2.06
3-Aminobiphenyl	2.08	Pyridine	2.00
Acrylonitrite	2.05	Acrylonitrite	1.97
Crotonaldehyde	2.00	Nicotine	1.96
1,3-Butadiene	1.92	1,3-Butadiene	1.87
Resorcinol	1.90	3-Aminobiphenyl	1.87
Benzo[*a*]pyrene	1.89	Resorcinol	1.86

Constituent per mg of nicotine		Constituent per mg of tar	
Constituent	Ratio of brand CV to replicate CV	Constituent	Ratio of brand CV to replicate CV
Methyl ethyl ketone	1.88	Crotonaldehyde	1.80
Tar	1.74	Methyl ethyl ketone	1.77
2-Aminonaphthalene	1.73	Toluene	1.73
Toluene	1.72	2-Aminonaphthalene	1.65
Mercury	1.62	Mercury	1.60
Benzene	1.55	Benzene	1.57
1-Aminonaphthalene	1.53	1-Aminonaphthalene	1.46
Arsenic	0.88	Arsenic	1.08

CV, coefficient of variation.
Source: adapted, with the permission of the publisher, from reference *9*.

5.5 Specific regulatory recommendations for TSNAs

Existing data offer a sufficient range of brands and constituent yields to define what the industry is capable of now and to enable us to set standards that will reduce the levels of toxic constituents in smoke now.

The WHO Study Group on Tobacco Product Regulation recommends that Member States should establish and/or strengthen the regulatory framework for tobacco products in their respective jurisdictions. In terms of the Study Group's recommendations for the setting of upper limits to tobacco smoke toxicants, these should apply to all cigarettes manufactured and/or sold in, or imported from or exported to those countries that implemented such regulatory limits.

The initial smoke constituents proposed for regulation are the tobacco-specific *N'*-nitrosamines NNN (*N*-nitrosonornicotine) and NNK (4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone). These TSNAs are potent carcinogens, and there is clear evidence that alteration of the curing methods for tobacco and other manufacturing approaches can substantially lower the levels present in smoke (*10*, *14*). In addition, there is marked variation across brands in the level of these nitrosamines within countries, as demonstrated in the Massachusetts Benchmark data, internationally, as demonstrated by the comparison of international brands published by Counts and colleagues, and between countries, as demonstrated by data from Canada and Australia as compared to data from the United States and the international data.

The carcinogenic activities of NNK and NNN have been firmly established by extensive studies in laboratory animals. In rats, NNK induces tumours of

the lung, nasal cavity, liver and pancreas. The lung is the major target tissue, and detailed dose-response studies have been performed by subcutaneous injection of NNK. Administration of NNK in the drinking water (5 ppm) results in lung tumour production in 90% of the treated rats versus 8% in control rats. Other studies show that NNK induces tumours of the lung in rats independent of the route of administration. Clearly, NNK is a potent systemic lung carcinogen in the rat. NNK also induces primarily lung tumours in multiple strains of mice of varying susceptibility and by various routes of administration. NNK causes lung and tracheal tumours in hamsters, nasal cavity and lung tumours in mink, and lung tumours in ferrets. NNN is also a recognized carcinogen. It causes tumours of the oesophagus and nasal cavity in rats. NNN causes tracheal and nasal cavity tumours in hamsters, and lung tumours in mice. A mixture of NNK and NNN, swabbed repeatedly in the rat oral cavity, induced oral tumours as well as tumours of the lung. Based on these animal carcinogenicity data, human exposure data and mechanistic studies, NNN and NNK together are classified as a human carcinogen (Group 1) by IARC (*15*).

Levels of NNK and NNN in unburned tobacco contribute significantly to, and are correlated with, levels in smoke. NNK and NNN are scarcely present in uncured tobacco leaf. It has been known for over 25 years that these carcinogens form during the curing and processing of tobacco. Modifications of these processes are now available that can substantially reduce the levels of NNK and NNN in tobacco, and therefore in smoke (*10*, *14*). It is also known that NNK and NNN levels vary significantly with tobacco type, being higher in air-cured/processed Burley tobacco than in flue-cured bright tobacco. Other studies show that tobacco nitrate contributes to smoke levels of NNK and NNN. Collectively, the available evidence strongly indicates that the technology is available to significantly reduce NNK and NNN levels in cigarette smoke.

