

Toxicity Testing Plan

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**U.S. CONSUMER PRODUCT
SAFETY COMMISSION
IN CONSULTATION WITH THE
U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
AUGUST 1993**



Fire Safe Cigarette Act of 1990

Under the Cigarette Safety Act of 1984 (P.L. 98-567), the Technical Study Group on Cigarette and Little Cigar Fire Safety (TSG) found that it is technically feasible and may be commercially feasible to develop a cigarette that will have a significantly reduced propensity to ignite furniture and mattresses. Furthermore, they found that the overall impact of such a cigarette on other aspects of the United States society and economy may be minimal.

Recognizing that cigarette-ignited fires continue to be the leading cause of fire deaths in the United States, the Fire Safe Cigarette Act of 1990 (P.L. 101-352) was passed by the 101st Congress and signed into law on August 10, 1990. The Act deemed it appropriate for the U.S. Consumer Product Safety Commission to complete the research recommended by the TSG and provide, by August 10, 1993, an assessment of the practicality of a cigarette fire safety performance standard.

Three particular tasks were assigned to the National Institute of Standards and Technology's Building and Fire Research Laboratory:

- develop a standard test method to determine cigarette ignition propensity,
- compile performance data for cigarettes using the standard test method, and
- conduct laboratory studies on and computer modeling of ignition physics to develop valid, user-friendly predictive capability.

Three tasks were assigned to the Consumer Product Safety Commission:

- design and implement a study to collect baseline and follow-up data about the characteristics of cigarettes, products ignited, and smokers involved in fires,
- develop information on societal costs of cigarette-ignited fires, and
- in consultation with the Secretary of Health and Human Services, develop information on changes in the toxicity of smoke and resultant health effects from cigarette prototypes.

The Act also established a Technical Advisory Group to advise and work with the two agencies.

This report is one of six describing the research performed and the results obtained. Copies of these reports may be obtained from the **U.S. Consumer Product Safety Commission, Washington, DC 20207.**

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Part I

**VOLUME 5
PART I**

**TOXICITY TESTING PLAN FOR
LOW IGNITION-POTENTIAL CIGARETTES**

**U.S. Consumer Product Safety Commission and its
Expert Panel, in consultation with the
U.S. Department of Health and Human Services**

May 5, 1993

HEALTH EFFECTS ASSESSMENT PLAN

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EXECUTIVE SUMMARY

I. Introduction

The Fire-Safe Cigarette Act of 1990 requires the U.S. Consumer Product Safety Commission (CPSC), in consultation with the Secretary of the U.S. Department of Health and Human Services (DHHS), to develop information on changes in the toxicity of smoke and resultant health effects of cigarettes with a reduced ability to start fires. The Act states that CPSC "shall not obligate more than \$50,000 to develop such information." The Technical Advisory Group (TAG) established by the Act agreed that this amount precluded any significant testing of prototypes. The Act succeeds the Cigarette Safety Act of 1984 which established a Technical Study Group to examine the feasibility of developing cigarettes with lowered ignition potential. The Technical Study Group concluded it is technically feasible and may be commercially feasible to develop cigarettes that will have a significantly reduced propensity to ignite upholstered furniture or mattresses.

The Act expresses a consideration for the possible nationwide health implications of changes resulting from the market substitution/entrance of low-ignition cigarette types. There were about 50 million smokers in the U.S. in 1991, according to the National Cancer Institute. The primary concern is that a small increase in the risk of a serious health effect, due to new cigarette types, could result in a great increase in human mortality and morbidity and thus overbalance the benefits that would be achieved from the reduction of fires.

CPSC staff, in consultation with DHHS and with the concurrence of the TAG, decided that in view of the statutory \$50,000 limitation, a plan must be developed for the toxicological work needed. CPSC convened an expert panel to assist in the development of the plan. The panel was composed of knowledgeable scientists in the field of cigarette toxicity testing. These members were nominated by TAG members and selected by the CPSC staff.

This report discusses significant issues and recommends testing necessary for the comprehensive assessment of health effects of low-ignition potential cigarette smoke. It is not intended to be a detailed manual of cigarette toxicity testing, although some necessary technical information is presented.

II. General Discussion

Several adverse health effects of serious concern are the basis for considering the various existing toxicity tests. These effects include: lung and throat cancer, chronic obstructive lung disease, heart and vessel disease, male and female reproductive effects, fetal growth retardation, and psychophysiological addiction, as indicated in Chapter A. Not all of these health effects can be addressed at this time due to the impracticality or non-existence of adequate tests, expenses, or time needed for testing. Therefore, only the tests believed to be practical are recommended. Estimates of costs and times needed for testing are included in Chapters B and D-F.

Major issues surrounding the testing include sidestream smoke, bases of comparisons, analytical vs. *in vitro* vs. *in vivo* testing, machine reflection of human smoking behavior, design or performance-based testing, screening paradigms, and disclosure of new additives or increased levels of existing additives, as discussed in Chapter A. Since low ignition-potential cigarettes might cause changes in smoking behaviors and therefore modify the toxicity, altered human behavior may become a significant factor in exposure, as discussed in Chapter C. Since the smoke is collected by mechanically smoking the cigarettes, the apparatus should be set to reflect smoking behavior as closely as technically feasible.

Two methods presently exist for the mechanical smoking of cigarettes, as noted in Chapter B. The Federal Trade Commission (FTC) method, established in 1969, is used in the United States, and the CORESTA method (ISO 3308-1991) is mainly used in Europe. The FTC method is described in Chapter B and is very similar to the CORESTA method. Both methods analyze for tar, nicotine, carbon monoxide, and moisture content.

In light of present knowledge on the adverse health effects and toxic constituents of cigarette smoke, further testing beyond the Federally mandated requirements for tar, nicotine, and carbon monoxide levels is needed to evaluate the toxicity. Levels of key chemical constituents known to be associated with adverse health effects need to be measured, as described in Chapter D. Cigarette smoke is a complex mixture of more than 3,500 chemicals containing at least 35 known carcinogens, and analysis of a limited number of individual chemicals may not predict the net toxic effects of the smoke. In order to address certain conglomerative toxicities of the non-gaseous constituents, *in vitro* and animal testing are needed, as described in Chapters E and F. Limited whole-animal testing is necessary because of the complexity of the biological systems and a variety of toxic reactions caused by cigarette smoke. As an example, pulmonary inflammation testing requires intact immune, respiratory, and circulatory systems to be simultaneously present.

The CPSC staff recommends the following guidance plan after reviewing the considerations of its expert panel and DHHS.

III. Assessment Plan

This plan provides guidance for the development of data needed to evaluate the changes in toxicity associated with low ignition-potential cigarettes. Performance-based, rather than design-based, testing will be used to provide data specific to cigarette prototypes. A screening paradigm that requires acceptable performance levels by a candidate cigarette type at one tier of tests before proceeding with the next tier is recommended. This would allow early rejection of candidates evaluated as unacceptable. However, definition of acceptable levels of performance is beyond the scope of this plan and the direction given by the Act. Therefore, the tests are presented in a sequence of tiers for screening without ascribing acceptable levels of performance at each tier.

Results of the recommended testing will be used to assess the relative toxicity of low-ignition potential cigarettes. The toxicity of a candidate low ignition cigarette should be compared to:

- 1) the specific marketed brand/type intended for replacement, or comparable marketed brands/types for a non-replacement candidate, and
- 2) standard reference cigarettes, such as the University of Kentucky standard cigarettes mentioned in Chapter E, for quality control.

There are insufficient test methods and data on exposure to cigarette smoke and resultant effects for the direct translation of the results into absolute risks to humans. Since the overall health goal is to avoid the production of greater or perhaps new toxicities than that caused by existing cigarettes, a comparative approach of assessing toxicity is appropriate.

Selection of the guidance plan tests assumes that no new additives would be present in the candidate cigarettes and that presently used additives would not exceed the levels in the current cigarettes. Since toxic effects not considered by this guidance plan could also occur, it is recommended that additives exceeding the current maximum levels of use on a per unit weight of tobacco basis must be disclosed to the U.S. Department of Health and Human Services. Confidential business information status may be requested for the data disclosed.

A. Smoking machine

The FTC method described in Chapter B is the basis for the mechanical generation of smoke constituents. Puff volume,

frequency, and draw velocity may be modified as dictated by behavioral data developed from human testing (Tier III), as described in Chapter C. Unless consistent correlation of testing results of mainstream and sidestream smokes can be shown, both must be separately collected and tested.

B. Description of Tiers

An outline of four tiers is presented in Table 1. A description of the tiers follows.

Tier I - Analyses of chemicals

All constituents will be reported as per unit weight of tobacco burned and per cigarette. Moisture, nicotine, tar (total particulate matter- dry), and carbon monoxide will be measured according to the FTC method, as described in Chapter B. Nitric oxide will also be measured using the detector attachment to the smoking machine. The gaseous phase will be analyzed for acidity, reduction/oxidation potential, hydrogen cyanide, volatile hydrocarbons, aldehydes, and volatile nitrosamines, as described in Chapter D. The tar will be analyzed for phenols, catechols, polyaromatic hydrocarbons, and tobacco-specific nitrosamines (Chapter D).

Tier II - In vitro tests

The tar will be assayed for mutagenic activity with Ames' Salmonella test with strains TA98, 100, and 1535. The tar will also be assayed for malignant cell transforming activity, using C3H/10T1/2 mouse embryo fibroblast cells. Both mutagenicity and cell transformation assays are described in Chapter E.

Tier III - Human smoking behavior

Humans are typically the last experimental tier in testing products with potential human health effects. An example is the premarket testing of new drugs. Human testing to collect topographical data is limited to a couple of weeks of exposure.

Smoking behavior, including puff volume, frequency, and draw velocity of a selected group of human volunteers would be monitored, as outlined in Chapter C. Carbon monoxide (breath or blood) and cotinine (urinary, salivary, or blood) will serve as biological markers of exposure to the smoke. If the smoking behavior data is significantly different from the FTC smoking machine settings such that an increase in exposure to the analyzed chemicals might result, then the machine must be set to reflect these data before generating smoke constituents for further Tier I and II testing and then animal testing.

Tier IV - Animal tests

Inflammatory lung response to cigarette smoke in C57Bl mice will be assayed as described in Chapter E. Tumor formation in the upper respiratory tract of random-bred golden Syrian hamsters from inhalation exposure and the skin, lungs, and other tissues of Swiss albino Ha/ICR/Mil strain mice from skin painting exposure will be examined. These two carcinogenicity tests are described in Chapter F.

All testing must conform to good laboratory practices, humane laboratory animal methods, and informed human consent procedures accepted within the scientific community. Evaluations of toxicity must be conducted by scientists possessing appropriate toxicological qualifications.

IV. First implementation step

Table 2 is a collection of direct cost estimates for Tiers I, II, and IV. No estimates are available for Tier III. Completion of all four testing tiers by successful low ignition potential cigarette candidates might be considered expensive relative to the present level of testing required by FTC (\$330K for Tiers I, II, and IV vs. \$3.5K for FTC; Table 2). Therefore, a stepwise implementation of the plan is suggested.

