5.3 Measures to assess the effectiveness of tobacco product regulation

Introduction

Tobacco product regulation is a rapidly emerging area in tobacco control. Scientists, policy makers, and international public health organisations have called for comprehensive regulation of tobacco products with the aim of protecting public health. A handful of countries and jurisdictions have already adopted legislation requiring reporting and testing of tobacco product contents and emissions. Articles 9 and 10 of the WHO Framework Convention on Tobacco Control (FCTC) contain the requirements for regulation of tobacco product contents and emissions, as well as manufacturers' disclosures about the product (Figure 5.6).

As the regulatory landscape evolves around the world, it is essential to evaluate the effectiveness of new regulations and their impact on the product itself and on the population, in order to determine whether regulations are meeting public health goals. The emergence of new legislation and regulatory standards for tobacco products provides a unique opportunity to study changes in the product and in health outcomes over time and across countries and regions. Because product regulations cannot be assessed through randomised clinical trials, researchers and public health officials must employ quasi-experimental designs and utilise opportunities for "natural experiments" through making comparative observations (Fong *et al.*, 2006a). Additionally, it is important to begin collecting baseline data and developing measures and protocols for evaluation, so that the impact of future regulations can be assessed. In 1999, a WHO Conference on the Regulation of Tobacco Products concluded that "The regulatory process must be guided by the best available science and the effects tracked so as to maximize health benefits, minimize unintended consequences, and thereby foster self-correction." (WHO, 2000).

The ultimate test of the impact of a regulation intended to protect public health is to demonstrate a reduction in morbidity or mortality associated with the regulation. However, it can take decades for some effects, such as changes in cancer incidence, to be seen. Thus, measures to assess product regulation have historically focused on the product itself, although such measures have significant limitations for predicting human risk. The need for in-depth product evaluation under actual conditions of use is supported by the history of the development and promotion of "light" cigarettes. Based on standardised machine smoking measurements, the average sales-weighted tar and nicotine yield for US cigarettes decreased by about 70% between the 1950s and 1990s (Hoffman & Hoffman, 2001). Scientists and public health officials initially supported this trend in the 1960s and 1970s (Parascandola, 2005), and it took decades before epidemiologic studies provided definitive evidence that changes in cigarettes designed to lower smoke yields did not in fact lead to any significant decrease in the tobacco-related disease burden (Burns *et al.*, 2001). We now know that much of the apparent decline was due to the use of filter ventilation, which produces markedly reduced machine measured yields, but not necessarily on the amounts smokers actually take in (Kozlowski *et al.*, 1998a).

Laboratory-based product testing remains vitally important, despite its limitations for predicting human risk. First, it supports monitoring of adherence to laws intended to regulate features of product design and performance, such as emission limits based on machine measurements and low ignition propensity laws. Second, it allows for the measurement of differences between products or changes in products that may impact exposure, such as comparing cigarettes that

- **Regulation of the contents of tobacco products.** The Conference of the Parties, in consultation with competent international bodies, shall propose guidelines for testing and measuring the contents and emissions of tobacco products, and for the regulation of these contents and emissions. Each Party shall, where approved by competent national authorities, adopt and implement effective legislative, executive and administrative or other measures for such testing and measuring, and for such regulation.

- **Regulation of tobacco product disclosures**. Each Party shall, in accordance with its national law, adopt and implement effective legislative, executive, administrative or other measures requiring manufacturers and importers of tobacco products to disclose to governmental authorities information about the contents and emissions of tobacco products. Each Party shall further adopt and implement effective measures for public disclosure of information about the toxic constituents of the tobacco products and the emissions that they may produce.

WHO (2003)

Figure 5.6 WHO FCTC Articles 9 and 10: *Regulation of the contents of tobacco products* and *Regulation of tobacco product disclosures,* respectively

heat versus burn tobacco or cigarettes containing tobacco with high versus low tobacco-specific nitrosamine (TSNA) levels. Third, systematic product testing is important because it contributes to the development of general expertise and capacity for tobacco product regulation. Historically, most product-related expertise has been limited to the tobacco industry, and public health scientists have been at a disadvantage in understanding the relevance of product characteristics for health and behaviour, as in the case of "light" and low-tar cigarettes (Parascandola, 2005). While doubtless new, more sophisticated technologies and measures will be developed, such progress will be limited without a network of experienced, public health oriented scientists and technicians.

The task of tobacco product evaluation is complicated by the fact that regulatory requirements are still evolving; for many potential outcomes validated standard measures have not yet been identified. While the FCTC mandates regulation and reporting of tobacco product contents and emissions, guidance for implementation of these articles is still under development by the Conference of the Parties (COP) (http://www.who.int/tobacco/fctc/cop/en/). Thus, it is not clear yet which specific measures will be required in the implementation of the FCTC.

This section will review existing measures relevant to tobacco product regulation as well as discuss challenges and research needs. First, the characteristics of some existing tobacco product regulations will be described to illustrate the range and types of provisions used in current regulations. Second, the section will cover proximal measures for assessing tobacco product regulations, which focus on the product itself. Measures of product content, design and emissions will be discussed, including the limitations of smoking machine protocols for assessing actual human exposure. Third, the section will address distal measures as well, which focus on the impact of regulations for human exposure and risk, including biomarkers and surveillance activities.

Existing tobacco product regulations

Tobacco product regulation remains in its early stages but is evolving rapidly. A number of countries and jurisdictions have adopted product regulations, including ingredient disclosure laws, limits on tar and nicotine yields, low ignition propensity (fire safety) standards, or bans on additives, such as candy flavourings. However, there is little uniformity across jurisdictions in

the content of these laws. Some jurisdictions require constituent disclosure only, while others set standards or limits on content or emissions. Moreover, while some product standards target toxic properties directly (such as by establishing maximum tar or carbon monoxide limits), others target properties that, while not directly harmful, affect addictiveness or consumer appeal (such as by controlling flavour additives that affect the appeal of the product to children).

Currently, there is no centralized, systematic monitoring of tobacco product regulations. The data collected in *Tobacco Control Country Profiles 2003* includes some information on regulation for many countries (Shafey *et al.,* 2003). However, the available data does not specify the details of the regulations (i.e. which constituents are regulated, what product standards or limits are imposed) and it is not updated regularly. As countries continue to debate and enact new tobacco product regulations, there is a need for comprehensive tracking of the evolving regulatory environment.

A few countries and jurisdictions have adopted tobacco product regulations and provide early models of the types of regulatory mechanisms that may be implemented more widely. There are also a number of countries that have adopted International Organization for Standards (ISO) emission limits for tar and nicotine aimed at reducing tobacco related harm, including Brazil, Thailand, China, South Africa, and Malaysia, as well as the European Union (EU).

There are at least five main types of tobacco product regulations that can currently be observed: 1) regulations that require disclosure of product information (such as tar and nicotine content) (Figure 5.7); 2) regulations intended to reduce product toxicity and harm (such as maximum emission limits for tar and nicotine) (Figure 5.8); 3) regulations intended to reduce the addictiveness and/or attractiveness of tobacco products (such as bans on ingredients that impact nicotine delivery or bans on flavour additives that may make a product more attractive to children) (Figure 5.9); 4) regulations intended to prevent fires caused by cigarettes (ignition propensity laws) (Figure 5.10); and 5) bans (or removal of bans) on product categories (Figure 5.11). A few examples are provided in Table 5.15 to illustrate the range of different types of product regulations that are currently being implemented or discussed.

A more detailed presentation of country specific regulations follows:

Canada:

The Tobacco Reporting Regulations, developed under the authority of the 1997 Tobacco Act, require manufacturers and importers of tobacco products to Canada to submit to the Minister of Health information on tobacco product composition and emissions. This includes, for smoked products, information on more than 40 toxic emissions in both mainstream and sidestream smoke under two different smoking regimens, and information on more than 20 specific constituents of whole/unburned tobacco (http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/index_e.html).

Brazil:

The National Health Surveillance Agency (ANVISA) is charged with regulating a wide variety of consumer products in the interest of public health, including cigarettes and other tobacco products. ANVISA resolution No. 46 (March 21, 2001) establishes maximum tar, nicotine, and carbon monoxide yields for cigarettes, and the tobacco industry is required to submit annual reports that identify and list by brand all ingredients and additives in every tobacco product produced in Brazil (http://www. anvisa.gov.br/eng/tobacco/index.htm).

European Union:

In effect since 2004, a directive of the European Parliament to Member States limits the maximum yield of tar, nicotine, and carbon monoxide in cigarettes manufactured or marketed in the EU (10 mg tar, 1 mg nicotine, and 10 mg carbon monoxide). The directive also requires the tobacco industry to submit to Member States a list of ingredients, and quantities thereof, used in the manufacture of those tobacco products by brand name and type (http://ec.europa.eu/health/ph_determinants

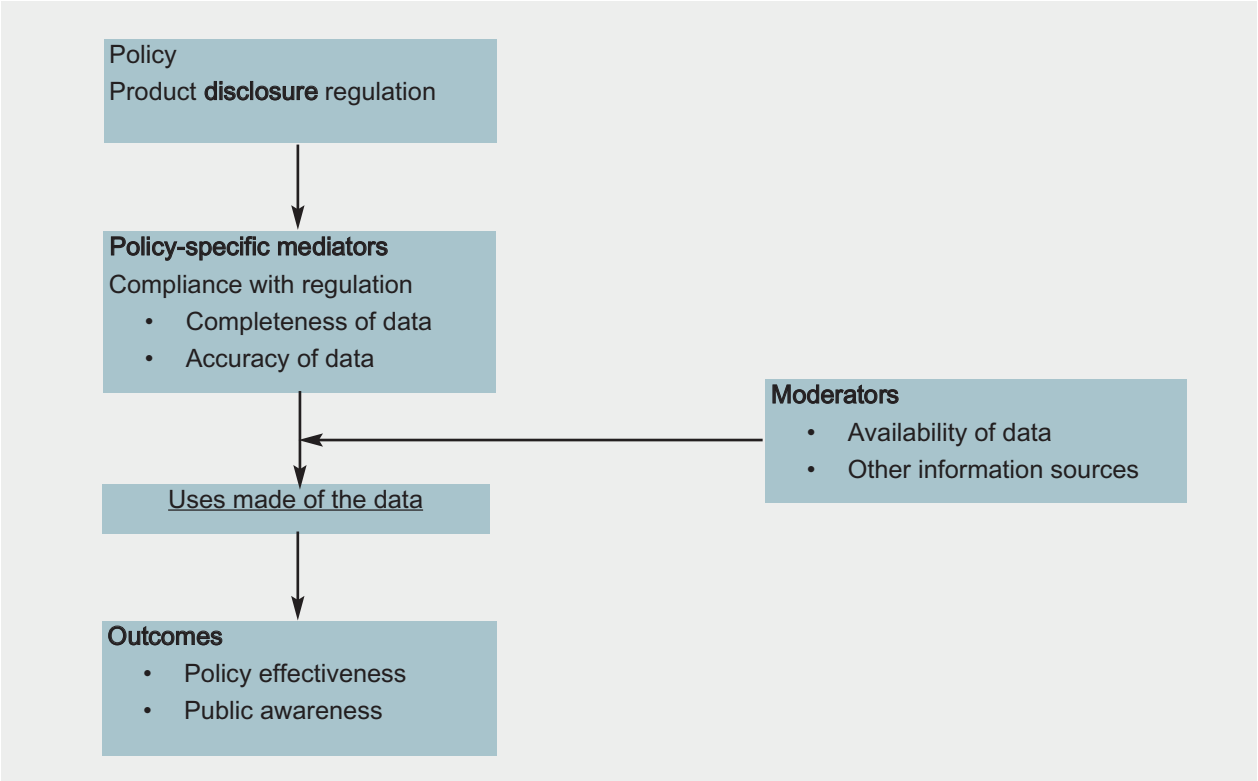

Figure 5.7 Conceptual framework for the evaluation of product disclosure requirements

/life_style/Tobacco/tobacco_en.htm).