The variation of NNN and NNK per mg of nicotine is defined in this report using data from an international sample of Philip Morris brands published by Counts and colleagues (*9*). While this appears to be the best international data currently available, it obviously reflects a selected sample of brands from a single manufacturer using tobacco blends that are much higher in nitrosamines than brands that are currently on the market in some countries, notably Australia, Canada, and the United Kingdom. Therefore, the guidance on levels per mg of nicotine presented in this report is intended to inform countries where data are not available or are inadequate. Countries where the principal brands on the market use flue-cured bright tobacco with low levels of nitrosamines may be well advised to establish their own regulatory maximum limits based on measurements made on the cigarettes actually sold in their market.

Examination of the levels of NNN and NNK measured using the Canadian intense machine-smoking protocol reveals that the median level of NNN in the Philip Morris brands tested is approximately 114 nanograms per mg of nicotine in smoke emissions, with a range in the brands tested from 16 to 189 nanograms per mg of nicotine (*9*). The median level for NNK in the Philip Morris brands tested was 72 nanograms per mg of nicotine with a range of 23 to 111 nanograms per mg of nicotine. These data suggest that a level for NNN in smoke emissions of 114 nanograms per mg of nicotine or lower has already been achieved by half of the cigarettes that Philip Morris has marketed internationally. This level therefore reflects a readily achievable maximum limit that can be established for this compound as an initial maximum limit above which cigarettes should be excluded from the market.

A maximum limit for NNK of 72 nanograms per mg of nicotine in smoke emissions is similarly proposed.

The basis on which these values were set is the median value reported for these international brands, which are American blended cigarettes having higher nitrosamine levels than blends used for cigarettes in many other countries, notably Australia, Canada, and the United Kingdom. Data on smoke constituent yields from these three countries demonstrate that the tobacco used in cigarettes smoked in these countries yields levels of NNN and NNK that are substantially lower than the median values measured by Counts and colleagues in their sample of brands (*9*). Data for 2004 on smoke constituent yields for Canadian cigarettes (*16*), using the Canadian intense testing method, reveal, once American and Gauloises brands are excluded, a mean level of NNN of 23.8 nanograms per mg of nicotine for the brands tested and a mean level of 50.5 nanograms per mg of nicotine for NNK. The Canadian mean NNN level is less than a quarter of the median value for the international brands used to establish the maximum limit recommendation, demonstrating both that cigarettes with substantially lower levels of NNN can be manufactured and successfully marketed and that individual countries may be well advised to set maximum limits based on the products sold in their own markets.

Data from Australia reveal similar results. Data on smoke constituent yields for Australian cigarettes (*17*), using the Canadian intense smoking testing method, reveal a mean level of 20.8 nanograms of NNN per mg of nicotine for the brands tested and a mean level of 27.3 nanograms per mg of nicotine for NNK. This demonstrates that there is substantial scope for further reductions in the levels of nitrosamines, and that it is possible to manufacture cigarettes with these low levels of nitrosamines that have broad market appeal.

5.6 Interpretation of the maximum limit values

The appropriate approach would be to apply the maximum limit values to the mean value measured for a given brand. In calculating that mean value, a sufficient number of replicate measures should be conducted to ensure a representative sample of the cigarettes of that brand on the market and to provide a narrow confidence band around the mean value for the brand. Specifically, it is not suggested that the maximum limit value be interpreted as the established maximum limit value plus two or more standard deviations of the replicate measurement.

5.7 Communication of the results of the testing to the public

The approach to regulation and setting maximum limit values presented in this report is based on the general principle of reducing to the maximum extent technologically possible toxic substances present in cigarette smoke. Current scientific knowledge does not allow us to conclude definitively that the reduction of nitrosamines, or of any other individual constituents in cigarette smoke, will reduce the rate of cancer incidence in smokers who use cigarettes with lower levels of these constituents, nor has existing science demonstrated that the specified changes in maximum values will result overall in a meaningful change in actual exposure for consumers. Setting maximum limits and removing some brands with higher levels from the market is not a statement that the remaining brands are safe or less hazardous than the brands that have been removed, nor does it represent government approval of the safety of the products that remain on the market.