Table 1
Health Effects Assessment Plan
Outline of Tiers

Tier I - Analyses of chemicals

Whole smoke
 acidity (pH)
 reduction/oxidation potential

Gas phase
 gases
 carbon monoxide
 hydrogen cyanide
 nitric oxide

 aldehydes
 acetaldehyde
 acrolein
 propionaldehyde

 volatile hydrocarbons
 benzene
 toluene
 1,3-butadiene
 isoprene

 volatile nitrosamines
 N-nitrosodiethylamine
 N-nitrosodimethylamine
 N-nitrosopyrrolidine

Particulate phase
 catechol
 nicotine
 phenols, as phenol
 polyaromatic hydrocarbon
 benzo(a)pyrene

 tar-FTC
 tobacco specific nitrosamines
 N'-nitrosornicotine
 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

Tier II- In Vitro Tests

Salmonella mutagenicity (Ames' assay)
mouse embryo fibroblast cell transformation assay

Tier III - Human Smoking Behavior

cotinine
carbon monoxide
topography

Tier IV - Animal Tests

mouse inflammatory lung response
hamster upper respiratory tract carcinogenicity
mouse skin painting carcinogenicity

Table 2
Estimated Direct Costs in 1993 U.S. Dollars
per brand or prototype

\$	
	Tier I - Analyses of chemicals = \$9,500
3500	FTC-required tar, nicotine, and carbon monoxide
250	Whole smoke
500	acidity (pH)
	reduction/oxidation potential
	Gas phase
	gases
350	hydrogen cyanide
400	nitric oxide
700	aldehydes
600	volatile hydrocarbons
800	volatile nitrosamines
	N-nitrosodiethylamine
	N-nitrosodimethylamine
	N-nitrosopyrrolidine
	Particulate phase
350	catechol
250	nicotine
500	phenols, as phenol
500	benzo(a)pyrene
800	tobacco specific nitrosamines
	Tier II- In Vitro Tests = \$9,350
1850	Salmonella mutagenicity (Ames' assay)
7500	mouse embryo fibroblast cell transformation assay
	Tier IV - Animal Tests = \$309,000
50K	mouse inflammatory lung response
220K	hamster upper respiratory tract carcinogenicity
39K	mouse skin painting carcinogenicity
=====	
\$327,850	total for Tiers I, II, and IV

A practical selection of recommended tests should comprise a first step in the implementation of this health effects assessment plan. Subsequent steps should consider the testing recommended by this plan. The first step should include:

Smoke and condensate generated by machine according to the FTC protocol

Tier I \$5,050

tar-FTC

nicotine

carbon monoxide

whole smoke pH

benzo(a)pyrene

tobacco specific nitrosamines

N'-nitrosonornicotine

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

Tier II \$1,850

Salmonella mutagenicity ("Ames") assay

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\$6,900

Estimated total per brand or prototype

The rationale for selecting these tests extends beyond cost and time duration considerations. Levels of specific chemicals (Tier I) as well as an indication of the genotoxicity of the mixture (Tier II) are needed. Tar, nicotine, and carbon monoxide are presently required by FTC. The pH of the whole smoke is relevant to nicotine uptake. Benzo(a)pyrene is a known animal and human carcinogen; however, cigarettes are not the only source of exposure. The tobacco-specific nitrosamines are potent animal carcinogens and tobacco is the only known source of human exposure. No data are available on the human carcinogenicity of these nitrosamines.

Chapter Λ

**OVERVIEW AND MAJOR CONSIDERATIONS IN THE TOXICITY
TESTING OF LOW IGNITION-POTENTIAL CIGARETTES**

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ABSTRACT

Both mainstream and sidestream cigarette smoke are complex chemical mixtures. In view of this chemical complexity, it should be no surprise that cigarette smoke has multiple, diverse effects on human health. Nor should it be unexpected that multiple chemicals in cigarette smoke contribute to any single adverse health effect.

The diverse human health consequences of cigarette smoking are briefly reviewed. Many experimental laboratory models have been developed to study the mechanisms of cigarette smoke-induced disease. These laboratory models are not always convertible into practical, standardized test systems that quantitatively compare one cigarette prototype with another. In view of the multiplicity of health effects and mechanisms of smoke-induced health damage, no single test or battery of tests can capture all possible health endpoints.

While analyses of smoke constituents and studies in laboratory animals are feasible, human epidemiological studies are not practical for short-term assessment of small differences in the toxic effects of various cigarette prototypes. Cigarette smoke samples for chemical analysis and biological testing need to be collected in a manner that approximates human cigarette puffing as closely as technically feasible.

In formulating a testing plan, the CPSC essentially has two options: a design-based testing plan, in which individual, pre-selected cigarette design parameters, such as paper porosity or percent expanded tobacco, are systematically varied and tested; and a performance-based testing plan, in which complete cigarette prototypes, and not individual design parameters, are evaluated.

Some testing protocols entail a "screening paradigm." Multiple tests are performed in sequence. If a prototype fails any particular test in the sequence, the prototype is rejected and no further tests are performed. Other multi-test protocols allow for tradeoffs among costs and benefits. An unfavorable result at any point along the testing sequence does not necessarily result in rejection.

The only governmentally-mandated, health-oriented testing of commercial cigarette brands is the measurement and reporting of "tar," nicotine and carbon monoxide in mainstream smoke by the Federal Trade Commission. With this exception, none of the toxicity tests described by the Expert Panel are routinely performed on existing cigarette brands by any governmental agency. The contents of currently marketed cigarettes are proprietary information. Specific additives, tobacco

composition, and other design features are not publicly disclosed.

SCOPE OF THE EXPERT PANEL REPORT

This report addresses: scientific aspects of the design of cigarette toxicity testing systems; the selection and sequencing of particular tests; the reliability, feasibility, and costs of particular tests; and the interpretation, limitations, uses and misuses of test results. In addition to the present Overview chapter, the report contains specific chapters on:

(i) collection of smoke samples from prototype cigarettes for toxicity testing, by Dr. Harold Pillsbury (Chapter B);

(ii) measuring the dosage of smoke constituents actually absorbed by human smokers of different cigarette prototypes, by Dr. David Burns (Chapter C);

(iii) measuring the amounts of specific chemicals contained in the collected smoke, by Dr. Dietrich Hoffmann (Chapter D);

(iv) toxicity testing in single-cell ("in vitro") systems, by Dr. Gary Gairola (Chapter E);

(v) toxicity testing in whole animal ("in vivo") systems, by Dr. Dietrich Hoffmann (Chapter F); and

(vi) research needs for developing methods to collect additional data (Chapter G, input needed).

The Expert Panel has not made policy recommendations. The Panel members did not perform any testing of prototypes in connection with this Report.

SOURCES OF INFORMATION

In preparing this Report, the Expert Panel relied upon: the Final Report of the Technical Study Group on Cigarette and Little Cigar Fire Safety under the Cigarette Safety Act of 1984 [28]; background papers issued in connection with the Technical Study Group Report [17;27]; reports issued by the National Institute for Standards and Technology (and its predecessor, the National Bureau of Standards) in connection with low-ignition potential cigarettes [11;18]; communications from members of the TAG, CPSC staff and DHHS Staff; the published scientific literature; as well as its own expertise and experience. No proprietary or confidential information was requested, offered, or considered.

MAINSTREAM VERSUS SIDESTREAM CIGARETTE SMOKE

Both smokers and nonsmokers can incur adverse health effects from the smoke of burning cigarettes. Smokers inhale mostly

"mainstream (MS) smoke" that is drawn through the burning tobacco column and filter tip and exits through the mouthpiece of the cigarette. Nonsmokers inhale mostly "sidestream (SS) smoke" that is emitted into the surrounding air between puffs from the end of the smoldering cigarette. Sidestream smoke is the major source of "environmental tobacco smoke (ETS)."

While SS and MS smoke have qualitatively similar chemical compositions, the respective quantities of individual smoke constituents can be quite different [35, Chapt.3; 37, p.88]. For example, in studies of nonfilter cigarettes smoked by machines, the yield of carbon monoxide (CO) in sidestream smoke was 2.5 to 4.7-fold that of MS smoke, while the corresponding SS/MS ratio for N-Nitrosodimethylamine (NDMA), an animal carcinogen, was 20 to 100 [35, pp.130-131]. In one compilation of toxic and tumorigenic agents in cigarette smoke, the SS/MS ratio ranged from 0.03 to 130 [14].

Cigarette modifications that reduce the yields of "tar," nicotine and CO in mainstream smoke do not necessarily reduce the corresponding yields in sidestream smoke. In one study of U.S. commercial cigarettes, the SS/MS ratios for carbon monoxide were 2.1 and 2.7, respectively, in two nonfilter cigarettes; 3.5 in a conventional filter cigarette; and 26.8 in a perforated filter cigarette. The SS/MS ratios for NDMA were 23.6 and 139 in the nonfilter cigarettes; 50.4 in the filter cigarette; and 167 in the perforated filter cigarette [35, p.131]. The exposure to sidestream smoke constituents, though, may be greatly reduced depending on distance from the cigarette and ventilation characteristics.

Modifications of cigarette design intended to reduce ignition potential may likewise have different effects on the compositions of MS and SS smoke. In principle, ignition-reducing chemical agents added to the tobacco column or paper wrapper, such as metals and silicates, may transfer differently into MS and SS smoke.

A number of devices have been developed to collect samples of SS smoke for chemical analysis [7]. However, there are no regularly published data on the composition of SS smoke of U.S. cigarette brands. By contrast, the Federal Trade Commission regularly publishes machine-measured yields of "tar," nicotine and CO of the MS smoke of U.S. commercial cigarettes, as described later in this Report. Still, a testing plan for low-ignition potential cigarette prototypes needs to consider both MS and SS smoke.

RANGE OF HUMAN HEALTH CONSEQUENCES

Cigarette smoke (whether MS or SS) is not a homogeneous entity, but a complex mixture of substances. Some smoke components, such as CO, hydrogen cyanide and nitrogen oxides, are gases. Others, such as nicotine and polyaromatic hydrocarbons (PAH), are contained in the submicron-sized solid particles that are suspended in the smoke. Still others, such as formaldehyde and benzene, are volatile chemicals contained in the liquid-vapor portion of the smoke aerosol [37, p.79; 39, Chapt.14]. In view of this chemical complexity, it should be no surprise that cigarette smoke has multiple, diverse effects on human health. Nor should it be unexpected that multiple chemicals in cigarette smoke contribute to any one adverse health effect.

Among the major health effects of cigarette smoke that need to be considered in the development of a toxicity testing plan are the following: cancer; non-cancerous lung diseases; atherosclerotic diseases of the heart and blood vessels; and toxicity to the human reproductive system.

Cancer

Cigarette smoking causes cancers of the lung, esophagus, larynx, oral cavity, bladder, and pancreas in male and female smokers. Smoking has reported to increase the risks of cancers of the kidney, liver, anus, male penis, and female uterine cervix, as well as leukemia [13;31;37;38]. Cigarette smoking is far and away the major cause of lung cancer in the U.S., accounting for 90 percent of cases in men and 79 percent in women [37, p.156].

Numerous epidemiological studies covering the experience of millions of men and women over many years show that smokers' risks of developing cancer increase with the number of cigarettes smoked daily, with the lifetime duration of smoking, and with early age of starting smoking. Smoking cessation gradually reduces cancer risk [37;38]. Filter-tipped and low "tar" cigarettes reduce cancer risk somewhat. Cigarette smoking interacts with other causative agents, including alcohol, asbestos, certain viruses, and certain workplace exposures, in the development of human cancers [31;34;37].

Mainstream cigarette smoke contains over three dozen distinct chemical species considered to be tumorigenic in humans or animals [14; 31, pp.192-218; 37, p.86]. Some of these chemicals are alone capable of initiating tumors in laboratory animals; others can promote the development of previously initiated cancers. As described later in this Report, condensates collected from cigarette smoke cause mutations and damage to DNA in laboratory assays of mutagenesis [12], as well as malignant transformation in laboratory tests of a chemical's ability to induce malignant changes in mammalian cells [3;8].

Undiluted mainstream cigarette smoke is too toxic to be tolerated by laboratory animals such as rodents. In long term experiments with diluted smoke, these animals still do not inhale the smoke in the same way as humans. In natural human smoking, the smoke is puffed in volumes of about 30 to 70 ml; the puffed smoke is temporarily retained in the smoker's mouth, after which it may be inhaled deeply into the lungs. By contrast, some laboratory animals breathe by panting, while others are obligate nose breathers. Even with installation of smoke through artificial airways, it can be quite difficult to get the animals to inhale deeply, as human smokers do. Accordingly, the distribution and retention of smoke components in the respiratory systems of laboratory animals may not mimic natural human smoking.