United States:

The Comprehensive Smoking Education Act of 1984 and Comprehensive Smokeless Tobacco Health Education Act of 1986 require cigarette and smokeless tobacco manufacturers to submit a list of ingredients added to tobacco to the Secretary of Health and Human Services. However, the law requires that the list not identify the specific brand or company using the ingredients. Smokeless tobacco manufacturers must also report the quantity of nicotine in each product according to standard measures. (Centers for Disease Control and Prevention, 1997a; http://www.cdc.gov/tobacco/FCLA/terms.htm).

Massachusetts:

Manufacturers of cigarettes and smokeless tobacco products sold in Massachusetts must report the product's nicotine yield according to a standardised protocol. The State also proposed a regulation requiring reporting of all ingredients added to cigarettes by brand, but this regulation was barred by a federal court (http://www.mass.gov/dph/mtcp/legal/prodreg.htm).

New York State:

In 2004, New York State became the first jurisdiction in the world to implement reduced ignition propensity (RIP) standards for cigarettes; Canada became the first country to do so in 2005. Both the New York State and Canadian laws stipulate that at least 75% of cigarettes must self-extinguish before burning the full length of their tobacco columns using a

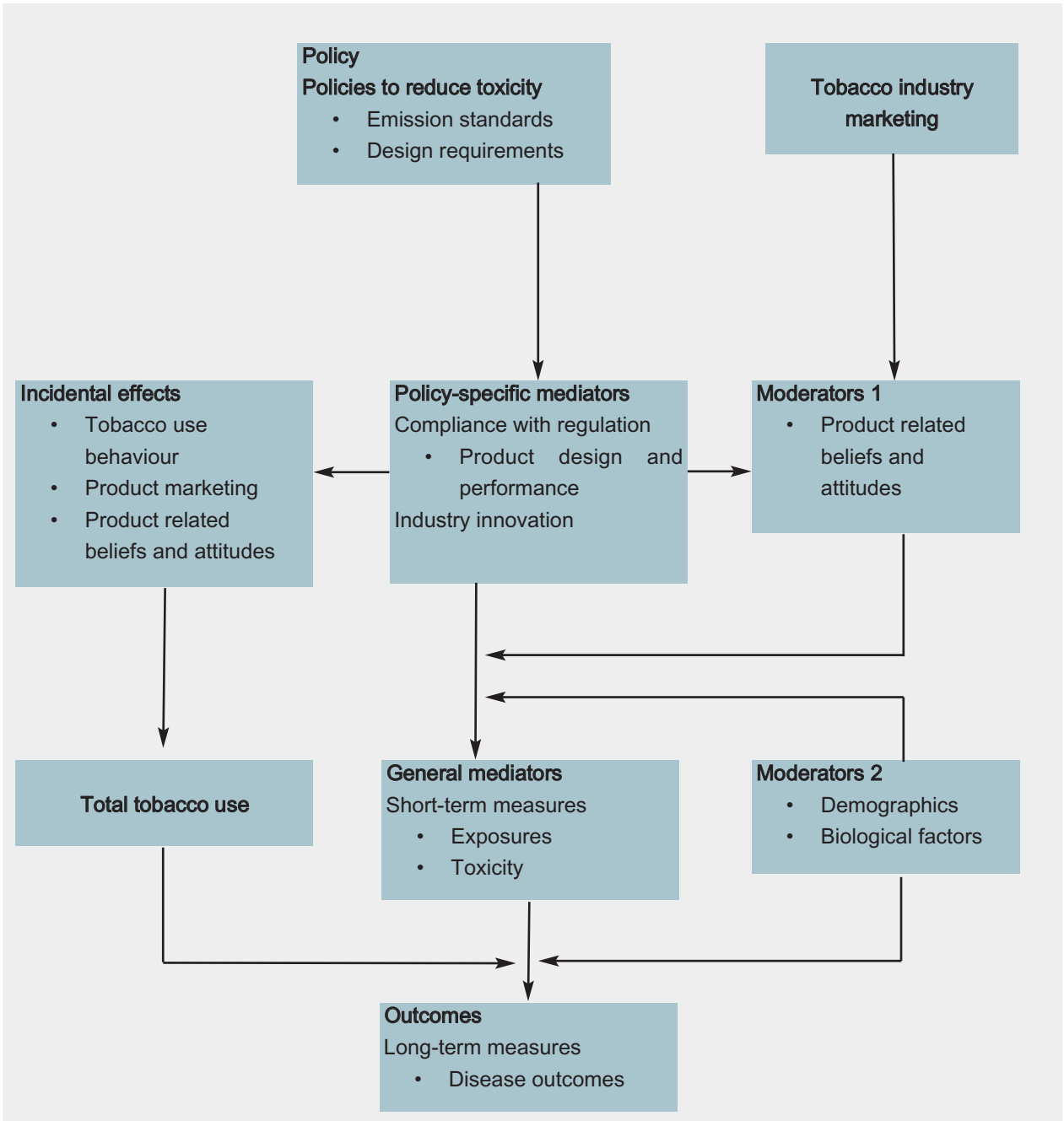

Figure 5.8 Conceptual framework for the evaluation of policies to reduce tobacco toxicity

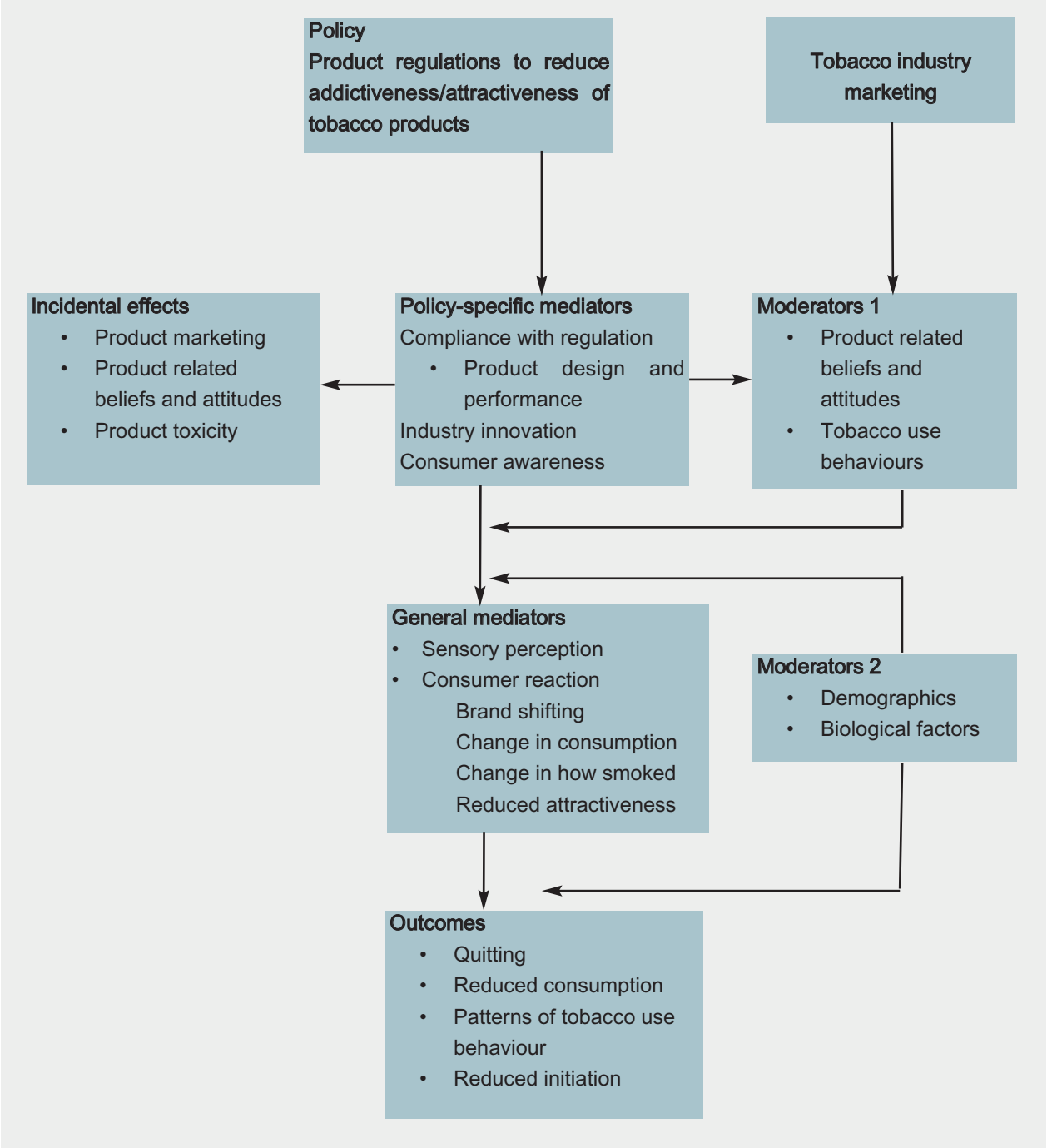

Figure 5.9 Conceptual framework for the evaluation of policies to reduce the attractiveness and/or addictiveness of tobacco products