Regulatory authorities have an obligation to ensure that the public is not misled by the results of the recommended machine-testing and maximum limit values, as the public was misled by the use of machine testing for tar and nicotine yields. The WHO Study Group on Tobacco Product Regulation recommends that any regulatory approach should specifically prohibit the use of the results of the testing in marketing or other communications to the consuming public, including product labelling. It is also recommended that communicating the relative ranking of brands by testing levels and/or the statement that the brand has met governmental regulatory standards should be prohibited. Because information is often transmitted to smokers through the kinds of news stories that accompany the implementation of new regulations, it is a responsibility of the regulatory structure to monitor tobacco industry marketing and smokers' understanding and interpretation of the new regulations in relation to the hazard of the products remaining on the market, and whether their understanding of the hazard of the remaining products is influencing initiation or cessation rates, and to take whatever corrective action is necessary to prevent consumers from being misled. The report by the WHO

Scientific Advisory Committee on Tobacco Product Regulation on the evaluation of new or modified tobacco products (*18*) deals with these monitoring and surveillance concerns in greater depth.

These recommendations are intended to reinforce the concerns expressed by the WHO Study Group on Tobacco Product Regulation in its recommendation 1, and specifically its concern that:

> Packaging labels should not carry statements such as, "These cigarettes contain reduced levels of nitrosamines" or "These cigarettes contain half the level of carbon monoxide compared to our regular brand". These are quantitative statements that imply that one brand is safer than another. The Study Group is very concerned that cigarette testing will be used by the tobacco industry to make claims that imply health benefits in order to market its products. Instead, health information should be disseminated by the display of qualitative facts on the packages, such as "These cigarettes contain nitrosamines that have been shown to cause cancer in laboratory animals" or "Smoke from these cigarettes contains benzene, a known carcinogen". It is important to publish qualitative information *only*, based on appropriate research that indicates the presence of toxic components in smoke (*19*).

5.8 Methods for measuring nitrosamines

Temporal and geographical variations are important sources of product variability that should be included in any examination of the differences within and across products. Samples should be collected using the standard method described in ISO 8243. Cigarettes should be obtained as part of a series of samplings at different times. The time period for reporting is divided into at least five separate time sub-periods. One sample of 20 cigarettes is taken in each sub-period. Each sample is drawn from separate sampling points.

In order to reduce variability in the product due to storage conditions, samples should be conditioned prior to smoking by machine for at least 24 hours, according to ISO standards as described in ISO 3402.

Cigarettes will be analysed using the Canadian intense testing regimen, using 55 ml puff volume, 2.0 seconds puff duration, 30 seconds puff interval, and 100% vent blocking, butt length 23 mm for non-filter cigarettes or the length of filter paper plus 3 mm for filter brands. For linear smoking machines, three cigarettes will be smoked on each of 30 Cambridge filter pads. For rotary smoking machines, 10 cigarettes will be smoked on each of 10 pads.

The most widely used method for analysis of TSNAs is gas chromatography/thermal energy analysis (TEA). This technique is specific for nitroso-containing compounds and has been used for many years successfully for measuring NNN and NNK in tobacco smoke. A description of this method, known as the "Health Canada Official Method", can be found at http://www.qp.gov.bc.ca/stat_reg/regs/health/oic_94.pdf (accessed

2 March 2007), beginning on page 119. Alternative methods, including liquid-chromatography/tandem mass spectrometry, found at http://www.aristalabs.com/pdf/MainstreamAnalysis.pdf (accessed 2 March 2007), or those described by Wu and colleagues (*20*) have also been used, but any alternative technique must show equivalent accuracy and reproducibility to TEA before use.

Individual measurements will be made for each pad, the number of pads analysed, and the mean and standard deviation for each brand reported. Dates and locations of each sampling should also be reported, along with the final results.

5.9 Considerations for modified cigarettes and potential reduced exposure products

The recommendations in this report are intended to apply to traditional machine-manufactured cigarettes that burn tobacco, and they should not be applied to cigarettes that heat tobacco or use technology other than combustion to deliver nicotine. The assessment of these unconventional tobacco products and other potential reduced exposure products (PREPs) are discussed in a previous report by the WHO Study Group on Tobacco Product Regulation (*18*).