Nevertheless, as described later in this Report, significant progress has been made in the design of inhalation devices that can expose laboratory animals, especially rodents, to diluted smoke for long periods. Long-term smoke inhalation regularly induces tumors of the larynx in Syrian golden hamsters. Direct installation of cigarette tar into the airways of laboratory animals causes lung cancers [14;31]. As discussed later in this Report, the most widely used experimental system is the mouse skin bioassay, in which cancers are induced by the repeated application of condensates of cigarette smoke to the shaved skins of mice.

Independent scientific agencies have concluded that environmental tobacco smoke causes lung cancer in nonsmokers [22;35]. SS smoke, like MS smoke, contains numerous tumorigenic agents.

Non-Cancerous Lung Diseases

Cigarette smoking is the main cause of chronic obstructive lung disease (COLD), also called chronic obstructive pulmonary disease (COPD) [33]. Smoking accounts for 84 percent of COLD deaths in men and 79 percent in women [37, Chapt.3].

COLD is a slowly progressive illness that develops after repeated insults to the lung over many years. In the early years after starting to smoke, an individual may report no symptoms. Even at this early stage, however, breathing tests can often detect abnormalities in the small, terminal airways of the lung [2;26;33], and these abnormalities have been directly observed in autopsy studies of young smokers who died suddenly [23]. For smokers in their twenties, there is already a dose-response relation between the extent of abnormal lung tests and the number of cigarettes smoked daily. In random population surveys, from 17 to 60 percent of adult smokers under age 55 have detectable small airways dysfunction [33, pp.27-32].

Over the course of two decades or more of smoking, a constellation of chronic respiratory changes develops. This picture of chronic lung injury includes: (i) mucus hypersecretion, with chronic cough and phlegm; (ii) airway thickening and narrowing, resulting in obstruction to airflow during expiration; and (iii) emphysema, i.e., abnormal dilation of the air spaces at the end of the respiratory tree, with destruction of the walls lining the air sacs, resulting in further airflow obstruction. These changes can cause significant respiratory impairment, disability, and death. While individual patients vary in the relative contribution of these three changes, those with clinically severe COLD typically have all three.

While a minority of cigarette smokers will develop clinically severe COLD, some chronic deterioration in lungstructure or function is demonstrable in the majority of long-term smokers [33, Chapt.2]. Some smokers show more chronic cough and phlegm, others more airway obstruction. In general, breathing function declines as a person's cumulative exposure to smoke, measured in pack-years, increases [6].

Cigarette smoke produces pathological changes in the lungs of smokers by a number of different mechanisms [38, pp.282-285]. Cigarette smoke is toxic to the small hairlike cilia that line the central breathing passages. These cilia, in combination with mucus secretions, defend against deep inhalation of foreign material [33, p.279]. Smoking also induces many abnormalities in the inflammatory and immune systems within the lung [34, p.256]. In particular, cigarette smoke causes inflammatory cells to produce an enzyme called elastase. The enzyme elastase in turn breaks down elastin, an important protein that lines the elastic walls of the air sacs [9; 33, p.431]. Moreover, oxidants present in cigarette smoke can inactivate a separate protective enzyme called alpha-1-antitrypsin, which inhibits the destructive action of elastase [16; 33, p.434].

Researchers have produced various types of acute and chronic lung injury in laboratory animals exposed to cigarette smoke [33, pp.286,428,432,436]. But they have had difficulty inducing genuine emphysema from cigarette smoke alone. As in experimental models of cancer, the laboratory animals do not inhale the smoke deeply. Moreover, very long smoke exposures may be required, as is the case in humans. In one experimental study, hamsters exposed either to low doses of elastase or low doses of smoke alone did not develop emphysema, but the combination of low doses of cigarette smoke and elastase caused emphysema-like changes [15]. A later chapter in this Report describes a laboratory test for the acute inflammatory effects of cigarette smoke on the lung, in which mice are exposed to cigarette smoke through a nose-only system.

A large number of organic and inorganic chemicals in the gaseous, volatile and particulate phases of cigarette smoke appear to contribute to its toxicity to the respiratory system [3, pp.289,415], including hydrocarbons, aldehydes, ketones, organic acids, phenols, cyanides, acrolein, and nitrogen oxides. Some components contribute to the development of chronic mucus hypersecretion in the central airways, while others play a major role in the production of small airway abnormalities and emphysematous injury to the peripheral air sacs [33, p.425]. As noted above, oxidizing agents in smoke inhibit the enzymes that defend against the destruction of lung elastin.

Passive exposure to environmental tobacco smoke produces respiratory irritation in nonsmokers, particularly in the children of smoking parents [33, Chapt.7; 35, p.37]. Infants and children of smoking parents are at increased risk of acute respiratory infections, chronic cough and wheezing, and measurable declines in lung function [35, pp.38-59]. These early-life infections can have long-term adverse effects. In adults passively exposed to ETS, some studies have reported measurable changes in lung function. Overall, the effect appears to be too small to implicate passive smoking alone as a cause of cold-blown COLD [35, p.62].

Atherosclerotic Cardiovascular Diseases

Cigarette smoking is a major contributing cause to coronary heart disease, stroke, and other atherosclerotic diseases of the circulatory system [32;37].

Atherosclerosis is a chronic disease that can affect the arterial blood vessels in virtually every part of the human body, including the coronary arteries that supply blood to the heart muscle; the aorta that carries the blood directly from the heart; carotid arteries that carry blood to the brain; and the iliac and femoral arteries that carry blood to the legs.

The common underlying lesion of atherosclerosis is the plaque, which occurs within the wall of the affected artery. As the plaque enlarges and matures, the artery becomes narrowed, and blood flow is reduced. If the narrowed artery carries blood to the heart, then chest pain on exertion (angina) is produced. If the affected artery carries blood to the leg, then calf pain on walking (claudication) is produced. If the affected artery carries blood to the brain, then transient neurological symptoms, such as fainting, loss of vision, movement, or speech (transient ischemic attacks) are produced. If the affected artery carries blood to a man's penis, impotence can result.

A sufficiently narrowed artery is susceptible to complete blockage by a superimposed blood clot. If the blocked artery carries blood to the heart, then a heart attack (myocardial

infarction) is produced. A blockage of an artery supplying a limb can produce gangrene. A blockage to the arteries supplying the brain can cause a stroke.

The most important form of atherosclerosis in the U.S. is coronary atherosclerosis. Its manifestations, which include angina, heart attack, heart failure, and sudden death, are described by the inclusive term coronary heart disease (CHD). Atherosclerosis involving the arteries supplying the brain is a form of cerebrovascular disease (CVD). Atherosclerosis involving the arteries to the limbs is called peripheral vascular disease (PVD).

Atherosclerotic plaques take years to develop. The earliest lesion is called a fatty streak, which consists of deposits of cholesterol within the arterial wall. These fatty streaks can be observed in young people with no symptoms, and even in children. There is a progressive inflammatory reaction to the fatty deposits, and a collection of fibrous debris, muscle cells, and more fatty deposits is incorporated into the developing plaque.

Cholesterol is a fatty substance that does not dissolve readily in water. It circulates in the blood mostly by attaching to specialized proteins. These cholesterol-protein complexes, which also contain other fatty substances, form particles of various sizes, which are called lipoproteins. The lipoprotein particles are classified by their density. There are very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles.

The fundamental event in the initiation of a fatty cholesterol deposit appears to be the transfer of LDL particles from the blood across the inner lining (endothelium) of the arterial wall. This transfer may require prior injury to the inner lining of the artery, in order to expose the raw surface to LDL transfer. When a person's blood cholesterol is measured, the amount that is specifically attached to LDL is called the LDL-cholesterol, or popularly the "bad cholesterol."

On the other hand, HDL particles work in the opposite direction, removing cholesterol from LDL and transporting it back to the liver. Because of this reverse-transport function of HDL, the amount of cholesterol attached to HDL is popularly termed the "good cholesterol."

In epidemiological studies of humans, certain measurable personal characteristics have been consistently found to be predictors of the risks of atherosclerotic disease. These predictors are sometimes called risk factors. For example, male gender is a risk factor for coronary heart disease. This does not mean that maleness per se causes CHD. Still, the fact that

women have lower rates of CHD, and that their risk of CHD increases after menopause, indicates that sex hormones are important in the development of the disease. Likewise, elevated blood pressure is a risk factor for CHD (and for strokes). Again, this does not mean that hypertension *per se* causes CHD. However, higher pressures in the arterial system tend to damage the inner lining (endothelium) of arteries, thus contributing to the development of plaque formation, arterial narrowing and blockage. Because atherosclerosis entails a sequence of pathological events over an extended period of time, it is to be expected that multiple environmental agents and personal characteristics can affect the course of the disease.

In numerous epidemiologic studies of millions of people, cigarette smokers have been found to have higher rates of heart attack, sudden death, and other manifestations of CHD. They also have higher rates of stroke, peripheral vascular disease, and other atherosclerotic lesions [32;37;39]. In a study of over one million people followed during 1982-1986, currently smoking men had a 94 percent greater risk of CHD than lifelong nonsmokers; while currently smoking women had a 78 percent greater risk. In smokers under age 65, men had a 181 percent greater risk, and women a 200 percent greater risk [37, Chapt.3].

Cigarette smoking is sometimes called an "independent risk factor" for CHD because smokers' CHD rates are found to be higher even when other risk factors such as gender, blood pressure, and cholesterol level are taken into account. It is sometimes called a "modifiable risk factor" because one can reduce or stop smoking. While smoking obviously cannot be a cause of CHD in someone who never smoked, it can be an important contributor to CHD in a smoker. Among 548 thousand deaths from CHD in the U.S. in 1985, an estimated 115 thousand would not have occurred but for the presence of cigarette smoking [37].

Cigarette smoke appears to enhance the atherosclerotic process by several different mechanisms [38, p.192]. Cigarette smoking affects cholesterol metabolism. Smokers have repeatedly been observed to have lower HDL-cholesterol levels [41]; and smoking cessation raises HDL-cholesterol [25]. In animal models, cigarette smoke can damage the inner lining of blood vessels, thus enhancing the transfer of LDL and the development of underlying plaques [19;42]. Cigarette smoking can also affect the blood clotting system, including the adherence of blood platelets to the lining of arterial blood vessels [24;32] and the formation of blood clots that block a narrowed artery. Cigarette smoke can also cause spasm of the coronary arteries.

Many chemical components of cigarette smoke have been implicated in the development of atherosclerotic disease. Nicotine, the major psychoactive component of smoke, causes powerful changes in heart rate and blood circulation. Nicotine

appears to cause injury to the arterial lining [19;42]. Carbon monoxide in cigarette smoke binds to the hemoglobin in red blood cells, thereby reducing the oxygen-carrying capacity of the blood. Hydrogen cyanide, nitrogen oxides, and chemical components of cigarette "tar" have also been implicated [32]. Oxidants in cigarette smoke may also promote plaque formation.

Cigarette Smoking and Human Reproduction

Cigarette smoking adversely affects sexual and reproductive function in women in a number of different ways.

Cigarette smoking appears to impair female fertility [1; 5; 21; 30, p.235]. Among the possible mechanisms are direct toxicity to female eggs, interference with motility in the female reproductive tract, and alterations in immunity that predispose female smokers to infections that block the Fallopian tubes [4].

Maternal cigarette smoking has serious adverse effects on the outcome of pregnancy. These include: retarded fetal growth; low birthweight; spontaneous abortion; certain complications of pregnancy, labor and delivery, such as bleeding during pregnancy and prolonged premature rupture of membranes; and infant death [30, p.188; 37, p.71; 38, Chapt.8; 39, Chapt.8]. Direct nicotine toxicity has been suggested as a mechanism for spontaneous abortion [38, p.372]. While a smoking-induced reduction in maternal weight gain contributes to fetal growth retardation [30, p.202; 40], the evidence points to oxygen starvation of the fetus and placenta as important factors. Carbon monoxide in cigarette smoke can cross the placenta and bind to the hemoglobin in fetal blood. Smoking causes constriction of the umbilical arteries, impairing placental blood flow. Nicotine, which also crosses the placenta, can have a number of toxic effects on the fetus [30, p.229]. Cyanide, another component of cigarette smoke, has also been implicated.