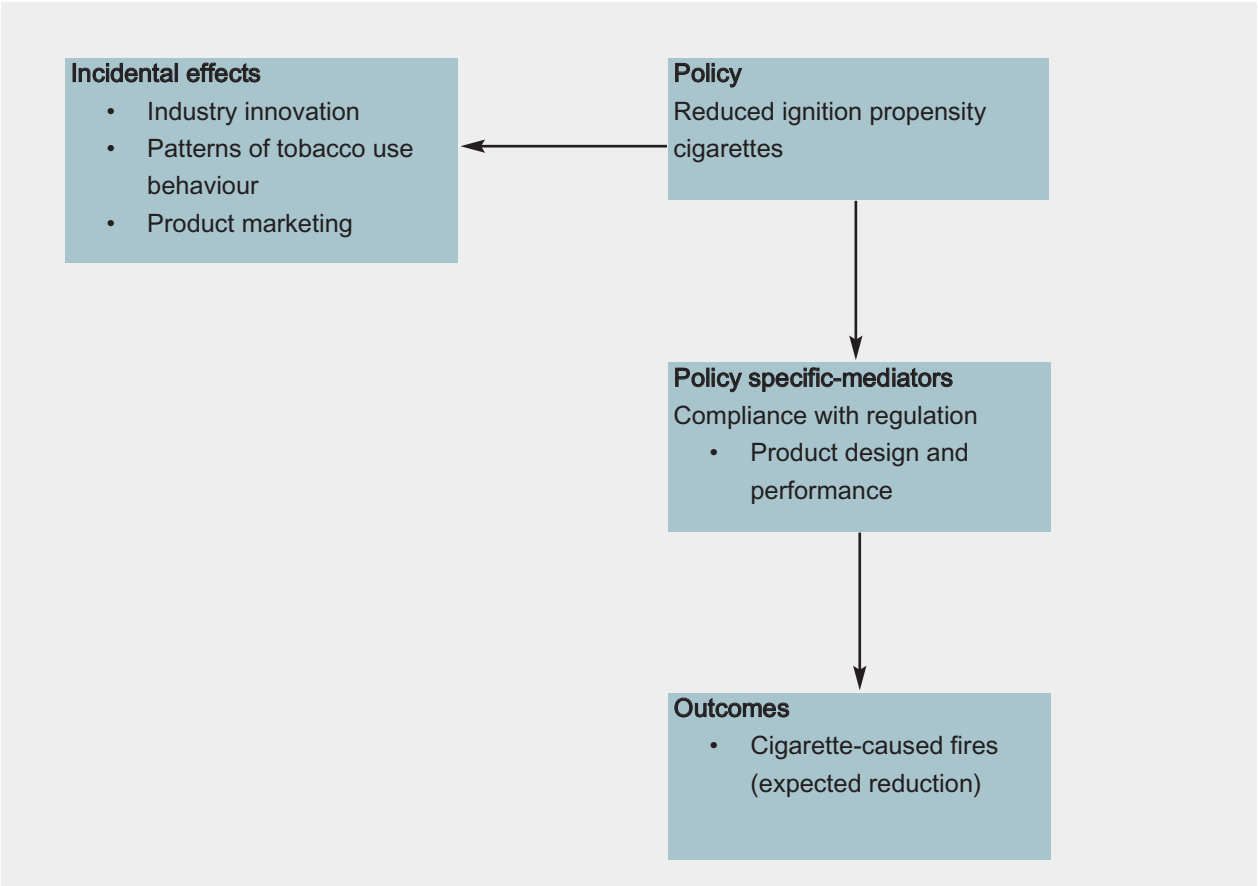

Figure 5.10 Conceptual framework for the evaluation of tobacco product regulation to reduce fires

standardised method for assessing ignition propensity. Both laws use the American Society for Testing and Materials (ASTM) method, which involves positioning a cigarette on one of three standard substrates to generate sufficient heat to continue burning, and thus potentially cause ignition of bedding or upholstered furniture (ASTM E2187-04 *Standard Test Method for Measuring the Ignition Strength of Cigarettes*; http://www.astm.org/cgi-bin/SoftCart.exe/database.cart/redline_pages/e2187.htm?E+mystore).

So far, no jurisdiction has successfully enacted comprehensive regulations governing the design, contents, and emissions of tobacco products. Product performance standards, for example, could be used to reduce known harmful emissions. Currently available data and methods are insufficient to allow for a quantitative estimate of the public health impact of reductions in specific constituents in tobacco smoke. However, evidence shows that there is a wide variation globally between countries and cigarette brands in emissions of tar, nicotine, and carbon monoxide, as well as major carcinogens, suggesting that reductions are feasible and are justifiable on a precautionary basis. A survey of transnational and locally-produced cigarettes in 35 countries found, when measured by a standardised machine

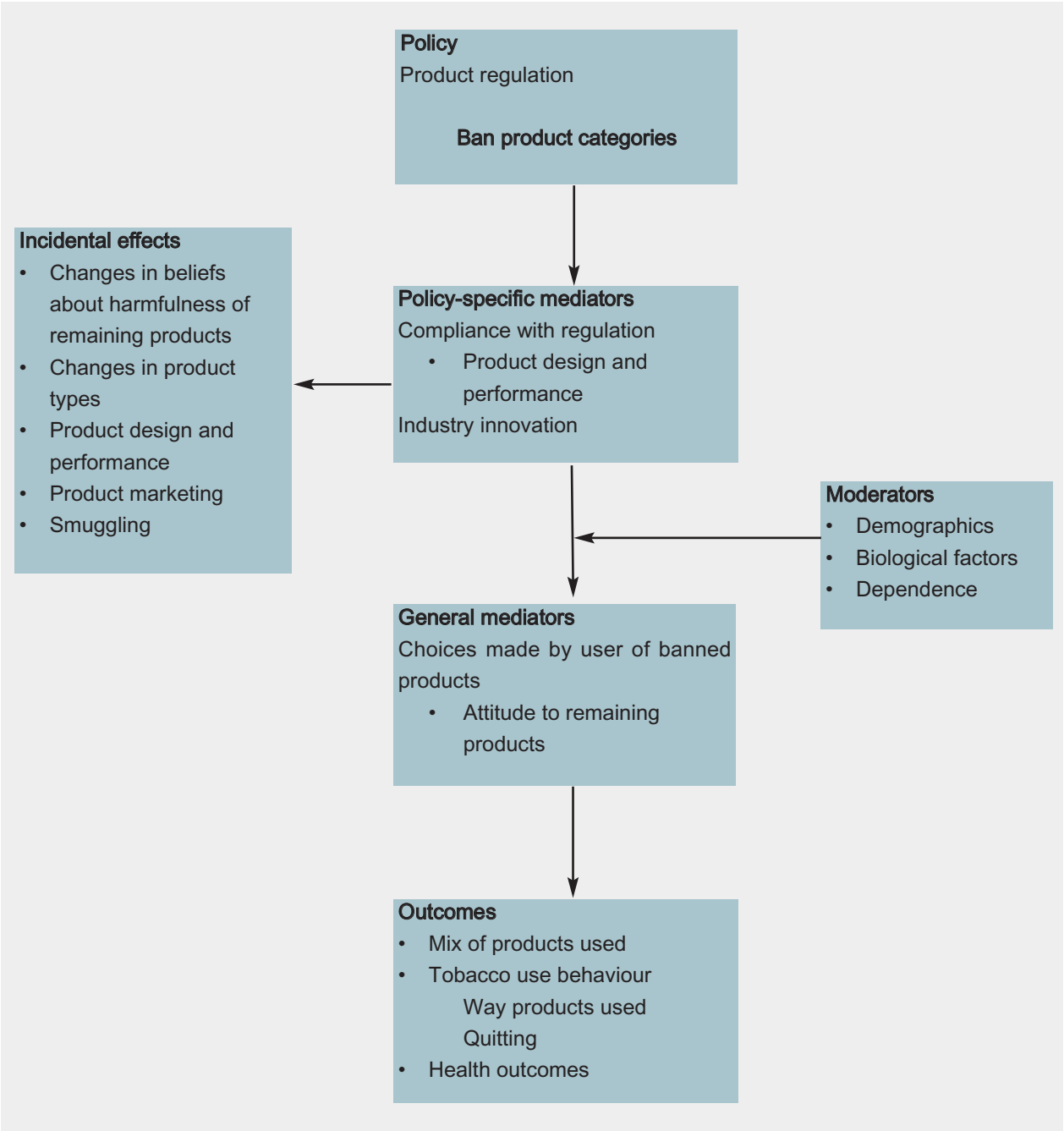

Figure 5.11 Conceptual framework for the evaluation of tobacco product regulation to ban specific product categories

Regulation Type	Requirements
Product Disclosure Example: Canada Tobacco Reporting Regulation	Reporting of 40 constituents in mainstream and sidestream smoke and 20 specific constituents of whole/burned tobacco according to specified protocols.
Reduce Harm Example: European Union Directive 2001/37/EC	Maximum cigarette emission yields: 10 mg tar, 1 mg nicotine, and 10 mg caron monoxide determined by specified machine testing method.
Reduce Addictiveness and/or Attractiveness	Proposed ban on additives that increase the addictiveness of tobacco products.
Reduce Cigarette-Caused Fires Example: New York State Fire Safety Standard for Cigarettes	Mandatory performance standards require that at least 75% of cigarettes must self-extinguish before burning the full length of their tobacco columns, utilizing the American Society for Testing and Materials (ASTM) method for measuring ignition propensity.
Product Bans Example: European Union Directives 2001/37/EC, 92/41/EEC	Prohibits sale and marketing of "all products for oral use, except those intended to be smoked or chewed, make wholly or partly of tobacco."

Table 5.15 Product Regulations

smoking protocol, wide variation in emissions of tar (6.8 to 21.6 mg), nicotine (0.5 to 1.6 mg), and carbon monoxide (5.9 to 17.4 mg), with cigarettes from the Eastern Mediterranean, Southeast Asia, and Western Pacific WHO regions reporting higher deliveries than those from other regions (Calafat *et al.*, 2004). Further analyses from this survey have revealed that mainstream smoke levels of tobacco-specific nitrosamines (TSNAs) and poly-cyclic aromatic hydrocarbons (PAHs) also vary widely across countries, including within the same multinational brand (Wu *et al.*, 2005; Ding *et al.*, 2006). Given the observed variation, one regulatory proposal involves a system for controlling toxins and carcinogens in cigarettes by the establishment of upper limits based on the median of the existing market (Gray & Boyle, 2002). There have also been proposals to reduce nicotine to non-addictive levels to prevent the development of nicotine addiction in young people (Benowitz & Henningfield, 1994). However, so far such proposals have not been implemented in any regulations, and currently there is insufficient evidence to predict what the potential impact of such regulations would be on health outcomes or smoking behaviour.

Regulations requiring tobacco product disclosure, as required in FCTC Article 10, also have an essential role. In order to effectively establish product standards and regulate manufacture of the product, regulators must have valid information about product design, contents, and emissions. Standardised reporting and disclosure by manufacturers assists regulators in monitoring changing trends in product design across the market that may impact public health. Additionally, such disclosures allow for more effective evaluation of the impact and potential unintended effects of new regulatory requirements on product design and emissions.