It is possible to alter the level of a constituent per mg of nicotine in cigarette smoke by changing the nicotine yield of the cigarette as well as by altering the level of the toxicant. It is also possible to increase the yield of nicotine in cigarette smoke by adding nicotine to the tobacco or the filter, as well as by altering the type of tobacco used to make high nicotine varieties, among other approaches. While these approaches may theoretically have independent utility in decreasing exposure to tobacco toxicants, their potential to do so remains uncertain. The potential for using increased nicotine yields as a method of lowering the level of toxicant per mg of nicotine to a level below the maximum limit value is therefore also unproven as being of value in reducing the toxicity of the smoke; and regulatory authorities should not encourage or allow increased nicotine yields as a method of complying with the regulations relating to maximum limit values.

Detection of increasing nicotine yields in brands can be facilitated by tracking machine-delivered nicotine yields per cigarette over time and by examining the distribution of tar to nicotine ratios for the brands within a given market. For those brands with increasing nicotine yields over time, and for those brands with tar to nicotine ratios in the bottom third of the brands on the market, regulators may choose to require that the brand be below a maximum limit value for nitrosamine per mg of tar as well as per mg of nicotine. This would make it more likely that the level of TSNAs was reduced per yield of

nicotine, the drug being sought in smoking, and per the mass of smoke generated. The median level of NNN per mg of tar in the sample of international brands examined using the Canadian intense method was 7.1 nanograms per mg of tar, and the median level for NNK per mg of tar was 4.6 nanograms per mg of tar (*9*).

It is also possible that some brands of cigarettes may be offered with reduced nicotine in the tobacco used to make the cigarette. Nicotine can be removed from the tobacco leaf, and genetically altered tobacco is available with very low nicotine content. Cigarettes made with these tobaccos will have low nicotine deliveries under any testing method and correspondingly may have very high levels of toxicants per mg of nicotine. Regulators may want to identify brands that are intentionally lowering nicotine in the tobacco as a separate category for evaluation. Once the regulatory authority is satisfied that the manufacturer is actually using low nicotine tobacco in the product, it may want to exempt those brands from the maximum limit value per mg of nicotine and use a maximum limit value per mg of tar as a substitute.

5.10 Future directions

The maximum limits for TSNAs are proposed for consideration by WHO Member States as they establish and/or strengthen the regulatory framework for tobacco products in their respective jurisdictions. As additional scientific information and data on constituents from a wider range of brands and geographical areas become available, these recommendations are likely to be modified and extended to other constituents.

Examination of available data to identify a more complete list of constituents for which additional maximum limits can be offered is currently under way. Analyses of existing data sets will be integrated with existing knowledge of the toxicity of tobacco smoke constituents to enable a list of constituents to be recommended to regulatory authorities, which can then require their measurement by the tobacco manufacturers. Special attention should be paid to the inclusion in this list of constituents that are thought to contribute to cardiovascular disease and chronic lung disease as well as cancer.

In making design changes in products in order to meet these requirements, the tobacco companies should be obliged to evaluate and report changes in levels of toxicants resulting from the new design and to provide a list of constituents as specified by the tobacco regulatory authority of British Columbia, Canada (*21*).

The list of constituents for which maximum limits are recommended will be tested for its impact on the number of brands restricted in order to assess the fraction of the brands on the market that may be affected. This question will

be addressed prior to making further recommendations for constituents to be measured for regulatory purposes so that the consequences of the regulation on the market can be anticipated.

The results of this effort will be a list of constituents recommended for monitoring, a plan for setting the level of those constituents per mg of nicotine for a country committed to undertaking measurement and regulation of the brands within its own market, and a list of levels per mg of nicotine based on existing data for brands marketed internationally that can be used for regulation by countries that have not developed their own testing structure. It is expected that most countries will require the testing of constituents to be carried out by the tobacco manufacturers, with periodic validation of the industry data by independent laboratories.