Currently smoking women enter nonsurgical menopause about one to two years earlier than nonsmokers [38, p.397]. Heavy smokers experience an even earlier menopause than light smokers. This effect has important consequences for women's health, because the rates of osteoporosis and atherosclerotic cardiovascular diseases increase after menopause. One proposed mechanism for early menopause is that polyaromatic hydrocarbons (PAH) in smoke are directly toxic to ovarian follicles [20].

Cigarette smoking may also affect male reproductive performance. In a number of studies, men who report impotence (i.e., the inability to maintain an erection sufficient for intercourse) were more likely to be cigarette smokers. This association between smoking and impotence is particularly common among men who have high blood pressure or diabetes, and appears to be a consequence of increased atherosclerotic disease in the

blood vessels supplying the genitalia, rather than an effect on sexual drive.

Nicotine as a psychoactive drug

The psychoactive drug in cigarette smoke is nicotine. Cigarette smoking is a highly controlled form of self-administration of this drug. Nicotine use is self-reinforcing. Attempts to stop smoking lead to craving, withdrawal symptoms, and high rates of relapse [36].

RESEARCH MODELS VERSUS STANDARDIZED TEST SYSTEMS

As the foregoing brief review indicates, there are many laboratory and animal models of the mechanisms of cigarette-induced human toxicity, and there are many methods of studying the health effects of smoking in humans. However, not all of these models and methods are easily converted into inexpensive, practical, standardized tests that quantitatively compare one cigarette prototype with another.

THE MULTIPLICITY OF TESTING PROTOCOLS

It is unlikely that any battery of standardized, practical tests will be able to gauge all important dimensions of human cigarette toxicity. Exhaustive testing of every conceivable dimension of toxicity is a "bottomless pit." From the scientific standpoint, there will necessarily be some stopping point to testing.

At present, there exists a wide range of testing protocols, reflecting different dimensions of human toxicity. These testing protocols will be considered in detail in later sections of this Report. In general, tests of cigarette toxicity include:

(1) Chemical and physical analyses of MS and SS smoke collected by smoking machines under standardized conditions. These tests include quantitative measurement of known smoke constituents, qualitative analyses for new chemicals, and studies of particle size distribution.

(2) Studies of the dosage of specific smoke constituents actually received by human smokers or by nonsmokers exposed passively to environmental tobacco smoke.

(3) Laboratory tests of the effects of whole smoke or fractions of smoke on individual cells and tissues. The individual cells can be single-cell organisms, such as bacteria. They can be cells extracted from a specific organ of an animal

and preserved in tissue culture. Tests that do not entail exposure to an entire living animal are called in vitro tests.

(4) Laboratory tests of the effects of smoke or smoke fractions in whole animals. These include short-term tests to study specific mechanisms of disease or to assess acute toxicity, and long-term tests to assess the effects of chronic exposure.

The multiplicity of human health endpoints, as well as the wide range of available tests, means that a particular cigarette prototype may appear more toxic in some tests, equally toxic in other tests, and less toxic in still others.

EPIDEMIOLOGY

Human epidemiological studies play a central role in generating and testing hypotheses about causation of disease; in identifying groups of people who at higher or lower risks of disease; in estimating quantitatively the risks of specific diseases in relation to different levels of toxic exposure; and in evaluating the effects of preventive measures.

Epidemiological studies are more limited in assessing the differences in the toxic effects of various types or brands of cigarettes. For example, to determine whether brand "A" causes less lung cancer than brand "B," a researcher would have to identify and compare long-term smokers exclusively of brand "A" with long-term smokers of brand "B" alone. If the expected differences in cancer rates are small, then large numbers of long-term smokers of each brand need to be identified.

Epidemiologic methods are impractical for testing the comparative effects of prototype cigarettes that have not already been marketed and smoked by consumers.

ABSOLUTE RISK VERSUS RELATIVE RISK

Human epidemiology can be used to estimate quantitatively the risk of specific diseases to human smokers. For example, in a study of smoking practices and mortality rates among 1.2 million U.S. adults followed during 1982-1986, about 0.8 percent of current male smokers aged 65 or more died of lung cancer each year [37, p.143]; while the comparable annual lung cancer death rate was about 0.04 percent among men aged 65 or more who never smoked. These quantitative risk estimates are often termed "absolute risks." The fact that the continuing smokers' risk of lung cancer was 20-fold that of nonsmokers is an expression of "relative risk."

Estimating absolute risks from nonhuman toxicity studies is much more complicated. For example, the smoke from prototype

cigarette "Z" might contain 0.05mg of benzo(a)pyrene (BaP), a known carcinogen, while the smoke from a control cigarette might contain 0.02mg of BaP. To estimate human lung cancer risks from these data alone would require a number of assumptions relating the dose of BaP to the incidence lung cancer in humans.

Toxicity studies can give estimates of relative risk, but applying these estimates directly to humans requires caution. While prototype "Z" had 2.5-fold as much BaP as the control cigarette, we cannot automatically conclude that their relative risks of lung cancer in humans is 2.5. The relative concentrations of benz(a)anthracene, another carcinogen in the "polyaromatic hydrocarbon" group, might be higher or lower. Estimating relative risks from toxicity studies entails combining estimates from different sources [8].

HUMAN BEHAVIORAL RESPONSES ARE IMPORTANT IN TESTING

Testing plans require samples of cigarette smoke, which can then be analyzed chemically or biologically. The results of such testing may hinge critically on the method of collecting the sample. Smoke samples from cigarettes are generally collected from smoking machines, not from living smokers. As discussed in more detail later in this Report, it is important that such samples be collected in a manner that mimics human smoking as closely as is technically feasible.

The study of the ways in which humans consume cigarette smoke is called smoking topography. Many variables are involved, even in the smoking of a single cigarette: the intensity of the draw on the column of smoke during a single puff; the duration of the puff; the volume of smoke in each puff; the intervals between puffs; and the number of puffs taken per cigarette. These variables, as well as other physiological factors, affect the actual dosages of smoke constituents that are inhaled, absorbed, and retained in the smoker's body. The study of the actual dosages of smoke constituents received by human smokers is called smoking dosimetry.

No two humans smoke cigarettes exactly the same way. Puffing intensity, duration and volume, as well as inter-puff intervals and puffs per cigarette, vary among human smokers. Accordingly, no protocol for machine-based collection of cigarette smoke can accurately mimic all human smoking. Toxicity testing of machine-collected smoke samples may not accurately gauge a particular smoker's risk, but rather an average or representative smoker's risk.

Toxicity testing ordinarily requires a uniform method of collecting smoke samples. To compare the "tar," nicotine and CO yields of the smoke of prototype "X" with those of a control

cigarette, one uses the same smoking machine to smoke both cigarettes under the same conditions according to the same protocol. For example, under the current FTC protocol, a smoking machine takes one puff each minute. The puff volume is 35ml; and the puff duration is 2 seconds. As described later in this Report, the smoking machine continues to take puffs on the test cigarette until a pre-specified butt length is achieved.

Different cigarette prototypes or design modifications may affect the ways that people smoke cigarettes. This can complicate the choice of test conditions for collecting smoke samples from prototype cigarettes. For example, when cigarette prototype "X" is smoked by machines under standard FTC conditions, the amount of nicotine in the smoke may appear to be reduced. But human smoking topography may show that smokers actually take deeper puffs on prototype "X" than the 35 ml-puffs taken by the smoking machines. Human dosimetry may further show that the amount of nicotine actually absorbed from prototype "X" is not reduced. Alternatively, prototype "Y" may contain more "tar" per machine-smoked puff. But human topography may show that smokers take fewer puffs on that prototype, so that the total yield of "tar" per cigarette is not increased.

For these reasons, human smoking topography and dosimetry may need to be a part of cigarette testing for increased toxicity.

DESIGN-BASED TESTS VERSUS PERFORMANCE-BASED TESTS

Section 2(c) of the Fire Safe Cigarette Act of 1990 mandates the development of information on "changes" in toxicity of smoke and resultant health effects of cigarette "prototypes." Such information can be acquired by design-based testing, which assesses the effects of a specific, known modification (or a combination of modifications) in cigarette design.

The "tar," nicotine and CO analyses of "Series 1" and "Series 2" experimental cigarettes performed by the National Bureau of Standards (NBS) are examples of design-based testing [11, Tables 2-5, 2-6, 2-7, 3-10, 3-11, and 3-12]. In Series 1, for instance, NBS analyzed five different dimensions of cigarette design: tobacco leaf composition (burley vs. flue cured); tobacco density (decreased by tobacco expansion); paper porosity; the presence of citrate additive to the cigarette wrapping paper; and the circumference of the tobacco column. Experimental cigarettes were produced that contained modifications in one or more of these design dimensions. The modified cigarettes could then be compared to each other and to control cigarettes with no modifications. By such comparison, NBS estimated that lower tobacco density decreased the "tar" yield per smoke puff; while

low paper permeability increased "tar" yield per puff [11, Table 3-12].

The NBS Series-1 tests did not encompass all possibilities in design-based testing. A specific newchemical additive could be incorporated into a test cigarette, whose smoke would be compared with that of a control cigarette that is otherwise identical. In the NBS Series 1, the experimental cigarettes were all filter-tipped, with the individual tows, plug wraps, and plasticizer levels selected by the participating cigarette manufacturers [11, p.33]. The effects of lower-porosity wrapping paper could also have been assessed in nonfilter cigarettes, or perforated filter-tip cigarettes. This might be important if the presence or type of filter affected ignition propensity [11, p.65]

From the scientific standpoint, design-based testing is advantageous when there is a limited practical range of cigarette design modifications, and when such design modifications are publicly known. For example, if changes in tobacco packing density and paper permeability were the only feasible design modifications under consideration, and if the proposed methods of tobacco expansion and paper manufacture were specifically disclosed, then the effects of such design changes could be assessed. However, if a specific cigarette prototype entailed tobacco expansion combined with proprietary changes in tobacco leaf composition, cigarette paper, and filter design, then design-based testing may be impractical.

The alternative is performance-based testing, in which individual cigarette prototypes-- not design technologies-- are assessed. Such testing may be more appropriate when there are many different cigarette prototypes, each with complex design changes, and when the specific changes are proprietary or not fully disclosed. NBS's analyses of patented cigarettes [11; Table 3-14] more closely resembles performance-based testing. In that case, inventors submitted their own prototypes, along with unmodified control cigarettes. While NBS appears to have tested these patented prototypes for ignition propensity only, analyses of "tar," nicotine and CO in such patented cigarettes would constitute performance-based testing.

Accordingly, in design-based testing, information might be acquired on the effects of changes in paper porosity on smoke carbon monoxide. By contrast, in performance-based testing, information is acquired on the CO delivery of prototype "X."

In performance-based testing, there is no unique or natural control cigarette. As in the NBS testing of patented cigarettes, the smoke of prototype "X" could be compared to a control cigarette that incorporates none of the proposed modifications. But this alternative is not necessarily so simple. Prototype "X"

could include modifications designed to: (i) reduce ignition potential; (ii) reduce smoke toxic constituents; and (iii) improve consumer acceptability. A comparable control cigarette may be unmodified in one or all of these dimensions. Such controls may not correspond to any currently marketed cigarette brand. Alternatively, the prototype "X" could be compared to other existing marketed cigarettes; to another prototype "Y;" or to pre-set standard cigarette. Thus, in performance-based testing, one could conclude that prototype "Z" delivered more or less nicotine than any other prototype; than the average marketed cigarette; or than some value set by a public or private standard-setting body.

TESTING PROTOCOLS: SCREENING VERSUS TRADEOFFS

Testing is expensive and time-consuming. Accordingly, most testing protocols entail a sequence of tests. The order of testing is usually influenced by the cost and time required. If human subjects are involved, then risk and ethical considerations are important.

For example, in the screening of environmental agents for their carcinogenic potential (e.g., under the Toxic Substances Control Act), bacterial mutagenesis and other short-term tests for genotoxicity are performed first. After that, whole animal exposure studies of acute toxicity may be considered. Thereafter, longer term whole-animal studies of carcinogenicity may be undertaken. In the screening of investigational new drugs, human studies are undertaken only after laboratory and whole animal studies are completed.