In order to guide evaluation of tobacco product regulations, it is important to have a conceptual model of the proximal and distal effects of the regulation, taking into account other factors that mediate or moderate those effects (policy-specific mediators, general

mediators, and outcomes). The model should also include other incidental effects of a regulation that are important to evaluating the impact of a regulation on public health. As a general framework, it is likely that the impact of tobacco product regulations on intended health outcomes will be moderated by changes in product design and performance, product marketing, product-related beliefs and attitudes, and tobacco use behaviour, which in turn are expected to influence exposures to tobacco constituents and emissions.

However, because tobacco product regulations can have a range of different goals, multiple conceptual models are needed to understand different types of regulations, just as a variety of methods and measures are needed for evaluating different regulations. Five generalized logic models are provided to guide the development of evaluations of tobacco product regulations (Figures 5.7 to 5.11). These five logic models reflect the five major types of tobacco product regulations identified above (disclosure, reducing product harm, reducing product addictiveness/ attractiveness, preventing fires, product bans). The logic models all start with the introduction of a new policy and then proceed to show a pathway to proximal and distal variables or constructs to be used in assessing the effects of the regulation. Key mediators and moderators, along with incidental effects, are also identified for inclusion in evaluations. For example, a regulation requiring manufacturers to disclose product information to consumers should be evaluated ultimately in terms of its impact on public awareness of the information communicated and the effectiveness of those communications in successfully informing the public about product characteristics. Those effects may be moderated by the availability of relevant data and the presence of other information sources. In contrast, a regulation that aims to reduce product toxicity and harm should be evaluated ultimately in terms of its impact on disease outcomes. Short-term measures of changes in exposures or toxicity, such as use of biomarkers, may substitute for actual measures of disease outcome. These outcomes are likely to be moderated by demographic and biological factors, as well as consumers' product related attitudes and behaviours. Thus, these two types of regulations require very different logic models for their evaluation. Before developing an evaluation plan or protocol, it is important to have a logic model that maps out the goals of the regulation and relevant factors that are expected to influence its effectiveness.

Proximal measures

The most proximal measures of the effectiveness of a tobacco product regulation include measures of the product itself. The first step in evaluating a performance standard regulation, for example, is to measure compliance through product testing. For reduced ignition propensity cigarette laws: does the product meet the full-length burn testing requirements specific in the regulation? For tar and nicotine limits: does the product meet the specified maximum tar and nicotine threshold according to the standardised test method required in the legislation? The specific testing that is required will depend on the requirements and goals of the law. There are a wide variety of product characteristics that could potentially be subject to or be affected by regulation. In addition to assessing compliance, assessment of tobacco product characteristics is important for informing the development of new or modified regulations and for identifying potential unanticipated product changes.

Both tobacco and tobacco smoke are very complex matrices, consisting of thousands of compounds. Over 3044 constituents have been isolated from tobacco (Roberts, 1988); it is estimated that there are over 4800 compounds in mainstream cigarette smoke (Green & Rodgman, 1996). At least 69 carcinogens have been identified in cigarette smoke, including 11 classified as Group 1 known human carcinogens by IARC (Hoffmann & Hoffmann, 1997). Moreover, the composition of cigarettes and cigarette smoke has changed substantially since the 1950s, as the product itself has changed, with changes in tobacco blend, processing techniques, cigarette design, the introduction of filters, and use of additives (Hoffmann & Hoffmann,

1997). At the same time, it is essential to study the product under actual conditions of use, because differences in smoking behaviour can have a substantial influence on product emissions.

However, because of the complexity of tobacco smoke, it is extremely difficult to estimate the health effects of specific constituents in tobacco and tobacco smoke. There have been efforts to quantify the relative contribution to risk of individual tobacco smoke constituents, particularly for cancer, but such estimates are fraught with uncertainty and numerous assumptions. Possibly the most comprehensive such risk assessment, including cancer and non-cancer risk indices for 158 known hazardous chemicals in cigarette smoke, found that these known risk agents underestimated observed cancer rates in cigarette smokers by 5-fold, suggesting that actual exposures were dramatically underestimated and/or that other important carcinogens or mechanisms of action exist that were not included in the risk assessment (Fowles & Dybing, 2003). Further research is needed to understand the individual and combined effects of the many constituents in tobacco and tobacco smoke.

Sampling and preparation

To effectively monitor products as used by consumers, it is essential to follow an effective protocol for obtaining product samples and storing and preparing the product for analysis. Products should be purchased from a range of retail vendors to ensure that the product tested is representative of the product available to consumers and that different manufacturing lot numbers are represented in the sample. In addition, a rigorous protocol should be employed for storing samples. For example, cigarettes and smokeless tobacco should be stored at –70° Celsius in vacuum sealed bags to prevent the effects of aging. Sources of guidelines and protocols for sampling and preparation are available in Table 5.16.

Product content

Official testing of cigarettes has generally focused on measurements of cigarette smoke constituents (i.e. tar, nicotine, and carbon monoxide) using standard machine smoking protocols rather than of the unburned tobacco itself. However, the composition of smoke is directly dependent on the profile of constituents in the tobacco (Fischer *et al.,* 1990). While cigarette design features and human smoking behaviour can dramatically vary the content (both qualitatively and quantitatively) of the smoke and the smoker's exposure, the characteristics of the tobacco are equally important. Moreover, there is wide variation in the concentration of nicotine and other important constituents in the tobacco filter in cigarettes from different brands and countries around the world (IARC, 2004). Additionally, trends in tobacco processing and blending over time may impact public health. For example, while increasing tobacco nitrate levels was seen as a way of reducing

Sampling	**ISO 8243: 2006 Cigarettes: Sampling**
Sample Preparation	ISO 3402: 1999 Tobacco and Tobacco Products: Atmosphere for Conditioning and Testing
	Health Canada: Preparation of Cigarettes from Packaged Leaf Tobacco for Testing (Health Canada, 1999a)
	US Centers for Disease Control and Prevention: Protocol to Measure the Quantity of Nicotine Contained in Smokeless Tobacco Products Manufactured, Imported, or Packaged in the United States (Centers for Disease Control and Prevention, 1997a).

ISO: International Organization for Standardization (www.ISO.org)

Table 5.16 Sampling and Preparations Standards

PAHs in tobacco smoke in the 1960s, in the 1980s scientists recognized that increased nitrate levels were also increasing the yield of nitrosamines in tobacco and smoke (Brunnemann & Hoffmann, 1982; Fischer *et al.*, 1989a). Measurement of constituents in tobacco can provide the earliest point of monitoring for regulation and possible intervention.

There are a range of well established methods for measuring the chemical characteristics of tobacco that have long been in use by tobacco manufacturers and agricultural scientists. Since the 1950s, there have been significant developments in analytical methods for studying tobacco products with the introduction of technologies such as gas chromatography and mass spectrometry (Green & Rodgman, 1996). There are three standard setting organisations that have developed and adopted methods for analysis of tobacco and cigarette smoke: the International Organization for Standardization (ISO), the Association of Analytical Communities International (AOAC), and the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA). The CORESTA board is made up of 14 member companies from the tobacco industry (http://www.coresta.org/Home_Page/Presentation%20of %20CORESTA_April07.pdf).

Additionally, the tobacco-related efforts of ISO have historically been driven primarily by the needs of industry and, thus, they have not adopted methods for many areas of particular interest to public health (i.e. emissions as driven by users behaviour, free-base nicotine, presence of carcinogens) (Bialous & Yach, 2001). Additionally, Health Canada and the US Centers for Disease Control and Prevention (CDC) have published official methods for manufacturer reporting of tobacco constituents. Table 5.17 summarizes the existing methods for whole tobacco analysis and their sources. While an exhaustive discussion of tobacco constituents and associated methods is beyond the scope of this section, a few key agents are discussed here which have particular relevance and importance for product regulation.

Nicotine:

Standardised protocols for extracting and measuring nicotine in whole tobacco using gas chromatographic analysis have been adopted and widely used by industrial and professional organisations (ISO (15152: 2003), CORESTA (No. 62, Feb 2005), AOAC (920.35)), as well as public health agencies (Health Canada, Massachusetts Department of Public Health, CDC). It is important to measure nicotine levels in tobacco as nicotine is the primary driver of smoking behaviour and addiction, and the level of nicotine in tobacco is an essential predictor of nicotine levels in smoke emissions delivered to the tobacco user. A recent report found that nicotine levels in US cigarettes have increased from 1998 to 2005 by about 11%, and concluded that this trend was due primarily to an increase in nicotine in the raw tobacco used in cigarettes (Connolly *et al.*, 2007).

The Massachusetts Department of Health and the CDC also require reporting of the amount of nicotine that is present in the unprotonated, free-base form in smokeless tobacco. This form of nicotine is absorbed more easily through the mucosal membranes in the mouth (Brunnemann & Hoffmann, 1974). Measurements of unprotonated nicotine content in tobacco provide a more accurate assessment of the quantity of nicotine in the product that is delivered to the user (Hoffmann *et al.*, 1995). Free nicotine content in tobacco can be calculated using the Henderson-Hasselbalch equation, which is based on measured pH and nicotine content. This information is important for understanding trends in product use and for providing a basis for monitoring and regulating nicotine content in the product. A CDC study that measured free nicotine in popular brands of smokeless tobacco, found that the brands with the largest amount of unprotonated nicotine also are the most frequently sold (Richter & Spierto, 2003). In smokeless tobacco products, manipulation of tobacco pH and free-base nicotine levels has also been used by the tobacco industry as part of a “graduation strategy,” whereby novice users are introduced to products with lower nicotine

Analyte	Analysis Method	Protocols
Nicotine	Gas chromatographic analysis	Health Canada; CORESTA No. 62, Feb 2005; CDC; AOAC 920.35
Total Moisture	Weight before and after heating in oven at 99° C	CDC; AOAC 966.02
pH	pH meter	Health Canada; CDC
Free Nicotine	Calculated from pH and nicotine using the Henderson-Hasselbalch equation	Centers for Disease Control and Prevention (1997a): Massachusetts Department of Public Health
Nitrosamines	Gas chromatographic analysis	Health Canada; CORESTA (under development); CDC (Song & Ashley, 1999)
Nitrates	Continuous Flow Analysis	Health Canada; CORESTA No. 36, Nov 1994
Metals	Atomic absorption spectroscopy (AAS) analysis	Health Canada; IARC (1986)
Ammonia	High Performance Liquid Chromatography (HPLC)	Health Canada
Humectants	Gas chromatographic analysis	
Pesticide residues	Gas chromatographic analysis	CORESTA No. 2, May 1997; ISO 4389:2000

ISO: http://www.iso.org
CORESTA: http://www.coresta.org/
AOAC: http://eoma.aoac.org/methods
Health Canada: http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/index_e.html
Massachusetts: http://www.mass.gov/dph/mtcp/legal/prodreg.htm
CDC: US Centers for Disease Control and Prevention: Protocol to Measure the Quantity of Nicotine Contained in Smokeless Tobacco Products Manufactured, Imported, or Packaged in the United States.
Federal Register. Vol. 62, No. 85, Friday, May 2, 1997. p. 24115 - 24117 (recommended method for determination of organochlorine pesticide residues on tobacco)

Table 5.17 Whole Tobacco Analysis Methods

delivery and eventually progress to higher delivery products (Connolly, 1995; Tomar *et al.*, 1995). Thus, continued monitoring of pH levels and free-base nicotine in tobacco is important for monitoring the addiction potential of products (see following subsection on constituents in mainstream and sidestream tobacco smoke).