Independent or government laboratories with demonstrated competency in the analysis of TSNAs, and in the analysis of other constituents if they are recommended for future regulatory consideration, should carry out an analysis of a subset of the brands and varieties. These laboratories should be members of the World Health Organization Tobacco Laboratory Network (TobLabNet).

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6. Overall recommendations

6.1 Contents and design features of tobacco products: their relationship to dependence potential and consumer appeal

Main recommendations

The harm caused by tobacco products is a function of their toxic emissions as well as the extent and patterns of their use. Patterns of use, in turn, are related to dependence potential and consumer appeal. The tobacco industry's documents and expert evaluation reveal extensive manipulation of contents and designs to increase dependence potential and appeal. For example, the dependence-causing effects of nicotine can be increased by contents and designs that increase the free base fraction of nicotine, and flavourings such as cherry and cloves can be used to appeal to target populations.

It is recommended that tobacco product contents and designs be evaluated from the perspective of dependence potential and consumer appeal to provide the foundation for potential restrictions on designs and ingredients that enhance such potential and appeal.

Significance for public health policies

The significance for public health policies includes maintaining, increasing, and implementing standards for the contents, designs and emissions of tobacco products that relate to dependence potential and consumer appeal, thereby supporting efforts to reduce the prevalence of use and possibly toxicant intake among users. Combined with other elements and actions of tobacco control, such policies should lead to a reduction in tobacco use and associated disease.

Implications for the Organization's programmes

Given the variety of ingredients and design features of tobacco products, WHO will need to undertake surveillance of and research efforts into the effects on health of tobacco initiation and cessation. Research will need to be conducted into the contents and design features that may contribute to

dependence potential, consumer appeal, and hence a more prevalent, persistent and deadly use of tobacco products. WHO will also need to develop a timetable for the implementation of its goals, which should take into account its resources and capacity, as well as the need for revising existing goals and setting new targets as its objectives are achieved.

6.2 Candy-flavoured tobacco products: research needs and regulatory recommendations

Main recommendations

The use and marketing of candy-like additives in tobacco products should be restricted. This report identifies current packaging styles and flavour varieties and provides guideline recommendations for tobacco product manufacturers and health professionals involved in programmes aimed at promoting the cessation of tobacco use.

Tobacco manufacturers should be required to disclose additives, including candy-like additives, in tobacco products by brand and level. Any claim of supposed health risk reduction should be prohibited. The use of candy-flavoured additives in new tobacco brands should be prohibited. For tobacco companies or brands currently using flavoured additives, limits should be set on any additive that contributes to dependence, initiation, or increase in second-hand smoke exposure, or that discourages cessation. These recommendations and other strategies to regulate candy-flavoured tobacco products should be part of an overall strategy to regulate the contents, emissions and design of tobacco products and to promote disease reduction.

Significance for public health policies

Analyses of the tobacco industry's internal documents indicate the common use of additives to change the perception and impact of tobacco smoke delivery and environmental tobacco smoke. The basic principles of public health stipulate that candy-like flavoured additives should not be used to make dependence-causing drugs more appealing or to mask the harmful effects of product use and exposure. While tobacco companies deny targeting youths, published research suggests that candy-flavoured additives are a significant factor in attracting young and inexperienced smokers.

Published research has also revealed the use of new flavour-delivery mechanisms associated with candy-flavoured products (such as plastic pellets embedded within cigarette filters). The failure to disclose the use of flavour-delivery technologies raises additional health concerns and emphasizes the frequently unrecognized role of flavour and additive delivery in product design. These findings support the need for appropriate government regulations

to identify and evaluate the potential for increased individual-based as well as population-based harm.

Implication for the Organization's programmes

A WHO policy recommendation to encourage the regulation of candy-flavoured additives is an essential component of a comprehensive plan to regulate tobacco products. WHO needs to stimulate and promote research to evaluate the effects and toxicity of new delivery mechanisms such as the flavoured pellet hidden inside cigarettes. More population-based research is needed on the effect that candy-like flavours and other additives have on initiation, dependence, use and exposure.