Some testing protocols entail a "screening paradigm." If a substance or product fails any particular test in the sequence, the product is rejected and no further tests are performed. For example, in the testing of cigarette prototypes, analytical studies of smoke components might be performed initially, followed by short-term mutagenicity studies, followed then by long-term bioassays of carcinogenicity in animals, followed by studies of smoke dosimetry in humans.

In the screening paradigm, a cigarette prototype "Z" that initially yielded an excess of carcinogenic polyaromatic hydrocarbons (PAH) might be rejected, and no further testing performed. Alternatively, if cigarette prototype "Q" yielded no excess of toxic compounds on chemical analysis, then testing of prototype "Q" would proceed to the next level.

In contrast to screening protocols, other testing protocols allow for tradeoffs among costs and benefits. A positive test at any point along the testing sequence does not necessarily result in rejection. For example, prototype "R" may have performed

exceptionally in tests of low- ignition potential, but it yielded an excess of tobacco-specific nitrosamines in chemical analysis. Such a finding might not lead to automatic rejection of prototype "R." Instead, testing would continue, and the positive analytical test result would be weighed against other evidence.

Conservatively designed protocols may be appropriate when the potential adverse health effects of a new product or new design are more important than its potential benefits for fire safety. On the other hand, if a relatively small increase in "tar" or nicotine delivery is to be gauged against a major reduction in ignition potential, then some form of cost-benefit analysis will be required.

SPECIAL ASPECTS OF CIGARETTE PROTOTYPE TESTING

The only governmentally-mandated, health-oriented testing of the finished cigarette product is the measurement and reporting of tar, nicotine and carbon monoxide by the Federal Trade Commission. While USDA and DHHS may conduct research programs on the health effects of smoking, no other federal or state agency is currently required to perform tests for toxicity on various brands of marketed cigarettes. With the exception of standardized machine measurements of "tar," nicotine and CO, none of the toxicity tests described by the expert panel are routinely performed on existing cigarette brands by any governmental agency.

Some low-ignition prototype cigarettes may contain additives that are not in currently marketed cigarettes. Such additives may have qualitatively different health effects than those discussed above. Neither performance-based nor design-based testing solves the problem of evaluating the health effects of new, undisclosed cigarette additives. For example, if a new inorganic compound, such as a metal salt, were added to the cigarette tobacco, then one might have supplement the test battery with additional studies of acute or chronic toxicity to kidney, liver and other organs. In cases where new additives are involved, and not merely a quantitative change in existing design parameters, disclosure of contents is required for adequate toxicity testing.

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GLOSSARY AND ABBREVIATIONS

benzo(a)pyrene (BaP): a carcinogenic chemical in cigarette smoke, a member of the class of polyaromatic hydrocarbons.

benzo(a)anthracene: a carcinogenic chemical in cigarette smoke, a member of the class of polyaromatic hydrocarbons.

carbon monoxide (CO): a gas found in cigarette smoke.

condensate: the portion of whole smoke that condenses upon passage of the smoke through a cold trap.

dosimetry of smoking: study of the actual dosages of smoke constituents inhaled, absorbed and retained by human smokers.

environmental tobacco smoke (ETS): mostly sidestream smoke, but also exhaled mainstream smoke, as well as some gaseous and

vapor-phase constituents of smoke that diffuse through the cigarette paper wrapper into the surrounding air.

genotoxicity tests: tests of the propensity of cigarette smoke, smoke particles, or smoke condensate to damage the genetic material (DNA) of the test cell; a more general term than mutagenicity tests.

mainstream (MS) smoke: smoke that is drawn through the burning tobacco column and filter tip and exits through the mouthpiece of the cigarette.

"in vitro" test: a test that is performed on single cells or organs derived from an animal (or human), as opposed to an "in vivo" test that is performed on an entire living animal (or human). Tests performed on primitive single-celled organisms, such as bacteria or yeast, are classified as "in vitro" tests.

"in vivo" test: a test that is performed in a whole, living animal (or human), as opposed to an "in vitro" test.

mutagenesis tests: tests for the propensity of cigarette smoke, smoke particulates, or smoke condensate to cause mutations in the genetic material (DNA) of the test cell. A widely used mutagenesis test is the Ames test, which is performed on special strains of the Salmonella bacterium.

N-Nitrosodimethylamine (NDMA): an animal carcinogen.

particulate phase: the portion of cigarette smoke that is trapped by a standard Cambridge filter at room temperature.

polyaromatic hydrocarbons: a class of carcinogenic chemicals found in cigarette smoke. An example is benzo(a)pyrene.

sidestream (SS) smoke: smoke that is emitted into the surrounding air between puffs from the end of the smoldering cigarette.

topography of smoking: study of the ways that humans consume cigarette smoke, including the intensity of the draw on the column of smoke during a single puff; the duration of the puff; the volume of smoke in each puff; the intervals between puffs; the number of puffs taken per cigarette; and the number of cigarettes smoked daily.

tumorigenic: causing tumors or cancers in laboratory animals or humans; used synonymously here with "carcinogenic."

vapor phase: the gaseous and vaporizable chemicals in cigarette smoke that pass through a standard Cambridge filter at room temperature.

Chapter B



**SMOKING MACHINE PARAMETERS FOR COLLECTION OF TOTAL
PARTICULATE MATTER AND GASES FROM LOW IGNITION-
POTENTIAL CIGARETTES**

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**Under contract to the U.S. Consumer Product Safety Commission
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Introduction

The official Federal Trade Commission method (FTC, 1969, 1979, 1980) to determine tar, nicotine, and carbon monoxide in cigarette smoke is presented as a basis for the method of collecting total particulate matter and gases from low-ignition potential cigarettes. The FTC method is very similar to the *Centre de Coopération pour les Recherches Scientifiques au Tabac* (CORESTA) Standard Method (CORESTA, 1968) used in Europe. One of these two methods or a slight modification thereof is used in all countries that test cigarettes.

Differences between the FTC and the CORESTA methods are small. The environmental rooms under the CORESTA method are maintained at 22 ± 3 °C and 55-65% relative humidity. The FTC method requires conditions of 75 ± 2 °F (23.9 ± 1.1 °C) and relative humidity of $60 \pm 2\%$. Under the FTC method, cigarettes are smoked to a butt length of 23 mm or the overwrap plus 3 mm, whichever is longer. Using the CORESTA method, cigarettes with a filter length exceeding 15 mm are smoked to the length of filter plus 8 mm and cigarettes with extra long filter tips are smoked to the length of the tipping plus 3 mm.

The Filtrona-400 smoking machine used in the CORESTA method requires an increased draft over the burning cigarette, whereas the FTC method does not. The increased draft is not encountered under normal smoking conditions. It causes the cigarette to burn faster, reducing the number of puffs and lowering the yield of total particulate matter (TPM) and gases. This air flow over the cigarette is needed to match the results of the Filtrona smoking machine to that of the Borgwaldt smoking machine. The Filtrona and Borgwaldt are the only two existing commercial manufacturers of smoking machines.

The following is a summary of the FTC protocol.

Materials and Methods

- 1) **Environmental Room:** A room where cigarette conditioning and smoking is conducted. The room should be maintained at 75 ± 2 °F (23.9 ± 1.1 °C) and $60\% \pm 2\%$ relative humidity.
- 2) **Smoking Machine:** The cigarette smoking machine should be similar to the Filtrona machine used by FTC (Pillsbury, 1969). This machine can smoke 20 cigarettes at one time, one in each port. Each port can be fitted with a filter holder and filter pad for the collection of TPM. Gases pass through the pad and are collected in specially designed plastic bags (Filtrona).

3) Smoke Collection Trap: The polyacrylic plastic holders (Wartman, 1959) can be obtained from the manufacturer of the smoking machine (Filtrona).

4) Filter Disks: Filter disks (pads) are made from a fiberglass sheet pre-cut to a diameter of approximately 44 mm. The filters collect at least 99.9% of all particles larger than 0.3 μm in diameter (Ogg, 1964). The filter disks fit into plastic holders. The particles collected on the pad are referred to as total particulate matter (TPM).

5) Standard solutions:

A) Extraction solution: This solution contains extractant and internal standards- 2-propanol containing 1 mg anethole (p-propenylanisole, 1-methoxy-4-propenylbenzene) per mL as an internal standard for nicotine and 20 mg ethanol per mL as an internal standard for water.

B) Moisture content: Standards are prepared by adding measured amounts of water into measured volumes of extraction solution. A standard curve is constructed from the ratio of peak heights of the water to the peak height of ethanol against the amount of water added to the extraction solution, after correcting with a solvent blank.

C) Nicotine: A stock solution contains 2.500 g nicotine in 100 mL of extraction solution. Working solutions are made from 1, 2, 3, 4, and 5 mL of the stock solution diluted and brought to 100 mL volume with extraction solution. A standard curve is constructed as with moisture content.

6) Carbon monoxide: Gases from the plastic bags in the smoking machine are passed into an infrared detector. The detector is calibrated using a carbon monoxide gas standard.

7) Gas chromatograph:

A) Moisture content analysis: The 6 ft x 1/8" (1.8 m x 0.32 cm) diameter column is packed with 80-100 mesh porous polymer (Porapak Q). Operating temperature for the column is set at 200 °C, injection port at 240 °C, and thermal conductivity detector at 210 °C. The helium carrier gas flow is about 100 mL per minute.

B) Nicotine analysis: The 6 ft x 1/8" (1.8 m x 0.32 cm) diameter column is packed with 2% KOH and 10% polyethylene glycol (Carbowax 20M) on 45-60 mesh acid washed diatomaceous earth. The column temperature is set at 165 °C, and the injection port and the flame ionization detector are set at 200-250 °C. Helium carrier gas flow is about 40 mL per minute.

8) "Monitor" cigarettes: These are cigarettes with known tar, nicotine and carbon monoxide yields. Monitor cigarettes serve as "standards" to ensure that the smoking machine is operating properly. True standard reference cigarettes are mentioned in Dr. Gairola's chapter on Short-term Toxicity Tests. No fewer than four ports should be used for monitors per 20 port machine on each run.

9) Run: This is a complete smoking of 100 cigarettes- five of the same type in each of the 20 ports (4 monitor and 16 test cigarettes).

Samples

Cigarette quantities: A minimum of 150 cigarettes and preferably 200 cigarettes of each type are needed for the FTC specified tests. This would ensure that at least 100 cigarettes of each type were successfully smoked for one run. Typically, some test pads are discarded due to cigarette lighting failures, port leaks, or other technical problems.

Sample preparation and selection: Store all cigarette samples and monitors in an environmental room or chamber for not less than 24 hours before marking or smoking. Cigarettes should remain in the environmental room until they are smoked. Select only cigarettes without physical damage. Cigarettes should be marked to either a butt length of 23 mm or the overwrap plus 3 mm, whichever is longer. The insertion depth of about 9 mm is also marked. Mark the perforations for easy identification by the technician during placement into the holder. The perforations must not be occluded or compressed by the holder since this would affect the smoke yield.

Machine Smoking of Cigarettes

- 1) Puff volume: 35 mL \pm 0.5 mL
- 2) Puff duration: 2 sec \pm 0.2 sec, measured under actual machine smoking conditions. Resulting draw velocity is about 17.5 mL per sec.
- 3) Puff frequency: One puff per 60 sec \pm 1 sec.

Weigh the filter assembly to the nearest 0.05 mg and connect it to the smoking machine so that the cigarette and filter assembly are held horizontally. Test the smoking apparatus and filter assembly for leaks. Insert a cigarette through the hole in the rubber membrane until the butt end is inserted approximately 9 mm, such that the butt end does not contact the filter disk. Light the cigarette at the beginning of the first

puff. Smoke five cigarettes per pad. If the cigarettes are very low in tar, more cigarettes may be smoked per pad providing the pad does not wet through. The cigarettes should be protected from drafts, other than normal convection, during smoking.