Nitrosamines:

Tobacco-specific *N*-nitrosamines (TSNAs), *N*-nitrosonornicotine (NNN), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), and *N*-nitrosoanabasine (NAB) are present in both unburned tobacco and tobacco smoke. NNN and NNK play a significant role in cancer induction by tobacco products (Hecht, 1998). The TSNAs are formed from tobacco alkaloids during the curing and

processing of tobacco. Studies have suggested that the tobacco blend may be the most important determinant of TSNAs (UK Laboratory of the Government Chemist, 2000; Harris, 2001). Oriental and flue-cured Virginia tobaccos contain lower levels of nitrate and TSNAs, while higher levels are found in air-cured burley tobaccos (Fischer *et al.*, 1989a; Bush *et al.*, 2001; Peele *et al.*, 2001).

NNN and NNK make a likely target for surveillance and regulation as they play a significant role in tobacco-related cancer, are measurable even in trace quantities, and are specific to tobacco. Moreover, in recent years it has been demonstrated that use of new curing technologies can considerably reduce the levels of TSNA, especially NNK, or even completely eliminate them (Bush *et al.*, 2001; Peele *et al.*, 2001). A study conducted by the CDC comparing TSNA levels in cigarettes purchased in 13 countries and the USA, found that in 11 of the 13 countries locally-purchased Marlboro cigarettes had significantly higher TSNA levels than locally popular non-US brands purchased in the same country (Ashley *et al.*, 2003). Methods for measuring NNN and NNK have been adopted by Health Canada for regulation.

Additives/flavourings:

Additives may include both natural and synthetic agents that impart or enhance flavour. There are hundreds of additives that are used in tobacco products. While in some countries agents may be screened for their direct toxicity, little is known about the fate of these agents after the combustion process. Additionally, additives are used to make tobacco smoke less harsh and to increase nicotine delivery, thus impacting the physiological effects of smoking and resulting behaviours. Ammonium compounds raise the alkalinity of smoke, which increases the level of "free" nicotine delivered to the smoker, and have been employed as an additive in cigarettes (Henningfield *et al.*, 2004). Menthol, a chemical compound which acts as a mild local anesthetic, has been added to cigarettes beginning in the 1920s and 1930s to mask the harshness of tobacco smoke (Reid, 1993).

Detecting flavouring compounds and other additives is complicated by the fact that they may be present in very small quantities and, more importantly, researchers and regulators may lack specific information about their presence. Regulators rely on information from annual reports of additives used and their quantities by cigarette brand, such as in the EU, but many countries do not yet have such requirements. Because of the hundreds of additives that may be in use, testing for many of them is impractical. At least one study has quantified the presence of 12 potentially toxic flavour-related compounds in cigarette tobacco, including coumarine and safrole, and found that 62% of 68 brands tested contained one or more of these 12 compounds (Stanfill & Ashley, 2000). The UK Department of Health maintains a list of permitted additives to tobacco products (now numbering over 600) along with maximum inclusion limits, although their effects after combustion have generally not been tested (http://www.advisorybodies.doh.gov.uk/scoth/technicaladvisorygroup/additiveslist.pdf).

Evaluation of the impact of product regulations that control additives is limited by inadequate information and scientific data about the presence of additives in products by brand, and their potential effects on behaviour and health outcomes.

Product design

Cigarette design has evolved over the past half century, with the introduction of filters, changes in tobacco processing techniques, and the introduction of new materials and technologies. The resulting changes in product design and characteristics can have a substantial impact on the exposure a smoker receives. The types of materials used in filters and filter design can alter the chemical composition of the smoke that is inhaled, including the levels of carbon monoxide and other harmful constituents. Additionally, use of expanded or reconstituted tobacco in cigarettes can affect tar and nicotine yields and the profile of constituents. Cigarette length, circumference, and packing density can also alter the chemical composition of the smoke (Hoffmann & Hoffmann,

1997). Specific design features have also been employed to reduce cigarette ignition propensity, such as reduced tobacco density, reduced paper porosity, decreased circumference, and the removal or reduction of burn additives.

Physical characteristics of tobacco products should be measured in order to inform the development and implementation of tobacco product regulations and to support evaluation of regulations. The WHO Study Group on Tobacco Product Regulation (TobReg) has provided a recommended list for product characteristics to be reported by manufacturers for all brands on an annual basis (WHO Study Group on Tobacco Product Regulation, 2004; http://www.who.int/tobacco/global_interaction/tobreg/goa_2003_principles/en/index.html).

Table 5.18 includes the TobReg recommendations and additional product characteristics that should be measured to assess the impact of regulation on product design; reference numbers are provided for official laboratory protocols where they exist. This list is not exhaustive and should be revised regularly to account for new types of products and design innovations, such as new potential reduced exposure products (PREPs) that employ unconventional technology. These product characteristics are not necessarily direct targets of regulation or indicators of effectiveness of regulations in all cases. They should be considered, however, as useful measures for supporting the development and implementation of regulations, such as by revealing unexpected product changes in response to regulations (see following section on ventilation). Because most of the measures are routinely used by manufacturers in product characterization and quality control, such information should be requested from manufacturers by regulators where possible.

Cigarette ventilation:

Since the 1960s, cigarette filter ventilation has been the dominant design feature employed by manufacturers to reduce machine measured tar and nicotine yields (Kozlowski *et al.*, 2006). Small pinholes on cigarette filters allow

Product Characteristics	Measurements
Raw Materials	Tobacco blend, weight of tobacco, percentage of reconstituted tobacco, percentage of expanded tobacco, moisture content, firmness, contaminants (i.e. glass, pesticides, heavy metals).
Filter	Type, length, weight, density, ventilation[a], draw resistance[b], fiber residues, charcoal content.
Cigarette Body	Rod length, tipping paper length, diameter[c], air permeability[d].
Emission	Aerosol particle size with and without filter.
Ignition Propensity	Percent self-extinguishing.

[a]ISO 9512: 2002 Cigarettes - Determination of ventilation - Definitions and measurement principles
[b]ISO 6565: 2002 Tobacco and tobacco products - Draw resistance of cigarettes and pressure drop of filter rods - Standard conditions and measurement
[c]ISO 2971: 1998 Cigarettes and filter rods - Determination of nominal diameter - Method using a laser beam measuring apparatus
[d]ISO 2965: 1997 Materials used as cigarette papers, filter plug wrap and filter joining paper, including materials having an oriented permeable zone - Determination of air permeability

Table 5.18 Product Characteristics to be Measured to Assess Impact of Product Regulation

the smoke to be diluted by air drawn in by the smoker. However, studies have shown that smokers tend to place their fingers over these vent holes in order to derive a desired level of nicotine (Kozlowski *et al.,* 1980). Additionally, smokers puff harder to compensate and the greater flow through the cylinder also reduces the proportion of air that comes in through the vent holes. Because of this flexibility in the cigarette design, machine measured ISO/FTC tar yields do not reflect the actual range of exposures smokers receive. A study comparing ventilation (measured as the percentage of air drawn through the filter vents) across 32 brands of US cigarettes, with FTC tar yields ranging from 1 mg to 18 mg, found that the degree of ventilation (from 0 to 83%) varied inversely with standard tar, nicotine, and CO yields, suggesting that ventilation is a key determinant of machine measured yields (Centers for Disease Control and Prevention, 1997b). Similarly, another study accounted for 95% of the variance in ISO measured levels as a function of extent of filter venting (King & Borland, 2004).

A recent study assessed how UK cigarette manufacturers modified their products in order to comply with the EC 10-1-10 maximum yield regulation. Comparing 10 cigarette brands before and after the regulation was imposed, they found that machine measured tar was reduced from 11-13 mg to 10 mg for each brand, carbon monoxide yields dropped significantly from a median of 13 to 10 mg, as well as nicotine from a median of 1.0 mg to 0.9 mg. However, the only product design feature that showed consistent change was the amount of filter ventilation, as the median increased by 479% from 1999 to 2005. In contrast, other product design characteristics that were measured in the study, including filter weight, filter length, and tobacco length, showed no changes (O'Connor *et al*., 2006a). This study illustrates the importance of monitoring product design over time against a baseline level to understand how products are modified in response to new regulations, and whether the public health objectives of the regulation are being met. An alternative proposal involves imposing maximum tar, nicotine, and carbon monoxide yields along with a ban on filter vents (Kozlowski & O'Connor, 2002; Kozlowski *et al*., 2006).

Amount of ventilation should be measured in cigarettes, particularly for evaluating the introduction of new regulatory limits on emissions. Additionally, given the elasticity in exposures from ventilated cigarettes, measurements of emissions should take this variability into account, such as by measuring emissions in relation to a fixed amount of nicotine or per milligram of nicotine.

Reduced ignition propensity:

Reduced ignition propensity (RIP) regulations are relatively new, so limited data is available on their impact and effectiveness. One study conducted to evaluate the impact of the New York law, found that the average percentage of full-length burns was 10% for five leading brands sold in New York after the law went into effect, compared with 99.8% for cigarette brands from California and Massachusetts (Connolly *et al*., 2005). These findings confirm that the law did result in changes to the product design that achieved the aims of the legislation. Product testing can be used to assess compliance and product performance following the introduction of RIP laws. It is also important to evaluate smokers' reactions to changes in cigarette design to identify potential unintended effects on smoking behaviour. A survey of adult smokers' reactions to RIP cigarettes found that while smokers in New York State were more likely to report that their cigarettes went out between puffs, they were no more likely than smokers in states without RIP laws to report differences in cigarette taste, suggesting that RIP cigarette laws do not substantially impact consumer acceptability (O'Connor *et al*., 2006b). Moreover, proximal measures of the product itself cannot assess more distal outcomes, such as changes in the number of fires caused by cigarettes. Distal measures and surveillance are discussed in the following section.

Product Emissions

Measuring the contents and characteristics of tobacco smoke has been the primary focus of

tobacco product testing and regulation efforts since the 1960s. Measuring the contents of tobacco smoke provides direct information about the agents the smoker is exposed to. However, these measures also have substantial limitations; while they allow for the identification of important constituents in tobacco smoke, they do not necessarily reflect exposure under actual smoking conditions. Measurements of product emissions have typically relied on machine collection of tobacco smoke, which does not reflect actual human smoking behaviour. This section will review various machine smoking protocols, and their limitations, and will then discuss specific constituents in tobacco smoke that have been proposed for surveillance and regulation.