6.3 Biomarkers of tobacco exposure and of tobacco smoke-induced health effects

Main recommendations

Tobacco-related biomarkers can be a measure either of exposure to the emissions of tobacco products or of the potential or actual biological changes or harm in the human body as a consequence of such exposure. Genetic biomarkers for disease susceptibility also exist that may play a significant role in whether or not a smoker develops a disease. Although no currently existing biomarkers or panels of biomarkers are sufficiently robust to support a claim of risk or harm reduction in a regulatory setting, biomarkers have substantive value in some regulatory contexts. Biomarkers of exposure should be required in studies submitted for regulatory approval of tobacco-use cessation interventions, in support of exposure reduction claims, and in studies defining the dependence potential of different products. They can also be useful in evaluating or monitoring the effectiveness of individual-level tobacco cessation interventions.

Significance for public health policies

Self-reported smoking status and self-reported daily cigarette consumption remain the recommended methods for quantifying and evaluating trends in tobacco exposures for the general population, and both have been validated in epidemiological studies as predictors of disease outcomes. However, differences in cigarette design, as well as in variations in individual smokers' patterns of use, limit the ability of self-reported daily consumption of cigarettes to reflect accurately the exposure received by individuals from the use of different tobacco products. Biomarkers offer the potential to quantify more accurately individual exposure to nicotine and other specific tobacco emissions, and are valuable in situations where increased accuracy in the

definition of smoking status or more precise measures of intensities of exposure are needed. Caution needs to be exercised in extrapolating from biomarker measurement of exposure to one tobacco smoke constituent to either whole-smoke exposure or disease risk when comparing exposures from different tobacco products.

Biomarkers of exposure are also useful in evaluating specific public policy questions about the effect of policy changes on exposures in the general population, notably whether restrictions on smoking in general or in specific locations reduce exposure.

Implication for the Organization's programmes

Regulatory actions may need to be implemented despite the limitations of scientific knowledge or in the absence of scientific certainty. Faced with this scientific uncertainty, regulators may seek the advice of panels of experts who can review all of the evidence on a given product. In assessing the toxicities of different tobacco products, information from the biomarkers of biological processes should be combined with the chemical measurement of emissions, information from biomarkers of tobacco exposure, design characteristics, and existing epidemiological and clinical data. In this area of tobacco control, WHO needs to be at the forefront of stimulating and supporting research on biomarkers of tobacco exposure and tobacco-induced harm.

6.4 Setting maximum limits for toxic constituents in cigarette smoke

Main recommendations

Smoke emissions contain a large number of potent toxicants, and levels of these toxicants per mg of nicotine vary substantially across existing brands. While it is not possible to eliminate all of these toxicants or to estimate reliably the reductions in risks that would result from lowering the level of a single toxicant, effective public health protection requires a cautionary approach, and lowering the levels of toxicants in smoke to the greatest extent possible is therefore a worthwhile and reasonable regulatory goal. This approach is similar to that of reducing the levels of contaminants in food products, even in the absence of clear evidence that reducing the contamination measurably alters disease risks. From a public health perspective, it is difficult to justify allowing very high levels of carcinogens in some cigarette brands when other brands have only a fraction of those levels, even if there is uncertainty about the magnitude of the benefit of reducing a single constituent.

The question of whether setting maximum limits for some potent smoke toxicants could reduce the toxicant levels produced by cigarette brands within

a given market is being examined. It is proposed that, initially, maximum limits should be set for the carcinogenic tobacco-specific nitrosamines NNN (*N*-nitrosonornicotine) and NNK (4-(*N*-nitroso-methylamino)-1-(3-pyridyl)-1-butanone).

The levels of NNN and NNK per mg of nicotine vary substantially across brands in all markets examined, and setting a maximum level at the mid-point of that range would substantially lower the levels for the brands remaining on the market. Other evidence establishes that NNN and NNK levels in tobacco can be readily lowered by changes in curing and other practices, which suggests that tobacco manufacturers could without difficulty or delay reduce the levels for all brands to below the maximum levels recommended. It is recommended that, after an appropriate reporting interval, brands with levels exceeding these maximum limits should be prohibited from being imported, exported, distributed and sold.