Results

After five or more cigarettes are smoked in each port, each pad is extracted with the extraction solution. The extracted material is analyzed for moisture content and nicotine levels. Other extracted materials, such as described in the Analyses chapter, may also be analyzed. Part of the gas phase, which has been accumulated in the bag, is passed through an infrared detector for the determination of carbon monoxide. Although not required by FTC, nitric oxide may be measured by a chemiluminescent detector designed by Filtrona, specifically for the smoking machine. The gas may also be analyzed for substances indicated in Dr. Hoffman's Analysis chapter. Tar, nicotine and carbon monoxide are reported as mg per cigarette.

TPM (total particulate matter): Immediately after smoking the cigarettes disconnect the filter assembly from the smoking machine. Record the weight gain of the filter assembly to the nearest 0.05 mg and divide this by the number of cigarettes smoked to determine TPM per cigarette.

Extraction: Immediately after weighing, place the filter pad in a dry, rubber-stoppered 25 mL flask. Wipe out the filter assembly with one-fourth of an unused pad and place this into the flask. Add 10.0 mL of extraction solution and shake for 30 minutes.

Water: A 1-10 uL aliquot of the extract is withdrawn through the stopper and injected into the chromatograph. Compare the resulting peak against the standard curve to determine the moisture content.

Nicotine: A 1-10 uL aliquot of the extract is withdrawn through the stopper and injected into the chromatograph. Compare the resulting peak against the standard curve to determine the nicotine content.

Carbon Monoxide: The gaseous phase collected in the plastic bag is passed through an infrared detector for the determination of carbon monoxide.

Tar: Tar is the TPM minus the water and nicotine. This is sometimes referred to as "FTC tar" to distinguish it from other definitions of "tar".

Discussion

The Filtrona smoking machine can be modified by installing a collection funnel at each port to collect sidestream smoke, which may have different constituent levels (Johnson, 1973; see also discussion in Dr. Harris' Overview chapter). Filtrona's 8-port smoking machine is more easily modified than the 20-port model. The filter assembly can be replaced with a cold trap if this technique for collecting condensate is desired.

There may be a wide range of variability in smoking behaviors due to cigarette design, physiological, psychological, and pharmacological factors (see Dr. Burns' Topography chapter; Guyatt, 1989a,b; Kolonen, 1991, 1992; Nil, 1989; Zacny, 1988). Although the present testing methods are designed to produce comparative results, the smoking machine could be set up as closely as technically feasible to reflect future data on smoking behavior. The machine has sufficient range to accommodate possible changes, for example, puff frequency from one puff per minute to six puffs per minute or volume from 20 mL to 50 mL puffs. The draw velocity would also change since it is related to the frequency and volume.

A high degree of replicability for tar, nicotine, and carbon monoxide was found in parallel testing between FTC and most other laboratories over a period of 20 years. Unfortunately, all the data from these tests were destroyed when FTC closed the laboratory in 1987. Attached to this chapter is a typical graph from my files that illustrates the close correlation of tar levels found by the FTC and a private laboratory.

Cost

The approximate cost of machine smoking and analyses for tar, nicotine, and carbon monoxide for one run with a 20-port machine would be \$3,000-\$4,000 (4 monitor ports + 16 test ports = 20 monitor cigarettes + 80 test cigarettes, minimum). The current capacity of the Tobacco Institute Laboratory is six runs per day.

Recommendations

The FTC method should be used as the basis for the smoking machine setup in the collection of gases and total particulate matter for low ignition-potential cigarette testing. The apparatus and methodology is adaptable to changes that may be indicated by new and future data on human smoking behavior and smoke exposure. The FTC method is replicable and well-established among the US industry.

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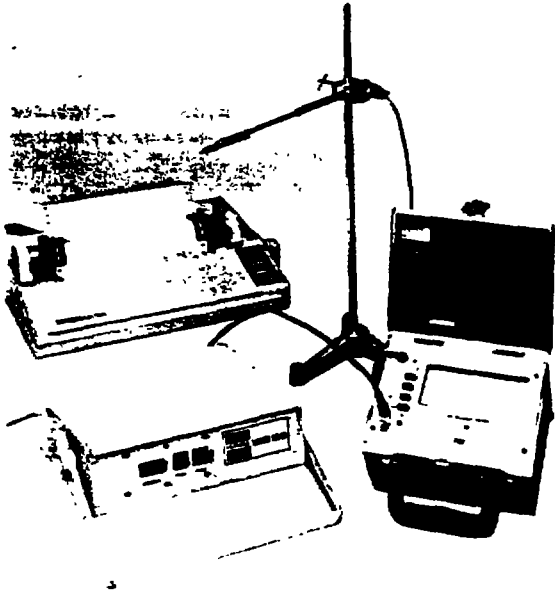
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AIR FLOW VELOCITY MEASUREMENT FOR HARMONISED SMOKING MACHINES



TSI 1640 and VMD100
(Serial Printer is optional)

- The VMD100 provides a clear digital display enabling easy measurement of time averaged air flow velocity.
- Printed velocity measurements and graphical plot of values against time.
- TSI 1640 measurement probe specially designed for maximum accuracy of low velocity air flows.
- Omnidirectional probe measures velocity as independently of direction as possible.
- Traceable calibration.
- Calibration matched units.

The combination of the TSI 1640 and VMD100 completely supports the measurement requirement detailed in ISO 3308 (1991) Annex A, and CORESTA recommended Method No.25.

The TSI 1640 was selected as the most suitable measurement device available for the measurement of air flow velocities in the smoke hood and extraction/ducting systems of smoking equipment.

The VMD100 Digitiser has been specially designed to be programmed with the calibration coordinates of the TSI 1640 providing accurate digital measurements of air flow velocity.

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TSI 1640 Omnidirectional Air Flow Velocity Meter

The Model TSI 1640, Omnidirectional Air Velocity Meter was selected for the measurement of air flow velocities in the smoke hood and extraction/ducting system for the following reasons:

- It is a battery powered, portable unit, with mains battery charger.
- The TSI 1640 scale ranges are:
 - 0.00 - 300 mms^{-1} For Smoke Hood Velocities.
 - 250 - 1200 mms^{-1} } For Extraction/Ducting Velocities.
 - 1000 - 3000 mms^{-1} }
- The probe is specially designed for maximum accuracy of low velocity flows.
- Omnidirectional probe designed to measure velocity as independently of direction as possible.
- Provides signal averaging. The thermal capacitance of the copper sensor ball approximates a time constant of about 2s.
- The accuracy of measurement is +/- 2% of full scale deflection.
- Each sensor is individually calibrated with a system that has been verified using Laser Doppler Velocimetry.
- A certificate of traceability to the National Institute of Standards & Technology, Maryland, (USA), is supplied with each probe.

VMD 100 Airflow Measurement Digitiser/Results Plotter.

- Purpose designed to interface directly with the analogue output of the TSI 1640.
- Calibration coordinates can be re-programmed by user.
- The TSI 1640 is specially calibrated in a TSI wind tunnel. The calibration coordinates obtained are programmed into the VMD 100 which provides a clear and simple display of flow velocity in mms^{-1} .
- The flow integration time in the VMD 100 is selectable from 10 to 120s in 10s steps.
- A serial output printer port is provided which gives a formatted report of elapsed time, average velocity and a graphical plot of flow variation.
- Mains powered 110V; 60HZ & 220V; 50HZ.

Product Ordering Description/Code:

TSI 1640 (110V; 60HZ) — Stock Code: 64054
TSI 1640 (220V; 50HZ) — Stock Code: 64053
VMD 100 — Stock Code: 91580

Although the information in this publication is given in good faith, our policy of continuous product improvement means that we reserve the right to alter specifications without notice.



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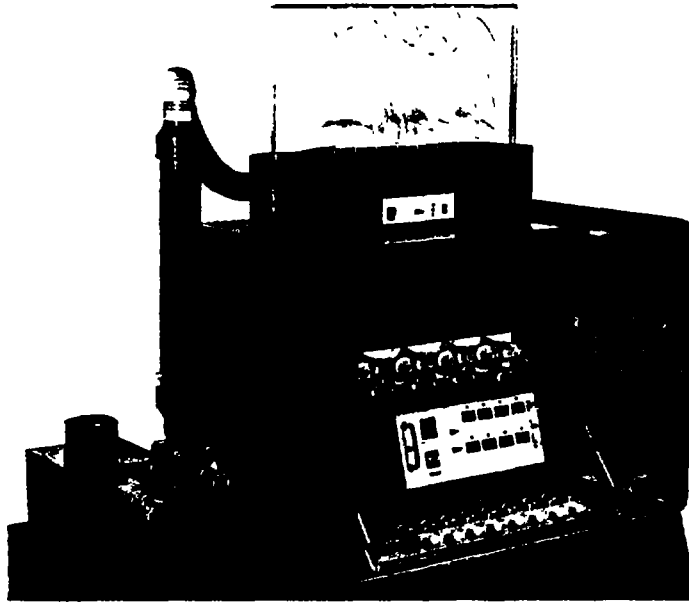
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SM342 Eight Channel Harmonised Smoking Machine



SM342 shown with vapour phase attachment (COM302) mounted on top of the unit.

Conforms to ISO 3308; 1991 and Coresta Methods.

- ▷ Simple adjustment of flow at cigarette level with single ball valve.

Easy installation and operation — no need for special laboratory enclosures.

Extended butt length adjustment.

Automatic lighting sequence — ideal for routine smoking.

The Filtrona Model SM342 is an eight channel smoking machine designed for the collection of particulate matter from cigarette or cigar smoke and the collection of vapour phase.

This instrument is designed to have minimum dead volume for enhanced vapour phase measurement; i.e. total or puff by puff measurement of CO, or puff by puff measurement of CO and/or NO

Volume, duration and frequency of puff can be varied to suit individual requirements. It has a motor driven lighter ignition system.

SM304 Up-Grade Packages are available to convert existing SM302's in the field to the new harmonised standard.

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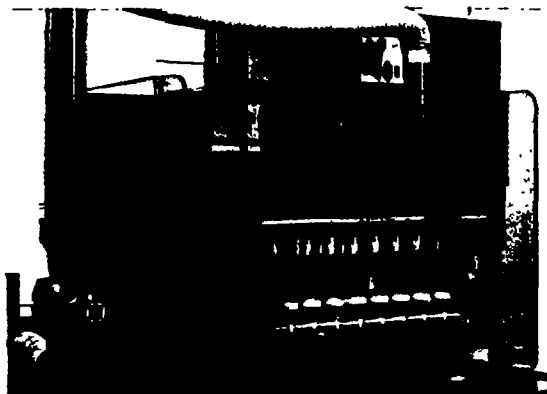
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SM342 Smoking Machine



A special feature of the SM342 Smoking Machine and SM304 Up-Grade Package is the new base assembly, which enables the Smoking Machine to rotate, providing ease of access to the rear of the unit for the adjustment of the positive displacement pistons and for routine maintenance.

Specification for SM342 (and SM302 up-grade to SM304 standard)

Number of smoking channels:	8
Type:	Restricted smoking circuit comprising separate volumetric displacement pump and change-over solenoid.
Collection media:	
Particulate matter:	Glass fibre pad (Cambridge filter)
Vapour phase:	Collection bags (one per channel using COM 302) and suitable analysers connected to sampling system.
Dead volume:	Not more than 5ml per channel, measured from the front face of the inlet port to the top of the syringe.
Puff volume:	Normally set to 35 ml. (variable over the range 20—40 ml)
Puff duration:	Normally set to 2.0s (variable from 1.6 to 6.0s).
Puff frequency:	Normally set at 1 per 60s (variable from 10 to 999s).
Sample Range:	
Cigarettes:	
Length:	66—120 mm (85 mm burn length).
Diameter:	4.5—9.5 mm
Cigars:	
Length:	66—120 mm (85 mm burn length).
Diameter:	With alternative holder, any diameter up to 19 mm.
Mains services (operating voltages):	110/115/220/240 V; 50/60HZ.
Dimensions (bench area required):	
Width:	1500 mm
Depth:	850 mm
Net weight installed:	100 Kg.