Machine smoking methods:

Machine smoking methods for measuring tar, nicotine, carbon monoxide, and other constituents in cigarette smoke have been widely used in many countries over the past 30 years. The procedure involves having a machine "smoke" cigarettes according to fixed parameters that determine the frequency, duration, and volume of puffs, as well as the butt length. The particulate matter is collected onto a Cambridge filter pad made of extremely fine diameter glass fibers. Mainstream smoke particulates are collected on filter pads located behind the cigarette port, while sidestream smoke is collected with the use of BAT "fishtail" devices, which allow smoke from the end of the cigarette to travel up a glass enclosure to a filter pad located at the top. Filter pads are weighed before and after a "smoking" run to determine the Total Particulate Matter (TPM) (the amount of particulates accumulated on the filter pad). A solvent is used to remove the chemicals from the filter pads, and once this extraction is complete, various chemical and physical separation techniques are used to isolate the desired component(s). Once the desired chemical has been isolated, various analytical methods (such as gas chromatography with mass spectrometry) are used to determine the amount of chemical collected. Gas phase chemicals, such as carbon monoxide, may pass through the filter pads and into collection bags for measurement.

To ensure consistency across measurements, standard parameters are used to control the machine's puffing activity. The parameters most widely in use were based on a protocol outlined by the US Department of Agriculture (Ogg, 1964); a similar protocol had been proposed by American Tobacco Company researchers in 1936 (Bradford *et al.*, 1936). The protocol called for 2-second, 35-mL puffs to be taken until a 23-mm butt length remained on the cigarette. These parameters were somewhat arbitrarily selected based on informal observations; Ogg reportedly stated that he arrived at the parameters he chose by informally observing people smoking, timing them with the aid of a stopwatch, and measuring the length of the "unsmoked" cigarette left in the ashtray (Harold & Pillsbury, 1996). When the US Federal Trade Commission adopted this method for use in its testing laboratory, the agency acknowledged that these parameters were not intended to mimic the smoking behaviour of any particular individual or even an "average" smoker, but the application of a uniform standard would, they stated, allow for meaningful comparisons across products (Press release, August 1, 1967). ISO adopted a similar set of parameters in their cigarette testing method (ISO Standard 3308: 2000 (4th edition), *Routine Analytical Cigarette-Smoking Machine: Definitions and Standard Conditions*).

However, beginning in the 1980s, a more profound understanding of smoking behaviour revealed that smokers who switched to cigarettes with lower machine measured tar and nicotine yields modified their smoking behaviour to compensate by taking more frequent puffs, inhaling the smoke more deeply, covering up filter ventilation holes, and smoking more of each cigarette (Benowitz *et al.*, 1983; National Cancer Institute, 2001). More accurate measures of the actual smoke exposure of a given individual can be obtained through the study of smoking topography, where the smoker uses a mouthpiece connected to a device that measures parameters of smoking behaviour (such as number of puffs, puff volume, duration, velocity, and the intervals between puffs) (Djordjevic *et al.*, 2000; Lee

et al., 2003). However, while smoking topography measurements are valid for assessing individual exposure, the parameters vary widely across the population and no single set of smoking parameters can effectively represent this variation.

Because of growing concerns about the validity of the FTC/ISO parameters, alternative machine smoking regimens have been proposed. In particular, the FTC and ISO smoking regimens do not account for the fact that smokers may cover ventilation holes with their fingers, and alternative smoking regimens have attempted to address this. The Commonwealth of Massachusetts in the USA currently tests cigarettes with a 45 mL puff drawn twice per minute with 50% of the filter vent holes blocked (Commonwealth of Massachusetts, 2007). Canadian government testing standards require a more intensive smoking regimen, where 55 mL puffs are drawn twice per minute with 100% of the vent holes blocked (Health Canada, 1999b). While these regimens also cannot represent the wide variation in human smoking patterns, they may be less likely to underestimate actual human exposure by using more intense puffing parameters. This may be especially important for lower yield products for which smokers may compensate with more intense puffing behaviour.

A "compensatory" machine smoking regimen was proposed; rather than smoking all brands using the same puffing regimen, the compensatory regimen attempts to mimic the systematic differences in human smoking across different products, whereby lower nicotine yield brands are smoked more intensely. It was suggested that the puff volume and puff frequency be varied according to the ISO nicotine yield. For brands with <10 mg tar, a 40 mL puff is taken every 60 seconds. With every decrease of 0.1 mg nicotine, the puff volume rises by 4 mL and the puff frequency falls by 4 seconds. For example, a cigarette with 0.5 mg nicotine under the ISO method would be smoked at 60 mL puffs every 40 seconds, whereas a 0.1 mg cigarette would be smoked at 76 mL puffs every 24 seconds (Kozlowski & O'Connor, 2000). Another alternative is to tie analysis of constituents to a fixed nicotine level whereby cigarettes are smoked to predetermined nicotine yields and the levels of other constituents assessed from that (Hammond *et al.*, 2007b). Alternatively, TobReg of WHO has recommended use of yields per mg of nicotine, using standard puffing regimens. (WHO Study Group on Tobacco Product Regulation, 2004).

A recent study compared the performance of these four smoking regimens against actual human smoking patterns and biological measures of exposure to assess how well they reflect actual exposures smokers are likely to receive (Table 5.19) (Hammond *et al.*, 2006b). The aim of the study was to compare measures of smoke volume and nicotine uptake among human smokers against the puffing variables and nicotine yields generated by the four smoking regimens, as well as a Human Mimic regimen where brands were machine smoked using puffing behaviour recorded from human smokers in the study. Participants in the study smoked cigarettes through a portable smoking topography device to record their smoking behaviour, and they also provided saliva samples to be analyzed for cotinine. The study found that, using the Human Mimic condition as a benchmark, subjects were exposed to tar, nicotine, and carbon monoxide levels that were 2 to 4 times greater than the ISO yields, suggesting that the ISO standard seriously underestimates actual human exposure. Moreover, while the Canadian intense smoking conditions are considered to represent the maximum emissions to which a smoker is likely to be exposed, the study found that total smoke volume was not significantly different from the actual smoke volume as measured in the participants when smoking their usual brand. Among those subjects who were experimentally switched to a lower yield brand, all four smoking regimens produced a lower volume of smoke than the Human Mimic. Comparing these findings to the measured salivary cotinine levels further reveals the limitations of machine smoking methods. The yields from the Massachusetts, Canadian, and Compensatory regimens were no better at predicting measures of

	FTC	ISO	Massachusetts	Canadian	Compensatory
Puff Volume (mL)	35	35	45	55	40
Puff Duration (seconds)	2	2	2	2	2
Interpuff Interval (seconds)	60	60	30	30	30
Ventilation Hole Blockage (%)	0	0	50	100	50
Butt Length	23 mm or filter + 3 mm	Filter length + 8 mm or filter over-wrap+ 3mm	Filter length + 8 mm or filter overwrap + 3 mm	Filter length + 8 mm or filter overwrap + 3 mm	Filter length + 8 mm or filter overwap + 3 mm

Adapted from Hammond *et al.*, (2006b)

Table 5.19 Recommended Machine-Smoking Regimes for Cigarette Testing

nicotine uptake than the ISO yields. Even the Human Mimic condition was only moderately correlated with salivary cotinine levels, reflecting the wide variability in uptake based on nicotine metabolism among smokers even when smoking the same brand. A subsequent study comparing emissions data from 238 Canadian cigarette brands tested under ISO and "Canadian intense" machine smoking conditions, found that the more intense protocol was not necessarily more representative of actual human smoking behaviour and exposure (Hammond *et al.*, 2007b).

Standardised machine testing regimens lack validity as measures of actual human exposure. Despite its limitations, however, machine testing using ISO and alternative parameters remains valuable for informing the development and implementation of product regulations and, where relevant, for measuring basic compliance with constituent limits based on standardised machine testing regimens. The WHO Study Group on Tobacco Product Regulation (TobReg) has recommended that standardised machine smoking tests be used by scientists and regulators "to the extent that it provides a basis for a comparison of the results with new testing protocols until protocols that reflect variations in human smoking behaviour according to different cigarette designs are developed." (WHO Study Group on Tobacco Product Regulation, 2004). Despite its limitations for predicting actual human exposures, machine testing can provide important information on cigarette engineering and how differences in cigarette design may affect smoke emissions.

There remains a need for further development of methods for collecting smoke emissions that are more representative of actual human smoking exposures. Additionally, some promising approaches to account for variations in smoking behaviour based on nicotine titration warrant further development, including measurement of constituent yields per milligram of nicotine and analysis of cigarette filter stains (Strasser *et al.*, 2006)

Constituents in mainstream and sidestream tobacco smoke

Mainstream cigarette smoke is a complex and dynamic mixture of thousands of constituents that are distributed between a vapour phase and a particulate phase (Jenkins *et al.*, 2000). Since the 1950s, following the first epidemiologic studies linking smoking and lung cancer, dozens of

carcinogens and other harmful constituents have been identified in tobacco smoke. The primary focus has been on PAHs, such as benzo [*a*]pyrene and TSNAs, such as NNK, which are considered to be major lung carcinogens (Hecht, 1999). Carbon monoxide in cigarette smoke has also been extensively studied and is likely to contribute to atherosclerosis, and other cardiovascular diseases, by reducing delivery of oxygen through the body (US Department of Health and Human Services, 2004). It is not possible to discuss the significance of each constituent in this section, but a thorough list of major toxic and carcinogenic constituents in the vapour phase and particulate matter of cigarette smoke is provided in IARC Monograph 83 (IARC, 2004). The WHO TobReg study group has developed a recommended list of constituents to be reported or measured in mainstream and sidestream smoke (2004). Additionally, Health Canada requires manufacturers to report more than 40 specific constituents annually for each brand in both mainstream and sidestream smoke. Though essentially the same list of constituents is measured for both mainstream and sidestream smoke, it is important to do measurements for both types of emissions because their quantities may differ. These constituents are listed in Table 5.20.

A few compounds believed to be particularly important are briefly discussed here:

Nicotine:

Measuring nicotine emissions is central to evaluating the addictive potential of tobacco products. Standardised methods for measuring nicotine in machine collected smoke have been widely used, but their ability to predict actual nicotine intake is restricted by the limitations of standardised machine smoking parameters. It is also important to measure the proportion of nicotine that is available in the unprotonated, free-base form, which is more easily absorbed by the body. Research has shown that levels of free-base nicotine vary substantially across different types of tobacco and tobacco product brands, and that the tobacco industry has manipulated the free-nicotine content of tobacco products through additives, such as ammonia (Ferris *et al.*, 2006). A laboratory smoking device and a gas chromatograph-mass spectrometer were used to measure the amount of free-base nicotine in the particulate matter of mainstream cigarette smoke, and found that significant amounts of nicotine in the particulate matter can be in free-base form (Pankow *et al.*, 2003). Similarly, a research group from the CDC found that the measured ranges of free-base nicotine in smoke particulate matter were remarkably similar over the different tar and nicotine delivery categories of full-flavoured, light, and ultra-light cigarette brands, sug-gesting that standard tar and nicotine yields do not provide a valid estimate of actual nicotine emissions (Watson *et al.*, 2004b).