Significance for public health policies

Cigarettes are the most toxic consumer product and, partly because of that extreme toxicity, they have escaped effective product regulation. Adoption of the proposed maximum limits, banning brands above those limits, and prohibiting claims based on meeting the limit would allow regulation of tobacco emissions and a lowering of the levels of toxicants in the smoke of the remaining brands without misleading the public about the relative risk of smoking different brands. This regulatory approach is directed at manufacturers, encouraging them to reduce the toxicants in their products to the maximum extent possible; and as such, it is a strategy for regulating products rather than reducing harm. It is therefore suggested that Member States should require maximum limit values.

Maximum limits for a more complete list of constituents, which will include those that are thought to contribute to chronic lung disease and cardiovascular disease, as well as to cancer, are currently being developed.

Since previous machine-testing measures, notably of tar and nicotine, have been misrepresented to consumers as representing differences in exposure or risk, values measured using this approach should not be the basis of marketing claims by manufacturers, the ranking of brands within a market, or decisions by consumers concerned about their disease risks.

Implications for the Organization's programmes

As the capacity of the tobacco manufacturers to achieve lower levels of toxicants increases, the maximum levels can be progressively lowered to ensure that the toxicant yields of cigarettes are progressively reduced to a minimum.

It is expected that most countries will require that tobacco contents and emissions testing be provided by the tobacco manufacturers, with periodic validation by independent laboratories that have demonstrated competency in the analysis of tobacco constituents, such as those that are members of WHO's Tobacco Laboratory Network (TobLabNet). In order for TobLabNet to succeed in its role as the counterbalance against the industry's tobacco testing and research capabilities, WHO needs to continue to support it. It is only by gaining a comprehensive understanding of the characteristics of tobacco products, including their contents, emissions, and design features, that public health and regulatory agencies will be in a position to regulate effectively a product that kills half its regular consumers.

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Annex 1

Reports and other documents arising from meetings of the WHO Scientific Advisory Committee on Tobacco Product Regulation (SACTob)

Statement of principles guiding the evaluation of new or modified tobacco products (2003)

This publication sheds light on the existing scientific understanding of risks caused by tobacco use. It provides a framework of questions to be considered in evaluating the harm reduction potential of new tobacco products. Its main points are as follows:

- Existing scientific evidence is not sufficient to assess the differences in health risk potential between newly engineered tobacco products and existing products.
- Regulatory oversight of cigarette and cigarette-like products should include examination of separate aspects of the new products.
- Claims of reduced exposure or reduced harm should be supported by adequate scientific data provided by the manufacturer that intends to make the claim.
- Each type of claim requires a substantive body of evidence.
- Regulatory oversight is necessary to assess and monitor changes in newly modified tobacco products.
- Claims of reductions in smoke emissions or reduced uptake of toxicants need to be supported by evidence.

Recommendation on nicotine and its regulation in tobacco and non-tobacco products (2002)

Research findings over two decades have pointed to nicotine as the key pharmacological factor underlying tobacco use. This report makes recommendations based on existing science concerning the regulation of tobacco and non-tobacco products. They are as follows:

- The present situation in which the most toxic form of nicotine delivery is the least regulated, is unacceptable from a public health perspective.

- Because nicotine, compared with other tobacco constituents and emissions, appears to be responsible for a small proportion of tobacco-caused diseases, there is considerable scope for developments that reduce the risks experienced by users of tobacco, without undermining efforts to prevent initiation into tobacco use and to promote cessation among established users.
- In the absence of firm contrary data, those responsible for public-policy decisions are justified in using the conservative assumption that smokers' preferences for a nicotine dose are persistent over time and are not influenced by changes in the product used, and that smokers will compensate for reductions in yield to maintain a relatively consistent dose of nicotine.
- A broad and comprehensive regulatory framework is required to enable policy options for controlling nicotine to minimize risks.

Recommendation on tobacco product ingredients and emissions (2003)

The purpose of this publication is to provide recommendations to support the development of protocols for assessing tobacco product ingredients and associated emissions with the intent to reduce tobacco-caused disease. The central premise is that tobacco product ingredients and their emissions, including nicotine, should be regulated. The preferred focus for regulation is the emission from the product when it is used as intended (exceptions may include certain cigarette ingredients such as nicotine and ammonia). These principles apply to all smoked products, including novel cigarette substitutes and smokeless tobacco products, in recognition of the fact that all tobacco products have ingredients and emissions.