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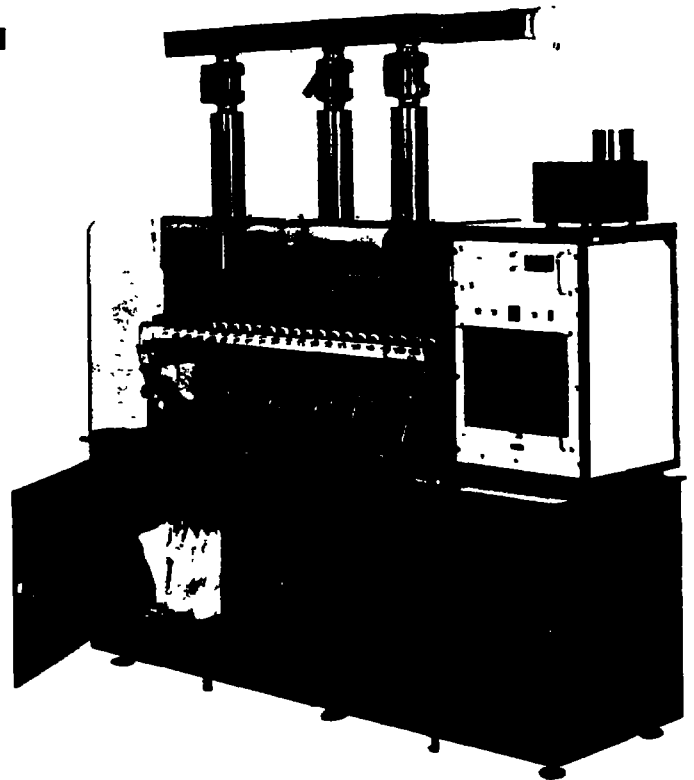
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SM400 Twenty Channel Harmonised Smoking Machine

- Conforms to ISO3308-1991 and CORESTA Methods.
- Improved operator access by the use of a smoking bar which moves forward for easy cottoning and loading.
- Computer controlled, high torque motor drive system produces excellent puff profiles.

With the new design of hood and versatile ducting arrangement, the air flow at the cigarette position is adjustable to meet the new ISO standards.

- SM400 upgrade packages are available for the earlier SM350 and SM300 Smoking Machines.



The Harmonisation Task Force of the CORESTA Smoke Study Group has been working to develop one set of standard methods which may be used worldwide.

Part of the work has been concerned with the control and standardisation of the air flow at the cigarette smoking position, and FILTRONA'S participation in this work has led to the introduction of a new smoking machine — Model SM400.

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SM400 Smoking Machine

<i>PRODUCT CODE</i>	<i>DESCRIPTION</i>
SM400	Harmonised smoking machine complete with new style (SMK401) 'operator friendly' CF Adjustors.
SM435	Up-grade package for SM350
SM430	①Up-grade package for SM300

Both up-grade packages consist of:

- SM400 Harmonised Smoke Hood.
- Extraction Ducting System.
- First Stage Extraction Fan.
- CF Adjustor (old style) Retaining Kit.
- Air Velocity Setting Jig.
- Installation Instructions.
- Air Flow Setting Procedures.

OPTIONS:

SMK401	New butt length and eccentricity adjustors (set of 20), for SM350 smoking bars only (93mm cotton pillars/micro switch arms.)
TSI 1640	Air Velocity Meter.
VMD100	Air Velocity Digitiser and Plotter

Note 1: The installation of a new smoking bar is recommended.

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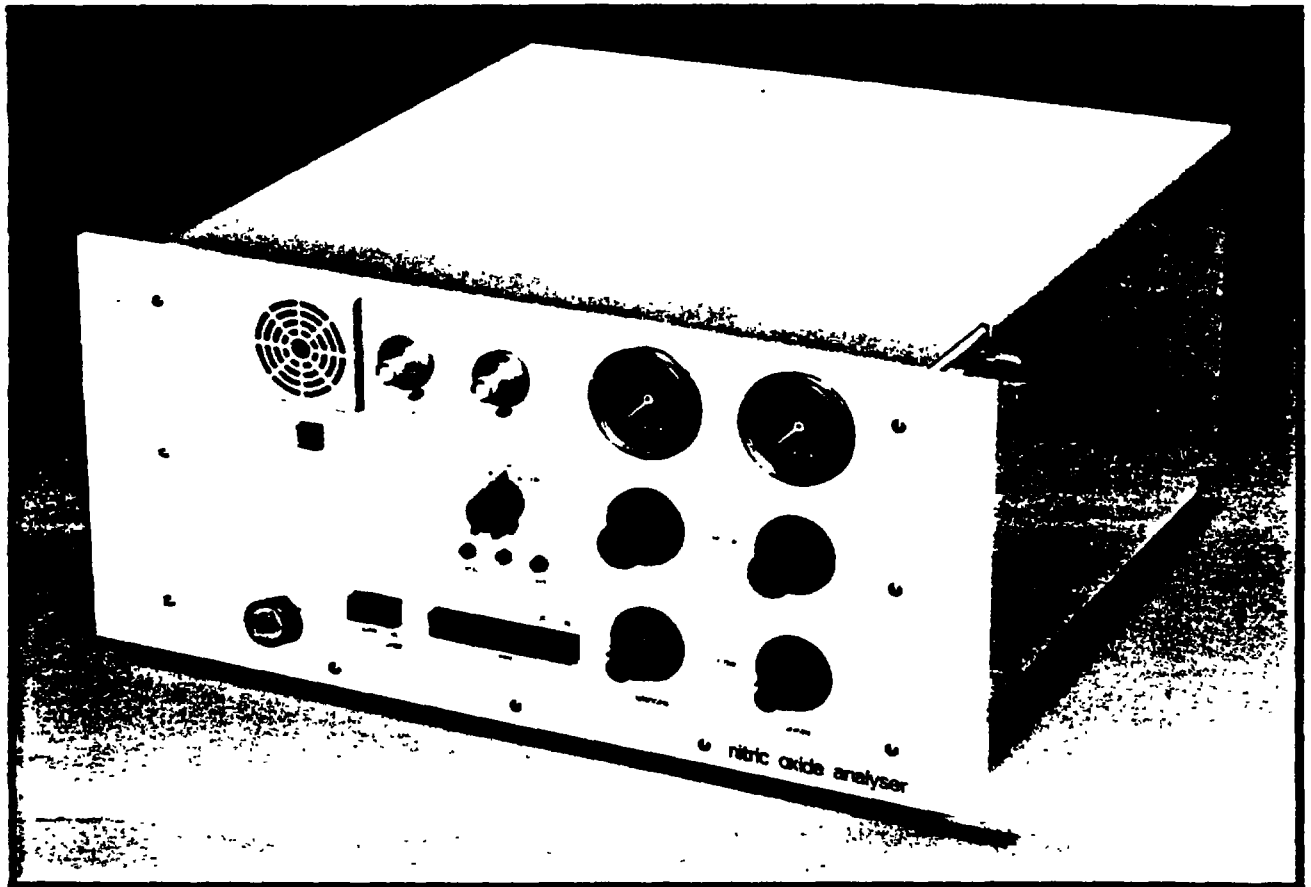
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Nitric Oxide Analyser



Nitric Oxide Analyser (NOA 100)

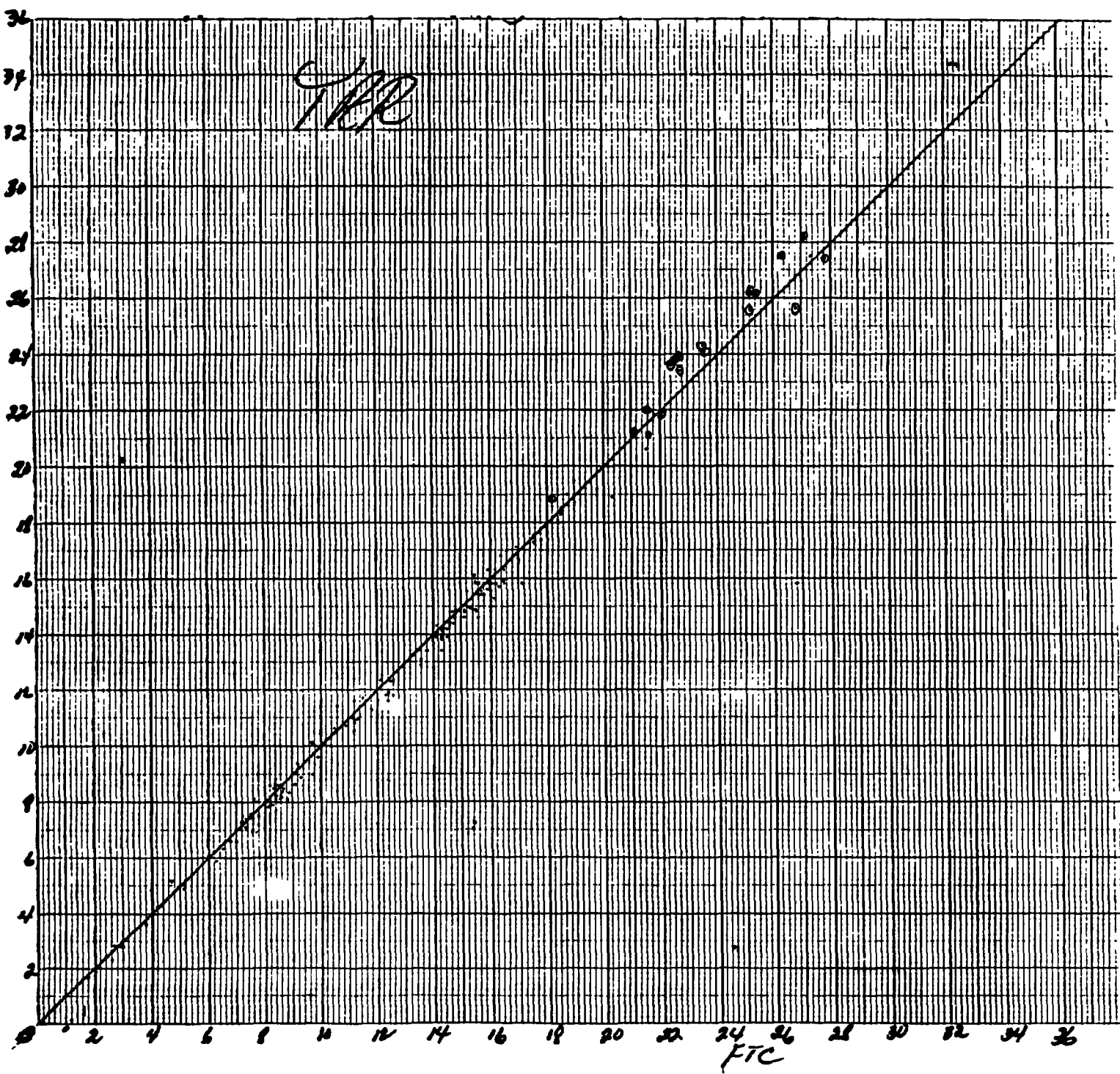
Analysis of Nitric Oxide in Cigarette Smoke

Nitric oxide is a physiologically important constituent of the vapour phase of cigarette smoke, and its measurement may become the subject of future legislation. However, one of the problems of isolating and analysing nitric oxide in cigarette smoke is that it reacts rapidly with other smoke constituents and atmospheric oxygen to give other oxides of nitrogen. It is therefore essential that samples are analysed on a puff-by-puff basis and are diluted with an inert gas in order to prevent secondary reactions and interference from other smoke constituents.

The Filtrona Nitric Oxide Analyser is connected to a suitable smoking machine and automatically measures the amount of nitric oxide on a puff-by-puff basis.