Polycyclic aromatic hydrocarbons:

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of carcinogens formed during the incomplete combustion of organic material, such as tobacco. They are found in tobacco smoke, broiled foods, and in occupational settings, such as iron and steel foundries. Benzo[*a*]pyrene is the best known member of this class of compounds and has been classified by an IARC expert panel as “carcinogenic to humans” (Straif *et al.*, 2005).

N-Nitrosamines:

Tobacco smoke nitrosamines (TSNAs) include a large group of carcinogens that are known to induce tumours in a variety of animal species. TSNAs, such as NNN and NNK, are chemically related to nicotine and nornicotine, a secondary amine tobacco alkaloid, and are thus only found in tobacco products. An IARC working group on smokeless tobacco and tobacco-related nitrosamines concluded that exposure to NNN and NNK is “carcinogenic to humans” (Cogliano *et al.*, 2004).

Aromatic amines:

Aromatic amines were first identified as carcinogens in workers in the dye industry. Of these, 4-aminobiphemyl and 2-naphthylamine are well-established human bladder carcinogens (IARC, 1987).

The 1999 Massachusetts Benchmark Study provided the

Health Canada	TobReg Mimimum
Nitrosamines	NNN, NNK, NAT, NAB
Acrylonitrile	
3, 4 Aminobiphenyl	
1,2 Aminonaphthalene	
Ammonia	
Arsenic	Arsenic
Benzene	
Benzo[*a*]pyrene	
1,3-Butadiene	
Cadmium	Cadmium
Carbonyls	
Chromium	Chromium
Eugenol	
	Formeldahyde
Hydrogen Cyanide	Hydrogen Cyanide
Isoprene	
Lead	Lead
Mercury	Mercury
Nickel	Nickel
Nitrogen Oxides	Nitrogen Oxides
Phenolics	
Pyridine	
Quinoline	
Selenium	Selenium
Styrene	
Toluene	
Filter efficiency	
pH	
Tar, nicotine, carbon monoxide	Tar, nicotine/free nicotine, carbon monoxide
	Ratio of nicotine-free dry particulate matter to nicotine yield

Table 5.20 Emissions Candidates for Surveillance

most comprehensive data to date on the profile of smoke emissions of contemporary cigarettes. Eighteen leading cigarette brands from the USA delivering a range of tar values (from 1 mg to 26 mg per cigarette according to FTC parameters) were screened for 44 constituents using both the FTC and Massachusetts machine smoking methods. The primary constituents varied dramatically across the brands, including total tar (6.1 mg to 48.7 mg per cigarette), carbon monoxide (11.0 mg to 40.7 mg per cigarette), and nicotine (0.50 mg to 3.32 mg per cigarette) (Borgerding *et al.*, 2000; IARC, 2004). The study also illustrated the limitations of ISO tar and nicotine yields for predicting doses of specific toxins and carcinogens in tobacco smoke. One analysis of the Benchmark data showed that FTC tar, nicotine, and carbon monoxide yields were poor predictors of TSNA

yield per cigarette, suggesting that information about the tobacco blend could be more informative for predicting TSNA emissions (Harris, 2001).

Indeed, measured yields of constituents can vary substantially depending on the smoking parameters used for machine measurements. One analysis found that the yields of six IARC Group I carcinogens (benzene, cadmium, 2-aminonaphthalene, nickel, chromium, and 4-aminobiphenyl) in mainstream smoke, were an average of 2-4 times higher when measured by the more intense Health Canada parameters than by ISO parameters (IARC, 2004). Another study of mainstream smoke from three popular brands of US cigarettes purchased on the open market in 29 countries worldwide, showed little variation in tar and nicotine, but substantial differences in the yields of NNN and NNK within each brand (Gray *et al.,* 2000). Additionally, analyses have shown that blocking filter ventilation holes can alter the characteristics of mainstream smoke, including increasing the delivered doses of specific carcinogens and hazardous agents (Brunnemann *et al.*, 1990). These analyses suggest that standard ISO machine measured tar and nicotine ratings cannot be relied upon to estimate emissions of toxic constituents. Further research is needed to understand how varying smoking parameters may affect the contents of cigarette smoke.

Cigarette smoke is also highly dynamic, and the profile of smoke constituents varies over time and across puffs in response to changes in temperature and dilution of smoke and other factors. The distribution of individual constituents across the particulate and gas phases of smoke also changes over time; volatile and semi-volatile compounds, such as benzene and 1,3-butadiene, can be present in significant quantities in both phases. Recently, a high throughput method for analyzing volatile organic compounds in smoke was published (Polzin *et al.*, 2007). However, measuring this dynamic mix in real-time to determine how exposure varies over a series of puffs, for example, is extremely complex. Efforts have been made to characterize volatile compounds in smoke in real-time using time-of-flight mass spectrometry, but this application is experimental and requires state of the art equipment (Adam *et al.,* 2006).

Distal measures

Biological Impact:

The ultimate test of the success of tobacco product regulations in protecting public health would be to observe actual reductions in tobacco-related disease incidence. Population level trends in lung cancer incidence, for example, have reflected changes in cigarette smoking over time. However, such long-term health outcomes do not represent an effective target for regulation, because of the delay between exposure and the appearance of disease symptoms, which can, as with cancer, take decades.

Biomarkers of exposure and biological impact show substantial promise for assessing early effects of tobacco use that are relevant for later disease outcomes. Disease risk is presumed to be a function of the amount, site, and duration of the exposures. Thus, biomarkers of exposure may provide more accurate prediction of disease outcomes than standard measures of tobacco consumption. In particular, there are substantial differences in how individuals use tobacco products, and how their bodies respond to chemical agents in tobacco smoke that are not reflected by simply measuring number of cigarettes per day or use of standardised machine smoking to predict exposures. Additionally, biomarkers may play a particularly important role in the assessment of how differences between products or changes in product design or constituents may impact health. For example, biomarkers of toxic effects or biological damage can provide early indications of the impact of potential reduced exposure products or constituent limits on disease outcomes.

Biomarkers can be divided into at least two major categories (Hatsukami *et al.*, 2006):

- Biomarkers of internal exposure: biomarkers that provide a direct or indirect measure of the quantity of a tobacco-derived constituent or

metabolite in the body. These will not always be closely related to intake because of differences in rates of metabolism.

- Biomarkers of potential harm: biomarkers that measure a biological effect or binding of a tobacco constituent or metabolite in a target organ or tissue. For example, carcinogen-DNA adducts can be used to measure the presence and activity of a specific carcinogen in target tissue. Further along, this also includes biomarkers that measure actual damage to organs or tissues, such as genetic mutations or chromosomal aberrations, which may or may not lead to disease.

It is important to distinguish between biomarkers of exposure versus biomarkers of biologic effects or disease; it may be possible to show a reduction in exposure while the impact on disease outcomes remains uncertain. Additionally, it is helpful to distinguish between biomarkers specific to a particular chemical, such as NNAL, and biomarkers that assess the impact of complex exposures, such as urine mutagenicity.

With the rise of genomics and advances in molecular biology the field of cancer-related biomarker research has advanced considerably over the past 25 years (Schmidt, 2006), but to date there is "no comprehensive set of biomarkers of carcinogen exposure or biological effects as a predictive measure of the total carcinogenicity related to exposure to tobacco or tobacco smoke" (Hatsukami *et al.*, 2006). The Institute of Medicine report *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction,* cited the need for biomarker development in their principal research recommendations: "Although candidate disease-specific surrogate markers are currently available, further validation of these markers is needed. In addition, other biomarkers that accurately reflect mechanisms of disease must be developed to serve as intermediate indicators of disease and disease risk." (Institute of Medicine, 2001). Another expert committee, that assembled to identify key research needs related to tobacco harm reduction, also included among its recommendations the need to identify and validate biomarkers that are predictive of later disease development (Hatsukami *et al.*, 2002). Many biomarkers are currently used in research to study biologic effects of tobacco products or potential reductions in exposure from modified products. Table 5.21 lists a panel of biomarkers that have been recommended as the most promising for use in research on potential reduced exposure products. However, these biomarkers are not necessarily ready for use in a regulatory setting as they require better characterization of their relation to health risks and disease.

A candidate biomarker must go through a process of validation that establishes the qualitative and quantitative relationship of the biomarker to a specific exposure (i.e. a chemical in tobacco smoke) and to a selected end-point (i.e. cancer) (International Programme on Chemical Safety, 1993). There are several issues to consider in evaluating a candidate biomarker including: understanding of the role of the biomarker along a disease pathway, amount of supportive dose-response data (e.g. quantitative data correlating levels of the biomarker with smoking status and with disease endpoints), specificity (is it specific for exposure to tobacco toxicants?), sensitivity (are available tests sufficiently sensitive to detect quantities within a range encountered in the population and to detect meaningful changes), and reproducibility (e.g. intra-subject reliability) (Institute of Medicine, 2001). Supportive data for a biomarker's association with tobacco use should ideally include differences between tobacco users and non-users, a decrease with cessation of tobacco use, a dose-response relationship with quantity or frequency of use, and a decrease with reduced smoking (Hatsukami *et al.,* 2006). Additionally, identification of multiple biomarkers along a continuum from exposure to early disease effects can provide a more robust profile of the relationship between exposure and disease risk.

Biomarkers of internal exposure:

Biomarkers of internal exposure can potentially provide a more

General Tobacco Exposure
Nicotine/Cotinine
Carbon Monoxide
Cancer
NNAL
NNAL Glucs
3-Aminobiphenyl
4-Aminobiphenyl
Sister chromatid exchange
Nonmalignant Lung Disease
Macrophages
Cardiovascular Disease
Flow-mediated dilation
Circulating endothelial precursor cells
Fibrinogen
Homocysteine
White blood cell count
C-reactive protein
sICAM1
Glucose-clamping studies

Adapted from Hatsukami *et. al.* (2006)

*Held in February, 2004, and sponsored by the National Cancer Institute, the National Institute on Drug Abuse, the National Institute on Alcohol Abuse and Alcoholism, and the Centers for Disease Control and Prevention.

Table 5.21 Panel of Biomarkers: Recommended by 2004 Conference* on Methods and Biomarkers to Assess Potential Reduced Exposure Tobacco Products (PREPS)

accurate estimate of actual exposure received by the smoker than can be inferred from machine-based cigarette ratings or number of cigarettes smoked. For example, it was found that over an approximately 10-fold range in FTC cigarette ratings there was little or no significant difference in blood nicotine levels in several studies, demonstrating that FTC ratings do not reflect actual uptake of nicotine by the smoker (Benowitz, 1996b).