SACTob recommendations on health claims derived from ISO/FTC method to measure cigarette yield (2003)

This publication deals with the validity of health claims based on the standardized testing methods for the measurement of tar, nicotine and carbon monoxide yields of tobacco smoke adopted by the International Organization for Standardization (ISO) and the United States Federal Trade Commission (FTC). It contains the following conclusions and recommendations.

- The numerical ratings for tar, nicotine and carbon monoxide based on the current ISO/FTC testing methods and presented on cigarette packages and in advertising as single numerical values are misleading and should not be displayed.
- All misleading health and exposure claims should be banned.

- The ban should apply to packaging, brand names, advertising and other promotional activities.
- Banned terms should include "light", "ultra-light", "mild" and "low tar", and may be extended to other misleading terms. The ban should include not only misleading terms and claims, but also names, trademarks, imagery and other means of conveying the impression that the product provides a health benefit.

Recommendation on smokeless tobacco products (2003)

This recommendation emphasizes the lack of research on the health risks that smokeless tobacco poses and the considerable caution that needs to be exercised when smokeless tobacco products are marketed. The use of smokeless tobacco, highly prevalent in many countries, is a significant part of the global tobacco problem. There is conclusive evidence that certain smokeless tobacco products, namely, betel quid with tobacco, tobacco with lime, and other tobacco mixtures in South Asia, and smokeless tobacco in the United States of America, increase the risk of cancer. The designation of smokeless tobacco products as harm-reducing agents may promote a false perception of safety. This publication also notes with concern that in most countries there is no specific mechanism for regulating smokeless tobacco products. Often, smokeless tobacco products are not required to carry any health warnings. The recommendation emphasizes the need to regulate the ingredients and emissions of smokeless and smoked tobacco products.

Annex 2

Reports and other documents arising from meetings of the WHO Study Group on Tobacco Product Regulation (TobReg)

Guiding principles for the development of tobacco product research and testing capacity and proposed protocols for the initiation of tobacco product testing: recommendation 1 (2004)

The implementation of Articles 9, 10 and 11 of the Framework Convention requires the empirical testing of tobacco products. This publication provides the rationale and recommended protocols for the implementation of such testing. It is recognized that there may be a variety of options that may be considered in selecting specific parameters. However, the WHO Study Group on Tobacco Product Regulation recommends that these options be based on the current state of the science and that due consideration be paid to the limitations of product testing methods already discussed in this recommendation as well as to the foregoing principles. Among the areas of product testing encompassed are laboratory capacity; tobacco product diversity; potential providers of laboratory research and testing; funding the development of laboratory research and testing capacity and operation; protocols for tobacco product testing; issues and limitations in establishing product testing protocols; regulatory considerations for product testing protocol development; and scientific considerations for product testing protocol development.

Best practices in tobacco control: regulation of tobacco products: Canada report (2005)

This report highlights Canadian tobacco product regulation. The Canadian tobacco regulatory regime, identified as one of the best by WHO's Tobacco Free Initiative and the WHO Study Group on Tobacco Product Regulation, incorporates mandatory periodic emissions testing, emissions disclosure based on all characteristics of the tobacco product, and labelling requirements which mandate large, clear health warnings and informational messages. And most noteworthy, this best practice shows how Canada, in an effort to promote public health goals, creatively manoeuvred around the limitations of the ISO smoking machine-testing protocol by amending its regulation to require manufacturers to additionally test using a more intense testing regimen. This Canadian intense testing regimen has since been adopted by the WHO Study

Group on Tobacco Product Regulation in its first recommendation: *Guiding principles for the development of tobacco product research and testing capacity and proposed protocols for the initiation of tobacco product testing*. The WHO Tobacco Free Initiative hopes that Member States will glean valuable insights and inspiration from Canada's experience.

Advisory note: waterpipe tobacco smoking: health effects, research needs and recommended actions by regulators (2005)

This advisory note addresses the growing concerns about the increasing prevalence and potential health effects of tobacco smoking using waterpipes, a practice that dates back at least four centuries in Africa and Asia. The note will provide guidance to WHO Member States and other research agencies interested in a more thorough understanding of the health effects of waterpipe smoking.