Benefits

- * Designed to work with Filtrona Model 302 Smoking Machine
- * Can be used with other piston-operated smoking machines with low dead volume.
- * Puff-by-puff dilution with nitrogen prevents secondary reactions.
- * Measurement by chemiluminescence technique which is specific for NO and does not require skilled operators
- * High-sensitivity photo-multiplier measures a wide range of NO concentrations
- * Automatic operation
- * Built-in ozone generator
- * High-quality flow controllers with clear vernier dials
- * Easy calibration



Correlation Between Tar Levels Recorded in FTC Test and a Private Laboratory

Principle of operation:

The measurement technique used is chemiluminescence, which is a widely accepted method for the analysis of nitric oxide in cigarette smoke. The principle is to react the sample of cigarette smoke with ozone and to observe the photo-emission using a photo-multiplier tube behind a dark-red optical filter. This reaction has a direct relationship to the quantity of nitric oxide

present in the smoke. The photo-multiplier signal is processed and displayed on a chart recorder.

Interference from other smoke constituents, and secondary reactions, are avoided by dilution of the smoke sample with a large volume of nitrogen before reaction with the ozone.

Method of operation:

NOA 100, developed by British-American Tobacco Company in consultation with other UK tobacco companies, is designed for automatic sampling of vapour phase with Filtrona Model 302 8-channel Smoking Machine. It can also be used manually with some other piston-operated smoking machines.

After each puff, the vapour phase is exhausted from the smoking machine into a gas sampling valve in the analyser. Nitrogen is fed into the sampling valve and sweeps the sample into the reaction cell. Ozone is there added to the sample, and the photo-multiplier tube measures the resulting photo-emission. Results are displayed on a chart

recorder, and the readings are compared with those obtained from standard mixtures of nitric oxide in nitrogen. This gives the concentration (volumetric parts per million) of nitric oxide, and a simple calculation is used to convert to delivery in μg per cigarette.

By attachment of a simple valving system, that part of the vapour phase not required for NO analysis can be transferred to a Filtrona CO analyser, for simultaneous analysis of NO and CO of the same sample.

Note that this instrument must be used with a chart recorder. This must have a response time of better than 0.6 seconds for full-scale deflection.

Specifications

Range	up to 5000 volumetric parts per million (VPM)
Attenuation settings	1, 2, 5, 10, 20, 50 (low, medium and high range for each setting)
Sensitivity	1 VPM NO
Linearity	$\pm 1\%$
Accuracy	$\pm 1\%$ of full scale after calibration
Reproducibility	$\pm 1\%$
Nitrogen supply pressure (external)	4 bar (60 psi) (Nitrogen to be supplied by user)
Oxygen supply pressure (external)	1.4 bar (20 psi) (Oxygen to be supplied by user)
Gas connections	1/8 inch Swagelok
Electrical supply	220-240V 50Hz supply (standard); versions for other supplies at extra cost
Dimensions (mm)	Width 483 (rack or case mounting) Depth 495 Height 223
Weight	20kg
Output	An external socket is provided for connecting to a 1 mV fast-response chart recorder. This can be supplied as an optional extra (see below)
Standard Equipment	All electrical and mechanical fittings needed to connect NOA 100 to a Filtrona 8-channel Smoking Machine (SM 302) are supplied.
Optional Extra Equipment	A suitable chart recorder can be supplied if required (Order Code NOA 10)

The logo for Filtrona, featuring the word "filtrona" in a bold, lowercase, sans-serif font. The letter "o" is replaced by a stylized circular graphic consisting of concentric circles and a central dot, resembling a target or a filter.

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Chapter C

**ASSESSING CHANGES IN TOPOGRAPHY (INHALATION PROFILE)
AND BIOLOGICAL EFFECTS OF TOBACCO SMOKE IN HUMANS**

David M. Burns, MD

University of California at San Diego Medical Center

**Under Contract to the U.S. Consumer Product Safety Commission
#CPSC-S-92-5475**

16 Mar 1993

INTRODUCTION

The most useful approaches in the evaluation of differences in risks between presently marketed and low-ignition-potential cigarettes focus on chemical analyses of the cigarette smoke, measures of smoke absorption, and assays in biological systems. Because of practical constraints, human epidemiologic studies would be of very limited practical value. Although the ideal database would derive from prospective human epidemiologic studies, at least twenty years of observation would be needed to collect sufficient data on seriously adverse health effects. Also, if small differences in toxicity are expected, then large sample populations would be needed.

The toxicity of cigarette smoke is a function of the toxic constituents present in the smoke, the levels of the constituents in the smoke, and the dosage of the constituents to the smoker. Thus these data must also be collected for the evaluation of the relative risks of low ignition-potential cigarettes in comparison to current brands.

Smoke production, as quantified by tar yield, varies substantially among the current cigarette brands. It also varies for a single brand when different patterns of inhalation are used. Moreover, the relative concentrations of toxic constituents also vary with brand of cigarette and pattern of inhalation, at least as measured by tar and nicotine yield. These differences are related to the health risks among different brands of cigarettes (DHHS 1981). Since it is possible that ignition-potential reducing designs in cigarette manufacturing might quantitatively and qualitatively alter the smoke produced, there is concern that the health risks might be increased.

The inhalation profile of a smoker as he or she smokes a cigarette is termed "topography". Ignition-potential reducing designs may alter the topography in ways that lead to greater inhalation and retention of the smoke. Differences in the depth and pattern of inhalation may change the amount of smoke that is deposited and retained in the airway. Therefore, machine generation of smoke from low ignition-potential cigarettes for testing should reflect the human patterns of inhalation for the specific brand of cigarette. This will ensure that the smoke being tested is similar in composition to that being inhaled by human smokers.

Assessment of differences in the risks of smoking low ignition-potential cigarettes in comparison to current brands of cigarettes should address:

- Differences in chemical composition of mainstream and sidestream smoke produced by these cigarettes

- Differences in the amount of the mainstream and sidestream smoke produced by these cigarettes
- Differences in the amount of smoke inhaled and retained by smokers, and
- Toxicity of the smoke produced by these cigarettes, as tested in biological systems.

FACTORS INFLUENCING SMOKE YIELD AND COMPOSITION

Different brands of cigarettes currently manufactured in the US vary markedly in yields of tar and nicotine (DHHS 1981) when smoked using the standard puff profile developed by the Federal Trade Commission (Chapter B). When the same brand of cigarette is smoked using different puff profiles, the yields of tar and nicotine also vary substantially (Zacny 1992). Individuals smoking the same brand of cigarette may inhale using markedly different patterns (Nil 1989). When smokers of a high yield cigarette switch to a lower yield cigarette, their inhalation pattern often changes (Kolonen 1991; Woodman 1987; Guyatt 1989). Interactions have been demonstrated between the yield of a cigarette and the pattern with which the smoker smokes the cigarette (DHHS 1988; Benowitz 1983; Kolonen 1991; Hofer 1992). These changes in yields and smoking patterns should be considered during the evaluation of the health effects.

A number of the manufacturing changes under consideration in the effort to reduce the ignition potential of cigarettes (e.g., higher porosity paper, less densely packed tobacco, different tobacco blends) may alter the amount and chemical composition of the smoke produced (Gann 1991), potentially changing its toxicity. The same changes in cigarette manufacturing processes may also alter the pattern of inhalation of the cigarette (Bridges 1990; Kolonen 1991; Armitage 1988). This, in turn, may change the chemical composition of the smoke (Kozlowski 1988; Fischer 1989), influence the retention of toxic and carcinogenic compounds from the smoke in the lungs of smokers (Zacny 1992; Hofer 1991; Battig 1982; Bridges 1986), and alter the composition and toxicity of the environmental tobacco smoke (Adams et al 1985).

A single set of machine smoking parameters, such as the current FTC protocol (Chapter B), could be followed for the generation of cigarette smoke for testing. However, this single set would ignore possible differences in patterns of smoking (and resultant constituent yield) of low-ignition potential cigarettes. For example, a drop in the draw resistance of a cigarette may lead to a puff volume greater than that specified in the FTC protocol. The larger puff volume could then lead to a deeper inhalation of the smoke and a greater fraction of the total particulate matter being deposited in the lung.

The complexity of the interaction of smoke yield and pattern of inhalation suggests that chemical and biological approaches are needed. Chemical analyses of the differences in whole smoke exposure of the smoker can be assessed from measures of the amount and composition of the smoke produced when cigarettes are smoked by machine using a variety of inhalation patterns, and these quantitative estimates can be compared to measures of the absorption of smoke constituents obtained from human smokers of these brands of cigarettes.

Biological assays of relative carcinogenicity and toxicity of the smoke produced by low ignition-potential cigarettes can be accomplished using a combination of chemical analytic techniques to measure the relative yields of individual compounds produced by different cigarettes and bioassay techniques to assess the relative toxicity of the smoke produced.

The toxicity of cigarette additives is of concern. A new additive, or its pyrolysis products, could increase the known toxicity of the smoke. It might also cause toxicities that are qualitatively different than those presently associated with cigarette smoke. Direct toxicity testing of additives and their combustion products should also be required.

The following section describes how smoking patterns can be measured and explores what is known about the variation in topography of smoking among smokers of the same type of cigarette, among smokers of cigarettes with different yields and among those who switch to cigarettes with different yields. A subsequent section defines what is known about the absorption of smoke constituents; and finally, an approach will be recommended for use in assessing the changes in risks and exposures that may occur with implementation of the proposed technologies to reduce the ignition potential of cigarettes.

PATTERN OF SMOKING

The first step in the process of assessing the relative risk of low ignition-potential cigarettes would be to establish how the patterns of smoking differ for low ignition-potential cigarettes compared to current cigarette brands. The major determinants of disease risks from smoking are the duration of smoking and the intensity of smoke exposure (DHHS 1982; 1983; 1984). Several measures of the intensity of smoke exposure correlate with increased disease risks, including: number of cigarettes smoked per day (DHHS 1982; 1983; 1984), depth of smoke inhalation (DHHS 1982), and tar and nicotine content of the cigarette (DHHS 1981). Each of these measures of intensity of exposure might change when a smoker switches from smoking conventional brands of cigarettes to low ignition-potential cigarettes.

Topographical characteristics, such as puff volume, draw rate, puff duration, and draw pressure differ between smokers and may alter the composition of the smoke. Smokers differ in the number of puffs per cigarette, length of time between puffs, depth of inhalation, and holding of the puff in the mouth before inhaling (Nil 1986; Guyatt 1989; Bridges 1990; Russell 1982; Battig 1982; DHHS 1988). Ignition potential-reducing changes in the blend or amount of tobacco in a cigarette, packing density of the cigarette, and porosity of the paper wrapper are changes under consideration (Gann 1988) and may also alter the topography.

The major purpose of examining topography when smoking low ignition-potential cigarettes is to determine appropriate smoking profiles for machine smoking of these cigarettes. Once the range of smoking topography is established for each brand of low ignition-potential cigarette, then the yields and chemical composition of mainstream and sidestream smoke likely to be generated by human smokers can be estimated.

Measurement of Smoking Pattern

Number of cigarettes smoked per day is typically estimated by self-report (by the smoker) through an interview or a questionnaire. This measure has been shown to be closely correlated with risks of serious disease (DHHS 1982; DHHS 1983, DHHS 1984).

Inhalation depth has also been assessed by self-report and is associated with disease risk in most, but not all, epidemiologic studies (DHHS 1982). A number of other methods have also been used to estimate depth of inhalation including measures of chest wall motion with strain gauges, impedance, magnetometers and whole body or inductance plethysmography (DHHS 1988). Depth of inhalation has also been assessed by measurement of blood carboxyhemoglobin to estimate exposure of the lung alveolar surface to the carbon monoxide in smoke (Herling 1988).

Draw characteristics of the topography have been measured using a variety of techniques including self-report and third person observation (Hofer 1991). The most common approach has been to use a flowmeter attached to the butt end of the cigarette as it is smoked (Creighton 1978; Puustinen 1987). This device allows direct measurement of the flow of smoke drawn into the mouth, draw pressure, and flow duration. The flow rate is integrated to calculate puff volume. The inter-puff interval is calculated from the time between periods of flow. The limitation of using a flowmeter is that it is placed between the smoker and the cigarette and may affect the pattern of smoking.

Variation in the Pattern of Smoking with Existing Cigarettes