Nicotine metabolites have been widely used as biomarkers of general exposure to tobacco products, including exposure to smokeless tobacco and to environmental tobacco smoke (ETS [referred to in this volume as secondhand smoke (SHS)]) among nonsmokers (Benowitz *et al.*, 1994; Benowitz, 1999). Cotinine is the most widely used metabolite, as it has a relatively long elimination half-life of 16 hours (compared to only two hours for nicotine) and can be easily measured in urine, serum, or saliva. Nicotine has also been measured in hair and toenails as a means of assessing exposure to SHS in large scale epidemiologic studies, although the reliability of these measures may be influenced by hair treatment, and other factors, and requires further evaluation (Al-Delaimy, 2002; Al Delaimy *et al.*, 2002). Nicotine and its metabolites also make effective biomarkers because they are highly specific to tobacco exposure (unless the subject is using nicotine replacement therapy).

Carbon monoxide (CO) exposure has also been used as a biomarker for exposure to tobacco smoke. CO can be measured in exhaled air, as CO boost before and after cigarette smoking, and in blood as carboxyhemoglobin (Benowitz, 2003). While CO is not specific to tobacco, it can serve as a reliable short-term measure of smoking. The minor tobacco alkaloids anabasine and anatabine, which are specific to tobacco products and can be measured in urine, have also been used in studies for verifying smoking status (Jacob *et al.*, 2002).

Chemically-specific biomarkers can be used to assess exposure to particular toxins and carcinogens in tobacco and smoke, which may be valuable for evaluating the impact of product performance standards targeting specific constituents. Among the

chemical biomarkers, NNAL and its glucuronides (NNAL-Glucs), which are metabolites of NNK, are particularly useful because they are specific for exposure to tobacco products (as NNK is a tobacco-specific carcinogen) (Hecht, 2002). NNAL and NNAL-Glucs are measured in urine and have been used to quantify levels of NNK uptake in smokers and smokeless tobacco users, and to assess changes following cessation or product switching (Hecht *et al.*, 2002; Hatsukami *et al.,* 2004; Joseph *et al.*, 2005; Lemmonds *et al.*, 2005).

Biomarkers of potential harm:

DNA adducts potentially provide a direct measure of tobacco-induced DNA damage. Adducts are formed when chemical carcinogens bind to DNA, which can alter the structure of the DNA and is believed to be an important step in the pathway to cancer. Protein adducts have also been used to determine levels of carcinogen exposure and activity, since most carcinogen metabolites that react with DNA will also react with proteins, such as hemoglobin, and they are more readily measured than DNA adducts (Ogawa *et al.*, 2006). Hemoglobin (Hb) adducts of aromatic amines, particularly 3- and 4-aminobiphenyl, have shown promise for use in studies of tobacco-related carcinogen exposure. They have been shown to be higher in smokers than nonsmokers (Bryant *et al.*, 1987; Phillips, 2002), and have also been used to measure exposure to carcinogens in secondhand smoke (Hammond *et al.*, 1993). Aromatic amines are not specific to cigarette smoke exposure, however, and can also be associated with occupational and other chemical exposures.

Among the complex biomarkers of DNA damage and potential harm, urine mutagenicity and sister chromatid exchanges are the most promising as indicators of potential cancer effects. Both of these measures have been found to be higher in smokers than nonsmokers and to decrease on cessation (Vijayalaxmi & Evans, 1982; De Marini, 2004). However, the measured effects may be caused by diet or other factors, as well as cigarette smoke, and these differences may reflect other risk behaviour patterns associated with smoking. Development of complex measures that assess the combined effects of tobacco toxins and carcinogens is important because chemically-specific biomarkers, while they may have greater specificity in relation to exposure, may be misleading as a measure of disease risk. A reduction in uptake of a single tobacco smoke constituent in smokers, such as NNAL, may not necessarily provide any meaningful reduction in risk. Consumers may interpret a claim of reduction in a single chemical exposure as indicating a health benefit. Thus, such measures should be put in the context of overall hazard from a complex product.

Biomarkers of potential harm have been used in the research context, such as in clinical studies of potential reduced exposure tobacco products (Breland *et al.*, 2006). However, at this point, none of these biomarkers have been recommended for widespread use in regulation because their relationship to risk and health outcomes has not been sufficiently characterized.

Surveillance

Comprehensive surveillance is essential to assess the impact of regulation on tobacco product use and effects across the population. However, this remains a challenge because capacity and infrastructure for surveillance is limited in many countries (Jha & Chaloupka, 2000). Thus, the extent of surveillance efforts and available infrastructure is likely to vary widely between countries. A comprehensive surveillance programme could potentially cover an enormous range of information. Broadly, surveillance efforts should address changes in the design and performance of the product itself, marketing activity, beliefs and attitudes around tobacco product use, tobacco use behaviours, including initiation and cessation, and health outcomes. Suggested construct areas for post-marketing surveillance are drawn from published recommendations and are listed in Table 5.22 (Institute of Medicine, 2001; Hatsukami *et al.*, 2005).

In addition to measuring potential changes in specific tobacco constituent exposures, it is important to track tobacco

product use and risk beliefs in relation to product regulations. Product modifications in response to regulation may impact tobacco use behaviour. Additionally, experience with "light" cigarettes has provided substantial evidence that smokers believe these products to be less harmful (Cohen, 1996a; Giovino *et al.*, 2000; Ashley *et al.*, 2001; Shiffman *et al.*, 2001). Establishment of regulatory performance standards or constituent upper limits, for example, may be misinterpreted as "safe" levels of exposure. While laboratory evaluation of product design and emissions can provide early warning of potential adverse effects, comprehensive post-marketing surveillance is essential to ensure that regulations are achieving their aims. Additionally, independent technical and research capacity and infrastructure are needed to track changes in tobacco products and users' behaviour.

Establishing laboratory research and testing capacity is a crucial step in supporting surveillance activities to inform evaluation of tobacco product regulation. In addition to tobacco product regulations, governments may have research capacity for studying other aspects of tobacco products. The objective of standardised product testing is to assess product performance and characterize the delivery of particular constituents known to be important for public health, such as carbon monoxide, nicotine, and nitrosamines. In contrast, the goals of research efforts are to understand better the nature of tobacco products, how they work, their effects, and how they might be modified to alter their effects. While testing operations adhere to standardised protocols, research endeavors aim for flexibility and development of new methods and measures for ongoing scientific discovery and analysis. The WHO Study Group on Tobacco Product Regulation has highlighted how both research and testing capacity are essential and must be coordinated (WHO Study Group on Tobacco Product Regulation, 2004). For example, as tobacco products change, new products are introduced, and new scientific methods become available; therefore, it may be necessary to develop new performance standards. Additionally, previous efforts to promote product modification to protect public health, through lowering measured tar and nicotine yields in cigarettes, were undermined by a lack of expertise on tobacco products and smoking behaviour in the public health community (Parascandola, 2005). Thus far, tobacco testing and measurement standards have been primarily driven by the interests of the tobacco industry; thus it is important that the public health community develop capacity and expertise in this area to ensure that product regulations serve the aims of public health (Bialous & Yach, 2001).

In 2005, WHO convened the first meeting of the Tobacco Laboratory Network (TobLabNet), which included more than 25 laboratories from 20 countries. The primary goal of the meeting was to establish a global network of government, university, and independent laboratories to strengthen national and regional capacity for the testing and research of the contents and emissions of tobacco products pursuant to Article 9 of the WHO FCTC. Future activities of the network may include training programmes and development of common measures and protocols (http://www.who.int/tobacco/global_interaction/tobreg/ laboratory/en/ index.html). More details about recommended equipment, personnel, and resources for operating a tobacco product testing laboratory are provided by TobReg (2004). There is a substantial need for support and development of laboratory capacity independent of the tobacco industry in countries around the world with the purpose of achieving public health goals.

Summary

Articles 9 and 10 of the WHO FCTC call for ratifying nations to adopt policies for the regulation and disclosure of tobacco product contents and emissions. This chapter focuses on a review of the methods and measures for evaluating policies that are intended to regulate tobacco products. There are currently five main types: 1) regulations that require disclosure of product information; 2) regulations intended to reduce product toxicity and

Tobacco Product Design and Performance	Product contents, design features (filter, cigarette body), emissions of constituents that modify toxicity and addiction, additives, ignition propensity.
Marketing Activity	Product packaging and labelling, advertising content, promotional materials.
Beliefs and Attitudes	Product awareness, understanding of product design and regulation, risk perception, sensory responses.
Tobacco Use Behaviours	History, current use, brand use, quit attempts/history, addiction/dependence, readiness and intentions to quit, demographics, smoking topography.
Health Outcomes	Biomarkers of toxin exposures, biomarkers of early biological effects, tobacco-related disease incidence.
Other Outcomes	Fires caused by cigarettes.

Table 5.22 Surveillance Construct Categories

harm; 3) regulations intended to reduce the addictiveness and/or attractiveness of tobacco products; 4) regulations intended to prevent fires caused by cigarettes; and 5) bans (or removal of bans) on product categories. The selection of specific constructs and methods for evaluation will vary depending on the goals of the specific policy. However, as a general framework, it is likely that the impact of tobacco product regulations on intended health outcomes will be moderated by changes in product design, performance, marketing, product-related beliefs and attitudes, and tobacco use behaviour, which in turn are expected to influence exposures to tobacco constituents and emissions. Thus, evaluations should not be limited to assessing compliance within the intended effects of a regulation, but should also consider unintended effects of responses, such as tobacco industry innovation, that may interfere with the impact of the regulation.

There is a need for a centralized database that would, at a minimum, characterize different product regulations so that the effects of different policies can be compared. Additionally, as a condition permitting tobacco product sales, governments should require (if they do not already) tobacco product manufacturers to regularly disclose information about their products at the finest level of brand subcategory, including sales and marketing data, product content, and design features. This is needed to inform the development, implementation, and evaluation of effective regulations. Additionally, ongoing surveillance is required to assess the impact of tobacco product regulation on the tobacco product market and on the population, as well as to detect industry responses and other unanticipated consequences of regulation. The challenges of measurement associated with evaluating the effects of tobacco product regulations should not be underestimated. For example, many governments have enacted maximum smoke emissions standards (i.e. tar, nicotine, and carbon monoxide) based on standardised machine testing protocols for the purpose of reducing exposure to the constituents in tobacco products and resultant harm. However, based on the evidence reviewed in this Handbook, it is not recommended that yields from standard machine testing protocols, such as the ISO cigarette testing method (ISO Standard 3308:2000 (4th edition)), be used to assess or predict human

exposure. Emission yields derived from these protocols are not valid measures of actual human exposure. In order to evaluate the effectiveness of product regulations aimed at reducing harm, measures of human use and exposure are essential. There is an urgent need to identify valid methods and measures for assessing human exposure and harm that have practical utility for evaluating tobacco product regulations.